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Science as a Way of Knowing—Genetics[1]

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SYNOPSIS. This essay is part of the third presentation of an educational project of the American Society of Zoologists. The purpose is to offer suggestions for improving the first-year biology courses in the universities. The method consists of emphasizing the conceptual framework of the biological sciences, showing how scientific information is obtained and evaluated, pointing out the strengths and limitations of scientific procedures, and above all showing the relevance of science for human hopes and well being. This is done annually with a major symposium, an essay distributed at the symposium, a film program, and, finally, the published proceedings, which are widely distributed to scientist-teachers throughout the world. Each year a major topic is considered. In 1983 it was *Evolutionary Biology* and in 1984 it was *Human Ecology*. This year it is *Genetics*.

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[1] From the Symposium on *Science as a Way of Knowing—Genetics* presented at the Annual Meeting of the American Society of Zoologists, 27–30 December 1985, at Baltimore, Maryland.

INTRODUCTION

During the past year the educational establishment has passed from the stage of producing innumerable reports documenting the Decline and Fall of (precollege) Education in America—the Chicken Little Stage—to that of serious attempts at remedial actions. There are moves to increase rigor in the classrooms; a greater appreciation by students that education is a serious and difficult matter; an acceptance that the nation's welfare depends on drastic and dramatic educational reform; moves to eliminate the educational fluff that was blown in during the post-war years and to increase the competence and professionalism of the teachers, and the reluctant admission that the schools may be better at education than they are in dealing with social problems that society as a whole has been unable and often unwilling to solve.

In the many discussions and programs concerned with educational reform, the colleges and universities have generally escaped scrutiny and condemnation. This is most surprising, since it is the universities that define the fields of learning, add notably to new knowledge, and educate those who will become the teachers in the schools. Just as parents must assume responsibility for their biological children, so must the university assume responsibility for those of their educational children who will teach in the schools.

The *Science as a Way of Knowing* project *is* concerned with higher education. It seeks to offer suggestions for improving the introductory courses in biology at colleges and universities and, by extension, to other courses in biology and the sister sciences. Each year a major field of biology is considered. In 1983 it was *Evolutionary Biology*; in 1984 it was *Human Ecology*. This year's topic is *Genetics*. Tentative plans schedule *Developmental Biology* for 1986, *Form & Function* for 1987, *Cell Biology* for 1988, and *Brain & Behavior* for 1989.

Our *modus operandi* consists of organizing a symposium at the Annual Meeting of the American Society of Zoologists where outstanding scientists deal with various aspects of the topic for the year. We also provide those attending the symposium with a long essay (this being an example), present a film program and, finally, the published proceedings are distributed throughout the world.

The relationship between the papers by the symposium speakers and this essay should be noted. The greater length of the essay permits a broader treatment of the conceptual foundations of the topic being considered and the inclusion of more extensive references. The individual papers, while dealing also with broad conceptual schemes, tend to emphasize the current state of knowledge. This dichotomy will be especially noticeable this year. There is so much to cover in the vast field of genetics that the essay will deal only with the fundamental developments up to the early days of molecular genetics when one could conclude that, beyond all reasonable doubt, DNA is the primary substance of inheritance. The accompanying papers of the symposium speakers will carry the analysis to the present day.

There is one notable omission from this year's symposium—a discussion of the genetic basis of development. That topic will be postponed until next year when the topic is *Developmental Biology*.

We must emphasize, once again, that the *Science as a Way of Knowing* project intends to provide *background* materials. Every year we have presented far more material than could or should be included in an introductory course occupying a full academic year. There is a reason for this apparent surfeit. It can be argued, correctly I believe, that there is an inverse relation between what a teacher must know and the length of time that can be allowed for the development of the topic. One must know a very great deal to be able to say properly a very little. That, of course, is the main difficulty and the main challenge for those who teach first-year courses in science.

THE PARAMETERS OF OUR APPROACH

Our title, *Science as a Way of Knowing*, defines our basic approach. We emphasize the conceptual framework of the biological sciences in the belief that the introductory courses will be more effective and satisfying if they deal primarily with ideas and the data supporting these ideas and not

mainly with vocabulary. We seek to show how scientific information is obtained and evaluated—something that citizens of a democracy must come to understand.

While we will be concerned almost exclusively with science as a way of knowing, we must not forget that there are other ways of knowing with great human appeal. In science we seek to understand natural phenomena using data derived from observations and experiments on those natural phenomena. Great pains are taken to exclude rigidity of thought, emotion, acceptance of *a priori* statements, personal opinions not based on scientific data, and supernatural explanations. Ideally we seek to believe only what nature tells us, not what we might wish to believe for personal, religious, political, or other reasons.

The power of the scientific way of knowing is that whatever answers are obtained must be verifiable by all other scientists with equal wisdom, skill, and open-mindedness. Thus the procedures of science are self-correcting.

This way of knowing is to be contrasted with that of philosophy, religion, and many of the humanistic disciplines where opinion often takes the place of verifiable conclusions. But having said that, we must recognize that many people prefer those answers derived from non-scientific modes of thought—as do scientists for many aspects of human life. The contrast between these two modes of thought becomes important when we ask what is the purpose of the answer being sought. Science has proved a powerful device for solving many of the problems that stem from the interactions of human beings and the non-human world around them and even some of the problems of the interrelations of human beings themselves. Nevertheless, we cannot look to science to tell us what is good, just, beautiful, or even enjoyable. In many instances, however, we may find that scientific information can help us predict the outcome of various human-based decisions and, once the decisions have been made, scientific procedures may help us to achieve the desired goals.

University courses in science must provide an effective understanding of the strengths and limitations of scientific procedures. The power and possibilities of science are shaping our lives and future prospects more than any other aspect of civilization. We cannot allow its control to reside with a detached elite.

There is wide acceptance that one important goal of education is to give students experience in solving problems. If this is accepted, then it is necessary to deal explicitly with problem solving and not just with solutions.

Our students, the future leaders of society, must come to understand that scientific knowledge is the *sine qua non* for developing new and necessary relationships with the natural world. Our world is no longer boundless of space and resources. We must adjust to living on the interest it provides, not the capital. We must develop this new relationship if humanity is to avoid an unparalleled and possibly terminal disaster.

But above all our students must come to accept science as a powerful device for achieving human goals while realizing that it is both inappropriate and impossible for science to define those goals. Science is an expression of what it is to be human.

In the field of education it is far easier to say what should be done than to do it. You must judge the degree to which this essay, and the symposium of which it is a part, achieve our goals. We will profit greatly from your suggestions and verdict.

A NOTE ON THE REFERENCES

Most of the references are grouped at the ends of the major sections of this essay. I have been able to check nearly all of them but, where this was not possible, there may be errors in the citations. The references are primarily those that are of interest to teachers but they range from those that would be appropriate for first-year students to the fundamental research literature. Those titles identified with an * provide excellent introductions to the topic being considered.

THE CENTRALITY OF GENETIC THEORY

In 1973 Th. Dobzhansky issued his famous challenge to the creationists, "Nothing in biology makes sense except in the light of evolution." True enough, but

there is something even more fundamental from which all the other major concepts of biology are derived.

The fundamental characteristic of life is its ability to replicate almost exactly by transforming the materials and energy of the non-living world into more of itself. Genetics is the field of investigation that seeks to understand this phenomenon of replication and, hence, must be considered basic to all biology. Replication and all other aspects of life are reflections of the structure and functioning of the genetic materials—the nucleic acids. Evolutionary biology is the field that investigates the long-term aspects of replication. Developmental biology is the field of investigation dealing with those aspects of replication that occur within the lifetimes of individual organisms. Systematic biology studies the diversity of life that is a consequence of replication being modulated by the environment over time. Ecology deals with the interactions of the environment with the genetically-programmed individual and groups of individuals. Morphology and physiology are the structural and functional consequences of the activities of the genetic materials at all levels from cell to organism.

Thus, there can be no more fundamental field of biology than genetic replication. Genetics first and foremost, including its long-term manifestation—evolutionary biology—is the integrator of all biological concepts and data.

This concept of the unity of biology based on genetics may be useful if presented to students at the onset of their study of genetics but its full significance will be better appreciated if it is repeated as the course progresses.

Today there are special problems in teaching genetics to first-year students. The science is developing so spectacularly and rapidly that there is a great temptation to present mainly the latest results—when there are so many exciting things to say, it is difficult not to say them. When this is done without first providing students with the conceptual framework of the field, the "latest" may be information that can be memorized but may be impossible to understand or appreciate. What is "old" for scientists can be "new" for students. Thus, learning about sex chromosomes or how the substance of heredity was discovered to be DNA will be heroic, important, and exciting stories to those unfamiliar with how these puzzles were explicated. Or, as J. R. Baker (1955, p. 450) put it:

> In many fields of science we must recognize an embryology of ideas: our modern outlook can only be fully grasped and assessed if we understand the causes that make us think as we do.

This advice of a generation ago is even more important today. The rate of progress in fleshing out the conceptual framework of biology is so great that there is danger that with an overload of information we will forget the conceptual framework itself. Students must not be overstuffed with information and starved for understanding.

WHAT IS THE QUESTION?

Science, as a way of knowing, is a powerful device for gaining an understanding of the natural world. Some will maintain that it is the only source of a systematic, verifiable, and conceptually adequate way of obtaining such understanding. Yet understanding rarely comes from attempts to answer what appear to be the obvious questions. Thus one obtains only a superficial understanding by employing the simple procedures of random observations of natural phenomena. Instead modern science has entered a Golden Age because it seeks answers to specific questions (hypotheses), which often seem trivial and not closely related to the "big questions."

Surprising as it may seem, *one of the greatest obstacles to understanding the natural world is not knowing what questions to ask.* This point can be brought home to students by projecting a color slide of a mountain, for example, and asking those students unfamiliar with geology to suggest some of the scientific questions one might ask about that mountain. Only a genius is likely to suggest anything very profound. A professional geologist would be able to supply much information about the age, composition, and method of formation but very little of the answers could be obtained by observations on that mountain alone. Instead,

understanding would have come from the synthesis of many observations and experiments in the fields of sedimentation, radioactive decay, erosion, vulcanism, chemistry, mineralogy, and plate tectonics.

This point can also be illustrated beautifully by a review of the difficulties scientists had in coming to understand what is involved in inheritance. Genetics, now the most rigorous and conceptually complete field of biology, has reached this stage only in our lifetime. For millennia human beings had no useful answers about inheritance because they were unable to formulate useful questions. In science, useful questions are those that are amenable to observation and experiment and, hence, susceptible of being answered.

Thus, for most of human history, inheritance was no more than a vague principle lacking precise rules and predictive value. Consider, for example, the sorts of data that people were likely to gather. The children of a human couple would, routinely, differ from one another in many ways. Some would be female, others male—a truly profound difference. Unless the children were identical twins, the sibs might differ strikingly in appearance and personality. At times the children would have little resemblance to the parents yet, at other times, there might be a strong familial resemblance. How could the same cause—reproduction by the same parents—lead to such diverse results?

Yet there were some regularities. The children of American Indians, Blacks, Orientals, and Caucasians were observed to have the general characteristics of their race. Until our century, observations on a variety of organisms produced no more precise answers than, only in the most general way, do offspring resemble parents. No precise rules were discovered that related the characteristics of offspring to those of the parents. Those vague answers were all that could be expected from the vague question, "What is the nature of inheritance?" There was no acceptable way to account for the observation that inheritance seemed to consist of the transmission of similarities, differences, and even novelty.

Since the educational value of science lies not only in the information that it provides but also in the manner of obtaining that information, there is value in exploring past attempts to understand the nature of inheritance. As with so many topics in biology, it is convenient to begin with the Greek philosophers. Not infrequently they defined the problem and suggested the main hypotheses that lasted into modern times. We will consider only two: Hippocrates and Aristotle.

HIPPOCRATES

Hippocrates, usually recognized as the Father of Medicine, might also be accepted as one of the Fathers of Genetics. Writing about 410 B.C., he proposed *pangenesis* as an explanatory hypothesis for inheritance. Pangenesis assumes that inheritance is based on the production of specific particles ("seeds") by all parts of the body and on the transmission of these particles to the offspring at the time of conception. Darwin was to adopt a similar explanatory hypothesis long afterwards and pangenesis was to remain the only general theory of inheritance until the end of the 19th century.

One of the observations that led Hippocrates to this belief concerned a race of people, the Macrocephali, who were characterized by very long heads. These long heads were thought to indicate nobility, so parents attempted to mold the soft skulls of their newborn to the desired shape.

> The characteristic was thus acquired at first by artificial means, but, as time passed, it became an inherited characteristic and the practice was no longer necessary. The seed comes from all parts of the body, healthy from the healthy parts and sickly from the sickly. If therefore bald parents usually have bald children, grey-eyed parents grey-eyed children, if squinting parents have squinting children, why should not long-headed parents have long-headed children. (Hippocrates, 1950, p. 103)

Hippocrates was also proposing the concept of the inheritance of acquired characteristics—a point of view that was to be adopted by Lamarck as the mechanism of evolutionary change—a theory accepted by many well into the 20th century.

Hippocrates's hypothesis for inheritance may not appear to be a monumental beginning—but it was. He identified a scientific problem (possibly the most difficult step of any), proposed an explanatory hypothesis, and wrote in a manner that we can understand. For such a scientific analysis to have occurred two and a half millennia ago is quite exceptional. The roots of the way we think about scientific phenomena go right back to the Greeks, even as much of our non-scientific mode of thought can be traced back to the ancient Hebrews (via the Hebrew and Christian bibles).

ARISTOTLE

Aristotle (384–322 B.C.) was active a century after Hippocrates. His *Generation of Animals* deals with problems of both genetics and development. This linking of two such seemingly disparate fields has a distinctly modern ring.

Aristotle assumed that there must be a *physical basis of inheritance* in the "semen" produced by the parents. This point, so obvious to us today, was basic to all future work. Inheritance need no longer be thought of as caused by some vague spirit or emotion but by a *substance* transmitted by the parent or parents. Little was known of the nature of this semen in the fourth century B.C. The modern understanding of gametes dates only to the 19th century A.D. Thus Aristotle used the term "semen" much as we would use the term "gametes," and not as a secretion of males that contains the sperm.

The problem, then, becomes understanding the nature of semen. Aristotle discusses the Pangenesis Theory of Inheritance in such a way that suggests the theory was widespread and probably generally accepted (however he was to reject it). He lists four major observations and arguments that support pangenesis as a probable hypothesis. First, noting that mating (in humans) gives pleasure to the whole body, he reasoned that the whole body must contribute to the semen. Second, there were observations suggesting that mutilations may be inherited. One such case came from Chalcedon (on the Bosporus in present-day Turkey) where a man was branded on the arm and his child, born subsequently, had a defect on the arm. Third, it was commonly observed that the offspring resemble parents not only in general but often in strikingly specific ways. Hence the specific characteristics could be assumed to produce specific substances that become part of the semen. And fourth, if semen for the whole can be produced, there is no reason why the specific parts of the body could not contribute to the semen as well.

Aristotle thought otherwise and rejected pangenesis. He suggested no comprehensive alternative but he did suggest a tentative hypothesis—that turns out to be the way we think today. Noting that children resemble parents not only in structure but also in such features as voice and gait, Aristotle asked how could non-structural features produce material for the semen. (You may find it interesting to ask students to deal with this argument of Aristotle as well as those to follow.) Then, too, babies of fathers with beards and gray hair are not similarly hirsute at birth. It had also been observed that children seem to inherit characteristics of more remote ancestors, who could hardly have contributed to the semen of their parents (how would we handle this observation today?). Thus a woman of Elis (in the northwest part of the Greek Peloponnese) had intercourse with a black-amoor (a term applied to any very dark-skinned individual). Her daughter was white but her grandson was black.

Even more important evidence that refuted pangenesis came from observations of the same general sort that were used to refute Darwin's Theory of Pangenesis more than two millennia later. It was well known that parts could easily be removed from plants, yet these mutilated plants could produce offspring that were perfect and entire. And then there was the awesome argument that if, as in humans, two parents produce the semen with the gemmules for all parts of the body, would we not expect offspring with two heads, four arms, etc.?

These and many other arguments and observations led Aristotle to reject pangenesis and to ask,

> Why not admit straight away that the semen . . . is such that out of it blood and flesh can be formed, instead of maintaining that semen is itself both blood and flesh? (Aristotle, 1943, p. 65)

This tentative hypothesis was to become the way we think today.

This is as far as Aristotle could advance our understanding of inheritance with the data and methodology of his time. He proposed a hypothesis, vague though it was, that in our day has proven to be true beyond all reasonable doubt. This hypothesis was to be the conceptual limit for the next two thousand years. The lack of progress in understanding inheritance was due mainly to an inability to formulate precise questions that could be studied with the available methodology.

After Aristotle

Interest in scientific questions almost ceased in the Western World throughout those long centuries when the Church held hegemony over the mind of man. It was not until well after the Renaissance that observation and experiment were applied in a systematic manner in an attempt to gain understanding of inheritance. Even then progress was exceedingly slow, again because it was impossible to find a productive question.

In the 18th and 19th centuries the standard way to seek information about inheritance was by cross-breeding. Individuals that differed from one another were crossed and offspring were studied. To this day this remains one of the most powerful procedures for obtaining information about inheritance. Nevertheless, it might be interesting to explore with your students what sorts of information could be expected from this approach. Such an exercise will be most valuable if one tries to answer the question, without considering what did in fact happen. It is hard to imagine discovering more than the pre-Mendelian breeders did discover: when differing individuals are crossed the offspring will usually be more or less intermediate or occasionally look more like one parent than the other.

In fact, so little progress was made before the closing decades of the 19th century in understanding inheritance that we may conclude that little of theoretical importance occurred between Aristotle (384–322 B.C.) and Charles Darwin (1809–1882 A.D.).

The Methods of Science

This may seem surprising considering the widespread notion that there are set procedures in science—the scientific method—that, if dutifully followed, will lead inexorably to new discoveries and deeper understanding. These methods were formulated slowly by philosophers over the centuries, but as usual, it will be possible to emphasize the contributions of only a few individuals. We will begin with Sir Francis Bacon (1561–1626), Lord Chancellor of England. As de Solla Price (1975) put it, with deliberate hyperbole, "Francis Bacon plotted the [scientific] revolution and codified the scientific method."

Francis Bacon and The Great Instauration

From Bacon on, biology lessened its emphasis on field work and natural history and increased its emphasis on laboratory observations and experimentation. There was much interest in the nature of science and in what came to be known as "the scientific method" for gaining knowledge of the natural world. The essence of the scientific method was its rejection of the classical and medieval-theological habit of starting the inquiry with a point of view that was accepted as true and then deducing the consequences. For example, the acceptance of the Judeo-Christian God as the creator of the universe and all its inhabitants provided a world view that had been accepted as adequate for centuries—and remains so for many today. It led to a very different view of nature than the one provided by modern science.

A diametrically opposed point of view began to develop as the Scientific Revolution emerged during the 16th and 17th centuries. Bacon's suggestion was to begin with data, not faith. That is, one should consider all known facts related to some

natural phenomenon and try to formulate general principles to explain the facts. This logical method of reasoning from the particulars to the general is known as induction—a procedure that was to give us the modern world but its adherents were usually found offensive by those who preferred the established traditions of society, church, and state.

Bacon's suggestions for doing science are described in his *Instauratio Magna* of 1620. This was planned as a multi-volume work but only a small portion of it was published, the most notable being the *Novum Organum or True Suggestions for the Interpretation of Nature.* Even this was a preliminary abstract and consists of 129 aphorisms in Book I and 52 in Book II. The old "Organon" (Aristotle, 1955; Ross, 1949) consisted of the logical treatises of Aristotle, the procedures of which Bacon wished to replace.

His argument begins by pointing out the ineffectiveness of earlier attempts to understand nature. Bacon notes that, unless great care is taken, the things that the human mind imbibes tend to be "false, confused, and overhastily abstracted from the facts." In good measure this is a consequence of our observing what we have already assumed to be true. The consequence of this *a priori* approach is that "philosophy and the other intellectual sciences . . . stand like statues, worshipped and celebrated, but not moved or advanced." It is no wonder that our understanding of nature is "badly built up, and like some magnificent structure, without any foundation."

> Though all the wits of all the ages should meet together and combine and transmit their labours, yet will no great progress ever be made in science by means of anticipations [that is, by relying totally on preconceived ideas]; because radical errors in the first concoction of the mind are not to be cured by the excellence of functions and remedies subsequent. (*Novum Organum,* Book I, Aphorism 30)

Thus reliable knowledge of the natural world comes from observing nature herself and not from probing the human mind. Nature was to be the arbiter in Bacon's plan "to commence the total reconstruction of sciences, arts, and all human knowledge"—his "Great Instauration."

Thus one was to begin an investigation by assembling all the data from observation and experiment that related to the topic of the investigation. Great care had to be exercised lest erroneous information be included. That, of course, would lead to erroneous conclusions. Not only must the observations be made as accurately as possible but often "neither the naked hand nor the understanding left to itself can effect much. It is by instruments and helps [for the mind] that the work is done."

Snares of the mind: Idols to be abhorred

The mind must guard against preconceived ideas if observations are to be accurately interpreted. This is extraordinarily difficult to achieve since what we are, think, and do depends so greatly on our acceptance of the belief systems of the society in which we live and of the science that we profess. These belief systems become the idols to which we may submit, and to the extent we do, may lead to erroneous conclusions. Bacon lists four: the Idols of the Tribe, Cave, Market-Place, and Theatre. (Bertrand Russell recognizes still another, the Idols of the Schools [1945, p. 544].)

The Idols of the Tribe consist of the erroneous preconceived ideas and fuzzy thinking common to all human beings.

The Idols of the Cave are the erroneous beliefs of each individual's mind—the individual mind being like an isolated cave. He notes especially how individuals tend to favor their own opinions and discoveries—a serious problem for us to this day.

> Men become attached to certain particular sciences and speculations, either because they fancy themselves the authors and inventors thereof, or because they have bestowed the greatest pains upon them and become most habituated to them. But men of this kind, if they betake themselves to philosophy and contemplations of a general character, distort and colour them in obedience to their former fancies . . . . (Book I, Aphorism 54)

Other Idols of the Cave are an undue reverence for antiquity or for novelty.

> This however turns to the great injury of the sciences and philosophy; since these affectations of antiquity and novelty are the humours of partisans rather than judgments; and truth is to be sought not in the felicity of any age, which is an unstable thing, but in the light of nature and experience, which is eternal .... (Book I, Aphorism 56)

> And generally let every student of nature take this as a rule,—that whatever his mind seizes and dwells upon with peculiar satisfaction is to be held in suspicion .... (Book I, Aphorism 58)

The Idols of the Market-Place are the semantic problems that arise when people try to communicate and use words differently. The words of our language were developed for everyday use and, not infrequently, they are unsuitable or insufficiently specific for use in science.

The Idols of the Theatre, that is, of philosophical systems, consist mainly of adhering to those modes of thought in philosophy and theology where "truth" is deduced from *a priori* premises. He notes, for example, that some have attempted to found a system of natural philosophy (that is, natural science) on the first book of Genesis. He advises, however, that "We be soberminded, and give to faith that only which is faith's."

And there are more general problems, for it is not

> to be forgotten that in every age Natural Philosophy has had a troublesome adversary and hard to deal with; namely, superstition, and the blind and immoderate zeal of religion.

Or, most discouragingly, in schools and universities

> and similar bodies destined for the abode of learned men and the cultivation of learning, everything is found adverse to the progress of science .... But by far the greatest obstacle to the progress of science ... is found in this—that men despair and think things impossible.

How to b a b

After these lengthy discussions of what he regarded as the procedural and philosophical errors of the past that made progress in science difficult or impossible, Bacon introduces his new approach by this charming metaphor.

> Those who have handled sciences have been either men of experiment or men of dogmas. The men of experiment are like the ant; they only collect and use: the reasoners resemble spiders, who make cobwebs out of their own substance. But the bee takes a middle course; it gathers its material from the flowers of the garden and the field, but transforms and digests it by a power of its own. Not unlike this is the true business of philosophy; for it neither relies solely or chiefly on the powers of the mind, nor does it take the matter which it gathers from natural history and mechanical experiments and lay it up in the memory whole, as it finds it; but it lays it up in the understanding altered and digested. Therefore from a closer and purer league between these two faculties, the experimental and the rational (such as has never yet been made) much may be hoped. (Book I, Aphorism 95)

It is not at all obvious, however, how one should try to be a bee. In Book II of *Novum Organum* he provides an example of what he has in mind by an analysis of what could be the true nature of heat. How is one to understand this phenomenon that is a constant feature of our ambient environment?

First, one should assemble all the readily available information about heat (or any other phenomenon to be investigated), so Bacon gives three tables of data. The Table of Existence and Presence enumerates many phenomena associated with heat: rays of the sun, meteors, thunderbolts, volcanic eruptions, flames, sparks, burning solids, quicklime sprinkled with water, horse-dung when fresh, strong wines, some spices, acid poured on the skin, and even intense cold.

A second Table of Deviation and Absences lists phenomena where we might

expect heat but do not find it. For example he notes that although light in the form of the sun's rays are hot, light from the moon and stars is cold. Furthermore, there have been instances where a person's hair is surrounded by flames (now called St. Elmo's Fire) yet the hair does not burn.

The third Table of Degrees or Comparisons of Heat lists instances where the same item may differ in temperature. For example, plants are not warm to human touch but may become so if they are enclosed in a box or allowed to decay. The heat of animals is increased by exercise, wine, feasting, Venus, fever, and pain.

Induction

Now comes the truly extraordinary part of Bacon's analysis. It seems impossible that anyone could consider all these varied, often irrelevant, and dubious bits of data listed in his three tables and, by induction, arrive at an understanding of the basic phenomenon of heat. First he eliminates some possibilities. For example, light cannot be the basis of heat, since light from the moon and stars is not hot even though light from flames and the sun may be. Color cannot be the cause, since a red hot iron and the relatively cool flame of burning alcohol differ so much. Heat cannot be a substance, since iron and other materials may be made hot and not lose substance.

After ruling out many possibilities in this manner, Bacon reaches this astonishing conclusion:

> From instances taken collectively, as well as singly, the nature whose limit is heat appears to be *motion.* This is chiefly exhibited in flame, which is in constant motion, and in warm or boiling liquids, which are likewise in constant motion. It is also shown in the excitement or increase of heat by motion and by bellows and draughts . . . . It is also shown by the extinction of fire and heat upon any strong pressure, which restrains and puts a stop to motion . . . (thus is with tinder, or the burning snuff of a candle or lamp, or even hot charcoal cinders, for when they are squeezed by snuffers, or the foot, and the like, the effect of the fire instantly ceases) . . . . It is further shown by this circumstance, namely, that every substance is destroyed, or at least materially changed, by strong and powerful heat: whence it is clear that tumult and confusion are occasioned by heat, together with a violent motion in the internal parts of bodies, and this gradually tends to their dissolution . . . . It must not be thought that heat generates motion, or motion heat, (though in some respects this be true,) but that the very essence of heat . . . is motion and nothing else . . . . (*Novum Organum,* Book II, Aphorism 20)

The data available to Bacon were wholly inadequate for him to reach what we now accept as the correct view of the nature of heat. Furthermore, induction—the philosophical process that Bacon so valued—could not alone sort among all of Bacon's phenomena related to heat and conclude that heat is a form of motion. In this case a fine mind had made a lucky guess.

Perhaps the greatest weakness in Bacon's system was the lack of a clear indication of how to make the intellectual step from the isolated facts to the general statement. That remains the central difficulty of inductive reasoning to this day. It is here that genius, intuition, inspiration, serendipity, and luck—one or several—must assume control of the analysis.

Two and a half centuries later the great English scientist, John Tyndall (1863), had this to say:

> From the direct contemplation of some of the phenomena of heat, a profound mind is led almost instinctively to conclude that heat is a kind of motion. Bacon held a view of this kind . . . .

But the *sine qua non* is that profound mind.

Induction—hypothesis—deduction

Induction means no more than that one begins a study with observation and experimentation relating to some natural phenomena, and uses the data obtained in attempting to reach some understanding of fundamental causes or associations of seemingly unrelated events. Selected data are used to frame provisional hypotheses and from these hypotheses deductions are

made and tested. Deduction remains a powerful adjunct of analysis but the deduction of modern scientists is not the same as the deductive reasoning that Bacon found so repugnant. In science today deductions from a hypothesis are (hopefully) necessary conclusions from that hypothesis. Their value is to suggest what observations or experiments can be done in order to confirm or deny the hypothesis, nothing more. The deductions of the early philosophers and theologians were often regarded as eternal conclusions drawn from eternal truths, but in reality they were based on shared faith or bold imagination, not on evidence.

To this day scientists strive to start only with the most reliable and confirmable data, and thereafter employ a constant interplay of inductive and deductive procedures to reach a more fundamental level of understanding of the natural world. That understanding can be no more than "this is the most accurate statement that can be made with the evidence at hand." It must be emphasized to students that this does not mean that the science of the day is "wrong." It means that the science of today will be replaced by a better science tomorrow. Our analysis of the development of genetic concepts will provide an excellent example. The genetics of Mendel of 1900 was not wrong. It was expanded in the better genetics of Sutton in 1903 and then that of Morgan in 1912, and finally into the vastly better genetics of today.

Some philosophers of science may maintain that Bacon was seriously inadequate in not appreciating the value of deductive reasoning. To be sure he was not as explicit as the philosophers of today but, considering the pioneer nature of his effort, one can argue that he comes off fairly well. For example,

> The signs for the interpretation of nature comprehend two divisions: the first regards the eliciting or creating of axioms from experiment, the second the deducing or deriving of new experiments from axioms. (*Novum Organum,* Book II, Aphorism 10)

If one substitutes "hypotheses" for "axioms" we find here not only a brief and elementary description of what philosophers of today recognize as important components of scientific methodology but about as accurate a statement as can be made of what working scientists actually do. And far from expecting scientific understanding to come solely from those ants collecting facts, Bacon suggests that with his approach

> we have good reason, therefore, to derive hope from a closer and purer alliance of these faculties, (the experimental and rational) than has yet been attempted. (Book I, Aphorism 95)

The English astronomer-chemist-photographer Sir John Herschel (1792–1871), himself an important student of scientific methods, recognized in Bacon the antecedents of the modern hypothetico-deductive method. Consider this one sentence quote:

> It is to our immortal countryman Bacon that we owe the broad announcement of this grand and fertile principle [induction]; and the development of the idea, that the whole of natural philosophy consists entirely of a series of inductive generalizations, commencing with the most circumstantially stated particulars, and carried up to universal laws, or axioms, which comprehend in their statements every subordinate degree of generality, and of a corresponding series of inverted reasoning from generals to particulars, by which these axioms are traced back to their remotest consequences, and all particular propositions deduced from them .... (Herschel, 1830, p. 104)

The fundamental difference between Bacon's approach and the approaches that he attacked was that scientific statements must be based on the data derived from observations and experiments of natural phenomena and not on preconceived principles, or beliefs of classical authors, or imagination, or superstition. As Bacon advises we

> should not arrogantly search for the sci-

ences in the narrow cells of human wit, but humbly in the greater world.

Thus it is incorrect to say that Bacon believed that induction is the only effective procedure for arriving at acceptable scientific statements. His emphasis on induction was to counter the seemingly total reliance of philosophers and theologians on deductive reasoning from broadly-inclusive *a priori* beliefs. Induction is not an automatic procedure for advancing science. It depends absolutely on the brilliance, perseverance, knowledge, and luck of the scientist. And deduction is an effective and powerful procedure when one uses it to make testable deductions from provisional hypotheses.

The legacy of Sir Francis Bacon, Lord Chancellor of England, is that we must study nature, not books alone, and cease the worship of those four Idols. Scientists of the 17th century—William Gilbert, Andreas Vesalius, Galileo Galilei, Johann Kepler, and William Harvey—were attempting to do these things. Bacon was most valuable in being a publicist and codifer of the Great Instauration—a new way for obtaining reliable information about the natural world.

References to Bacon

The standard edition of Bacon's works is that of Spedding, Ellis, and Heath (1857–1874) but Montagu (1851) is clearer at times. For a Latin edition of *Novum Organum* see also Fowler (1889). Selections, often with notes, are provided by Crombie (1959), Dick (1955), McClure (1928), and Robertson (1905). For evaluations of Bacon see W. T. Jones (1952), Liebig (1863), Macaulay (1837), Randall (1926), Russell (1945), and A. E. Taylor (1927, 1934).

DARWIN AND THE REBIRTH OF PANGENESIS

Darwin is an especially instructive example of a pre-Mendelian attempting to explain inheritance and we can see some of the reasons why so little progress was made. Darwin is recognized, after all, as a person of tremendous ability, not only for his *On the Origin of Species* but for fundamental investigations on a broad range of biological subjects as varied as coral reefs, habits of earthworms, taxonomy of barnacles both living and fossil, and the fertilization of orchids. His attempts to understand inheritance gave us the longest of his works—the two volumes of *The Variation of Animals and Plants under Domestication* (1868).

Initiating the inquiry

Darwin's problem was the same as for all who sought a rent in the veil of ignorance that enmeshed the subject of inheritance: how could one properly initiate the inquiry?

It was exceedingly important that he do so and achieve some level of success. His momentous theory of the origin of species by means of natural selection depended totally on a constant supply of new variants that persisted generation after generation and upon which selection could act. In the absence of new variants, evolutionary change would be impossible. And Darwin notes, "it is obvious that a variation which is not inherited throws no light on the derivation of species, nor is of any service to man" (vol. 2, p. 1; unless noted otherwise all of the references in this section will be to the 1868 edition of *The Variation of Animals and Plants under Domestication*).

Not everyone in the mid-19th century believed that the inheritance of minute differences was either of much importance or subject to rigorous rules. There was no need to do so if one accepted the prevailing and socially-mandated view that each kind of organism had been separately created by the Judeo-Christian God and if one noted the seemingly stochastic nature of inheritance. Possibly the main reason that individuals differed from one another was due to the environment.

The subject was thought fuzzy even by Darwin. He wrote:

> When a new character arises, whatever its nature may be, it generally tends to be inherited, at least in a temporary and sometimes in a most persistent manner. (vol. 2, p. 2)

Darwin had considerable first-hand expe-

FIG. 1. The hand of the Porcupine Man. (H. Baker, 1756)

rience with crossing plants and animals, especially pigeons, and through correspondence and reading he had an extensive knowledge of the results of others. He was convinced that inheritance must be a phenomenon that is widespread, somewhat precise, and important.

The Porcupine Man

Darwin's fine eye for the critical observation or test led him to lay great emphasis on some remarkable examples of inheritance that were so unusual that neither chance nor environment seemed adequate explanations. One of the more spectacular instances was the strange story of the "Porcupine Man" (Fig. 1).

In 1733 Machin reported to the Royal Society a strange case of Edward Lambert, then in his teens. He was the son of a laborer who lived in Suffolk.

> His skin (if it might be so called) seemed rather like a dusky coloured thick case, exactly fitting every part of his body, made of a rugged bark, or hide, with bristles in some places, which case covered the whole excepting the face, the palms of the hands, and the soles of the feet, caused an appearance as if those

> parts were naked, and the rest clothed. It did not bleed when cut or scarified, being callous and insensible. It said he shed it once every year, about autumn, at which time it usually grows to a thickness of three quarters of an inch, and then is thrust off by the new skin which is coming up underneath.

Young Edward seemed entirely healthy and normal in all other respects. His father reported that Edward had normal skin at birth but at about two months it began to change. The baby had not been sick and there was no obvious cause. The mother had received no fright while with child. None of the sibs exhibited the abnormality.

In 1756 H. Baker provided a later report. By then Edward Lambert was a married man. He had one surviving son with the defect. A total of six sons had shown the defect but five had died. Baker reported that when the hand is drawn across the victim's skin it made a rustling noise. Subsequently two of the grandchildren showed the same defect and, according to Darwin, eventually four generations were observed with the defect—always restricted to males (vol. 2, p. 4).

As an aside, it can be noted that the modern medical term for this defect is ichthyosis hystrix gravior. Until recently it was believed that the condition is controlled by an allele on the Y-chromosome, since it was thought to affect males only. Stern reviewed the case and found that there may have been a daughter with the abnormality, hence the case for Y-chromosome transmission is in doubt. See Gates (1946), Stern (1957, 1973), F. Vogel and Motulsky (1979), and especially Penrose and Stern (1958) for illustrations and the account of the effort to discover more about the Lambert family. Thus the evidence available to Darwin may have been faulty in some respects but it was adequate for his purposes, namely to suggest that there is something that is inherited.

What is inherited?

How is one to account for these exceedingly rare events, so atypical of what is usually observed? Was it merely a matter of chance (whatever that might mean) or was it the result of some undetected environmental influence? Darwin regarded this and similar instances as evidence that "something" was transmitted from parent to offspring:

> When we reflect that certain extraordinary peculiarities have thus appeared in a single individual out of many millions, all exposed in the same country to the same general conditions of life, and, again, that the same extraordinary peculiarity has sometimes appeared in individuals living under widely different conditions of life, we are driven to conclude that such peculiarities are not directly due to the action of surrounding conditions, but to unknown laws acting on the organisation or constitution of the individual;—that their production stands in hardly closer relation to the conditions than does life itself. If this be so, and the occurrence of the same unusual character in the child and parent cannot be attributed to both having been exposed to the same unusual conditions, then the following problem is worth consideration, as showing that the result cannot be due, as some authors have supposed, to mere coincidence, but must be consequent on the members of the same family inheriting something in common in their constitution. Let it be assumed that, in a large population, a particular affection occurs on an average in one out of a million, so that *a priori* chance that an individual taken at random will be so affected is only one in a million. Let the population consist of sixty million, composed, we will assume, of ten million families, each containing six members. On these data, Professor Stokes has calculated for me that the odds will be no less than 8333 millions to 1 that in the ten million families there will not be even a single family in which one parent and two children will be affected by the peculiarity in question. But numerous cases could be given, in which several children have been affected by the same rare peculiarity with one of their parents; and

> in this case, more especially if the grandchildren be included in the calculation, the odds against mere coincidence become something prodigious, almost beyond enumeration. (vol. 2, pp. 4–5)

Even today it would be hard to supply better arguments that "something" had been transmitted from Edward Lambert to his sons. It was most unlikely that the skin condition was a consequence of an environmental stimulus or of chance. If there was a physical basis for the inheritance of the porcupine-skin condition and similar variations, it should be possible to discover laws governing their transmission.

Assembling the data

Darwin set about to discover these laws with the accepted procedures of his time but, as we shall see, entirely different approaches would be needed to illuminate that black-box of inheritance. He tells us in his autobiography how he began his great study:

> After my return to England [in 1836 at the end of the voyage of the *Beagle*] it appeared to me that by following the example of Lyell in Geology, and by collecting all facts which bore in any way on the variation of animals and plants under domestication and nature, some light might perhaps be thrown on the whole subject. My first note-book was opened in July 1837. I worked on true Baconian principles, and without any theory collected facts on a wholesale scale, more especially with respect to domesticated productions, by printed enquiries, by conversations with skilful breeders and gardeners, and by extensive reading. (Barlow, 1958, p. 119)

And he did record a prodigious amount of information related to "domesticated productions." Roughly half of *Variation* provides information on the presumed origin of domesticated plants and animals from wild ancestors. It was assumed that this had involved the selection by human beings of the hereditary variations that were thought desirable. Starting with domesticated dogs and cats, he went on to assemble the available data for horses, asses, pigs, cattle, sheep, goats, rabbits, pigeons, chickens, ducks, geese, peacocks, turkeys, canaries, goldfish, honey bees, silk moths, the common cereals, garden vegetables, and fruits. All made sense with the assumption that rapidly-acting artificial selection by human beings was a counterpart of the excruciatingly slow natural selection that accounted for the origin of species.

Then there is a wealth of observations on inheritance in the more restricted sense. The following quotations will show the flavor of the results of his Baconian collection of data.

> Brothers and sisters of the same family are frequently affected, often at about the same age, by the same peculiar disease, not known to have previously occurred in the family. (vol. 2, p. 17)

> A rabbit produced in a litter a young animal having only one ear; and from this animal a breed was formed which steadily produced one-eared rabbits. (vol. 2, p. 12)

> I have been assured by breeders of the canary-bird that to get a good jonquil-coloured bird it does not answer to pair two jonquils, as the colour then comes out too strong, or is even brown. (vol. 2, pp. 21–22)

After your students have mastered Mendelian genetics, it may be interesting to them to suggest hypotheses to account for observations such as the one just given and that on chickens below.

> I have been assured by three medical men of the Jewish faith that circumcision, which has been practiced for so many ages, has produced no inherited effect. (vol. 2, p. 23)

But then Darwin goes on to quote an authority who suggests that there has been an effect.

> Nevertheless, Dr. Prosper Lucas has given, on good authorities, such a long list of inherited injuries, that it is difficult not to believe in them. (vol. 2, p. 23)

> In one lot of eleven mixed [chicken] eggs from the white Game and white Cochin by the black Spanish cock, seven of the chickens were white, and only four black: I mention this fact to show that whiteness of plumage is strongly inherited. (vol. 1, p. 240)

Darwin recorded numerous examples of observations that, in the years after 1900, were to be critical to the advancement of our understanding of inheritance. The next two quotations give accurate descriptions of what came to be known as sex-linked inheritance.

> Colour-blindness, from some unknown cause, shows itself much oftener in males than in females; . . . but it is eminently liable to be transmitted through women. (vol. 2, p. 72)

> Generally with the haemorrhagic diathesis [=hemophilia], and often with colour-blindness, and in some other cases, the sons never inherit the peculiarity directly from their fathers, but the daughters, and the daughters alone, transmit the latent tendency, so that the sons of the daughters alone exhibit it. Thus, the father, grandson, and the great-great-grandson will exhibit the peculiarity,—the grandmother, daughter, and great-granddaughter having transmitted it in a latent state. (vol. 2, p. 73)

The following quotation is of extraordinary interest in describing phenomena that were to become the core of Mendelian inheritance.

> As a general rule, crossed offspring in the first generation are nearly intermediate between their parents, but the grandchildren and succeeding generations continually revert, in a greater or lesser degree, to one or both of their progenitors. Several authors have maintained that hybrids and mongrels include all the characters of both parents, not fused together, but merely mingled in different proportions in different parts of the body; or, as Naudin has expressed it, a hybrid is a living mosaic-work, in which the eye cannot distinguish the discordant elements, so completely are they intermingled. We can hardly doubt that, in a certain sense, this is true, as when we behold in a hybrid the elements of both species segregating themselves . . . . Naudin further believes that the segregation of the two specific elements or essences is eminently liable to occur in the male and female reproductive matter; and he thus explains the almost universal tendency to reversion in successive hybrid generations. (vol. 2, pp. 48–49)

The phenomena to be explained

After this systematic and extensive survey of the information on inheritance, Darwin attempted to formulate a hypothesis to account for all the data. The main classes of phenomena that must be explained by a comprehensive hypothesis of inheritance were as follows.

1. *Some characteristics are inherited.* Most of these inherited features involved structures such as body size, color patterns, and an endless list of minor variations. Physiological characteristics were also inherited—such as color blindness and hemophilia. The inherited characteristic might be large or small, important or unimportant. He concluded, as quoted before, "When a new character arises, whatever its nature may be, it generally tends to be inherited, at least in a temporary and sometimes in a most persistent manner" (vol. 2, p. 2); in short, a capricious phenomenon. Any useful hypothesis would have to explain why features are inherited sometimes but not always.

2. *The inheritance, or not, of mutilations.* Some human societies habitually knock out teeth, perforate ears or nostrils, circumcise male babies, cut off a finger or two, yet their children do not show corresponding defects. There were other cases where it appeared that mutilations were inherited and they were given on such good authority that Darwin found "it difficult not to believe them." Several times Darwin referred to the case of "a cow that had lost a horn from an accident with consequent suppuration, produced three calves which

were hornless on the same side of the head" (vol. 2, p. 23). He concluded, "with respect to the inheritance of structures mutilated by injuries or altered by disease it is difficult to come to any definite conclusion" (vol. 2, pp. 22–23).

3. *Atavism.* This is the occurrence in an individual of some characteristic not expressed in the immediate forebears but believed to have been present in remote ancestors. For example, it was believed that the wild ancestors of the domesticated sheep had been black. Thus, when a black lamb appeared in a flock of carefully bred white sheep, it was explained as the persistence of some long-dormant hereditary feature.

4. *Sex-linked inheritance.* So far as the data went, it appeared that in most cases characters appeared to be inherited with equal facility from either parent. Nevertheless, Darwin knew of some where this was not the case. The examples of color blindness and hemophilia have been given already. Darwin drew an interesting conclusion from these cases, "We thus learn, and the fact is an important one, that transmission and development are distinct powers" (vol. 2, p. 84).

5. *Inbreeding.* If two organisms are crossed and their offspring bred with one another generation after generation, we speak of this as inbreeding. The usual result was the production of a relatively homogeneous population:

> When two breeds are crossed their characteristics usually become intimately fused together; but some characters refuse to blend, and are transmitted in an unmodified state either from both parents or from one. When grey mice are paired, the young are not piebald nor of an intermediate tint, but are pure white or of the ordinary grey colour . . . . In breeding Game fowls, a great authority, Mr. J. Douglas, remarks, "I may here state a strange fact: if you cross a black with a white game, you get both breeds of the clearest colours." Sir R. Heron crossed during many years white, black, brown, and fawn-coloured Angora rabbits, and never once got these colours mingled in the same animal, but often all four colours in the same litter. (vol. 2, p. 92)

Once again, the data of inheritance seemed to conform to no obvious rules or regularities. Any comprehensive hypothesis to explain the data would have to be adjusted to this difficult fact.

6. *Artificial selection.* Selection, either deliberate or unintentional, is a method that produces varieties of plants and animals of greater usefulness to human beings. It has been employed since the earliest days of agriculture. If a farmer desires to increase the size of chickens, and hence the quantity of food, he selects the largest hens and cocks to be the parents. In each generation he continues the same selection. With this procedure it is usually possible to develop animals or plants with the desired characteristics in a few generations. One of the most puzzling aspects of selection was the ability to produce individuals with characteristics not present in the ancestral population. For example, Darwin's favorite material, pigeons, had been selected to produce the most unusual breeds—entirely different from the ancestral rock dove of Europe. Selection could create something new. It was clear that artificial selection could, in a few generations, produce varieties that differed as much from one another in structural details as did the various wild species of the same genus or even species of different genera.

7. *The causes of variability.* "The subject is an obscure one; but it may be useful to probe our ignorance. Some authors . . . look at variability as a necessary contingent on reproduction, as much an aboriginal law, as growth or inheritance" (vol. 2, p. 250). Darwin believed that all domestic and wild species are variable. The differences are especially obvious in the domestic species where many unique varieties have been selected (Darwin found a report in the literature of a Dutch florist who kept 1,200 varieties of hyacinth). "Changes of any kind in the conditions of life, even extremely slight changes, often suffice to cause variability. Excess of nutriment is perhaps the most efficient single exciting cause" (vol.

2, p. 270). The kind of variation depends "in a far higher degree on the nature or constitution of the being, than on the nature of the changed conditions" (vol. 2, p. 250). (This last quote is one of numerous examples of the uncanny ability of Darwin to rise above the confusion of his time and see clearly what future research would establish.

8. *Regeneration.* When the tail or legs of salamanders are cut off, the lost structures are replaced. The ability to regenerate lost parts is common in many animals and plants and the events often resemble those in embryonic development. Darwin realized that both the formation of structures in development and the replacement of lost parts must have a hereditary basis since in both phenomena the final structures were characteristic of the species.

9. *Mode of reproduction.* Some organisms, such as *Hydra,* reproduce by asexual and sexual means. The *Hydra* that develops from a fertilized ovum is identical with that originating from an asexual bud. Thus, what is passed from parents to the offspring could not be restricted to the eggs and sperm—the ordinary cells of the *Hydra*'s body wall that formed the new individual asexually could also transmit the hereditary information. It is noteworthy that Darwin realized that any comprehensive theory of inheritance would have to account both for regeneration and the various patterns of development, including those species where the life cycle consists of two or more different stages.

10. *Delayed-action inheritance.* The details of fertilization in plants were poorly understood in Darwin's time and he was puzzled by the fact that the pollen affected not only the "germ" but some of the material tissues as well.

> If we could imagine the same flower to yield seeds during successive years, then it would not be very surprising that a flower of which the ovarium had been modified by foreign pollen should next year produce, when self-fertilised, offspring modified by the previous male influence. (vol. 1, p. 403)

Then there was the case of Lord Morton's Arabian chestnut mare. This mare was crossed to a species of zebra, the now extinct quagga. The foal of this union was intermediate in form and color. The mare was then sent to another farm where she was bred with a black Arabian stallion. There were two offspring.

> These colts were partially dun-coloured, and were striped on the legs more plainly than the real hybrid, or even than the quagga. One of the two colts had its neck and some other parts of its body plainly marked with stripes. Stripes on the body, not to mention those on the legs, and dun-colour, are extremely rare . . . . But what makes the case still more striking is that the hair of the mane in these colts resembled that of the quagga, being short, stiff, and upright. Hence there could be no doubt that the quagga affected the character of the offspring subsequently begot by the black Arabian horse. (vol. 1, p. 404)

There would certainly be a great deal of doubt about this observation today and, if true, the explanation would be entirely different. Darwin regarded these examples of delayed-action inheritance as "of the highest theoretical importance" and they, as much as anything else, were to be the cause of his flawed hypothesis for inheritance.

Formulating the hypothesis by induction

Any useful hypothesis to explain inheritance would have to account for the ten classes of data just enumerated. No obvious hypothesis emerges from the data, so one must engage in an exercise not unlike Bacon's arranging his data in three tables, eliminating some of the data, and of formulating the hypothesis from what remained.

It should be an interesting experience to have your students see what tentative conclusions they could reach solely on the basis of the ten classes of observations just given. Here are examples of how one might reason.

A. Since there are so many observations showing that offspring may resemble parents not only in general features but at times in very specific characteristics, one

must conclude that there is some physical basis for inheritance. This is suggested by class 1 above and is not negated by any of the other nine classes of data.

B. Since the only physical link between generations of those organisms that liberate eggs and sperm that unite outside of the body are these gametes, all of the hereditary factors must be contained in the gametes.

C. The gametes cannot be the sole possessor of the hereditary factors, since in some organisms apparently identical offspring can be produced by sexual and asexual means (class 9).

D. The observations on the regeneration of lost parts (class 8), together with point C, suggest that many (most?, all?) cells of the body contain all of the hereditary factors.

E. The hereditary factors may be present but are not expressed either on a short-term basis (parents not exhibiting the features while grandparents and grandchildren do) or on a long-term basis (atavism, class 3). This strongly suggests that the hereditary factors are relatively permanent and stable even when they are latent.

F. The hereditary factors may change or entirely new ones may be formed, as in cases of the sudden appearance of entirely new variations.

G. Since the hereditary factors are present generation after generation, there must be some mechanism for their replication.

H. The hereditary factors may act in a manner similar to infectious agents in that those of one individual may invade the cells of another—as with Lord Morton's mare (class 10).

Thus we may tentatively conclude that there are hereditary factors and that these are present in at least many of the cells; they may be transmitted via the gametes, may be expressed or remain dormant in a given generation, can persist unchanged for generations, may change under some unknown conditions, and are capable of increasing in number. All of this agrees with what we know today about genes.

Class H, however, is clearly not part of modern genetics—the hereditary factors, the genes, of higher plants and animals do not normally go wandering around the body. We now know that the case was misinterpreted—there was no contamination of Lord Morton's mare by the semen of the quagga. Similar barring was found to occur in the offspring of Arabian and English race horses (Ewart, 1901; Burkhardt, 1979; Gould, 1983, ch. 30). Darwin was unaware of this and believing the report to be true was one important factor in making his hypothesis defective.

The hypothesis of pangenesis

How could one unite the heterogeneous data on inheritance into a single conceptual scheme? Darwin made the attempt.

> As Whewell, the historian of the inductive sciences, remarks:—"Hypotheses may often be of service to science, when they involve a certain portion of incompleteness, and even of error." Under this point of view I venture to advance the hypothesis of Pangenesis, which implies that the whole organization, in the sense of every separate atom or unit, reproduces itself. (vol. 2, pp. 357–358)

Darwin calls these minute units of reproduction the gemmules. Gemmules were assumed to possess the following characteristics: each and every part of an organism, and even parts of cells, were assumed to produce gemmules of specific types. These were capable of moving throughout the body so that all parts of the body, including the eggs and sperm, would contain gemmules of all types, *i.e.*, they would contain all of the hereditary factors. During development the gemmules unite with one another or with partially formed cells to produce new cells of the sort that had produced them. New gemmules were assumed to be produced continually. Gemmules were usually active in the offspring but they might remain dormant for generations.

Explaining the data

It was possible to explain each of the ten classes of phenomena that required explanation with Darwin's hypothesis of pangenesis as follows.

1. The transmission of characteristics

from parent to offspring was explained as a consequence of the production of the specific gemmules in the parental body, their incorporation in gametes, and their development in the offspring. Edward Lambert's skin cells had produced porcupine-skin gemmules and these were passed to his offspring via his sperm.

2. Mutilations are usually not inherited since gemmules for the normal structure would have been produced before the mutilation. Thus, the regeneration of a salamander's leg is possible because the leg gemmules were already present throughout the body, and after amputation could be assembled to produce a new leg. The few cases in which mutilations appeared to be inherited seemed to involve diseased parts. Darwin explained these cases as follows:

> In this case it may be conjectured that the gemmules of the lost part were gradually all attracted by the partially diseased surface, and thus perished. (vol. 2, p. 398)

3. Atavism was explained as a consequence of long-dormant gemmules becoming active after the passage of many generations. This was an especially gratuitous assumption. No more is being said than the following—since some characteristics seem to reappear in a lineage after not having been present for many generations, and if characteristics are determined by gemmules, then the gemmules must have been dormant—certainly a circular argument.

4. Sex-linked inheritance is a consequence of gemmules being dormant in one sex. Thus a color-blind man transmits color-blind gemmules to his daughter, where they remain dormant. She transmits the color-blind gemmules to her sons where they develop and result in color blindness.

5. The usually observed blending when two different forms are crossed is a consequence of the gemmules of each parent being mixed in the offspring. Those cases in which the characteristics of one parent dominate is a consequence of that parent's gemmules "having some advantage in number, affinity, or vigour over those derived from the other parent."

6. Artificial selection is possible because, by choosing individuals with desirable characteristics, one chooses as parents those individuals with the desirable gemmules. By continuous inbreeding of parents with the desired characteristics, one can slowly perfect a variety of the sort required.

7. The origins of variability were obscure but somehow the environment must be the cause—but not in a simplistic Lamarckian sense. But once a new variation appeared, new sorts of gemmules would then be formed. This implies that somatic cells can influence the hereditary composition of the germ cells—a point of view that, much later, was to be regarded as a most serious genetic heresy.

8. Regeneration could be accounted for since the gemmules for all structures are found throughout the body so any portion has the gemmules to replace the lost parts.

9. The identical outcome of sexual and asexual reproduction finds the explanation also in that all parts of the body having gemmules for all parts. The gametes of *Hydra*, as well as the cells of the body wall that are about to produce a bud, have the same library of gemmules.

10. Gemmules from that quagga male passed to Lord Morton's mare via the semen, entered the ovary, and reappeared and expressed themselves when the mare was mated to the Arabian stallion.

What can we say? Darwin had performed a great service in assembling a huge mass of data and, in a real sense, he had defined the field of heredity. His hypothesis of pangenesis was a notable advance over the hypothesis of pangenesis proposed by Hippocrates more than two millennia earlier. His most important contribution may have been his emphasis that inheritance has a physical basis and perhaps rules could be discovered for its mechanisms. He realized the weakness of his hypothesis of pangenesis but he had tried to bring order where none existed. If his efforts served no other purpose they at least gave other scientists a place to start, a catalog of the sorts of data to be explained by any comprehensive theory of inheritance, a discussion of the main problems, and a hypothesis that could be tested.

Galton's rabbits

Charles Darwin's nephew, Francis Galton (1822–1911), had long been interested in his uncle's work and saw a way to test the hypothesis of pangenesis. In 1871, three years after the publication of *Variation . . .*, he presented a fascinating paper before the Royal Society. He began,

> Darwin's provisional theory of Pangenesis claims our belief on the ground that it is the only theory which explains, by a single law, the numerous phenomena allied to simple reproduction, such as reversion, growth, and repair of injuries. On the other hand, its postulates are hypothetical and large, so that few naturalists seem willing to grant them. To myself, as a student of Heredity, it seemed of pressing importance that these postulates should be tested. If their truth could be established, the influence of Pangenesis on the study of heredity would be immense; if otherwise the negative conclusion would still be a positive gain. (Galton, 1871*a*)

His test was simple and direct. He knew that blood could be transferred from one animal to another and that "it was not a cruel operation." He proposed to transfer blood between different strains of anesthetized rabbits and then study their offspring. Thus, if blood of black rabbits was injected into silver-grey rabbits and the silver-greys then bred with one another, one could ascertain if the blood of the black rabbits had any effect.

> If Pangenesis were true . . . the results would be startling in their novelty, and of no small practical use; for it would be possible to modify varieties of animals, by introducing slight dashes of new blood, in ways important to breeders.

Galton found that his experimental rabbits all bred true. There was no evidence that the injected blood modified the offspring of those receiving it.

Darwin reacted promptly (1871) to this attack on his hypothesis, maintaining that Galton's experiments were no test at all since he "had not said one word about the blood" and "It is, indeed, obvious that the presence of gemmules in the blood can form no necessary part of my hypothesis," since gemmules were assumed to exist in creatures lacking a circulatory system.

This was, indeed, a strange rejoinder: if gemmules were present throughout the body, surely they would be present in blood. Possibly Darwin was having troubles with the Idols of the Cave.

Galton replied (1871*b*) with mock contrition, saying how sorry he was to have misinterpreted what his uncle had said.

Pangenesis evaluated

Darwin's hypothesis of pangenesis was based on gemmules but he had no evidence for their existence. They were invented to account for the observed phenomena of inheritance. This is legitimate scientific procedure. Atoms were invented to account for the data of chemistry; a planet later named Pluto was invented to account for irregularities in the orbits of the known planets. Atom and Pluto were useful hypotheses long before their reality was established.

The weakness of the hypothesis of pangenesis was that it did not simplify heredity. Darwin listed the ways that inheritance works and then held that it works that way because the gemmules acted that way. This was the same as saying that "heredity" = "gemmules" and, since "gemmules" were entirely hypothetical, little was gained by ascribing such important functions to them. In Darwin's time the term "heredity" would have sufficed. The hypothesis was not well regarded, even though there was not a better one to take its place. As Vorzimmer (1970, p. 257) was to write years later, the hypothesis of pangenesis was "so *ad hoc* as to withstand any criticism which sought to point up any fact inconsistent with it."

It can also be maintained that, when Darwin wrote, there was no possibility of anyone developing a concept to explain all of the data of inheritance. This was especially true when some of the "facts" that Darwin thought most important were later found to be erroneous. Biologists would have to reach the stage of genetic engineering before they could do to Lord Morton's

mare what that quagga was thought to have done.

As this story unfolds we will find that after 1900 genetics made great progress by first trying to explain very little and then, as confirmable hypotheses were developed, more and more puzzlements were studied, explained, and incorporated into the corpus of genetic theory. A remark of Hardin (1985, p. 4), made in another connection is fully relevant here: "What began as knowledge about very little turns out to be wisdom about a great deal." Darwin began by trying to explain a great deal and ended by explaining very little.

CROP IMPROVEMENT BEFORE GENETICS

Genetics is much cherished today because it provides so much practical knowledge and methods for producing better varieties of cultivated plants and domesticated animals. Genetics began to become a rigorous science only in 1900, so it is astonishing to note that all of the important animal and plant crops had been domesticated and largely perfected before Darwin published *The Variation of Animals and Plants under Domestication* in 1868. In fact, far more was accomplished in the long ages of seeming ignorance of genetics than in the first half of the 20th century when genetics blossomed with new concepts and techniques.

There was great interest in England, in Darwin's time and earlier, in improving agriculture. This was a period when social status depended primarily on the ownership of land and far less on one's position in business or industry. Many of the lords of the manor, when not otherwise occupied producing episodes for *Masterpiece Theater,* paid special attention to the careful breeding of plants and animals. Much of the data in *Variation* came from these efforts. Darwin describes the approach,

> The effects of free or uncontrolled breeding between the members of the same variety or of closely allied varieties are important; but are so obvious that they need not be discussed at length. It is free intercrossing which chiefly gives uniformity, both under nature and under domestication, to the individuals of the same species or variety, when they live mingled together and are not exposed to any cause inducing excessive variability. The prevention of free crossing, and the intentional matching of individual animals, are the corner-stones of the breeder's art. No man in his senses would expect to improve or modify a breed in any particular manner, or keep an old breed true and distinct, unless he separated his animals. (vol. 2, p. 85)

Thus, with care, a breeder could perfect varieties for different conditions of soil and climate. In the case of sheep,

> The several races have become adapted to different kinds of pasture and climate: for instance, no one can rear Leicester sheep on mountainous regions, where Cheviots flourish. As Youatt has remarked, "in all the different districts of Great Britain we find various breeds of sheep beautifully adapted to the locality which they occupy. No one knows their origin; they are indigenous to the soil, climate, pasturage, and the locality on which they graze; they seem to have been formed for it and by it." Marshall relates that a flock of heavy Lincolnshire and light Norfolk sheep which had been bred together in a large sheep-walk, part of which was low, rich, and moist, and another part high and dry, with benty grass, when turned out, regularly separated from each other; the heavy sheep drawing off to the rich soil, and the lighter sheep to their own soil; so that "whilst there was plenty of grass the two breeds kept themselves as distinct as rooks and pigeons." (vol. 1, p. 96)

The development of domesticated varieties from wild ancestors is no more than evolution guided by both natural and artificial selection. Furthermore it involves the primary factor leading to speciation in nature—geographic isolation. To be sure, the cause of the geographic isolation might be no more than the fence surrounding a farm but a well-kept fence is entirely effective in preventing gene flow.

Evolution in nature is normally exceedingly slow, except in those cases where the

natural population is confronted by an entirely new environmental challenge such as pesticides, antibiotics, and industrial pollution. It is slow only because we normally observe the natural population *after* it has been subjected to selection for generations. By the time we observe the natural population the possibilities of what can be accomplished with the gene pool of the moment interacting with the environment of the moment will have been explored and the population will have achieved a level of perfection that permits survival.

While natural selection need do no more for the population than permit survival, artificial selection can accomplish much else. In domestication, evolution is driven by human goals. Domestication does not result in a better sheep for sheep's sake. It results in sheep with better wool, better flesh, greater ability to survive where the farmer wishes it to survive, and greater fecundity—all for the farmer's benefit. It may even produce a type of animal having great difficulty surviving under natural conditions. Hogs are bred for what must be uncomfortable obesity. Some varieties of dogs are bred for abnormalities of the skull that make breathing difficult and noisy. Some varieties of pigeons are selected for central nervous system defects that produce highly abnormal behavior patterns.

Evolution under domestication is rapid because new goals are set for the genome and selection is exceedingly severe—generation after generation for a few desirable parents become the sole progenitors for the subsequent generations. The pre-Mendelian breeders also knew how to increase variability over that as we know today to be associated with chromosomal crossing-over. They did this by crossing markedly different varieties (sometimes even different species) knowing from experience that the second and later generations would usually be highly variable. Not infrequently seemingly new types appeared and these would offer new possibilities for selection.

Thus human beings had been using sound genetic and evolutionary principles since the Neolithic when our ancestors began to abandon the chase and started to settle down. It required neither the insights of Darwin nor those of Mendel for our ancestors to have given us *all* the plant species that we now commonly use for food, fiber, work, and pleasure.

Nevertheless we are, at this moment, about to enter a new era when the techniques of modern biology can permit a notable improvement in our ability to mold animals and plants to human needs.

REFERENCES

The following references introduce the vast literature of attempts to understand inheritance in the pre-Mendelian centuries. These references are mainly those concerned with using the data of animal and plant breeding to understanding inheritance. References to those who attempted to understand inheritance through studies of gametes and fertilization will be given later.

Babcock (1949–1951), Bailey (1895), Barthelmess (1952), Cole (1930), Darlington (1969), Darwin (1868), Dunn (1965*a*, 1965*b*, 1969), Focke (1881), Froggatt and Nevin (1972), Galton (1889), Gasking (1967), Ghiselin (1969), Glass (1947, 1959*a*, 1959*b*), Lithgow (1889), Mayr (*1973 [included in 1976], 1976, *1982), Mitchell (1910–1911), Moore (1972*a*, 1972*b*), Needham (1959), Olby (1963, *1966), Pearson (1924), Roberts (1929), Sachs (1890), Stubbe (*1965), Sturtevant (1965*a*), Vernon (1903), Vorzimmer (1963, 1970), and Zirkle (1935*a*, 1935*b*, 1936, 1946, 1951*a*, 1951*b*).

TWIN APPROACHES TO STUDYING INHERITANCE

Two main research approaches were of prime importance in gaining an understanding of inheritance. So far we have considered only one—breeding. Organisms were crossed and the offspring studied. One then attempted to develop hypotheses about the mechanisms of inheritance from the data obtained. This was Darwin's approach but neither he nor others in the last half of the 19th century were able to advance our understanding very much.

The other line of research was based on

this analysis: There is a structural bottleneck in the life cycle of both animals and plants. The two sexes usually produce small eggs and always very small sperm and these combine to produce the individuals of the next generation. At least in some species, there is no further contact between parents and offspring so whatever hereditary information is transmitted must be in the gametes.

This last argument could not be extended to all species. In mammals and seed plants, for example, the early stages of development of the offspring occur in close relation to the maternal tissues. There would be a distinct possibility, therefore, of a maternal influence during early development. Nevertheless, one could follow Bacon and decide that, since there can be no maternal influence after fertilization in some species, such maternal influence cannot be regarded as a universal requirement. One could hypothesize further, with less confidence to be sure, that there may not be *any* maternal influence after fertilization in any species.

Therefore, if our working hypothesis was that all the hereditary information must be contained in the gametes, we might expect that a detailed study of gametes and fertilization could shed light on inheritance. Whether or not this proved to be a rewarding approach, we would have to accept that any comprehensive theory of inheritance must be compatible with whatever was discovered about the behavior of gametes.

This second approach, cytology, was to make such astonishing progress in the last half of the 19th century that, five years before geneticists began to understand Mendel's work, E. B. Wilson was able to suggest that the nucleic acids were the physical basis of inheritance.

One might simplify these two research approaches for studying inheritance by saying that breeding sought to discover the process of inheritance and cytology sought to discover the substance of inheritance. The two approaches were to be combined in 1902–1903 by W. S. Sutton and, thereafter, the interplay of experimental breeding and cytology was to be the reason for the ensuing rapid and rewarding increase in our understanding of inheritance.

THE DISCOVERY OF CELLS: ROBERT HOOKE

The birthday of cytology can be fixed with considerable accuracy. On April 15, 1663 Robert Hooke (1635–1703) placed a piece of cork under his microscope and demonstrated its otherwise invisible structure to fellow members of the Royal Society of London.

The Royal Society had been started the previous year for the purpose of "Improving Natural Knowledge" (Birch, 1756–1757; Sprat, 1722; Thomson, 1812; Lyons, 1944; Stimson, 1949; Purver, 1967). It consisted of a few learned men of London who met on a regular basis, often weekly, to discuss scientific matters and how knowledge could be used to improve the useful arts. The inspiration for the formation of the Royal Society had come from an earlier suggestion of Francis Bacon.

Hooke, a polymath of exceptional ability (Gunther, 1930–1938; 'Espinasse, 1962) was a very active member of the Royal Society. It was the custom for members not only to hold discussions but also to perform experiments and provide demonstrations. There was great interest in the new microscope that Hooke had constructed. He let the members look at parts of a moss plant on April 8, 1663. On April 15 "Mr. Hooke shewed two microscopical schemes, one representing the pores of cork, cut both transverse and perpendicular . . ." (Birch, 1756–1757, vol. 1, p. 218).

That was the beginning of two centuries of observation and experimentation that were to establish the Cell Theory.

Hooke's various observations were assembled and published in 1665, under the auspices of the Royal Society, as *Micrographia.* This was the world's first comprehensive view of a previously invisible part of nature.

Hooke and the other members were much influenced by Bacon's ideas and the Preface of *Micrographia* has a long and interesting discussion of how the old philosophy must be avoided:

> The Science of Nature has already too long made only a work of the Brain and Fancy: It is now high time that it should return to the plainness and soundness of Observations on material and obvious things.

The reader was not to expect "any infallible Deductions or certainty of Axioms" from him and Hooke asks that the reader

> Whereever he finds that I have ventur'd at any small conjectures, at the causes of the things that I have observed, I beseech him to look upon them only as doubtful Problems, and uncertain ghesses, and not as unquestionable Conclusions, or matters of unconfutable Science . . . [and] I desire him, not absolutely to rely upon these observations of my eyes, if he finds them contradicted by future Ocular Experiments of sober and impartial Discoverers. (Preface)

Hooke described and illustrated many objects in *Micrographia*: head of a pin, many small insects and their parts, feathers, vinegar eels, parts of many plants, hair, moulds, paper, petrified wood, fish scales, silk, sand, snow flakes, urine, and, of course, that piece of cork (Fig. 2).

Hooke imagined that cork consisted of a number of parallel tubes with cross partitions,

> These pores, or cells, were not very deep, but consisted of a great many little Boxes, separated out of one continuous long pore, by certain *diaphragms.* (p. 113)

He observed similar structures in many other kinds of plants. It is generally thought that Hooke described those boxes as empty and let it go at that. Not at all:

> Several of those Vegetables, whil'st green, I have with my *Microscope,* plainly enough discover'd these Cells or Pores fill'd with juices . . . as I have also observed in green Wood all these long *Microscopical* pores which appear in Charcoal perfectly empty of anything but Air. (p. 116)

This discovery of cells in cork and other plants could have been of general importance or it could have been a minor feature of a few kinds of organisms. Continued research was to show that the bodies of plants consisted entirely or almost entirely of similar box-like structures and, in time, the concept was extended to animals. Hooke had made an interesting observation that was not important at the time—it became an important discovery because of later research.

And it took a very long time for it to become important even though many other investigators observed cells in plants. For example, a fellow member of the Royal Society, Nehemiah Grew (1641–1712), published a monograph in 1682 that contained many beautiful plates showing the microscopic structure of plants (Fig. 3).

It took more than two centuries for it to be realized that knowledge of cells was essential for an understanding of inheritance. We can be certain that when Robert Hooke sat down to his microscope he was not intending to unravel the mysteries of inheritance. There was no more reason to believe that cells had anything to do with inheritance than, for example, did the bristles he observed on the surface of a flea he described in such detail.

Time and time again in science it turns out that the explanations in one field come to depend on those already made in entirely different fields. And we must remember that the entire field of cytology was impossible until knowledge of optics and the grinding of lenses, plus a genius or two, were to make microscopes feasible.

THE CELL THEORY

Cells became truly important only when the hypothesis was proposed that the bodies of all organisms are composed solely of cells or the products of cells. That hypothesis was formulated and tested early in the 19th century and it is associated mainly with three observers: R. J. H. Dutrochet, Matthias Jacob Schleiden, and Theodor Schwann.

But how could one possibly prove that "the bodies of all organisms are composed solely of cells or the products of cells?"

Obſerv. XVIII. *Of the* Schematiſme *or* Texture *of* Cork, *and of the Cells and Pores of ſome other ſuch frothy Bodies.*

I Took a good clear piece of Cork, and with a Pen-knife ſharpen'd as keen as a Razor, I cut a piece of it off, and thereby left the ſurface of it exceeding ſmooth, then examining it very diligently with a *Microſcope*, me thought I could perceive it to appear a little porous; but I could not ſo plainly diſtinguiſh them, as to be ſure that they were pores, much leſs what Figure they were of: But judging from the lightneſs and yielding quality of the Cork, that certainly the texture could not be ſo curious, but that poſſibly, if I could uſe ſome further diligence, I might find it to be diſcernable with a *Microſcope*, I with the ſame ſharp Pen-knife, cut off from the former ſmooth ſurface an exceeding thin piece of it, and placing it on a black object Plate, becauſe it was it ſelf a white body, and caſting the light on it with a deep *plano-convex Glaſs*, I could exceeding plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular; yet it was not unlike a Honey-comb in theſe particulars.

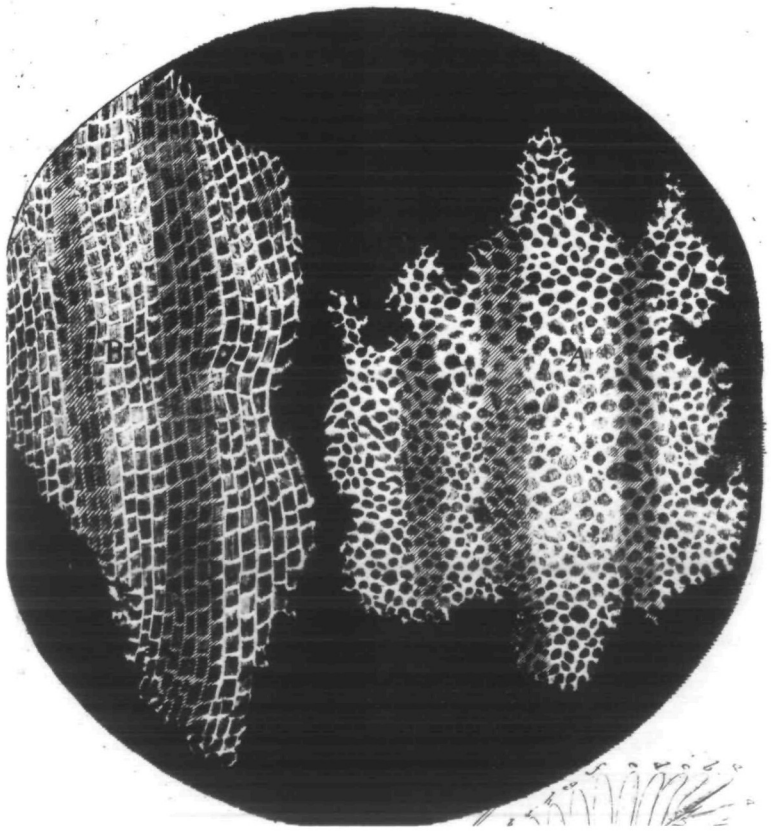


FIG. 2. Part of the text and the illustration from Robert Hooke's observations on cork. (Hooke, 1665)

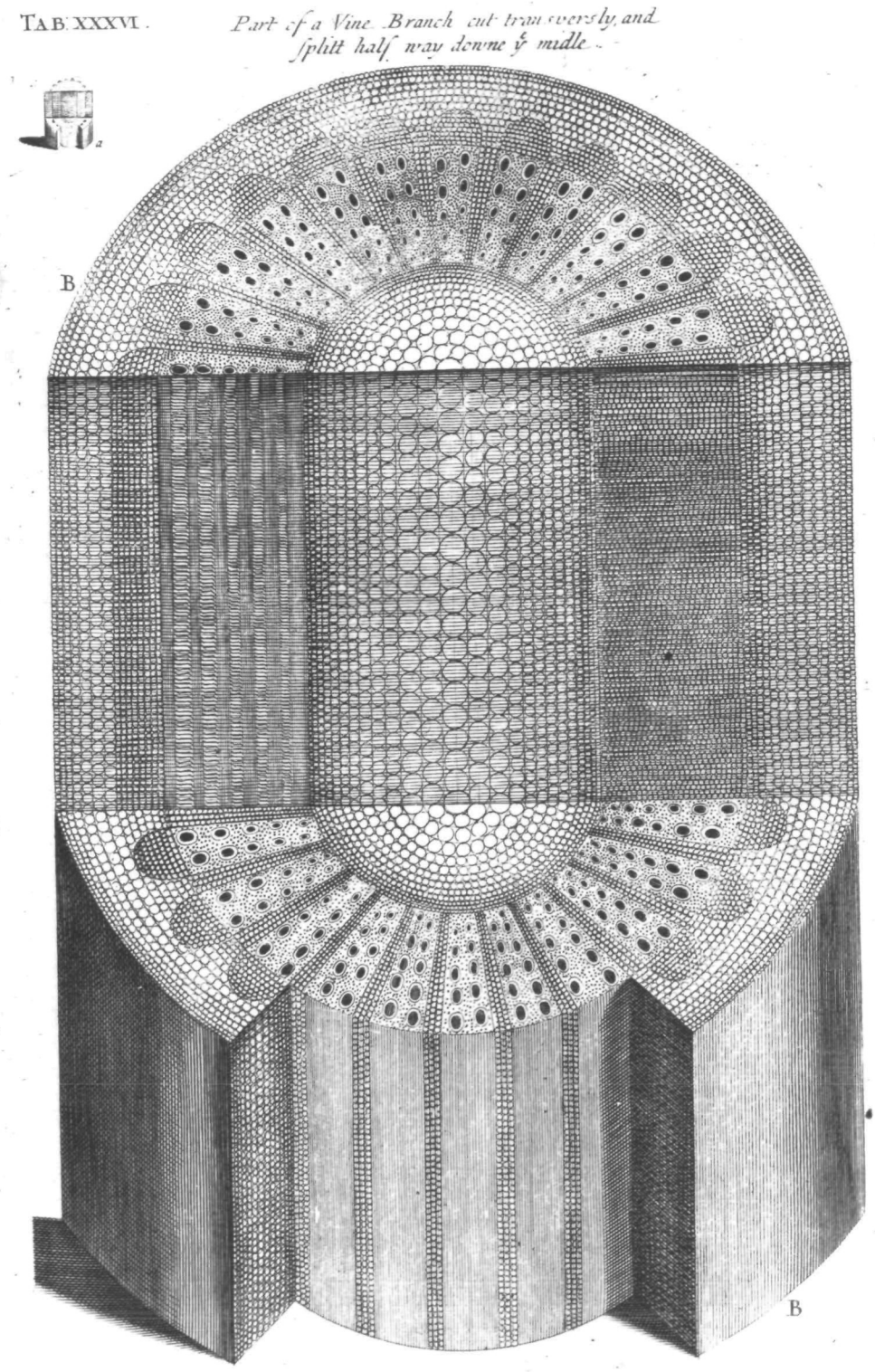


FIG. 3. The cellular structure of a vine branch. (Grew, 1682)

That would be a profitable question for your students to consider. In doing so they would come to learn something important about scientific concepts.

The answer is, of course, that such a statement could not possibly be proven. How could one study all organisms? Most are long gone from this earth. How might your students evaluate this statement: "The bodies of dinosaurs were composed of cells"? It would not even be practical to study one individual of all living species. All that one can hope for in science is that a statement is "true beyond all reasonable doubt." Following Hooke's initial observations, it was found that cells were a common feature of plants. More and more individual plants and more and more species were studied and all were found to have those cell-like structures. It was observed that these microscopic structures were not all the box-shaped cells of cork. Cells were discovered to come in various shapes and sizes (Fig. 3). We must not forget that these early microscopists were not observing cells as we understand them today but cell walls.

SCHWANN AND CELLS IN ANIMALS

With few exceptions the bodies of animals contain no structures resembling the "cells", *i.e.*, the cell walls, of plants. Thus it required a great deal of study and bold imagination before it became obvious that the concept of cells could be profitably applied to animals. This was first accomplished mainly by Theodor Schwann (1810–1882) in his monograph of 1839, published when he was 29 years old. Some of the illustrations are reproduced in Figure 4. He emphasizes the great difference between the cells of plants and the structures in animals but suggested that they are fundamentally the same.

> Though the variety in the external structure of plants is great, their internal structure is very simple. This extraordinary range in form is due only to a variation in the fitting together of elementary structures which, indeed, are subject to modification but are essentially identical—that is, they are cells. The entire class of cellular plants is composed solely of cells which can readily be recognized as such; some of them are composed merely of a series of similar or even only of a single cell.
>
> Animals being subject to a much greater range of variation in their external form than is found in plants also show (especially in the higher species) a much greater range of structure in their different tissues. A muscle differs greatly from a nerve, the latter from a cellular tissue (which shares only its name with the cellular tissue of plants), or elastic tissue, or horny tissue, etc. [This paragraph will be continued after the following suggestion.]

WHY CALL ALL THESE DIVERSE STRUCTURES "CELLS"?

It would be most valuable to stop in the middle of Schwann's paragraph and ask your students to consider this problem. Show them the data: modern slides of various types of cells in plants and especially animals. How can it be useful to maintain that neurons, muscle, and tissues of kidney, lung, blood, cartilage, bone, intestine wall, etc., are made of the same sort of structures? When they are obviously so different, why maintain that they are fundamentally the same? And what is to be gained by claiming that these diverse structures in animals can be equated with the very different looking structures in plants?

BACK TO SCHWANN

Part of the answer emerges

> If, however, we go back to the development of these tissues, then it will appear that all of these many forms of tissue are constituted solely of cells that are quite analogous to plant cells . . . . The purpose of the present treatise is to prove the foregoing by observations.

That is, in spite of the great diversity of structures that Schwann proposed to call cells, all develop from simpler structures that could be compared more readily with the cells of plants.

The speculation of your students may have raised the problem of the need for a

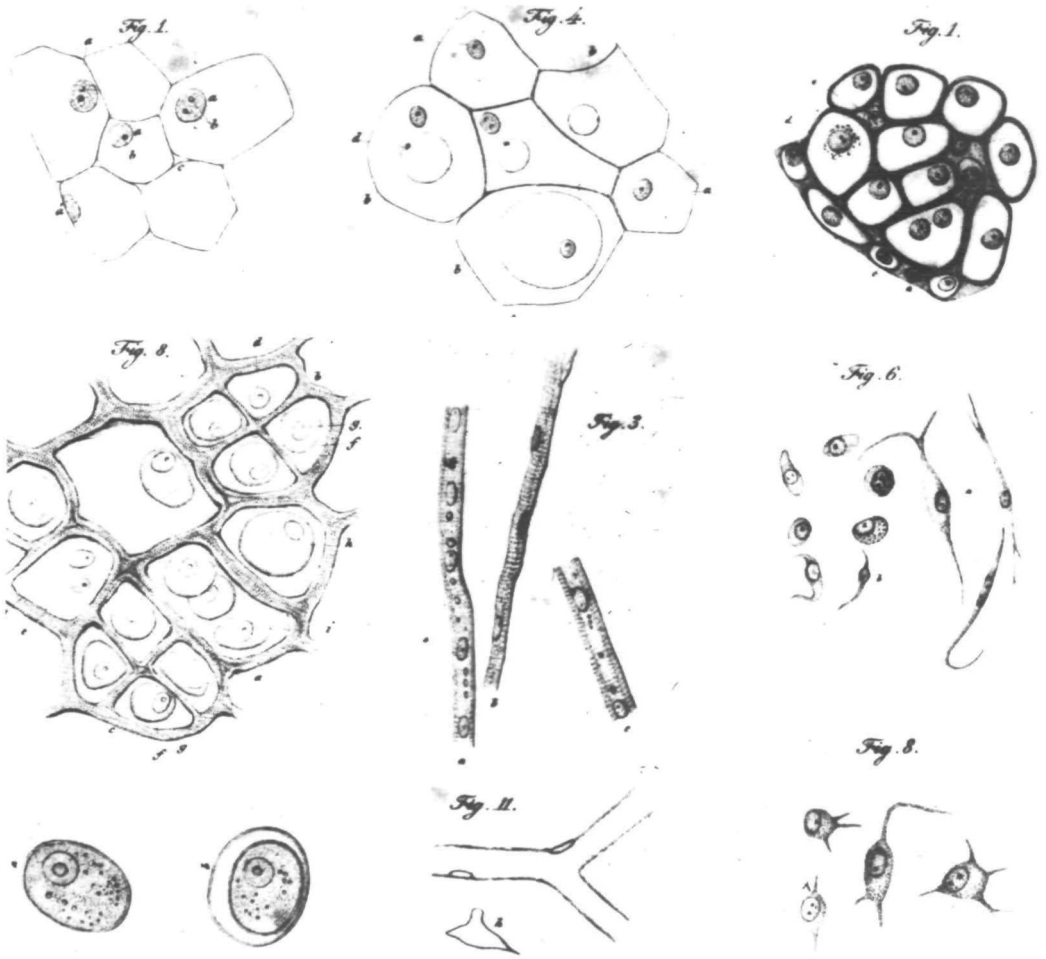


FIG. 4. Some of the illustrations from Schwann's monograph. Upper row, left to right: onion cells, notochord of a fish, cartilage of a frog. Middle row: cartilage of a tadpole, muscles of a fetal pig, areolar cells of a pig embryo. Bottom row: ganglion cells of a frog, capillary in a tadpole's tail, cells of a pig embryo. Note that nucleus and nucleoli are shown in nearly all cells. (Schwann, 1839)

way to define "cell." If neuron and leukocyte are both "cells," there must be a common basis for so classifying them. Schwann found a criterion, the presence of nuclei, that was more important than the origin of highly differentiated cells from simpler cells.

Only six years earlier, in 1833, Robert ("Brownian Motion") Brown (1773—1858) had described a single circular areola, or nucleus, in the cells of orchids and many other kinds of plants. Previous observers had noted these structures, illustrated them in their publications, but had attached no importance to them. Brown found that many kinds of cells contain nuclei but did not speculate on their significance.

Schwann then changed the rules for defining cells. Instead of relying on shape, which in plants meant the structure of the walls, he chose to base the definition on the presence of a nucleus.

> The most frequent and important basis for recognizing the existence of a cell is the presence or absence of the nucleus. Its sharp outline and its darker color make it easily recognizable in most cases

and its characteristic shape, especially if it contains nucleoli . . . identify the structure as a cell nucleus and make it analogous with the nucleus of the young cells contained in cartilage and plant cells . . . . More than nine-tenths of the structures thought to be cells show such a nucleus and in many of these a distinct cell membrane can be made out and in most it is more or less distinct. Under these circumstances it is perhaps permissible to conclude that in those spheres where no cell membrane be distinguished, but where a nucleus characteristic of its position and form is encountered, that a cell membrane is actually present but invisible.

Although Schwann was a careful observer, his contribution was not primarily what he saw but how he interpreted the observations. Previous investigators had emphasized the boxes. Schwann emphasized what was *inside* the box. For him the animal cell became a bit of living substance containing a nucleus and bounded by a membrane and, in the case of plants, further encased in cell walls.

What does this new view of cells have to do with inheritance? Very little, one must admit. Two other bits of information are required before cells can be considered to have an important relation to inheritance: the discovery that the gametes are cells and the realization that cells originate only from other cells.

GAMETES AS CELLS

Schwann recognized that ova are cells since they exhibited the structure required by his definition of cells. The nature of spermatozoa was less clear. Even the name, meaning "sperm animals," indicates uncertainty. In 1667 Leeuwenhoek, or one of his students, had discovered and reported to the Royal Society of London that seminal fluid contained some microscopic creatures that were imagined to enter the egg and achieve fertilization. That hypothesis was hotly contested and some imagined that the spermatozoa were parasites. In the 12th edition of *Systema Naturae* (1766–1768), Linnaeus tentatively listed the spermatic animalcules of Leeuwenhoek but felt that the determination of their proper place in the system of classification must be left for later research (Dobell, 1960, p. 377).

A little more than a century later, in 1784, Spallanzani conducted some remarkable experiments to ascertain the function of semen in the reproduction of frogs. During breeding the males clasp the females and, as we now know, deposit sperm on the eggs as they leave the female's cloacal opening. This was not known to Spallanzani but he discovered it. Another investigator with whom he corresponded had attempted, without much success, to discover the role of male frogs by putting trousers on them. Now to Spallanzani:

> The idea of the breeches, however whimsical and ridiculous it may appear, did not displease me, and I resolved to put it in practice. The males, notwithstanding this incumbrance, seek the females with equal eagerness, and perform, as well as they can, the act of generation; but the event is such as may be expected: the eggs are never prolific [that is, they do not develop], for want of having been bedewed with semen, which sometimes may be seen in the breeches in the form of drops. That these drops are real seed, appeared clearly from the artificial fecundation that was obtained by means of them. (vol. 2, p. 12)

In another experiment he filtered semen and found that it lost its fertilizing power. He saw what we now call sperm but did not regard them as essential for reproduction.

> Having often observed the seminal liquor of the toad, I found it very full of spermatic worms, which, like those of the frog, have an oblong shape, and writhe their body as they move. Upon two occasions I have been greatly surprised at finding this fluid totally destitute of inhabitants. I was induced to try, whether it is also destitute of fecundating virtue, but I found that it was just as effectual in this respect, as that which most abounds with these diminutive animals. (vol. 2, p. 118)

It was not until 1854 that George Newport was able to offer good evidence, using frogs, that the sperm cells enter the egg at fertilization.

(Here and elsewhere it is often difficult to give credit for the scientist who discovered an important biological phenomenon. After all, the discoverer of sperm, Leeuwenhoek, had thought sperm were the agents of fertilization. Others antedating Newport had the same opinion but it was Newport who made the first convincing observations. And, as was noted before, microscopists had observed and published illustrations of cells with globules that were later identified as nuclei before Brown emphasized their importance.)

In 1841 Kölliker studied the histology of the testis and found that some of the testis cells are converted into sperm. Sperm are so unusual in appearance that they are not likely to be considered cells. However when they can be shown to be derived from typical cells, their true nature becomes apparent. Sperm, then, are to be regarded as highly modified cells.

This is how our analysis stands:

1. Gametes are the only physical link between generations, at least in many organisms and possibly all.
2. Therefore the gametes must contain all of the hereditary information.
3. Since ova and sperm are cells, all of the hereditary information must be contained in these sex cells. Therefore, the physical basis of inheritance is the sex cells.

This does *not* mean that all cells contain hereditary information. One could still imagine that the gametes are specialized cells into which the factors responsible for inheritance, gemmules perhaps, somehow enter. We still need that second bit of information: "What is the origin of cells?"

Omnis Cellula e Cellula?

Cell division had been observed in 1835 but it was not realized that this is a general phenomenon. Schwann (1839) had a very different notion about the origin of cells.

> The general principles in the formation of cells may be given as follows. At first there is a structureless substance which may be either quite liquid or more or less gelatinous. This, depending on its chemical constitution and degree of vitality, has the inherent ability to bring about the formation of cells. It seems that usually the nucleus is formed first and then the cell around it. Cell formation is in the organic world what crystallization represents in the inorganic world. The cell, once formed, grows through its inherent energy, but in doing so it is guided by the organism as a whole in the way that conforms to the general organization. This is the phenomenon basic to all animal and plant growth. It is applicable to cases where the young cells originate in the mother cell, as well as those where they are formed outside of them. In both instances the origin of cells occurs in a liquid or in a structureless substance. We call this substance, in which cells are formed, a cell germinative substance or Cytoblastema. It can be compared figuratively, but only figuratively, with a solution from which crystals are precipitated.

This hypothesis for the origin of cells holds that they are episodic events in the life cycle of organisms. If true, the unit of inheritance must be the entire organism, not the cell. Schwann's hypothesis for the origin of cells was soon rejected by his contemporaries, since cell division was observed repeatedly in a variety of organisms and in different periods of development. More and more investigators began to suspect that cell division was the sole mechanism for producing new cells.

This was an exceedingly difficult hypothesis to prove beyond all reasonable doubt. The microscopes and the techniques for studying cells in the early 1800s were most inadequate by later standards and it took many observations on different sorts of organisms and tissues before Rudolph Virchow was to express the view in 1855 that *omnis cellula e cellula* ("all cells from cells") and have it generally accepted. In a lecture given in 1858 (Virchow, 1863) he put it thus:

> A new cell can [never] build itself up out of any non-cellular substance. Where a cell arises, there a cell must have previ-

ously existed (*omnis cellula e cellula*), just as an animal can spring only from an animal, a plant only from a plant. In this manner, although there are still a few spots in the body where absolute demonstration has not yet been afforded, the principle is nevertheless established, that in the whole series of living things, whether they be entire plants or animal organisms, or essential constituents of the same, an eternal law of *continuous development* prevails. There is no discontinuity of development of such a kind that a new generation can of itself give rise to a new series of developmental forms. (Virchow, 1863, Lecture II)

Of course not everyone agreed with Virchow that all cells and all organisms come from preexisting cells and organisms. Many observers continued to believe that cells could arise *de novo* and presented seemingly accurate observations to prove it. It was thought by some that whole organisms could arise *de novo* as well. Pasteur and the general acceptance that spontaneous generation cannot occur were still in the future. Nevertheless the twin hypotheses supported by Virchow were tested by more and more research and slowly it was established as true beyond all reasonable doubt that:

Omnis vivo e vivo
Omnis cellula e cellula

There was no question, then, that inheritance is based on cell continuity and we may now work with the hypothesis that all the hereditary information is contained not only in the germ cells but also, presumably, in the cells from which they arose—all the way back to the zygote. Also possible was the hypothesis that all cells contain the hereditary information necessary for the development of the individual and for its transmission, via the sex cells, to the next generation.

CYTOLOGY AND TECHNOLOGY

For most of human history we have relied almost entirely on our sense organs to tell us about the environment. Each sense organ detects only a narrow window in the range of possible stimuli. Our eyes, for example, can respond only to that portion of the electromagnetic spectrum between violet and red so we only see wavelengths between these two colors. Special instruments must be used if we are to detect the shorter ultraviolet, X-rays, and cosmic rays or the longer infra-red and radio waves.

Our unaided eyes fail also to tell us about objects that move very rapidly. The individual grey blades of a rapidly moving fan merge into a continuous circle that is less grey and the bullet leaving a rifle barrel is wholly invisible.

Nor can we see objects that are very small. The apparent uniformity of a half-tone illustration is a result of the individual dots of ink being too close together for the human eye to resolve. The twin headlights of an automobile appear as a single source of light when far away. As the automobile approaches, we become able to resolve the single light source into two.

Human eyes vary in their ability to resolve two objects, that is, to determine whether an object is single or multiple. The limit of resolution is about 100 microns at reading distance. Most individuals with normal eyes can distinguish two objects one millimeter apart at a distance of about 10 meters. (Should your students find this statement astonishing, suggest that they determine the value for their eyes.) A more general statement is that the human eye can resolve objects separated by an arc of 1 minute. That value was determined by Robert Hooke (1674) who wondered how far apart double stars had to be before they could be seen as two. When they were closer than 1 minute of arc, most people would see only a single point of light. Some people can do better and the maximum resolving power for the human eye is about 26 seconds of arc.

Nearly all cells are too small to be seen by the human eye so, obviously, cytology was not even theoretically possible before the invention of the microscope—probably in the 1590s. A long lag followed until 1663 when Hooke demonstrated those slices of cork to the members of the Royal Society. In fact, there was little serious and sustained work with microscopes before the

19th century. For most of their early history microscopes were little more than adult toys.

The small size of cells is not the only problem that makes studying them difficult. Most animals and their tissues are opaque and, since the compound microscope is most effective when objects are illuminated by transmitted light, the object to be studied must be either very thin or be sliced so thin that light can pass through. Imagine trying to cut liver into slices about 10 microns in thickness, which would be necessary to study the cells. Furthermore liver cells, consisting mostly of water, would soon dry out and be a shriveled mess. This is a special problem with animal cells, which lack the supporting walls of plant cells.

Very special methods had to be developed by the microscopists of the early 19th century if they were to learn about the cellular nature of organisms and, later on, the internal structure of cells themselves. It became common practice, therefore, to try to preserve tissues in such a manner that the cellular structure would remain intact and thin slices could be made.

The first step was fixation. This involved treating the material with alcohol, formaldehyde, or solutions of picric acid, potassium dichromate, mercuric chloride, or osmium tetroxide. These chemicals kill and harden cells, often by coagulating the proteins. It was hoped, of course, that this would be done in such a way that the parts of the cells would resemble the living state to an acceptable degree.

The fixed tissue could then be embedded in paraffin wax and slices made with a sharp razor or an instrument devised for this specific purpose—the microtome.

Even these thin slices might reveal very little. The cells and their internal structure might be indistinct. But the inventive microscopists tried everything and found that some dyes would stain some structures in cells but not others.

In 1858 Gerlach found that a dilute solution of carmine would stain the nucleus more intensely than the cytoplasm. This substance is derived from the dried bodies of female cochineal insects (*Coccus cacti*), which live on cactus plants in Central America and the southwestern United States.

In 1865 Böhmer found that hematoxylin, extracted from the logwood tree (*Haematoxylon campechianum*) of Central America, also had more affinity for the nucleus than the cytoplasm.

Later aniline dyes were manufactured in a vast variety for the textile industry and between 1875 and 1880 many were found to be useful in staining cells. One of these was eosin, which proved to have a great affinity for the cytoplasmic proteins. A common staining procedure was to use hematoxylin and eosin. This procedure stained the nucleus blue and the cytoplasm pink.

Similarly improvements were made in the last part of the 19th century in the microscopes available for cytological research. Many of these improvements were due to Ernst Abbe (1840–1905) and the Zeiss optical works in Jena, Germany. For most of his life Abbe was both professor of physics at the University in Jena and the principal lens designer for the Zeiss company and later its owner. In 1878 he developed his oil-immersion objective and, in 1886, the apochromatic objective. In the hands of a skilled microscopist magnifications of 2,500 diameters became possible. The light microscope was reaching the theoretical limit of its resolving power. This was a limitation due to the nature of light itself. That is, two objects can be resolved only if their distance apart is at least equal to half of the wavelength of light being used.

Although further opportunities for studying the fine structure of cells were to come with the phase-contrast and electron microscopes of the 20th century, we shall see that the cytologists of the last third of the 19th century were able to use the available technology to establish as highly probable the hypothesis that the physical basis of inheritance is the cell nucleus, or more specifically the chromosomes within it.

One must not imagine that these investigations involved no more than examining living or preserved cells with the best available optical equipment and describing as accurately as possible what was seen. The constant problem was whether or not a

given structure in a prepared slide closely resembled the living state or whether it was an artifact resulting from the very drastic treatment to which cells were usually subjected. Consider the saga of a cell subjected to the following procedure by a cytologist of the late 1800s.

> Sections of vegetable tissues present a beautiful appearance under the microscope when doubly stained. They should first be soaked in alcohol, if green, to deprive them of chlorophyll, then subjected to a solution of chloride of lime (¼ ounce to a pint of water) until thoroughly bleached. Soak then in a solution of hyposulphite of soda (1 drachm to 4 ounces of water) for one hour, and after thoroughly washing in several changes of water transfer them to alcohol. Prepare some red staining fluid by dissolving ½ a grain of magenta crystals in 1 ounce of alcohol. Soak the specimen in this for thirty minutes, then rapidly rinse it in alcohol and place in a blue fluid made by dissolving ½ grain of anilin blue in 1 drachm of distilled water, adding 10 minims of dilute nitric acid and alcohol enough to make 2 ounces. Let the specimen remain only two or three minutes in this, rapidly rinse in alcohol, put in oil of cajeput, thence to turpentine, and mount in balsam. (Wythe, 1880, p. 348)

Apart from giving thanks to the Muses for the metric system, one may wonder how accurately the final preparation reflected the structure of living cells. The answer might be "Not very much" but if the treatment produced constant results it was often possible to interpret the living state from the preparations. Nevertheless, no important discovery in cytology in the 19th century was accepted when first proposed. Observations would be repeated and the original assertions would be confirmed by some and vehemently denied by others. An original erroneous report might cause many cytologists to spend months in attempting to repeat the observations.

There were endless debates about the fine structure of protoplasm since, it was assumed, one was looking at the very basis of life itself.

> Since the fundamental activities of protoplasm are everywhere of the same nature, investigators have naturally sought to discover a corresponding fundamental morphological organization common to all forms of protoplasm and underlying all its special modifications. (E. B. Wilson, 1900, p. 23)

Wilson then goes on to discuss the many hypotheses for the structure of protoplasm (pp. 23–30). Various cytologists had maintained that protoplasm was either granular, or a fibrous reticulum, or alveolar (composed of droplets) or some combination thereof.

The difficulties of interpreting structures seen under the microscope had been long understood as shown by this cautionary advice given by Henry Baker in 1742.

> Beware of determining and declaring your Opinion suddenly on any Object; for Imagination often gets the Start of Judgment, and makes People believe they see Things, which better Observations will convince them could not possibly be seen: therefore assert nothing till after repeated Experiments and Examinations in all Lights and in all Positions. When you employ the Microscope, shake off all Prejudice, nor harbour any favourite Opinions; for, if you do, 'tis not unlikely Fancy will betray you into Error, and make you think you see what you would wish to see. Remember that Truth alone is the Matter you are in search after; and if you have been mistaken, let not Vanity seduce you to persist in your Mistake. (p. 62)

Shades of Sir Francis Bacon and his Idols but still first-rate advice.

Cytology as a way of knowing, especially in the 19th century, reveals that science does not progress in an orderly fashion but by the constant testing and retesting of observations, experiments, and hypotheses. Far from being a straight line to truth the path was more like that reticulum some saw as the basic structure of protoplasm.

(We might add that the term "protoplasm" is rarely used today. Since it meant no more than "living substance," Hardin

[1956] suggested that we could do without it.)

REFERENCES TO MICROSCOPES AND CYTOLOGICAL PROCEDURES

R. M. Allen (1940), H. Baker (1742), J. R. Baker (1948–1955), Belling (1930), Blumberg *et al.* (1967), Bracegirdle (1978), Bradbury (1967, 1968), Bradbury and Turner (1967), Burrells (1977), G. Clark (1981), Clark and Kasten (1983), Conn (1928–1933, 1961), Gage (1925), Gatenby and Beams (1950), Hogg (1867), Nicolson (1956), Power (1664), Singer (1915), Slayter (1970), Spencer (1982), Woodruff (1939), and Wythe (1880).

Many additional references dealing with cytology before 1900 will be given at the end of this section.

WHAT'S IN CELLS?

During the last half of the 19th century, the hypothesis that the bodies of animals and plants are composed solely of cells and cell products was established as true beyond all reasonable doubt in the minds of most competent microscopists. We can speak, therefore, of the Cell Theory, using the term "theory" to apply to an entire body of data, hypotheses, and concepts relating to an important natural phenomenon.

To this day the Cell Theory remains the most important concept relating to the structure of animals and plants and in the 20th century it gradually became accepted as the most important concept relating to function as well.

It would be profitable for your students to suggest why the Cell Theory is such an important concept. Hopefully some will suggest that cells are the basic units of structure and function, that they are the smallest units capable of independent life, that is, they are able to use substances acquired from the environment to maintain and produce the living state. Cells are the least common denominator of life.

There was another important reason for studying cells: analysis at a simpler level of organization contributes to understanding at more complex levels. The interactions of chemical substances are better understood when we know their molecular structure. The movements of the human body can be studied at many levels. One may observe and describe the complex movements of a ballet dancer or baseball pitcher, beautiful and important in their own right. Understanding is increased when we obtain information about the many muscles and their attachments that make the movements possible. Other sorts of understanding come when we study muscles at the cellular level. And finally still more information is obtained when we learn about the activity of myosin, actin, and the other molecules involved in the movement of muscles.

Knowledge obtained at each level of organization contributes to an understanding of the total phenomenon, while each level retains its own validity. One cannot completely understand either a Waslaw Nijinsky or a Fernando Valenzuela merely by knowing about actin and myosin any more than one can predict the properties of water from knowing about hydrogen and oxygen.

Nevertheless, one does understand better more complex levels by knowing simpler levels. Thus it was thought inevitable that more would be learned about the living state by learning about cells. Those cytologists working after the publication of Darwin's *Variation* in 1868 must have wondered if they could discover the gemmules that formed the basis of the hypothesis of pangenesis. Would they see those postulated tiny hereditary granules in all cells?

Again it would be a profitable exercise for your students to play the role of a cytologist in the 1870s seeking the physical basis of inheritance. When they examined cells they would find all sorts of spheres, granules, and fibers. How could one establish whether or not any of these organelles had a role in inheritance? Or, in fact, how might one establish the function of *any* intracellular structure?

It is unlikely that your students will have any profound and useful suggestions—and neither did the cytologists of the time. They could do no more than undertake a program of random investigations of cells. This was a necessary stage in the development of the field of cytology—the identification

of structures within cells and, where possible, learning something of their behavior. Seemingly cells from all available plants and animals were searched for examples of cell structures and one by one all the reagents from the chemical cabinet were dumped on cells and the consequences were observed—usually death of the cells. In cytology, then, this was the period of "Search and Destroy."

THE EPHEMERAL NUCLEUS

As noted before, the difficulty in studying living cells made fixed and stained preparations the favorite material. In such material the most prominent structure is Brown's nucleus. Many dyes, especially basic dyes such as carmine or hematoxylin, stain the nucleus heavily and this, together with its apparently universal presence suggested that it must be important.

But what is its origin? It took nearly a half century of observation and experiment by numerous cytologists to find out. In 1835 Valentin suggested that nuclei are formed by precipitation. Three years later Schleiden, followed by Schwann, also suggested a *de novo* origin. As late as the 1860s and 1870s some prominent cytologists continued to believe that at least some nuclei can have a non-nuclear origin.

Concurrently other equally competent cytologists were claiming that all nuclei originate from existing nuclei. Various methods were suggested—usually some form of pinching in two or fragmentation, a process that later came to be known as amitosis.

There was no necessary reason, of course, why there should be a single mechanism for the origin of nuclei. Considering the large amount of variability of natural phenomena, one should not be surprised if there were a variety of modes. Nevertheless, scientists seek the regularities in nature and it would be more intellectually satisfying if the concept of a constant mechanism of nuclear origin proved to be the case.

In retrospect we can see how even the most careful observation can lead to interpretations later shown to be incorrect. Thus, in what was considered favorable material—the early embryos of sea urchins—the nucleus appeared to pinch in half immediately before the cell as a whole divided. Figure 5 shows the first cell division and the beginning of the second in a sea urchin embryo. "Fig. 15" in this Figure 5 shows the zygote nucleus and in "Figs. 16–18" it forms a dumb-bell shaped object. In "Fig. 19" the egg has cleaved and each daughter cell has a nucleus. These drawings were published in 1876 by Oskar Hertwig. By that time he was aware that within the nucleus, which seemed to be dividing by amitosis, there were rods that could be seen after the eggs were fixed and stained.

There was another seemingly universal phenomenon that was difficult to explain for those maintaining that there is a fundamental type of continuity of the nucleus. The nucleus *does* disappear before the cell divides. That is, the spherical body that Brown and Schwann held to be a constant cell structure vanishes. In stained preparations it could be seen that, as the spherical nucleus vanishes, rod-shaped bodies not previously present made their appearance. Intensive study of these rods (later called chromosomes) led to the next major advances in cytology.

MITOSIS

In 1873 A. Schneider published what can now be taken as the first reasonable account of the complex nuclear changes, now called mitosis, that occurred at the time the cell divides. In the same year Otto Bütschli and Hermann Fol made similar reports.

Schneider's account was the most complete. His purpose was to describe the morphology of *Mesostoma,* one of the platyhelminths. Nearly all of his paper is devoted to the structure of this small flatworm but, being a careful observer, he described everything that he saw. Fertilization is internal in *Mesostoma* and early development takes place in the uterus. He provided illustrations of what he saw (Fig. 6).

The first drawing shows the egg surrounded by follicle cells. In the very center is the small nucleus with its even smaller nucleolus. The spiral structures are sperm. The egg is the clear central area of the

illustration and the much smaller globular structures surrounding it are the follicle cells, which are omitted in the succeeding drawings. Shortly before the cell divides the outline of the nucleus becomes indistinct. Schneider found, however, that by adding a little acetic acid it becomes visible, though folded and wrinkled. Later the nucleolus disappears and all that remained of the nucleus was a clear area in the cell. However, acetic acid treatment revealed a mass of delicate, curved fibers. The second drawing shows these strands, the chromosomes (a term not to be introduced until 1888), lined up on an equatorial plate. The strands seem to become more numerous, and when the cell divides they pass to the daughter cells.

What was one to make of this observation? The answer was far from clear. If one could not see the strands in the living cells and, if they appeared suddenly when acetic acid was added, would it not be reasonable to assume they were artifacts? Nevertheless, the fact that strands were observed repeatedly, and that they seemed to undertake these strange movements, argued that they might be present, though invisible, in the living state.

There is no evidence that Schneider realized he was giving a reasonably accurate first description of a process that was soon recognized to be of tremendous importance. His primary concern was the morphology of *Mesostoma* and to use the data obtained to ascertain the relation of these little animals to other invertebrates. In these post-Darwinian years one of the main preoccupations of biologists was to use morphology to try to sketch the broad outlines of organic evolution.

The events in cell division, and especially the changes in the nucleus, were immediately recognized as important phenomena to be studied. In fact, they seemed to be about the only constant changes that occurred in cells.

In 1881 Professor Mark of Harvard University published a comprehensive review of cell division. In his extensive bibliography there are 194 papers by 86 authors that were published between 1874 and 1878 alone. That is, in the five years immediately after the first reports of Schneider, Bütschli, and Fol. This was a period of more or less blind experimentation. The animal and plant kingdoms were combed for favorable material, which was studied while living as well as after various sorts of fixation and staining. There were few generally accepted conclusions. The persistent bugaboo of cytology—is it real or is it an artifact?—made progress slow and tentative.

Chromosomes *were* artifacts in most materials, that is, they could not be seen in living cells and became obvious only after the most drastic treatment. Their very name, meaning colored body, indicates their artifactual nature—no one maintained that the living cell possessed any colored rod-like structures. It could be, however, that such structures are present, though invisible, in the living cell and they could be made visible by appropriate techniques. The human eye, aided by the crude microscopes of the day, might not be able to "see" all of the cellular structures present.

What was necessary was to find material in which a direct comparison could be made between the structures of living cells and the same structures after fixation and staining. Could it be shown that the readily observed structures of fixed and stained cells closely resembled the difficult-to-see structures of living cells? If so, one could use fixed and stained preparations with the assurance that they reflected the living state and one could establish the degree of resemblance.

That was the accomplishment of Walther Flemming.

WALTHER FLEMMING

Flemming was successful in determining that the nuclear events observed in cell division in fixed and stained material have their counterparts in living cells. Although he did not discover mitosis, we owe to him more than to anyone else the concept of mitosis that we hold today. Only details were added after Flemming. His success was due to the material he selected for study, in being careful to check in living cells the things observed in fixed and

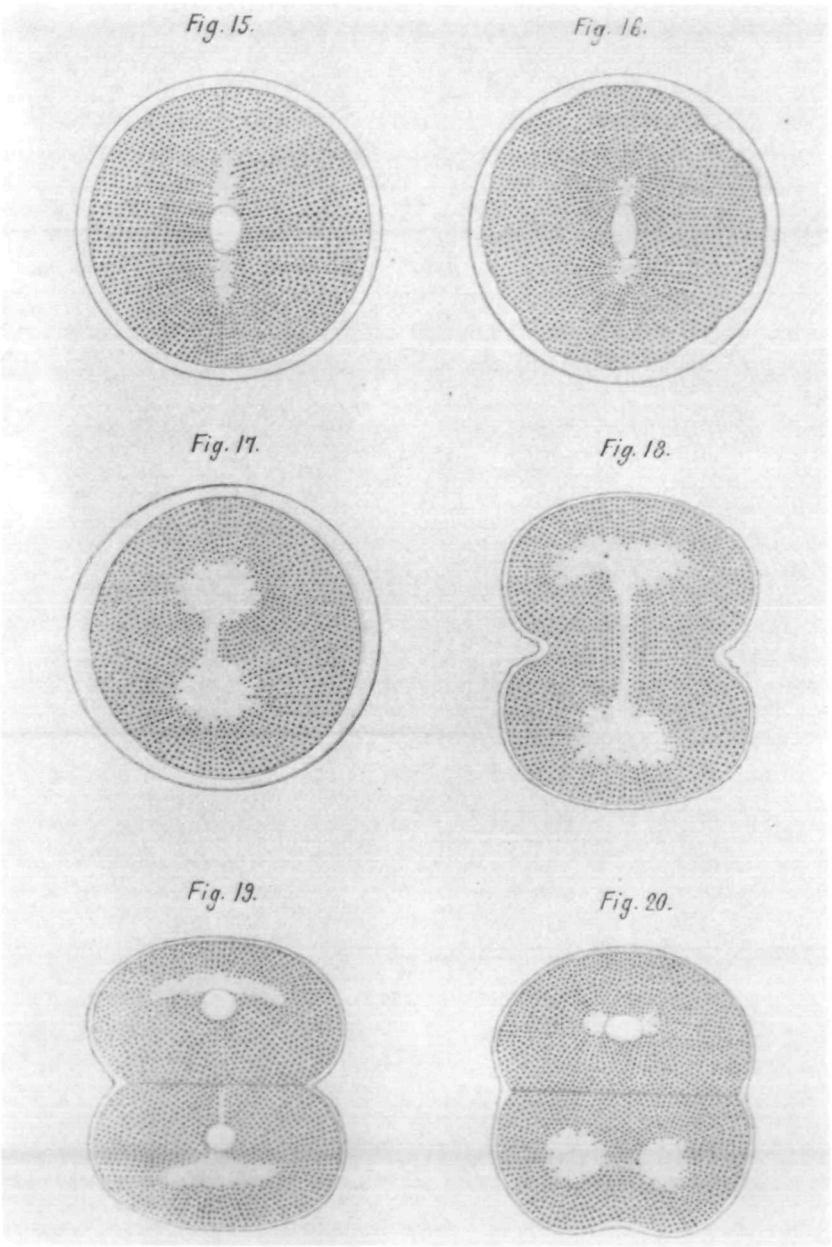
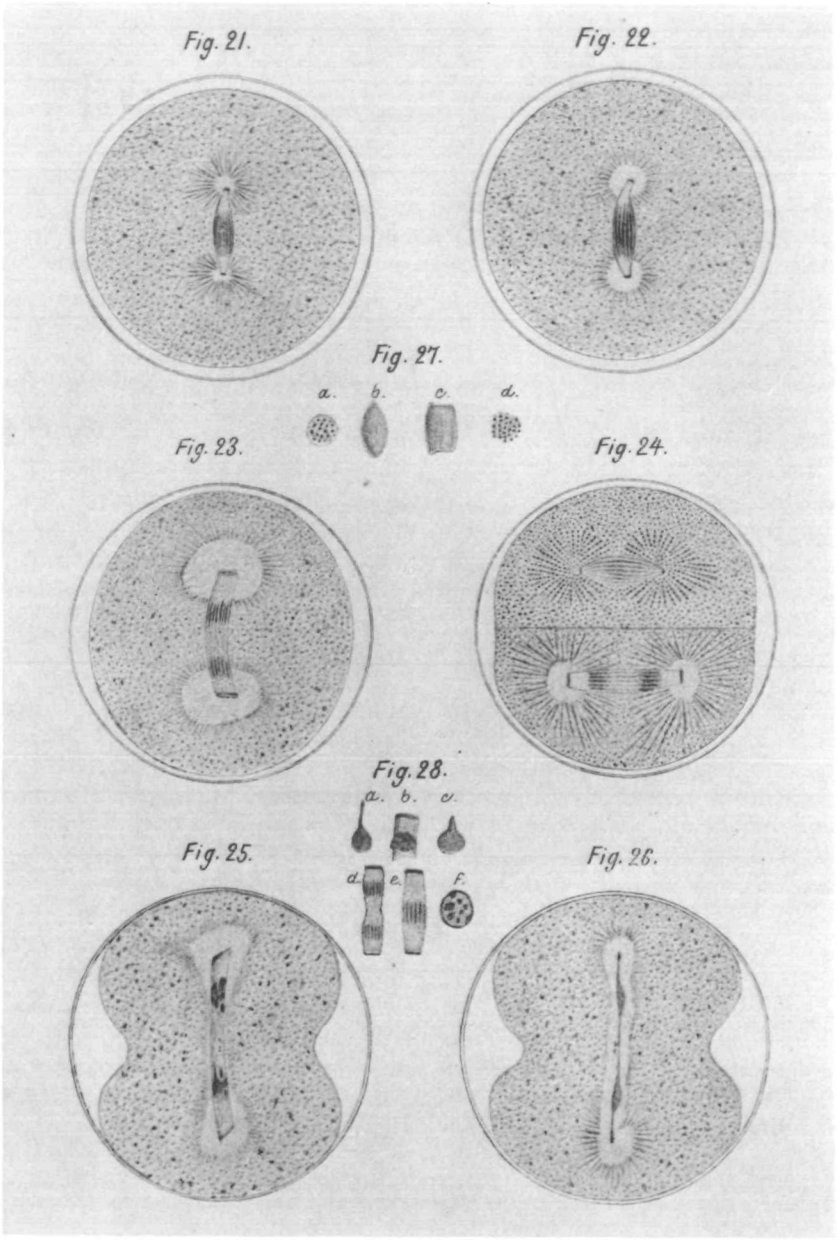


FIG. 5. Cell division in a sea urchin embryo. Hertwig's Figures 15–20 show what can be seen in living embryos. The nucleus appears to pinch in two. In the living embryo it is simple to determine the sequence of events. Shown are embryos at various times after fertilization: 30 minutes (Fig. 15), 45 minutes (Fig. 16), showing the two division centers, 60 minutes (Fig. 17), 65 minutes (Fig. 18), 70 minutes (Fig. 19). Figure 20 shows the beginning of the next division. Figures 21–26 show what can be seen after the embryos have been

stained cells, and in being able to use microscopes that were very much better than any available previously.

Not only does the use of living cells give one greater confidence that what is being observed is real, not artifact, but it also allows one to determine the sequence of events. This can be brought home to stu-



fixed in osmic acid and stained with carmine. The chromosomes, spindles, centrosomes, and asters have now become visible. The figures, in times after fertilization, are: 40 minutes (Fig. 21), 45 minutes (Fig. 22), 60 minutes (Fig. 23), second cleavage (Fig. 24), 65 minutes (Figs. 25, 26). (Hertwig, 1876) (Compare the rather vague rendition of the sea urchin chromosomes with Flemming's illustrations of amphibians in Fig. 7.)

dents by asking them to try to determine the sequence of mitotic events by studying the standard slides of onion root. Can one make an equally valid case for two nuclei fusing to form one as for one nucleus giving rise to two?

Early developmental stages provide a way to determine the sequence of nuclear

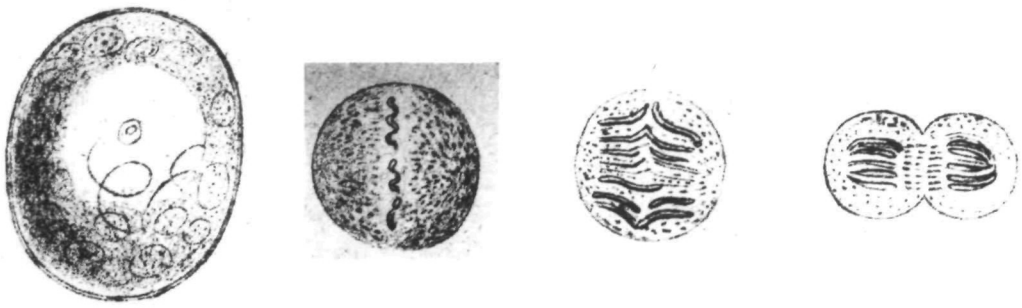


FIG. 6. Schneider's illustrations of the nuclear changes during cleavage in *Mesostoma* embryos. The left figure is of an uncleaved ovum (clear area with a nucleus and nucleous surrounded by follicle cells. The spiral structures are sperm). The other figures show the "strands," now chromosomes, and their movements during cell division. (Schneider, 1873)

changes—even in preserved cells. In fertilized echinoderm eggs, for example, cell divisions occur every half hour or so (depending on the temperature), and of greater importance, all the embryos develop synchronously. Thus, if small samples are preserved every few minutes and stained and studied later, one can be sure of the sequence of events. Embryos have rapid cell divisions—clearly a great advantage if one wishes to study the process. In most adult tissues a dividing cell is seen infrequently—except where there is rapid replacement or growth.

Flemming examined many sorts of cells and found that those in the epidermis of salamander embryos were worth detailed study. The chromosomes are huge by microscopic standards but, of much greater importance, with careful observation they can be seen in living cells. Figures 7 and 8 (from Flemming, 1882) show what he saw in living and preserved cells.

A nucleus not undergoing mitosis is said to be in the *resting stage.* This is an unfortunate term since it implies inactivity and it is now realized that great physiological activity is occurring during this stage. Flemming saw no chromosomes in the resting stage nuclei of living cells. The nucleus appeared to lack all internal structure. When such cells were fixed and stained the nucleus was seen to contain a dense and deeply staining network together with one or two large spherical granules, the nucleoli.

Changes in the nucleus are the first indications that mitosis is under way. In the apparently structureless living nucleus long delicate threads make their appearance. When they can first be seen, that is the start of *prophase.* (Mitosis is a continuous process that, for descriptive purposes, was divided into discrete stages by cytologists.) These threads condense into chromosomes that assemble in the middle of the cell at *metaphase,* at which time the nuclear membrane disappears. In stained cells the chromosomes were seen to be in an elongate fibrous structure—the spindle. Stained cells also revealed the presence of tiny granules, the centrioles, at the ends of the spindle. They also revealed another set of fibers, the astral rays, that radiate from the centrioles. In living cells during *anaphase* the chromosomes separate into two groups and move within the spindle area to opposite parts of the cell. When the chromosomes have reached the ends of the spindle, that is *telophase.* The chromosomes in living cells become less and less distinct and the nuclear membrane reforms. The nucleus is, once again, in the *resting stage.*

What is one to conclude about this process? Again, this might be a question for the class to consider.

It is obvious that *all* cell structures must be reproduced if the daughter cells are to

be essentially identical with the parent cell. Flemming was able to explain how this is accomplished for chromosomes. If the chromosomes of a single cell are to be divided equally between the daughter cells, the chromosomes must double in number at some stage in the cell cycle. Flemming observed that when the chromosomes first appear in early prophase they are double so, sometime between their disappearance in the previous telophase and their reappearance in prophase, each chromosome must have doubled (Figs. 7, 8).

Today, of course, we consider chromosomes to be permanent cell structures even though they are readily visible only during mitosis. We also recognize the individuality of chromosomes, that is, they usually exist in homologous pairs with each pair containing a specific set of genes. Could any of this be concluded from the observations of Flemming? Not really. In fact, could the following hypothesis be denied? We will assume, with Darwin, that inheritance and the functioning of cells are due to specific gemmules. The gemmules are widely distributed during the resting stage when they are, presumably, directing the activities of the cells. Before the start of mitosis the gemmules congregate in the nucleus and join one another, like beads on a string, to form long strands—the chromosomes. The gemmule-bearing chromosomes are then divided in mitosis and each daughter cell receives an allotment. The chromosomes then break down and the gemmules are dispersed throughout the cell, where they carry out their directing activities. Will Flemming's data support or deny this hypothesis?

Those students who already know that genes are parts of chromosomes may suggest that Flemming's observations suggest strongly that chromosomes are involved in inheritance. The argument may go something like this: since the mitotic process ensures that each daughter cell receives its allotment of chromosomes this must indicate, beyond much doubt, that such an elaborate and precise mechanism for duplication and distribution is of fundamental importance. And what can be more important than ensuring that the elements controlling inheritance and the life of each cell reach each cell?

But one might respond that, since the daughter cells come to be essentially identical with the parent cell, *all* cell products are reproduced. One might argue that it is merely an accident that the process of reproduction and distribution is more readily visible for the chromosomes. There is no reason, therefore, not to assume that chromosomes, cell membranes, and all those granules and globules in the cytoplasm have an equal chance of being involved in inheritance.

Those students who already know the outcome should be asked to suggest what sorts of cytological observations and experiments are required to show that chromosomes have individuality and permanence.

BUT MITOSIS CANNOT BE UNIVERSAL

Flemming and many other contemporary cytologists were making a strong case that mitotic divisions of the nucleus are a concomitant of cell division. This is a general statement that was based on putting together many observations on cells of numerous species of plants and animals. You might cite this to your students as an example of induction.

We can now use this general statement as a hypothesis to be tested. That is, we can switch to deductive reasoning. For example: if the hypothesis that the nucleus always divides by mitosis is true, then in each succeeding generation the number of chromosomes should double. This is inevitable, for if the nuclei of egg cells and sperm cells have been formed by mitosis, and if they unite in fertilization, the zygotes must have twice the number of chromosomes as the parents.

Yet they do not: Flemming and other cytologists were aware that the number of chromosomes seems to be about the same in all individuals and in all available generations of a species.

Obviously there is a problem with this hypothesis. There must be some mechanism for reducing the number of chro-

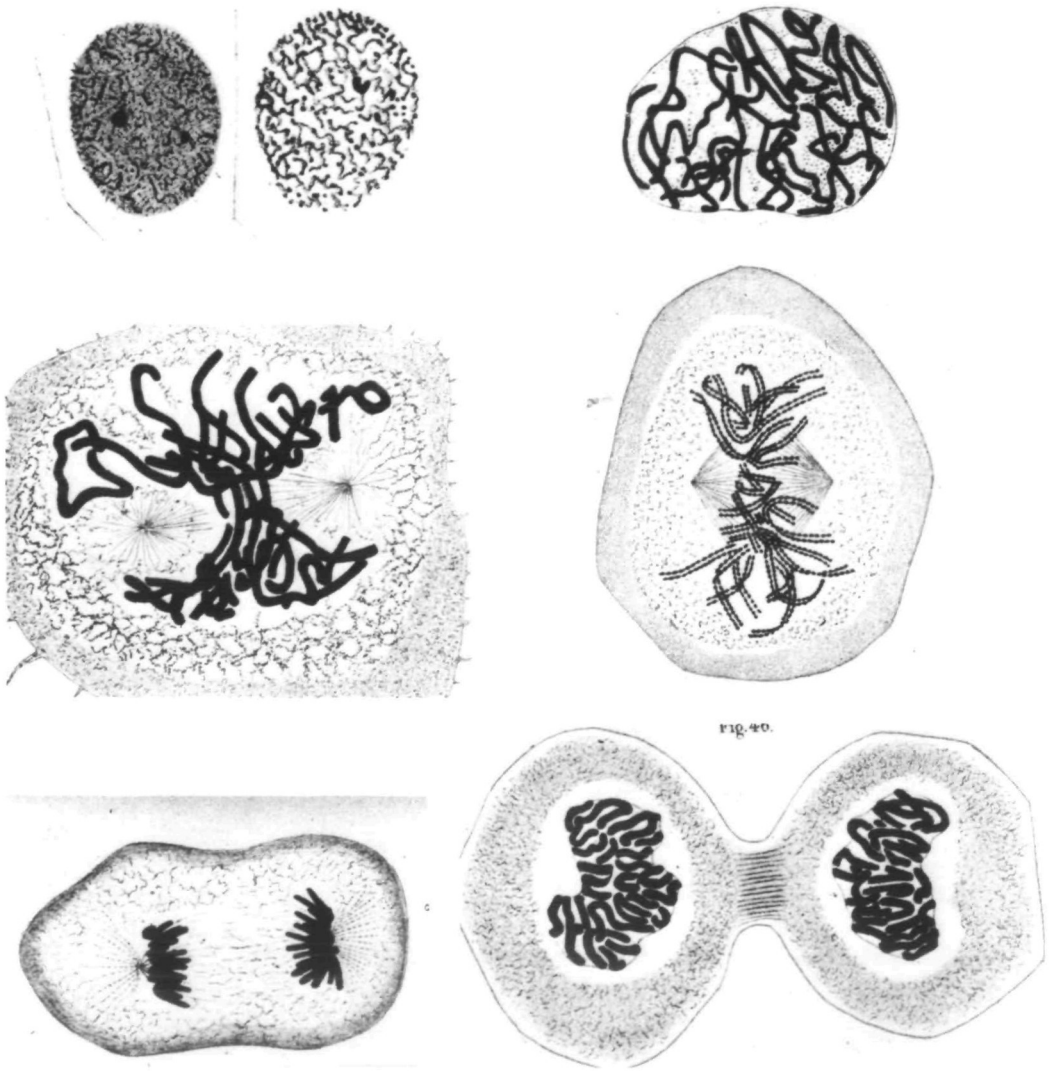


FIG. 7. Flemming's illustrations of mitosis in fixed and stained cells of a salamander embryo. The two cells at the left in the first row are in the resting stage. There are no chromosomes recognizable as such but there are two nucleoli. The right figure is of prophase. The nucleoli have disappeared but the nuclear membrane is still intact. The cytoplasm is not shown. The left figure in row two is of early metaphase. The nuclear membrane has disappeared and the centrosomes have moved apart. The right figure shows an especially fine preparation showing that the metaphase chromosomes are double, that is, each composed of two chromatids. The chromatids separate and move to the poles of the spindle, as in the bottom left figure. In the lower right figure the cell has divided and the chromosomes of the daughter nuclei are being surrounded by a nuclear membrane. (Flemming, 1882)

mosomes at or before fertilization. One might imagine that, when egg and sperm nuclei fused at fertilization, the chromosomes fused to one another or that half are destroyed. Or possibly there were some changes in chromosome number when the eggs and sperms were formed in the gonads.

THE SIGNIFICANCE OF POLAR BODIES

Various observers had noticed that, around the time of fertilization in the eggs of many species, tiny spheres were pinched off at the animal pole of the egg. They soon disappeared and, since no function was readily apparent, the non-committal term

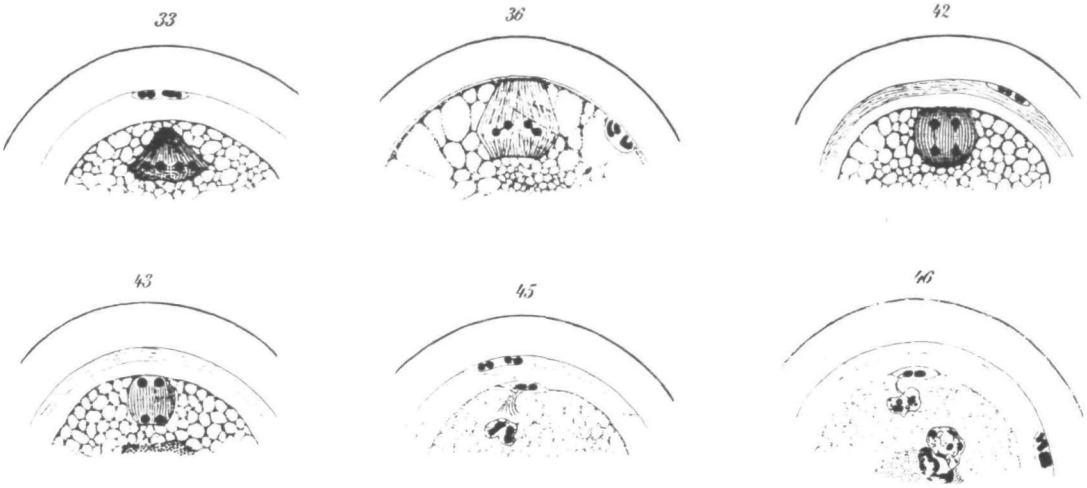


FIG. 9. Boveri's illustrations of meiosis is an *Ascaris* female. Previous to the figures shown here the four chromosomes had synapsed and replicated to form two tetrads. These were separated at the first meiotic division and two dyads went into the first polar body and two remained in the eggs. This is shown in Boveri's Figure 33. In Figure 36 the dyads are rotating prior to their separation in the second meiotic division. The second polar body is shown at 2 o'clock. Figures 42 and 43 show the dyads separating. In Figure 45 the second division is complete and the second polar body with its two chromosomes appear at the surface of the egg. The first polar body as above it. The two chromosomes in the egg are about to form the female pronucleus. Figure 46 shows the first polar body at 3 o'clock, the second polar body at the egg surface at 12 o'clock, the female pronucleus immediately below it, and the male pronucleus with the division apparatus at the bottom. (Boveri, 1887)

entered by a sperm. It is only then that meiosis begins and polar bodies are formed. Figure 9 from Boveri (1887) shows what happens.

At the onset of meiosis each of the four long chromosomes of the ovum shortens to form a tiny sphere. These four chromosomes then come together in pairs, a process known as synapsis. Then each chromosome is duplicated. Thus the cell will have two groups of four chromosomes each. Each group of four is known as a tetrad. The tetrads are divided by a highly unequal cell division that results in a small first polar body and a large egg cell. Each contains the diploid number of four chromosomes. These four chromosomes are not separate—they are in pairs. Thus, each tetrad has been divided into two dyads.

At the second meiotic division one observes a key feature of meiosis. The chromosomes are not duplicated. Thus each dyad enters the spindle and, at anaphase, its two chromosomes go to opposite poles and the cell divides unequally again. The result is a tiny second polar body with two chromosomes and a large ovum also with two chromosomes.

Thus meiosis in the female has, in two divisions, reduced the diploid number of four chromosomes to the monoploid number of two chromosomes. Weismann's hypothesis proved true, at least for the *Ascaris* female.

MEIOSIS IN THE *ASCARIS* MALE

Weismann's prediction for the male was found to be correct as well. When the testis was studied it was found that during early development its cells increase in number by mitotic divisions, that is, each son cell has the diploid number of four chromosomes (Bauer, 1893).

However, in the mature testis, the last two divisions before the cells differentiate into sperm are different. This is when the meiotic divisions occur in the male. As far as the chromosomes are concerned, the events are the same as in the female, but not so for the cell as a whole. Again the four chromosomes join in pairs, duplicate, and form the two tetrads. At the first

meiotic division each tetrad is divided, a dyad going to each pole. In contrast with the first meiotic division in the female, two cells of equal size are produced. At the second meiotic division there is no chromosomal replication and the dyads are divided, each son cell ending with two chromosomes.

Thus one original diploid cell, with four chromosomes, will form four cells after the two divisions—each with two chromosomes, the monoploid number. There are no further divisions of these cells and each differentiates into a sperm cell (Fig. 12).

An essential difference between meiosis and mitosis is this: in mitosis there is one duplication of each individual chromosome for each cell division; in meiosis there is only one duplication of each chromosome for the two subsequent divisions. Thus, mitosis is a mechanism for maintaining constancy of chromosomal number in cell division whereas meiosis is a mechanism for halving that number.

FERTILIZATION

The basic fact of fertilization, namely that a sperm rather than the seminal fluid is required to initiate development of the ovum, was discovered by J. L. Prévost and J. B. Dumas in 1824. However the actual role of the sperm was not established by their work. As noted before, George Newport (1854) proved that the sperm penetrate the ova of frogs. But what happens then?

The answer came from the observations of Oskar Hertwig (1876). He noted, as had others before him, that shortly after fertilization the ova of sea urchins appear to have two nuclei (Fig. 10). One of these first appears just under the surface of the ovum. Hertwig suggested that this was derived from the sperm. The other nucleus was near the center of the ovum. Hertwig suggested that it was the female nucleus. Five minutes after fertilization the putative sperm nucleus moved inward toward the center of the cell. By 10 minutes after fertilization the two nuclei were side by side in the center of the ovum. By 15 minutes there was a single nucleus.

Hertwig believed that he was observing the essential feature of fertilization: the union of paternal pronucleus formed from the sperm with a maternal pronucleus in the ovum. That union produces the zygote nucleus that would, by mitotic cell divisions, produce the cells of the new individual.

Ascaris provided much better material for studying the details of fertilization, again because of its few large chromosomes. Van Beneden and Boveri described the process in detail. Figure 11 is from Boveri (1888).

The first illustration, *a,* is of an entire ovum shortly after the sperm has entered. The paternal pronucleus is in the lower right-hand quadrant. The two darkly stained irregular masses are the two chromosomes—two is the monoploid number. The structure forming the wrinkled cap immediately above the paternal pronucleus is the acrosome, which is the portion of the sperm head composed of Golgi material. The dark granular mass in the center of the ovum is the centrosome. It, too, originated from the sperm. There are four black bodies near 12 o'clock. The upper two are the chromosomes of the second polar body. The lower two are the monoploid number of chromosomes of the maternal pronucleus. The second polar body is shown in the sectioned embryos of *b, c,* and *e* as well. Do your students understand why only some of the sections show the polar body?

In *b* the maternal and paternal pronuclei have moved closer to one another and their chromosomes have become indistinct. In *c* the chromosomes have elongated greatly and, although we now know there are only two in each pronucleus, this cannot be told from the illustration (this is a vivid example of the great difficulty cytologists had in coming to the realization that the chromosomes of any one species are constant in number and are individually unique—much of the time they seemed to be as confusing as spaghetti). One can distinguish two dark granules in the centrosome. These are the centrioles.

In *d* the chromosomes have become distinct once again (from *b* through *c* they were going through a modified resting stage) and each pronucleus shows two. The

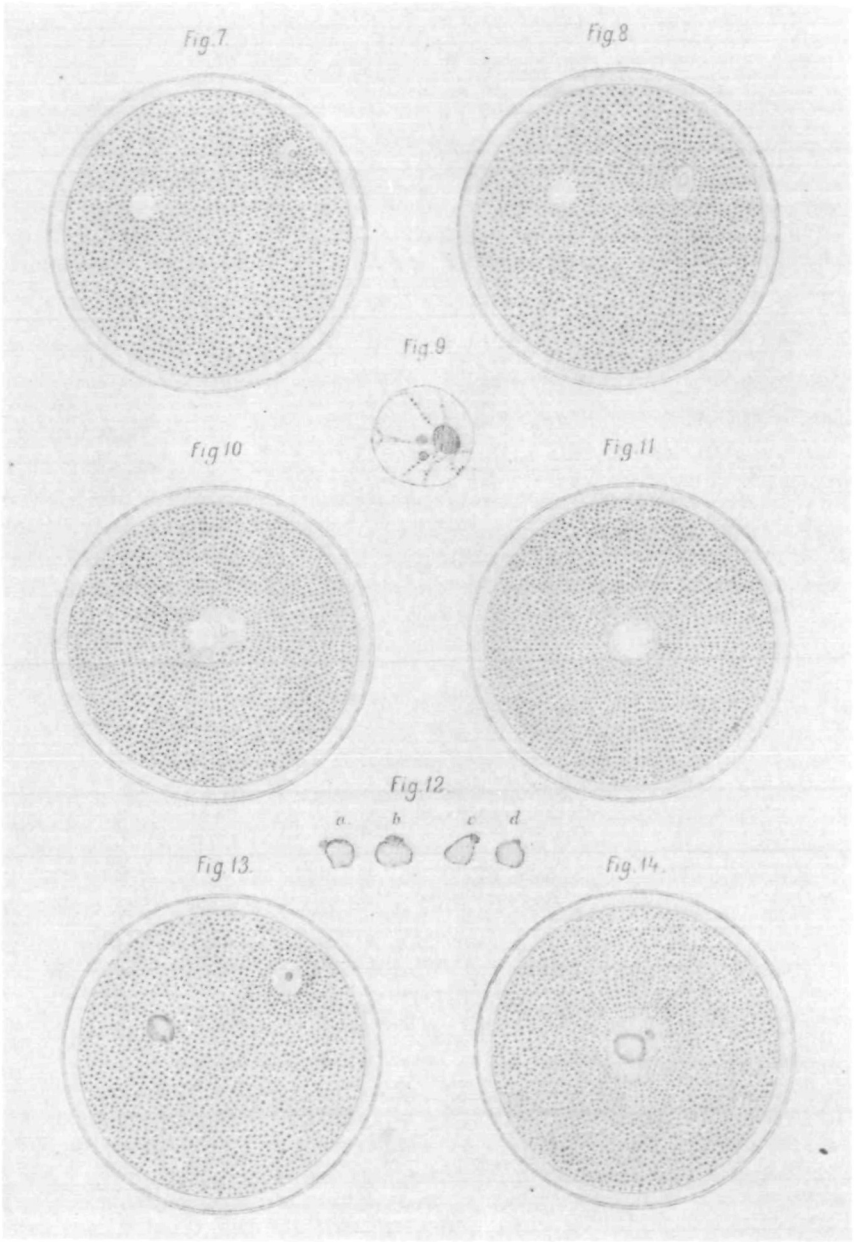


FIG. 10. Hertwig's illustrations of fertilization in the sea urchin. Figures 7, 8 (9 is of a mouse), 10 and 11 are of living embryos. His Figures 7 and 8 are 5 minutes after eggs and sperm were mixed. The female pronucleus is the clear area to the left and the male pronucleus is at the right. At 10 minutes (Fig. 10) the two pronuclei have come together in the center of the egg. At 15 minutes (Fig. 11) they appear to be completely fused. Figures 13 and 14 are of embryos fixed in osmic acid and stained with carmine 5 and 10 minutes, respectively, after the gametes were mixed. (Hertwig, 1876)

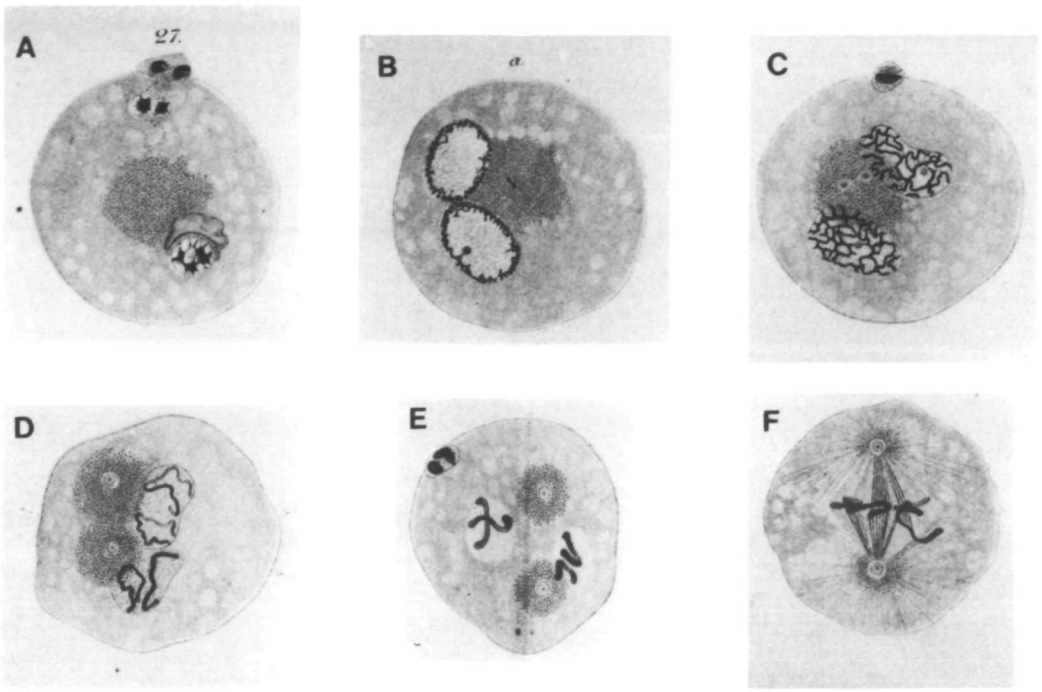


FIG. 11. Boveri's illustrations of fertilization in *Ascaris*. See text for details. (Boveri, 1888)

centrosome has divided in two, each with a centriole in the center. This process continues through *e*. In *f* four chromosomes, two from each pronucleus, are lined up on the spindle and shortly thereafter each is seen to be double, that is, be composed of two chromatids. The chromatids will separate to form independent chromosomes and one will go to each pole.

The form of the mitotic apparatus is shown well in *f*. At each end of the spindle one finds the tiny centriole, surrounded by a dark granular area—the centrosome. In preserved material fibers are seen to radiate from each centrosome, forming an aster. Other fibers extend from one centrosome to the other, forming the spindle. In *f* the cell is in metaphase of the first embryonic division and the chromosomes are lined up in an equatorial plate.

SIGNIFICANCE OF GAMETE FORMATION AND FERTILIZATION

It all turned out just as Weismann thought it must. The cells that were, many cell generations later, to form the gametes in both ovary and testis of *Ascaris* started out with four chromosomes, the diploid number. These cells divided repeatedly, always by mitosis.

In males the last two divisions of the sperm-forming cells of the testis, however, were meiotic, not mitotic. During these two divisions the cells divided twice but the chromosomes replicated only once. Each cell division was equal, that is, two cells of the same overall size were formed by each division (Fig. 12). This resulted in four cells of equal size, each with two chromosomes, the monoploid number. The four cells then differentiate into spermatozoa.

After many cell cycles of mitotic divisions, some of the ovarian cells enlarged greatly—forming the ova. As in the case with males, there were two meiotic divisions of the nuclear material with only a single replication of chromosomes. The first division of the cell was so unequal that most of the material remained in the cell that was to form the ovum and only a minute amount was included in the first polar body. This was repeated at the second division,

which produced the tiny second polar body and the large ovum. Nevertheless, the nuclei of the second polar body and of the ovum were identical—each with the monoploid number of two chromosomes.

Meiosis in *Ascaris* therefore produced monoploid sperm and monoploid ova. The union of one of each was the origin of the diploid zygote—the beginning of a fine new nematode worm. The processes are summarized in Figure 12.

It was clear from the work of van Benedenn, Boveri, and others that each parent transmits the same number of chromosomes to the zygote. Furthermore, the chromosomes in maternal and paternal nuclei appeared to be identical. These two observations could help explain the long-held belief that the hereditary contribution of each parent is roughly the same.

This was exciting and important research and soon many investigators were studying a large variety of plants and animals. With very few exceptions, what had been found for *Ascaris* was true for all other organisms. To be sure there were some minor variations, but an intensive study of these served only to increase the depth of our understanding of the entire process. A concept of universal application had been discovered.

PARADIGMS AND NORMAL SCIENCE

Thomas Kuhn (1970), in his *The Structure of Scientific Revolutions,* argues that science advances in two main ways which we might characterize as by fits and starts, or, to be more current, by punctuated equilibria. Kuhn points out that, from time to time, there is a revolution in the way scientists view their research problems and the sorts of observations and experiments that they undertake. Some bold, novel, and major new idea lets them see the existing data in a new perspective and suggests a new program of research. These major new ideas are, in Kuhn's terminology, *paradigms*—the "universally recognized scientific achievements that for a time provide model problems and solutions to a community of practitioners" (p. viii). The new paradigm is the central concern of the moment for a significant proportion of the scientists in a field. It determines the sorts of research they do and, in our times, whether or not they are likely to get grants. This work is the *normal science* that occupies most investigators most of the time. It consists of working out the consequences of the new paradigm.

We have discussed two major paradigms in cytology. The first was the Cell Theory, a new way of looking at the structure of organisms. That paradigm had a slow development but, in the first two-thirds of the 19th century, it occupied the attention of many cytologists. The normal science that was stimulated by the paradigm resulted in investigations of innumerable sorts of organisms and, almost always, their microscopic structure "made sense" in terms of Cell Theory.

These studies also extended the limits of what could be called "cell." The structure of tissues in human beings was investigated in great detail and soon this knowledge became of considerable importance in medicine as the basis of pathology. The structure of diseased cells and tissues became one of the most satisfactory criteria for identifying diseases. During the 19th century, diagnosis, not cure, was the crowning achievement of medicine. Physicians were far better in identifying diseases than in curing them.

Kuhn believes that in most instances one paradigm does not evolve into a new one. Instead, the field takes an entirely different approach with a new paradigm. Gradually the practitioners lose interest in the old paradigm and begin to work out the details of the new. Or most of the older scientists pass out of the picture with their old paradigm and the Young Turks do the normal science within the parameters of the new paradigm.

This happened in cytology. In the last third of the 19th century a new approach came into vogue. The new paradigm may be called the Theory of Chromosomal Continuity. It sought to trace the behavior of chromosomes in mitosis, meiosis, and fertilization. Many cytologists lost interest in establishing that yet another creature had a body composed of cells and, instead, sought to discover what that creature's

OUTLINE OF MEIOSIS AND

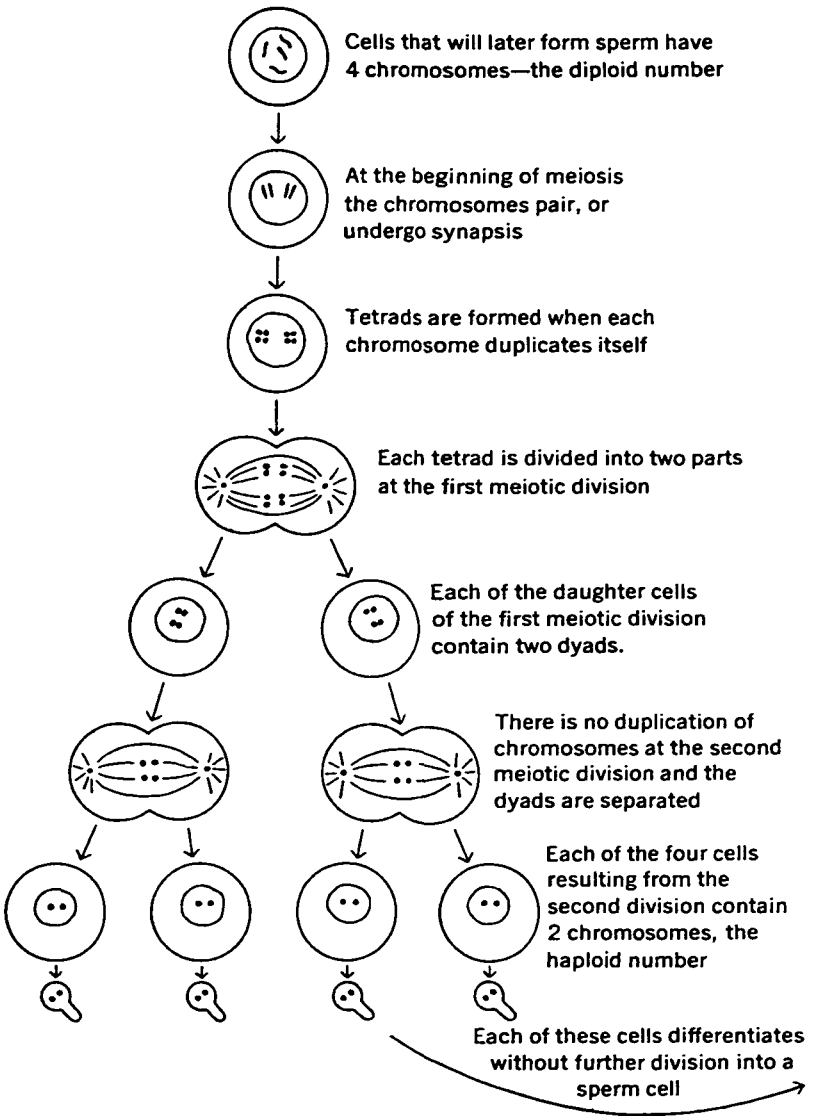


FIG. 12.

chromosomes did in the life cycle. Once again, the new paradigm provided a conceptual basis for important biological phenomena and guided the research, the normal science, that fleshed out the details.

Until fairly recent times, cytology was mainly a descriptive science. One's ability to manipulate cells to test hypotheses was severely limited. In many instances, however, it turned out that some species might exhibit the cellular condition sought by the investigator. Hence, nature had already arranged the experiment. It was mainly for this reason that cytologists studied a great variety of organisms to discover those that exhibited some variation in chromosomal behavior and, hence, might give tests of deductions when experimentation was not possible.

THE NUCLEUS AND INHERITANCE

The remarkable observations on the behavior of chromosomes in mitosis, meiosis, and fertilization made in the 1870s

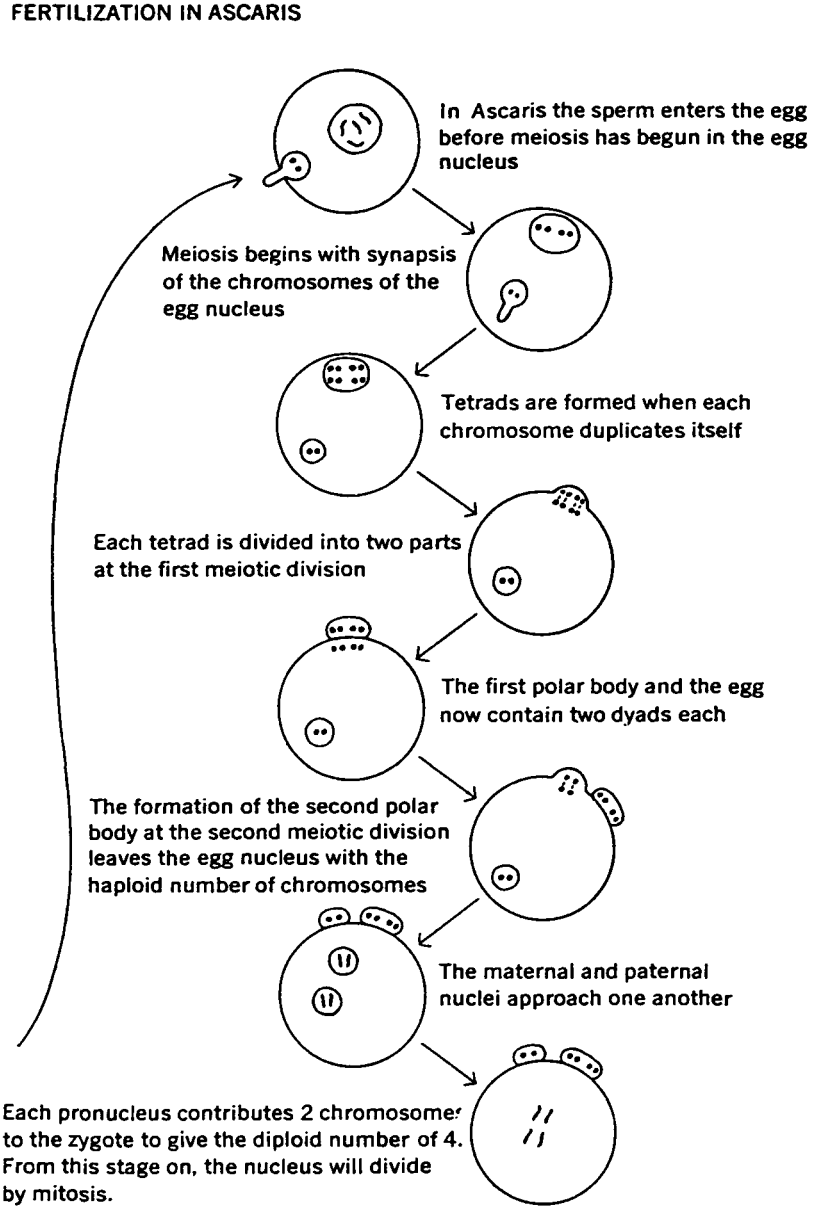


FIG. 12. Continued.

and 1880s, mainly in Germany, provided a general picture for the transmission from generation to generation of the fundamental structures responsible for inheritance.

But I trust that your students will observe that those studies provided no *critical* evidence that chromosomes are, indeed, the physical basis of inheritance. One could do no more than suggest that the chromosomes *could* play that role. Nor in the 1880s were they any closer to being able to say how one might establish the role of a cellular structure in heredity.

Nevertheless, many scientists believed that the nucleus and/or the chromosomes were the prime candidates for this important biological function. As early as 1866 Ernst Haeckel (1834–1919) suggested that the nucleus is responsible for the trans-

mission of what we call genetic information. This was really a long shot. It was still seven years before Schneider's description of mitosis in *Mesostoma,* and Haeckel had no data to support his hypothesis. Nevertheless Haeckel was one of the most prominent biologists of the day and any idea of his, no matter how slight the factual basis, would be noticed. Therefore, Haeckel's hypothesis of nuclear control of heredity would stimulate others to think along such lines.

Darwin's *Variation* was published two years later and, again, something was being said about inheritance by a very important scientist. The great difference between the hypotheses of Haeckel and Darwin indicated that the field was still wide open.

In 1884 still another important biologist, Carl Wilhelm von Nägeli (1817–1891), suggested a very different hypothesis. He postulated a material, the idioplasm, as the physical basis of inheritance. He proposed that it was an invisible chemical network extending throughout cells and from one cell to another. It could be imagined to be Darwin's gemmules joined to form a continuous web. He postulated that the idioplasm was somewhat unstable and could change during development but return to the original condition at the beginning of the next generation.

Nägeli expanded the hypothesis of the idioplasm into a huge theoretical structure without much basis in observation or experiment. In fact, the hypothesis was essentially impossible to test and, hence, of little use in furthering knowledge. It is mentioned here to show that, although some prominent biologists believed the nucleus to be the center of inheritance, other prominent biologists predicted other mechanisms. This is always the case at the frontiers of science. There will be a variety of competing explanatory hypotheses that will be tested, and, in time, one will remain the best explanatory hypothesis of the day. We mislead ourselves, and our students, if we treat the intellectual development of a field as a straight line to "truth." More often than not the model resembles not that straight line but Nägeli's idioplasm—going in all directions at the same time. But now back to the main line!

The Hypothesis of Chromosomal Control of Inheritance

In 1884–1885 four German biologists independently concluded that the physical basis of inheritance must be the chromosomes. They were Oskar Hertwig, Edouard Strasburger, Rudolf Kölliker, and August Weismann. The first three were active investigators of cellular events centering around fertilization. Weismann, in part because of poor eyesight, was concerned mainly with theory—recall his prediction of the reduction divisions.

The basic problem for the student of the cell who sought the physical basis of inheritance was to find some cellular phenomenon that could account for what was known about inheritance. That is, how could one find in cells something that accorded with the results of breeding experiments? Expressed still another way, one had to discover some cytological phenomenon that would parallel genetic phenomena—an especially difficult task when there were no precise genetic rules.

The sorts of data and argument that suggested the hypothesis of chromosomal involvement in inheritance to the four biologists mentioned above were as follows.

First, it was usually observed that both parents seem to have an equal share in transmitting their characteristics to the offspring. (Darwin had emphasized this even while recognizing a few cases of sex-linked inheritance as well as other exceptions.)

A study by Joseph Gottlieb Kölreuter, the great plant hybridizer, was an excellent example. More than a century earlier, he had crossed two very different species of the tobacco genus, *Nicotiana paniculata* and *Nicotiana rustica.* It was important to use plants that differed considerably so that he could test the influence of each parent. So far as Kölreuter could tell the hybrids were the same whether the cross was *Nicotiana paniculata* pollen × *Nicotiana rustica* ovules or the reverse. Neither parent had a dominant role. (See Roberts [1929, ch. 2].)

What could be the basis of this equality

found by Kölreuter and many others? In the case of animals there is a great difference in the quantity of material in ova and sperm. If the results of inheritance depended solely on the quantity of material transmitted by the female and by the male, and if their gametes were the sole physical link between the generations, then one would expect the female's influence on the offspring to be greater than the male's. Since this appears not to be so, the ovum as a whole and the sperm as a whole cannot be the physical basis of inheritance.

Is there, therefore, *any* cellular component of sperm and ovum that is equivalent? If so, it might be a candidate for a central role in inheritance. In the late 1880s a possible candidate was being suggested by the then most recent research. Hertwig and many others were finding that, shortly after fertilization, there were two nuclei—the female pronucleus and the male pronucleus. These appeared to be identical, apart from the centrioles and centrosome associated with the sperm pronucleus, so perhaps this equivalence in structure could be the basis of the equivalent importance of the two gametes in inheritance.

Second, there seemed to be both a stable and an unstable component to inheritance. In almost all ways offspring closely resembled their parents in general body structure. Thus, whatever was transmitted from parent to offspring via the gametes must have a high degree of stability. Yet offspring were rarely exactly like their parents and, moreover, the offspring might differ from one another.

There did seem to be a possible cellular basis for the stability—the chromosomes of the nucleus. During cell division the cytoplasm and its formed structures appear to be divided passively and by chance. That is, if a granule or globule happens to be at one end of the spindle, it ends up in the daughter cell including that material. The chromosomes, on the other hand, go through a complicated mitosis that results in each daughter cell apparently receiving an identical set of chromosomes. It seemed to Hertwig and the others that such precision in the distribution of the nuclear material during cell division could mean that the chromosomes were involved in transmitting the genetic information. There was no other likely candidate, except possibly the centrioles. But centrioles were so small and difficult to see that cytologists were not sure that they were present in all cells. They did not appear to be in ova just before fertilization and higher plants seemed to lack them.

Third, this same general argument could be applied to the chromosomal events during meiosis. The complex chromosomal changes during meiosis and fertilization could be looked upon as mechanisms for maintaining chromosomal constancy from generation to generation. Nothing like this was apparent for any other cell structure. Therefore, since inheritance is an intergeneration phenomenon and the chromosomes seem to be the only cell structures transmitted in such a precise manner as to maintain constancy, perhaps they are the key to inheritance.

Fourth, there was at least one type of experimental data. In some cases it was possible to cut protozoans into two parts—one part with the nucleus and the other without. Both parts may heal. The part with the nucleus was observed to regenerate any missing structures and to live as a normal, reproducing individual. On the other hand, the part without the nucleus did not regenerate to form a whole animal and it never reproduced. Its fate was death.

These observations were suggestive but they did not constitute complete proof that the nucleus, or its chromosomes, are the physical basis of inheritance. Furthermore, these observations could not explain many other aspects of inheritance—variation, for example. The fact that chromosomes appeared to be the only cell structures that remain constant from cell to cell, and from generation to generation, *could* mean that chromosomes are the structures of inheritance.

Weismann (1889) was willing to be far more definite than that. Recall the quotation previously given,

> . . . at least one certain result follows, viz.

> that there is an hereditary substance, a material bearer of hereditary tendencies, and that this substance is contained in the nucleus of the germ-cells, and in that part of it which forms the nuclear threads, which at certain periods appear in the form of loops or rods. (p. 355)

E. B. Wilson (1895, p. 4), in a most astonishing statement, foresaw the 1940s and 1950s.

> These facts justify the conclusion that the nuclei of the two germ-cells are in a morphological sense precisely equivalent, and they lend strong support to Hertwig's identification of the nucleus as the bearer of hereditary qualities. The precise equivalence of the chromosomes contributed by the two sexes is a physical correlative of the fact that the two sexes play, on the whole, equal parts in hereditary transmission, and it seems to show that the chromosomal substance, the *chromatin,* is to be regarded as the physical basis of inheritance. Now, chromatin is known to be closely similar to, if not identical with, a substance known as *nuclein* ($C_{29}H_{49}N_9P_3O_{22}$, according to Miescher), which analysis shows to be a tolerably definite chemical composed of nucleic acid (a complex organic acid rich in phosphorus) and albumin. And thus we reach the remarkable conclusion that inheritance may, perhaps, be effected by the physical transmission of a particular chemical compound from parent to offspring.

Prophetic yes, but at the time a hypothesis that could not be tested. In reality, attempts to use the data of cytology alone to understand inheritance appeared to have come to a dead end. How was one to establish a causal link between the data of inheritance derived from breeding experiments and the behavior of chromosomes? For that matter, the study of inheritance by breeding experiments had come to a dead end as well. Both cytology and, what we would now call genetics, were in the Kuhnian stage of normal science awaiting the arrival of a new paradigm. That was to occur, in a most dramatic fashion, in the year 1900.

But before we move into the 20th century we can conclude with this summary by E. B. Wilson of what had been accomplished in the flowering of cytology that occurred in the last quarter of the 19th century.

> The work of cytology in its period of foundation laid a broad and substantial basis for our more general conceptions of heredity and its physical substratum. It demonstrated the basic fact that heredity is a consequence of the genetic continuity of cells by division, and that the germ-cells are the vehicle of transmission from one generation to another. It accumulated strong evidence that the cell-nucleus plays an important role in heredity. It made known the significant fact that in all the ordinary forms of cell-division the nucleus does not divide *en masse* but first resolves itself into a definite number of chromosomes; that these bodies, originally formed as long threads, split lengthwise so as to effect a meristic division of the entire nuclear substance. It proved that fertilization of the egg everywhere involves the union or close association of two nuclei, one of maternal and one of paternal origin. It established the fact, sometimes designated as "Van Beneden's law" in honour of its discoverer, that these primary germ-nuclei give rise to similar groups of chromosomes, each containing half the number found in the body-cells. It demonstrated that when new germ-cells are formed each again receives only half the number characteristic of the body-cells. It steadily accumulated evidence, especially through the admirable studies of Boveri, that the chromosomes of successive generations of cells, though commonly lost to view in the resting nucleus, do not really lose their individuality, or that in some less obvious way they conform to the principle of genetic continuity. From these facts followed the far-reaching conclusion that the nuclei of the body-cells are diploid or duplex structures, descended equally from the original maternal and paternal chromosome-groups of the fertilized egg. Continually receiving confirmation by

> the labours of later years [*i.e.*, normal science], this result gradually took a central place in cytology; and about it all more specific discoveries relating to the chromosomes naturally group themselves . . . . Such, in bird's-eye view, were the most essential conclusions down to the close of the nineteenth century. A new era of discovery now opened [the new paradigm]. As soon as the Mendelian phenomena were made known it became evident that in broad outline they form a counterpart to those which cytology had already made known in respect to the chromosomes. (pp. 334–335)

The quote is from Wilson's famous Croonian Lecture to the Royal Society of London. It was given in 1914 by which time the hypotheses concerning the relations of chromosomes had been tested and proven true beyond all reasonable doubt.

REFERENCES TO 19TH CENTURY CYTOLOGY

The most useful single reference is J. R. Baker's (*1948–1955) series *The Cell Theory*. For a fine, though shorter, paper see Coleman (*1965). The *1900 edition of E. B. Wilson's *The Cell in Development and Inheritance* summarizes the field just before the Mendelian results became generally known. And for the grand sweep see Mayr (*1982).

See also Ackernecht (1953), Allen (1976), Baltzer (1964, 1967), Blumenbach (1742), Bracegirdle (1977, 1978), Carlson (1967*a*), Carpenter (1891), Chubb (1910–1911), Churchill (1968), Coleman (1971), Conklin (1939), Dobell (1960), Emblen (1970), 'Espinasse (1962), Gabriel and Fogel (1955), Gerlach (1858), Gerould (1922), Glass (1947), Grew (1682), Gunther (1930–1938), Haeckel (1866), Hall (1969), Hertwig (1895), Holmes (1963), Hooke (1665), Hughes (1959), Huxley (1853, 1868), Karling (1939), Kisch (1954), Kölliker (1853–1854), Mark (1881), Mazzeo (1967), Moore (1972*a*, 1972*b*), Nicolson (1956), Pickstone (1973), Power (1664), Rich (1926), Robinson (1979), Roget (1836), von Sachs (1890), Schleiden (1838, 1842), Schwann (1839, 1847), Scott (1891), Shadwell (1676), Singer (1915), Sirks (1952), Strasburger (1880), A. Thomson (1836–1839), Todd (1836–1839), Virchow (1863), Voeller (1968), Weismann (1889, 1891–1892, 1893), Wilkie (1960), E. B. Wilson (1895, 1899, 1900, 1914), J. W. Wilson (1944, 1947*a*, 1947*b*), and Woodruff (1939).

References to microscopes and cytological techniques were given earlier.

CYTOLOGY AND GENETICS: 1900–1910

Born again Mendelism

1900 is the year of the onset of modern genetics. It was then that a modest, unappreciated, and nearly-to-be-forgotten paper by a long-dead Augustinian monk became known to the scientific community at large. The field of animal and plant breeding had been in a long and unexciting Kuhnian period of "normal science" but in 1900 there was to be a notable paradigm shift and genetics was on its way to become a rigorous science with vast explanatory and predictive abilities. The new paradigm was to start with the discovery of a long-overlooked paper, *Versuche über Pflanzen-Hybriden*, based on lectures that Gregor Mendel delivered to the Natural History Society of Brünn (now Brno in Czechoslovakia) on 8 February and 8 March 1865 and published in 1866 (with a publication date of 1865).

The story is familiar to teachers of biology and a convenient source of the basic documents is provided by Stern and Sherwood (1966). Two scientists, Hugo de Vries (1900) and Carl Correns (1900), are credited as being the first to understand the importance of what Mendel had accomplished. A third scientist, Erik von Tschermak, is usually included as a co-first-appreciator but Stern and Sherwood (1966, pp. x–xi) give reasons why this is not merited.

De Vries had crossed numerous "species" and varieties of plants during the 1890s. In those days the term "species" was sometimes applied to different domesticated plants that we would now consider as belonging to the same species but differing by one or a few alleles with large effects. De Vries adopted the point of view that

these different "species" should be considered "as a composite of independent factors," or units, and that,

> The units of species-specific traits are to be seen in this connection as sharply separate entities and should be studied as such. They should be treated as independent of each other everywhere, as long as there is no basis for doing otherwise. In every crossing experiment only a single character or a definite number of them is to be taken into consideration. (Stern and Sherwood, p. 108)

De Vries spoke of antagonistic characters but noted that only one was expressed in the hybrid (*i.e.*, in the F_1). Nevertheless when the pollen and ovules were formed "the two antagonistic characteristics separate, following for the most part simple laws of probability" (Stern and Sherwood, p. 110).

De Vries stated that his essential conclusions had been reached long before by Mendel, whose work had been forgotten and its significance not understood.

The story of how de Vries came to know of Mendel's paper is of considerable interest (Stomps, 1954). He did not uncover it through a "literature search" but by one of those extraordinary accidents that seem to be of such importance in scientific discovery. A fellow Dutch scientist, Professor Beyerinck of Delft, knew that de Vries had been hybridizing plants and wrote wondering if he would be interested in an old reprint dealing with the same subject. It was Mendel's paper. The letter and reprint reached de Vries in 1900, just as he was preparing to publish his own experiments. He was able to do so knowing that he was confirming Mendel's earlier, and more extensive, experiments.

The story about Correns is equally interesting (Stern and Sherwood, 1966). He also had been performing genetic experiments with plants and was trying to develop a hypothesis to account for the data. In the autumn of 1899 the solution came to him in a "blind flash," which, more often than not, seems to be the origin of the truly important breakthroughs in science. A short time later he found a reference to Mendel's paper and looked it up. He published his own data and showed how it confirmed what Mendel had found.

Perhaps it is time for us, also, to see what Mendel had done.

Mendel 1865

Gregor Mendel's famous paper is not a scientific paper in the usual sense, but instead lectures that he presented to the Natural History Society of Brünn in 1865. The complete data were never published but the portion that he did include, coupled with his extraordinary analysis of the data, puts his contribution in the same class as *On the Origin of Species.*

Mendel was fully aware that experiments in plant breeding, usually called hybridization, had been conducted for years by many famous scientists. No general rules had emerged, as we have already seen from Darwin's lack of success in *Variation . . .*, published only two years after Mendel's paper.

Mendel had started his experiments trying to understand inheritance shortly after the publication of Darwin's *Origin* and one of the reasons for so doing was the need for "reaching the solution to a question whose significance for the evolutionary history of organic forms must not be underestimated." Thus, Mendel's work started out as normal science within the paradigm of the Theory of Evolution. Only later was it to become the beginning of a new paradigm—Mendelian Genetics. This is an interesting point for students: how a discovery in one field of science may be of great importance in another.

The experimental material

Plant hybridizers of the mid-19th century had a wealth of readily available material. Numerous varieties of the same species of both food and ornamental plants had been selected. Many of the varieties were very different from one another—so different that they might be given their own scientific names. Once varieties had been developed, continued selection was practiced so they would "breed true."

Mendel decided to work with garden peas and started with 34 varieties. He grew them

for two seasons to make sure that they bred true. Finally he reduced the number to 22 varieties.

Peas had important advantages. Not only were many varieties available, as already noted, but they were easy to grow and had short generation times. The offspring obtained by crossing the varieties were fertile. The structure of the flower was also important. The stamens and pistils are enclosed by the sepals and petals and, if the flowers were covered to prevent insects from reaching them, they self-fertilize, that is, pollen falls on the stigma of the same flower.

Nevertheless, experimental crosses could be made. This was done by removing the anthers before they matured and, later, placing pollen from another plant on the stigma. Thus, Mendel could cross any of his varieties or, if he left the flowers alone, the next generation would be a consequence of self-fertilization.

Mathematics for Mendel

Those who have taught Mendelian genetics will know that many students find the mathematics difficult. It may become easier for them when, in 1903, we will put the hereditary units on the chromosomes, but it is worth the effort to help students understand such critical aspects of the Mendelian model as how the 3:1 ratio for one pair of contrasting characters can be expanded to the 9:3:3:1 ratio for two pairs of contrasting characters.

It has been my experience that one of the most difficult principles that students have to learn is that ¼ of ¼ is neither ½ nor ⅛ but $\frac{1}{16}$. In addition, students must come to believe that a 3:1 ratio is the same as saying that ¾ of the sample is of one sort and ¼ of the sample is of another sort or that 75 percent is one and 25 percent is another. A determined but sympathetic teacher can usually bring about $\frac{15}{16}$ of the class to this level of achievement.

In the discussion of Mendel's experiment that follows a great deal of attention will be paid to presenting the material in a manner that may help students to understand, and truly appreciate, the elegance of what Mendel accomplished. It was first-rate science and it is a great pity that many can learn Mendel's method but not the workings of his mind.

Mendel's data

In genetic crosses we attempt to discover the hereditary basis of differences; at the same time we suspect that the information obtained will also help us to understand why individuals of the same species resemble each other so closely. One does not cross genetically identical individuals in the hope of discovering laws of inheritance. Thus the varieties of Mendel's peas differed from one another. The difference was in relation to the characteristics of other varieties. Thus some of his varieties had round seeds and in others the seeds were wrinkled ("angular" would be a better translation of his term); some of his varieties had yellow seeds and in others they were green. In all he used 7 pairs of contrasting characters as follows:

Character affected	Varieties
Seed shape	*round* or *wrinkled*
Seed color	*yellow* or *green*
Seed coat	*colored* or *white*
Pod shape	*inflated* or *wrinkled*
Pod color	*green* or *yellow*
Flower position	*axial* or *terminal*
Stem length	*long* or *short*

Varieties with the contrasting characters were crossed by removing the immature anthers from the flowers of one variety and placing pollen from the other variety on the stigma. The F_1, to use a term introduced subsequently, gave a uniform result: all of the F_1 exhibited the characteristic of only one parent. Mendel spoke of the characteristic that appeared in the F_1 as being *dominant,* in contrast the characteristic that did not appear—the *recessive.*

These results, so familiar to us today, were rather unexpected in the 1860s. Although there were similar instances, the general rule was that the F_1 individuals tended to be intermediate. And in most cases they are, for the simple reason that, if varieties differ in many ways, the F_1 will

usually be more or less intermediate. But Mendel concentrated on the inheritance of details, not of the totality. In a sense he forgot the whole plant and asked only if the peas had *round* or *wrinkled* seeds, etc.

The F_1 plants were protected from being cross-pollinated by insects and allowed to self. Again the results were uniform. For each of the original seven crosses of plants with contrasting characters, the F_2 offspring resembled one or the other parent of the P generation. They were never intermediate.

Whereas most plant breeders would have reported only that both varieties appeared in the F_2, Mendel did a simple and revolutionary thing. He counted the numbers of individuals with each characteristic. The results for the seven types of crosses were the same: a ratio of 3 plants with the dominant characteristic to 1 with the recessive. Or we might say ¾ (75 percent) showed the dominant characteristic and ¼ (25 percent) the recessive characteristic.

These ratios and percentages were derived from the data. In the case of a cross of pure breeding plants with *round* seeds with pure breeding *wrinkled* seeds, the F_2 produced 5,474 *round* and 1,850 *wrinkled*, a ratio of 2.96 to 1. The *yellow* × *green* cross gave an F_2 of 6,022 *yellow* and 2,001 *green*, a ratio of 3.01 to 1. As we shall see, Mendel had reason to suspect that the theoretical answer would be 3 to 1 and not 3.01 to 1. These are monohybrid crosses.

When Mendel followed the inheritance of two pairs of contrasting characteristics, the dihybrid cross, uniform results were again obtained. The F_1 exhibited the two dominant characteristics only and the F_2 exhibited all four characteristics in the now familiar 9:3:3:1 ratio. That is, $^9/_{16}$ of the F_2 showed both dominant characteristics, $^3/_{16}$ showed one dominant and one recessive, $^3/_{16}$ showed the other dominant and other recessive, and $^1/_{16}$ had both recessive characteristics.

Thus, if the original P generation cross had been *round-yellow* × *wrinkled-green*, all of the F_1 would be *round-yellow*. In the F_2 he obtained 315 *round-yellow*, 108 *round-green*, 101 *wrinkled-yellow*, and 32 *wrinkled-green*. For this total of 556, the ratios of different kinds are 9.8:3.4:3.2:1. Those ratios represent the real data but Mendel proposed a hypothesis that suggested in a theoretically ideal experiment the ratios would be 9:3:3:1.

Now our problem is to analyze how the 3:1 ratio is related to the 9:3:3:1 ratio.

In a cross of *yellow* × *green*, the F_2 will be ¾ *yellow* and ¼ *green*. Similarly, in a cross of *round* and *wrinkled*, the F_2 will be ¾ *round* and ¼ *wrinkled*. Many students asked to predict the F_2 ratios of a dihybrid cross, *round-yellow* × *wrinkled-green*, may find the problem insoluble at first.

The following analysis will usually work. When two, or even more, pairs of contrasting characteristics are involved, one must recognize that the 3:1 ratio still holds for the individual characteristics. In the cross already discussed that gave a 9:3:3:1 ratio for two pairs of characteristics, the ratio is still 3:1 for the single characteristics. Consider the cross that gives $^9/_{16}$ *round-yellow*, $^3/_{16}$ *round-green*, $^3/_{16}$ *wrinkled-yellow*, and $^1/_{16}$ *wrinkled-green*. Considering *round* and *wrinkled* separately, we find $^9/_{16} + {}^3/_{16} = {}^{12}/_{16}$ that are *round* and $^3/_{16} + {}^1/_{16} = {}^4/_{16}$ that are *wrinkled*. Since $^{12}/_{16} = {}^3/_4$ and $^4/_{16} = {}^1/_4$, we observe a 3:1 ratio for the single pair. The same holds true for the *yellow* and *green* pair.

If we then ask what are the fractions in the F_2 that will result from a dihybrid cross, the answer comes from a simple multiplication of the fractions for the separate characters. Thus, of the ¾ that will be *round*, ¾ of them will also be *yellow* and ¼ will also be *green*. Therefore ¾ × ¾, or $^9/_{16}$, will be both *round* and *yellow* and ¾ × ¼, or $^3/_{16}$, will be both *round* and *green*. Of the ¼ of the F_2 that are *wrinkled*, ¾ will also be *yellow* and ¼ will also be *green*. Therefore ¼ × ¾, or $^3/_{16}$, will be *wrinkled-yellow* and ¼ × ¼, or $^1/_{16}$, will be *wrinkled-green*. That is the derivation of the 9:3:3:1 ratio.

Students may be interested in using this method to find the ratios for crosses involving three or four pairs of contrasting characters.

These striking regularities were observed by Mendel in all of the crosses. Therefore, he thought there must be some underlying principle.

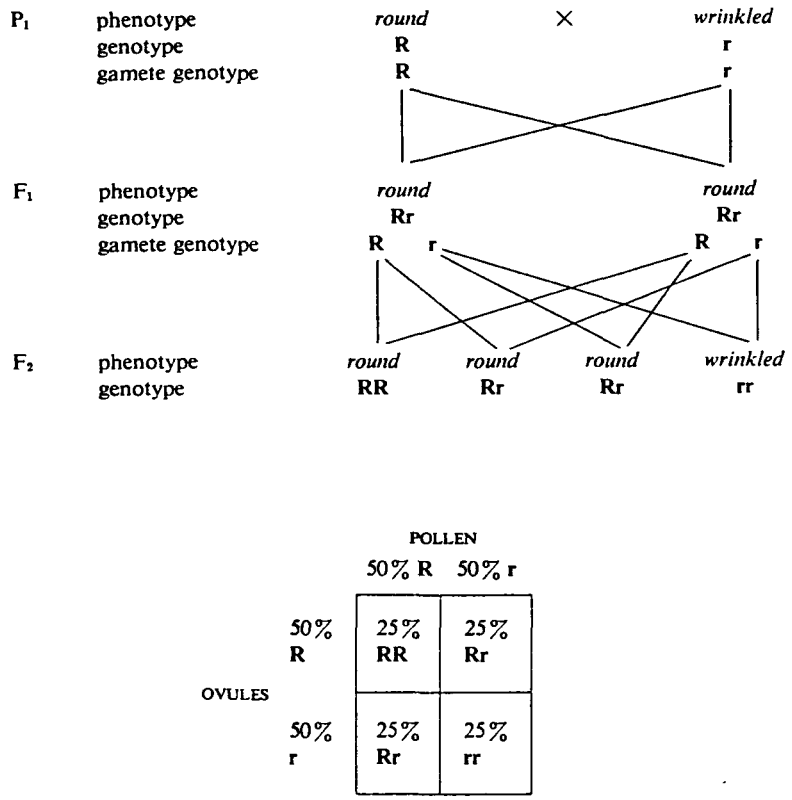


FIG. 13. Model for a Mendelian monohybrid cross. The genotypes of the P generation individuals are as Mendel would have shown them. The genetic checkerboard at the bottom shows the origin of the F_2 (genotypes as we would show them today) from the F_1 pollen and ovules.

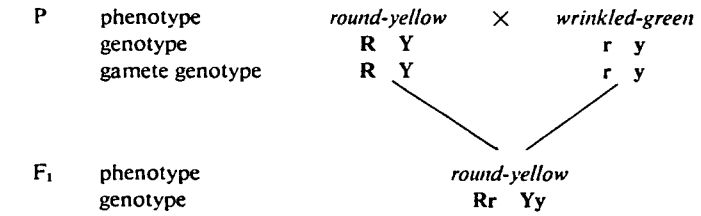
Model for the monohybrid cross

There was. Figure 13 is a model for the explanatory hypothesis that Mendel proposed to account for monohybrid crosses. Both the scheme and the terminology were to become standard half a century later in the early 1900s.

The first thing that an alert reader is likely to note is the "error" in the genotypes of the P generation. They are shown as monoploid instead of diploid and this brings up a very important point in our survey of genetic concepts. Mendel used the symbols for genotype to indicate *kinds* of hereditary factors, not *number* per gamete. The *round* parents' gametes could have contained innumerable **R** factors, not just 1 as we now believe. Thus the pure-breeding *round* plants produced only *round* offspring when selfed. The upper case and lower case letters indicate that the allele is dominant or recessive.

There can be only one type of offspring **Rr**, since there is only one type of pollen and one type of ovule. When these F_1 plants mature, each flower will produce ovules and pollen. Now comes one of the most important features of Mendel's model: he assumed that a gamete could have hereditary factors of only one kind, that is, in this cross a gamete would have **R** or **r** but not both (Fig. 13). This was to prove to be a very difficult problem for geneticists in the early 1900s. Their minds had been influenced by the concept of innumerable gemmules. How could a gamete be "pure" so to speak—have gemmules of only type **R** or type **r**?

Thus the F_1 individuals were assumed to produce gametes that had either **R** or **r**,



		POLLEN RY	Ry	rY	ry
OVULES	RY	RR YY *round-yellow*	RR Yy *round-yellow*	Rr YY *round-yellow*	Rr Yy *round-yellow*
	Ry	RR Yy *round-yellow*	RR yy *round-green*	Rr Yy *round-yellow*	Rr yy *round-green*
	rY	Rr YY *round-yellow*	Rr Yy *round-yellow*	rr YY *wrinkled-yellow*	rr Yy *wrinkled-yellow*
	ry	Rr Yy *round-yellow*	Rr yy *round-green*	rr Yy *wrinkled-yellow*	rr yy *wrinkled-green*

FIG. 14. Model for a Mendelian dihybrid cross. The genotypes of the P generation are as Mendel would have shown them. The genetic checkerboard shows the origin of the F_2 (genotypes as we would show them today) from the F_1 pollen and ovules.

but never both. Mendel next assumed that ovules and pollen grains would combine at random and that the kinds of offspring would be in frequencies determined by the frequencies of the different kinds of gametes.

It must be emphasized to students that the apparent simplicity of the scheme is shown in Figure 13, of the origin of the F_2 from the gametes of the F_1, works only because the genotypic classes of both ovules and pollen are in equal frequency. That is, each produces 50 percent **R** pollen (or ovules) and 50 percent **r** pollen (or ovules). The lines are so drawn that all possible combinations occur and they do so in equal frequency. The **R** gamete on the left, for example (let's pretend that it is a pollen grain), has an equal chance of combining with either an **R** or an **r** ovule. The same is true for the **r** pollen.

The genetic checkerboard at the bottom of Figure 13 is another conventional way of helping students to understand Mendel's hypothesis for the 3:1 ratio in the F_2 of a monohybrid cross.

The model applies to *all* of the crosses involving a single pair of contrasting characters. The model will account for the data only if the following conditions hold:

1. In each pair of contrasting hereditary units, one member of the pair is dominant and the other recessive. Dominance and recessiveness are operational definitions—determined by the phenotype of an individual that has both types of hereditary units.

2. The dominant and recessive hereditary characteristics do not modify one another in any permanent way when they exist together. Thus, in the F_1 the **r** factors from the *wrinkled* parent in the cross of Figure 13 are combined with **R** factors from the *round* parent. There is no expression

of the **r** factors in the F_1 but in the F_2 one quarter of the individuals are *wrinkled*—and just as wrinkled as the *wrinkled* grandparent.

3. By some mechanism unknown to Mendel the two sorts of factors in the F_1 segregate in such a manner that each gamete contains only one sort. Thus in the example, the gametes will contain **R** or **r**.

4. By still another unknown mechanism the **R** containing gametes and the **r** containing gametes are produced in equal numbers.

5. Combinations between the pollen and ovules are entirely at random and the frequencies of the offspring will depend on the frequencies of the different classes of gametes.

Testing the hypothesis

It should be emphasized that the congruence of data and model is not fortuitous. Although items 1 and 2 above could be accepted as true, items 3–5 were entirely hypothetical—they were invented to explain the data. This is perfectly acceptable scientific procedure. The model is to be regarded as a tentative explanation, a hypothesis, that will stand or fall on the basis of tests of deductions made from it.

One critical test was easy to make. The F_2 individuals in the Figure 13 cross consist of 3 plants showing the dominant *round* characteristic to every 1 showing the recessive *wrinkled* characteristic. However, if the hypothesis is true, the *round* seeds must be of two sorts and in a predictable ratio. Thus, for every seed that has the **R** genotype (remember we are still using the Mendelian scheme, so do not say **RR**), there will be two that are **Rr**.

There is no way of distinguishing visually between an **R** and an **Rr** seed, but if the seeds are planted and the flowers allowed to self-fertilize, the offspring will give the answer. Thus the **R** genotype should breed true and the **Rr** genotype should give a 3:1 ratio of *round* to *wrinkled*. Mendel planted the seeds and found this to be true.

Model for the dihybrid cross

Figure 14 shows the model for the dihybrid cross discussed before. A pure breeding *round-yellow* plant is crossed with a pure breeding *wrinkled-green*. The F_1 individuals are uniform—showing the dominant phenotype of both pairs of contrasting characters.

In the formation of gametes by the F_1, Mendel assumed, as for the monohybrid cross, that each gamete would receive only one type of the two contrasting units—either **R** or **r**. The same was assumed to be true for **Y** and **y**. At this point, still another assumption had to be made: there would be an independent assortment of both pairs. Thus each gamete would have either **R** or **r** and, in addition, either **Y** or **y**. There would then be four classes of both pollen and ovules: **RY**, **Ry**, **rY**, and **ry**. The model also demanded that these classes be in equal frequency—25 percent for each.

If students have trouble understanding the origin of the four classes of gametes, a simple game with two different coins—a penny and nickel perhaps—may help. Let each coin represent a gene and the "head" represent the dominant allele and the "tail" the recessive allele. The coins are then tossed and the results recorded. If enough throws are made, one expects ¼ to be heads for both coins (*i.e.*, the **RY** category above); ¼ will be tails for both coins (*i.e.*, the **ry** category above); ¼ will be heads for the penny and tails for the nickel (=**Ry**); and ¼ will be tails for the penny and heads for the nickel (=**rY**).

When there are four classes of gametes, it is not practical to use the lines, as in Figure 13, so in the lower part of Figure 14 there is a genetic checkerboard showing all possible combinations of pollen and ovules that produce the F_2 (for simplicity all genotypes are now shown as diploid). There are 16 boxes in the checkerboard and, if the phenotypes are combined, we find that 9 of the 16 are *round-yellow*, 3 are *round-green*, 3 are *wrinkled-yellow*, and 1 of the 16 is *wrinkled-green*. That accounts for the 9:3:3:1 ratio.

Notice that only *round-yellow* and *wrinkled-green* phenotypes were present in the P and F_1 generations. The model demands, however, that two *new* types of seeds appear: *round-green* and *wrinkled-yellow*.

Here are the data that Mendel reported for the F_2.

	Actual	Expected
round-yellow	315	313
round-green	108	104
wrinkled-yellow	101	104
wrinkled-green	32	35

The "actual" numbers are the counts of the seeds. The "expected" are the numbers in a perfect 9:3:3:1 ratio. The agreement of actual and expected is remarkably good, as Weldon and Fisher were to note years later.

Further tests of the hypothesis

The model for the dihybrid cross allowed even more elegant testing of the hypothesis. The hypothesis predicted that, except for the 32 *wrinkled-green* seeds, all other classes while looking the same consisted of genetically different individuals. This could be tested by planting the F_2 seeds, allowing the plants to self-fertilize, and then counting the F_3 seeds.

Consider first the 32 *wrinkled-green* seeds. The model predicts that these will breed true if selfed. The seeds were planted and 30 grew. All proved to be *wrinkled-green.*

The 101 *wrinkled-yellow* seeds were identical so far as the eye could tell. We can see from the model in Figure 14 that $\frac{3}{16}$ are in this category but two genotypes are represented: 1 of the 3 is **rrYY** and the other 2 are **rrYy** (Mendel would have listed the **rrYY** as **rY** but for simplicity I am using the genotypes as shown in the figure). Thus 1 out of every 3 of the seeds, the **rrYY** class, would be expected to breed true and produce only *wrinkled-yellow.* Two of the 3, the **rrYy** class, would be expected to produce offspring in a ratio of 3 *wrinkled-yellow* to 1 *wrinkled-green.* The 101 seeds were planted and 96 grew. Of these, 28 (32 expected) produced all *wrinkled-yellow* and 68 (64 expected) produced *wrinkled-yellow* and *wrinkled-green* in a ratio of 3:1. Thus the deduction from the hypothesis was found to be true.

The same analysis was done for *round-green.* The $\frac{3}{16}$ belonging to this class were predicted to consist of 1 **RRyy** and 2 **Rryy**. The $\frac{1}{3}$ that were **RRyy** should breed true. The $\frac{2}{3}$ that were **Rryy** should produce seeds in a ratio of 3 *round-green* and 1 *wrinkled-green.* The 108 seeds were planted and 102 grew. One would have expected 34 to breed true and 68 to give the 3:1 ratio. The actual numbers were 35 and 67.

The most complex test of the hypothesis was based on the $\frac{9}{16}$ of the F_2 that were *round-yellow.* A check of the checkerboard shows that 1 of the 9 is **RRYY**, 2 are **RRYy**, 2 are **RrYY**, and 4 are **RrYy**. Thus, only 1 of the 9, the **RRYY** class, should breed true. The **RRYy** should give offspring in a ratio of 3 *round-yellow* to 1 *round-green.* The **RrYY** should produce 3 *round-yellow* to 1 *wrinkled-yellow.* And finally the **RrYy**, which are the same as the F_1 in Figure 14, should give a 9:3:3:1 ratio. The 315 seeds were planted and 301 produced a crop. The model predicts that the actual numbers in each class (in the order just listed) should be 33, 67, 67, and 134. For example, $\frac{1}{9}$, or 33 seeds, should have bred true since the model predicts that number of seeds should have been **RRYY**. Mendel found the actual numbers to be 38, 65, 60, and 138.

The fact that the F_2 gave an F_3 that did not differ significantly from the hypothesis in these rather demanding tests of the deductions is strong support for the validity of the hypothesis. In every case the actual numbers are very close to the expected numbers. The expected numbers are based on the probability of the gametes behaving according to strict rules. The expected and actual numbers were never identical. We should not expect them to be so any more than we should always expect to get 5 heads and 5 tails for every 10 tosses of a coin.

Conclusions

Mendel's experiments on crossing varieties of peas and his remarkable analysis of the data permit the following eight major conclusions. It is important for students to realize that, in 1865, the conclusions were for peas and peas only. To be sure, Mendel had made some preliminary crosses with beans but the results were confusing.

First, the most important conclusion is that inheritance appears to follow definite and rather simple rules. Mendel proposed

a model that would account for the data of all his crosses. Furthermore, the model had great predictive value—a goal of all hypotheses and theories of science.

Second, when plants of two different types are crossed, there is no blending of the individual characteristics. Of the seven pairs of contrasting characteristics, one type was dominant and the other recessive. That is, in a hybrid formed by crossing a pure breeding plant having the dominant characteristic with a pure breeding plant having the recessive characteristic, the offspring are uniform in appearance and identical with the dominant parent.

Third, since the hybrid described above is identical in appearance with the pure breeding dominant parent, we can conclude that there is not an exact relation between genotype and phenotype. Thus, the *round* phenotype can be based on either an **RR** (Mendel would have said **R**) or **Rr** genotype.

Fourth, the hereditary factors responsible for the dominant and recessive condition are not modified by their occurrence together in a hybrid. If two such hybrids are crossed, both dominant- and recessive-appearing offspring will be produced, and these offspring show no evidence that the hereditary factors responsible for their appearance have been modified by their association in the parents. An F_2 individual with the recessive phenotype will be identical to the phenotype of the original P generation recessive.

Fifth, when hybrids such as **Rr** are crossed, the two types of hereditary units—**R** and **r**—segregate from one another, and at fertilization recombine at random. The offspring will be in a phenotypic ratio of 3:1 and genotypically there will be, using the modern convention for genotypes, 1 **RR**, 2 **Rr**, and 1 **r**. Segregation is often called "Mendel's First Law."

Sixth, this ratio can occur only if each gamete receives only one type of hereditary factor—in the example either **R** or **r**.

Seventh, when crosses involve two pairs of contrasting hereditary units, such as **RrYy** crossed with **RrYy**, each pair behaves independently. That is, the different types of hereditary units assort independently of one another, so the gametes can be only **RY**, **Ry**, **rY**, or **rr**. Thus all possible combinations will be obtained, with the strict rule that each gamete can have only one kind of each of the pairs of hereditary units. The different classes of gametes will be in equal frequency. This phenomenon of independent assortment is known as Mendel's Second Law.

Eighth, the Mendelian hypothesis, and its formulation in a model, was so specific that deductions could be made and these could be tested by observation and experiment. No other field of experimental biology had reached an equivalent stage of development in 1865.

But, as we have seen, at that time no biologist seemed to realize that such was the case. To be sure, Mendel's work would not have been important if it had applied only to garden peas—any more than Hooke's discovery of cells would have been important had cells been observed only in cork. The field of plant breeding was full of data from which no general conclusions could be drawn. Mendel wrote to a foremost scholar in the field, Nägeli, and explained his results. Nägeli must have regarded the data for peas as just one more example of the tremendous variation in the results obtained in hybridization experiments.

Nägeli suggested that Mendel try another plant—*Hieracium*, the hawkweed. Mendel did and failed to find consistent rules for inheritance. It turned out that Mendel had not been doing the experiments he assumed he was doing. It was exceedingly difficult to make experimental hybrids in *Hieracium* with its tiny flowers. Nevertheless Mendel thought he had done so in many instances and was surprised at the lack of uniformity in the results. The problem was with *Hieracium*, not Mendel. Long after Mendel's death it was discovered that a type of parthenogenetic development, apomixis, occurs in *Hieracium*. No uniform ratios are to be expected if some of the offspring are the result of fertilization and others of apomixis.

So even Mendel came to believe that his results had a restricted application and, in any event, his model was ignored during

the last third of the 19th century. During those decades the leading students of heredity had abandoned the paradigm of experimental breeding and concerned themselves mainly with the behavior of chromosomes in meiosis, mitosis, and fertilization. They believed that they were laying a physical basis for inheritance and further research was to prove them correct.

Mendel in retrospect

Much is usually made of the fact that Mendel's seminal work had been published in an obscure journal of an obscure society, so that it was either forgotten or unknown for 35 years—a 35 years that saw the flowering of cytology and intense interest in heredity. A more accurate statement would be, I suspect, that the paper was unappreciated rather than unknown. It was known to Focke (1881) who discussed it briefly in his standard treatment of plant hybridization and it was mentioned later by Bailey (1895). As already noted, Mendel had corresponded with one of the most prominent students of heredity at the time, Karl Wilhelm von Nägeli. Nägeli seemed not to be impressed with the data of Mendel's crosses of varieties of garden peas.

Bateson's explanation, taken from his introduction to Mendel's paper (Mendel, 1902, p. 2), was as follows:

> It may seem surprising that a work of such importance should so long have failed to find recognition and to become current in the world of science. It is true that the journal in which it appeared is scarce, but this circumstance has seldom long delayed general recognition. The cause is unquestionably to be found in the neglect of the experimental study of the problem of Species which supervened on the general acceptance of the Darwinian doctrines. The problem of Species, as Gartner, Kölreuter, Naudin, Mendel, and the other hybridists of the first half of the nineteenth century conceived it, attracted thenceforth no workers. The question, it was imagined, had been answered and the debate ended. No one felt any interest in the matter. [A paradigm shift!] A host of other lines of work were suddenly opened up, and in 1865 the more vigorous investigators naturally found those new methods of research more attractive than the tedious observations of the hybridisers, whose inquiries were supposed, moreover, to have led to no definite result. But if we are to make progress with the study of Heredity, and to proceed further with the problem "What is a Species?" as distinct from the other problem "How do Species survive?" we must go back and take up the thread of inquiry exactly where Mendel dropped it.

And, as we shall see, that is exactly what Bateson did.

Mendel's work on peas is not an isolated example of an important discovery being made but not understood by the scientific community at the time it was announced. New paradigms are not readily identified and adopted. Most scientists at any one time will be busy doing their normal science within the existing paradigm. The difficulty in changing what one does with hand and mind promotes resistance to new ideas and to the undertaking of new research programs.

This was not a problem for de Vries and Correns in 1900. The reason that they understood the importance of Mendel's conclusions is that they had done similar work and had developed a similar explanatory hypothesis before they read of Mendel's paper. They were working on the new paradigm before they knew of their paradigmatic progenitor.

The same point can be made for Bateson. He had been studying variation and hybridization for years, and although he had not observed the regularities of the Mendelian model, he knew the sorts of experiments that needed to be done. Consider the following.

On Tuesday and Wednesday, 11 and 12 July 1899, the Royal Horticultural Society held an "International Conference on Hybridisation (the Cross-Breeding of Species) and on the Cross-Breeding of Varieties" at Chiswick and London. Volume 24 of the Society's "Journal" consists

of the report of the conference. Thus we have the opinions of many of the world's outstanding plant hybridizers immediately before Mendel changed their science. Most of the articles in the journal describe the results of crosses but Bateson gave a more theoretical talk. This is part of what he had to say (Bateson, 1900*a*):

> What we first require is to know what happens when a variety is crossed with its *nearest allies.* If the result is to have a scientific value, it is almost absolutely necessary that the offspring of such crossing should then be examined *statistically.* It must be recorded how many of the offspring resembled each parent and how many showed the characters intermediate between those parents. If the parents differ in several characters, the offspring must be examined statistically, and marshalled, as it is called in respect of each of those characters separately.

It is almost as though Bateson is advising a graduate student, by the name of Mendel, how to plan his Ph.D. research program!

There are many aspects of the story about Mendel that may be of interest to students. One is the almost universal attention that is given to the scientist who makes the discovery. Until recently scientists, especially biologists, could hardly expect to "make their fortune" as scientists—that is, a big $$ fortune. The rewards to a scientist come from the joy of probing nature for her regularities and the approval of one's peers for research well done and for formulating bold and imaginative hypotheses. To this day scientists look at the Mendelian paper in awe. How could he have gone so far beyond the existing paradigm and made observations that were, well after his death, to revolutionize the biological sciences?

Another interesting point for students is that, time and time again, it seems that when the field is "ready" the discovery will be made. If Mendel had never lived, the history of genetics would not have been greatly different. About the year 1900 someone or another would have reached similar conclusions. It just happened that it was de Vries and Correns. Tschermak was so close that he is usually included with de Vries and Correns as a codiscoverer. In a year or so Bateson might have independently discovered the Mendelian rules for inheritance. There seems to be an element of inevitability in the progress of science.

Initial opposition to Mendelism

In the telling of the Mendelian story, students may be led to believe that in 1900, with the publication of the papers of de Vries and Correns, "pure science" had finally triumphed. Not at all. There was vigorous, at times vitriolic, opposition to Mendel's conclusions (Provine, 1971). This scientific donnybrook mainly involved three Englishmen—William Bateson *vs.* Karl Pearson and W. F. R. Weldon, each side with a camp of followers. The two schools were fundamentally different in their approaches. Bateson sought information about inheritance from experimental crosses. Weldon, Francis Galton, and Karl Pearson sought to apply mathematical, and especially statistical, methods to biological problems. The opposition of these biometricians is all the more surprising when we remember that Mendel had relied so heavily on mathematics.

The basic dispute began before 1900 and had to do with evolution. Once again it was a case of conflicting paradigms. Weldon, Galton, Pearson and others followed Darwin in believing that evolution is based on gradual phylogenetic changes. In natural populations the variation seems to be continuous. When arranged by size or essentially any other characteristic the individuals in a species seem to show continuous variation. Not surprisingly it was assumed that evolution involves changes so minute that, only with the passage of long intervals of time, would one observe any difference.

An important statement of this continuous variation school was Galton's Law of Ancestral Heredity. He viewed inheritance in its totality and pointed out that each individual's hereditary characteristics seem to come not only from the parents but also from more remote ancestors. On the basis of a careful study of the pedigrees of Basset hounds, Galton (1897) proposed his famous law.

> The law to be verified may seem at first sight too artificial to be true, but a closer examination shows that prejudice arising from the cursory impression is unfounded. This subject will be alluded to again, in the meantime the law shall be stated. It is that the two parents contribute between them on the average one half, or (0.5) of the total heritage of the offspring; the four grandparents, one quarter, or $(0.5)^2$; the eight great-grandparents, one eighth, or $(0.5)^3$, and so on. Thus the sum of the ancestral contribution is expressed by the series $\{(0.5) + (0.5)^2 + (0.5)^3, \&\}$, which, being equal to 1, accounts for the whole heritage.

Thus, a trace of even our most remote ancestors is found in ourselves and this past heritage would put a brake on sudden changes. The Darwinian demands for a mechanism for slow and imperceptible changes in evolution would be satisfied.

But how could Galton's Law work? Today our minds are so fixed with the notion that our genes come only from our parents, roughly half from each, that we cannot imagine a mechanism for inheritance from venerable ancestors that seems to bypass parents. The answer is that Galton was talking about phenotypes, not genotypes. It was, and still is, well known that phenotypic characteristics may be expressed in an individual whereas they were not expressed in the parents.

This notion of continuous variation in evolution and, of course, inheritance, was challenged by Bateson and others. He had been a student of evolution and heredity for many years. In 1894 he had produced a mammoth volume *Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of Species.* He wished to discover whether the evidence suggested that evolution is based on continuous variation or discontinuous variation and concluded that the latter was possible. In 1900 he felt much the same and wrote:

> We are taught that Evolution is a very slow process, going forward by infinitesimal steps. To the horticulturist it is rarely anything of the kind . . . . It is going at a gallop. Whenever, then, it can be shown that a variation comes discontinuously into being, it is no longer necessary to suppose that for its production long generations of selection and gradual accumulation of differences are needed, and the process of Evolution thus becomes much easier to conceive. According to what may be described as the generally received view, this process consists in the *gradual* transition from one normal form to another normal form. This supposition involves the almost impossible hypothesis that every intermediate form has successively been in its turn the normal. Wherever there is discontinuity the need for such a suggestion is wholly obviated. (1900*a*, p. 62)

No wonder Bateson found the Mendelian paradigm so acceptable. Hereditary differences could be striking; that is, variation appeared to be discontinuous. To Bateson and his followers the Mendelian model was compatible with their paradigm.

(As an aside it is of interest to note that a variant of this debate is still with us. Some evolutionists believe that the major pattern of evolution is based in small changes. Others believe the common pattern to be slow changes over long periods of time followed by short periods of rapid change—punctuated equilibria. Some of the confusion is a consequence of how large can a change be and still be small. Also, what is a long time and what is a short time? Some "short" times turn out to be about 10,000 years. The answer will probably be that some lineages are characterized by slow, relatively even, changes over the eons, others by stasis and jerks, and others with little appreciable change over very long periods of time. The two polar schools then are: Evolution by Creeps and Evolution by Jerks.)

The vehemence with which the debates raged indicated, quite clearly, that adherents to the older paradigm of continuous variation felt threatened. Yet those in the other camp who saw great promise in Mendel's approach, which supported discontinuous variation, had to admit that Mendel's conclusions could not account for the results of breeding experiments in all organisms and for all characteristics.

The consequence was that Bateson and the breeders continued to perform experiments that showed to what extent Mendel's principles could be extended, and Weldon and others continued to point out that not all could be explained on the original Mendelian hypothesis.

Weldon (1902) summarized Mendel's conclusions and wrote

> It is clearly important to test these remarkable statements by a careful study of the numerical results, and by the application of such tests as may be possible. It seems to me that by neglecting these precautions some writers have been led to overlook the wonderfully consistent way in which Mendel's results agree with his theory. (p. 232)

Weldon subjected the ratios to statistical tests and concluded that

> if the experiments were repeated a hundred times, we should expect to get a worse result about 95 times, or the odds against a result as good as this or better are 20 to 1. (p. 235)

Years later still another mathematically-inclined scientist, R. A. Fisher (1936), was to deal with the problem of Mendel's data being "too good." In any event there was abundant confirmation. Sinnott and Dunn (1925, p. 47) list the ratios found by Mendel and by six other plant breeders who attempted to check Mendel's results between 1900 and 1909. In the case of the *yellow* × *green*, for example, the total number of seeds was 179,399. Of these, 134,707 were *yellow* (75.09 percent) and 44,692 (24.91 percent) were *green*. Mendel had reported 75.05 percent *vs.* 24.95 percent. Apparently it was not all that difficult to obtain data that were "too good."

It bears repeating that Mendel never published his full data. His 1865 paper was based on lectures and it would have seemed reasonable for him to select the data from those crosses that best illustrated the hypothesis he was proposing. When giving public lectures scientists do not describe all of their experiments and give all of their data—even though it sometimes seems that they do. Then, too, Mendel was following the procedures of the 1860s, not those of today. But, when all is said and done, it has turned out that Mendel was right.

S. Wright (1966) studied the data again and concluded "I am confident, however, that there was no deliberate effort at falsification." See also Orel (1968).

Weldon went on to question the notion of dominance and recessiveness. He made the mistake, which Mendel was at pains to avoid, of assuming that the same phenotype implied the same genotype. Weldon knew of many varieties of peas that had characteristics similar to those Mendel had used. They did not always give the same results in crossing. Weldon did not seem to understand that a particular phenotype in one variety might not have the same genotypic basis as the apparently identical phenotype in another variety. In addition, Weldon did not seem to understand the importance of using parents of known constitution—whether, for example, the phenotype was produced by a homozygous or heterozygous genotype.

Nevertheless, he was able to cite cases where dominance was not complete and the "hybrids" were intermediate to some degree. This was to prove true in many cases, as Correns and others were to discover.

Weldon was attacking the notion that "Mendel's statements were universally valid" and summarizes,

> I think we can only conclude that segregation of seed-characters is not of universal occurrence among cross-bred Peas, and that when it does occur, it may or may not follow Mendel's laws. The law of segregation, like the law of dominance, appears therefore to hold for races of particular ancestry .... The fundamental mistake which vitiates all work based upon Mendel's method is the neglect of ancestry, and the attempt to regard the whole effect upon offspring, produced by a particular parent, as due to the existence in the parent of particular structural characters; while the contradictory results obtained by those who have observed the offspring of parents apparently identical in certain charac-

> ters show clearly enough that not only the parents themselves, but their race, that is their ancestry, must be taken into account before the results of pairing them can be predicted. (pp. 251–252)

The last objection is strange. Mendel had taken great pains to ensure that his original varieties bred true. It was one of the reasons for his success where so many others had failed. The last sentence in the quote just given shows that Weldon still thought that Galton's Law of Ancestral Heredity must be considered.

Mendelism was in clear competition with Galton's Law so, not surprisingly, the biometricians were anxious to challenge its data and conclusions—and that is what Weldon had done. Some indication of how highly this group regarded Galton is indicated by these quotations from Pearson (1898):

> In short if Mr. Galton's law can be firmly established, *it is a complete solution, at any rate to a first approximation, of the whole problem of heredity.* It throws back the question of inheritance upon two constants, which can be once and for all determined; herein lies its fundamental importance. (p. 393)

And Pearson closes his paper on this lofty note:

> At present I would merely state my opinion that, with all due reservations, it seems to me that the law of ancestral heredity is likely to prove one of the most brilliant of Mr. Galton's discoveries; it is highly probable that it is the simple descriptive statement which brings into a single focus all the complex lines of hereditary influence. If Darwinian evolution be natural selection combined with *heredity,* then the single statement which embraces the whole field of heredity must prove almost as epoch-making to the biologist as the law of gravitation to the astronomer. (p. 421)

Matters were moving very rapidly in the years 1900–1903. De Vries' (1900) "rediscovery" paper had been submitted for publication on 14 March 1900 and Corren's (1900) on 26 April. These created a great stir. Shortly thereafter Bateson (1900*b*) discussed these papers at a meeting of the Royal Horticultural Society. Subsequently a translation of Mendel's paper (Mendel, 1902) was made, thus making it readily available to the scientific world—few libraries would have possessed the original paper of 1865. Weldon's (1902) anti-Mendel paper was received by the editors of *Biometrika* on 9 December 1901.

Bateson set to work immediately and produced a book (1902), *Mendel's Principles of Heredity; A defence.* Not only does it provide a translation of Mendel's papers on *Pisum* and *Hieracium* but the data are discussed at length. The last half of Bateson's little book consists mainly in responding to Weldon's attack on Mendelism.

> At this juncture Professor Weldon intervenes as a professed exponent of Mendel's work. It is not perhaps to a devoted partisan of the Law of Ancestral Heredity that we should look for the most appreciative exposition of Mendel, but some bare measure of care and accuracy in representation is demanded no less in justice to fine work, than by the gravity of the issue. (p. 105)

Bateson then documents Weldon's errors and distortions of Mendel's work. Weldon, seeing his paradigm severely challenged, had responded in a manner we like to believe should not occur in science. He brought discredit upon himself and to the biometricians as a group. Bateson concludes as follows:

> I trust what I have written has convinced the reader that we are, [as a consequence of Mendel's work] at last beginning to move. Professor Weldon declares he has "no wish to belittle the importance of Mendel's achievement"; he desires "simply to call attention to a series of facts which seem to him to suggest fruitful lines of inquiry." In this purpose I venture to assist him, for I am disposed to think that unaided he is—to borrow Horace Walpole's phrase—about as likely to light a fire with a wet dish-clout as to kindle interest in Mendel's discov-

eries by his tempered appreciation. If I have helped a little in this cause my time has not been wasted.

> In these pages I have only touched the edge of that new country which is stretching out before us, whence in ten years' time we shall look back on the present days of our captivity. Soon every science that deals with animals and plants will be teeming with discovery, made possible by Mendel's work. The breeder, whether of plants or of animals, no longer trudging in the old paths of tradition, will be second only to the chemist in resource and in foresight. Each conception of life in which heredity bears a part—and which of them is exempt?—must change before the coming rush of facts. (p. 208)

Bateson's prediction of what might be seen ten years later was correct—Morgan would have established the groundwork for an astonishing development in genetics.

Bateson, the champion and the prophet, did much to protect and advance Mendelism in its infancy. He played a role similar to that of his countryman, Thomas Henry Huxley, who, a half-century earlier had been vigorous and effective in the defense of Darwinism.

We will return to the development of Mendelian genetics shortly but, also in 1902, a paper was published that was to unite the fields of animal and plant breeding with cytology. Thus the twin approaches to the study of inheritance were to become linked and mutually supportive—and require concordance.

SUTTON 1902: GENETICS + CYTOLOGY

Looking backwards at the conceptual development of the science of genetics, we can recognize 1902 as a year of momentous events. The young Walter Stanborough Sutton (1877–1916) was to demonstrate in a paper that year, and a second one in 1903, that there is an exact parallel in the behavior of the Mendelian hereditary units and of the chromosomes in meiosis and fertilization. The most economical hypothesis (Occam's Razor), therefore, was that the hereditary units were parts of the chromosomes. Alternatively the hereditary units might be parts of cell structures that behaved exactly like chromosomes in meiosis and fertilization.

This is obvious to us today—the unfailing clarity of hindsight. It was far from clear in 1902. The most prominent geneticist of the day, William Bateson, failed to be convinced by Sutton's data and analysis—in fact it was years before he became even partially convinced that genes *are* parts of chromosomes. E. B. Wilson, surely one of the world's outstanding cytologists, had great difficulty in understanding what Sutton was proposing. This is especially surprising since Sutton was working in Wilson's laboratory at Columbia University at the time. "Especially surprising" since we tend to think that the time of discovery is the time the significance of the discovery is understood by the scientific community. This is almost never so—it takes a long time for the "obvious" to become obvious.

Permanence and individuality of chromosomes?

Two of the premises of Sutton's hypothesis were that chromosomes persist in some form during the nuclear cycle, that is, they can be regarded as permanent structures and, furthermore, that chromosomes have individuality (that is, as we now realize, each pair of homologous chromosome has a unique cluster of genes).

In 1902 these premises had not been established beyond a reasonable doubt. The "disappearance" of the chromosomes at the time when the nucleus of a just divided cell entered the resting stage presented a serious problem for those who believed in the permanence and individuality of chromosomes. The most obvious interpretation was that chromosomes were temporary structures—a phenomenon of the mitotic period. Others believed that as the chromosomes entered the resting stage they joined, end to end, to form a continuous spireme. The spireme was thought to fracture into chromosomes at the onset of the next mitotic division. But need it fracture at the same place each mitotic division and, hence, maintain the individuality of chromosomes?

In the second edition of E. B. Wilson's *The Cell* (1900, pp. 294–304) there is strong support for the hypothesis of some type of permanence and individuality of the chromosomes. He notes that Rabl's observations, made in 1885, were evidence that

> *the chromosomes do not lose their individuality at the close of division, but persist in the chromatic reticulum of the resting nucleus.*

(The italics are Wilson's.) Wilson cites the studies of Boveri, van Beneden, and others on *Ascaris* as demonstrating that

> *whatever be the number of chromosomes entering into the formation of a reticular nucleus* [*i.e.*, a resting nucleus], *the same number afterward issues from it.*

The best evidence for this was again from *Ascaris.* At the end of telophase the nuclear membrane forms lobes that surround the ends of the chromosomes. These lobes persist and

> at the succeeding division the chromosomes reappear exactly in the same position, *their ends lying in the nuclear lobes as before* . . . . On the strength of these facts Boveri concluded that the chromosomes must be regarded as "individuals" or "elementary organisms," that have an independent existence in the cell. Boveri expressed his belief that "we may identify every chromatic element arising from a resting nucleus with a definite element that entered into the formation of that nucleus, from which the remarkable conclusion follows *that in all cells derived in the regular course of division from the fertilized egg, one-half of the chromosomes are of strictly paternal origin, the other half maternal.*"

Wilson was assembling evidence to make a point but, he noted, many cytologists did not accept this hypothesis. It is interesting for us to note today how slim the evidence may be at first for some truly basic concept—those lobes on the nuclear membrane of *Ascaris* being about the best evidence for the persistence of chromosomes during the resting stage. (During the late 1930s, when I was being taught cytology by Wilson's student and successor at Columbia University, Franz Schrader, those bumps on the *Ascaris* nucleus were *still* the prime evidence.)

In the third edition of *The Cell* Wilson (1928) notes that convincing (to some) evidence for chromosomal constancy was not available until 1901:

> That the chromosomes may show differences of sizes and shape in the same species was noted by Flemming, Strasburger and other earlier observers, but it did not at first occur to cytologists that such differences were other than fortuitous variations or fluctuations. Montgomery [1901] recognized the constancy of the differences of the chromosomes in respect to size and shape and in some cases also of behavior. His work in this field, carried out especially on the germ-cells of insects, formed the morphological counterpart of Boveri's [1902, 1907] epoch-making experimental demonstration of the physiological and qualitative differences of the chromosomes and thus contributed in an important way toward the demonstration of the genetic continuity of the chromosomes and the cytological explanation of Mendel's law. (p. 834)

The chromosomes of Brachystola

Sutton's 1902 paper was a study of the chromosomes in the testis of a grasshopper of the genus *Brachystola.*

> The chromosomes of *Brachystola,* like those of many amphibia, selachians and insects and certain flowering plants, exhibit a chromosome group, the members of which show distinct differences in size. Accordingly, one feature of [my] study has been a critical examination of large numbers of dividing cells (mainly from the testis) in order to determine whether, as has usually been taken for granted, these differences are merely a matter of chance, or whether, in accordance with the view recently expressed by Montgomery [1901], in regard to a certain pair of elements in the nuclei of one of the Hemiptera, characteristic size-relations are a constant attribute of the chromosomes individually considered.

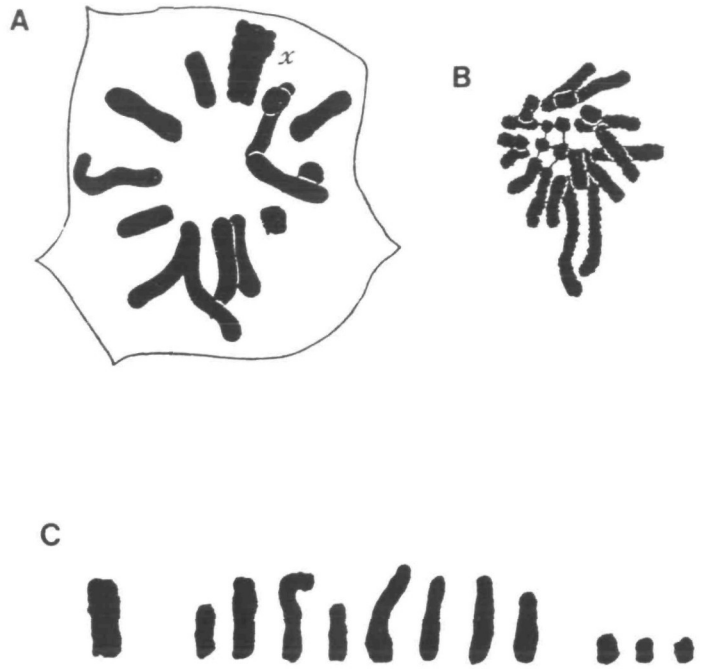


FIG. 15. Sutton's illustrations of *Brachystola* chromosomes. A shows the monoploid set of the male, B the diploid set of the female, and C are the chromosomes of A arranged by size. (A and B are from Sutton, 1920.)

> With the aid of camera drawings [*i.e.*, with a camera lucida] of the chromosome group in the various cell-generations, I will give a brief account of the evidence which has led me to adopt the latter conclusion. (p. 24)

Sutton's epoch-making analysis needed only two additional pieces of information: that chromosomes are permanent cell structures and that they are individually-specific cell structures (that is, each one is genetically different and not, as Weismann thought, each having all of the hereditary information). How was one to tell? He was working at a time long before it was possible to study the fine structure of chromosomes. The material dealt with was the deeply-stained, visually solid, chromosomes in mitosis or meiosis. The only practical means of identification was chromosomal size. Even this was beset with problems since the chromosomes change in size during mitosis, beginning as long delicate threads in prophase and becoming short and thick by metaphase. His solution was to use relative sizes, since the chromosomes seemed to change their sizes synchronously.

The spermatogonial cells in the testis of *Brachystola* undergo a series of mitotic divisions before the onset of meiosis. The youngest spermatogonia have 23 chromosomes. One of these is the so-called "accessory chromosome" that had been observed in other species and was something of a puzzle. Neglecting the accessory chromosome for a moment, camera lucida drawings showed that there were 22 other chromosomes of various sizes and shapes. When Sutton measured these carefully he found that there were not 22 different sizes but only 11. In other words there were 11 pairs of chromosomes, the chromosomes of each pair being of the same size (Fig. 15).

Whereas it was not easy to identify individual chromosomes, it was possible to recognize that the 11 pairs consisted of 8 large pairs and 3 small pairs. Careful study showed that the spermatogonia went through eight mitotic divisions and in the metaphase of each there were 8 large and 3 small pairs of chromosomes. This was the

evidence that Sutton accepted as indicating that the 22 chromosomes of *Brachystola* were of 11 kinds.

Meiosis and fertilization in Brachystola

The spermatogonia then differentiate into spermatocytes that undergo meiosis. The chromosomes of the same size synapse in pairs forming 11 tetrads—8 large and 3 small. After the second meiotic division each spermatocyte will have 1 each of the 8 long and 1 each of the 3 short chromosomes.

The upper left drawing of Figure 15 shows the monoploid number of chromosomes after the second meiotic division. The drawing below is of metaphase chromosomes showing the accessory at the left and the 8 long and 3 short chromosomes to the right.

The cells of the female were not as easy to study. Sutton reported, however, that the female had 22 chromosomes—again consisting of 8 pairs of long and 3 pairs of short chromosomes. The upper right drawing of Figure 15 is of the diploid set of chromosomes in an ovarian follicle cell.

The fact that both male and female nuclei have the same 8 pairs of long and 3 pairs of short chromosomes, was additional evidence for the specificity of chromosomes. Sutton was proposing that the size differences were real and not "as usually has been taken for granted, these differences are merely a matter of chance."

Thus it seemed that the diploid number for the male was 11 pairs plus the accessory and the female had only the 11 pairs. (Sutton made an error. Later workers found that the female has 24 chromosomes consisting of the 8 long pairs, 3 short pairs, and a pair of accessory chromosomes). The year before McClung (1901) had suggested that the X might be involved in the determination of maleness, a subject to which we shall return.

According to Sutton's observations, the mature ova of *Brachystola* would, therefore, have a monoploid number of 11 chromosomes. The sperm would be of two sorts, half would have the 11 chromosomes only and half would have 11 plus the accessory. Fertilization would result, therefore, in two sorts of offspring. Some would have 22 chromosomes, and be females, and others would have 22 chromosomes plus the accessory, and be males.

Analysis of the data

What does it all mean? Here is part of Sutton's extraordinary analysis:

> Taken as a whole, the evidence presented by the cells of *Brachystola* is such as to lend great weight to the conclusion that a chromosome may exist only by virtue of direct descent by longitudinal division from a preexisting chromosome and that the members of the daughter group bear to one another the same respective relations as did those of the mother group—in other words, that the chromosome in *Brachystola* is a distinct morphological individual.
>
> This conclusion inevitably raises the question whether there is also a physiological individuality, *i.e.,* whether the chromosomes represent respectively different series or groups of qualities or whether they are merely different-sized aggregations of the same material and, therefore, qualitatively alike.
>
> On this question my observations do not furnish direct evidence. But it is *a priori* improbable that the constant morphological differences we have seen should exist except by virtue of more fundamental differences of which they are an expression; and, further, by the unequal distribution of the accessory chromosome we are enabled to compare with developmental possibilities of cells containing it with those of cells which do not. Granting the normal constitution of the female cells examined and the similarity of the reduction process in the two sexes, such a comparison must show that this particular chromosome does possess a power not inherent in any of the others—the power of impressing on the contained cell the stamp of maleness, in accordance with McClung's hypothesis.
>
> The evidence advanced in the case of the ordinary chromosomes is obviously more in the nature of suggestion than of proof,

but it is offered in this connection as a morphological complement to the beautiful experimental researches of Boveri [we will get to them shortly] already referred to. In this paper Boveri shows how he has artificially accomplished for the various chromosomes of the sea-urchin, the same result that nature is constantly giving us in the case of the accessory chromosome of the Orthoptera. He has been able to produce and to study the development of blastomeres lacking certain of the chromosomes of the normal series.

By the normal series is here meant such a one as occurs in the nucleus of either the mature germinal products, since it has been clearly shown by the well-known work on the fertilization of enucleate egg-fragments and on chemically induced parthenogenesis, that either of the ripe germ-products possesses all the chromatin necessary for the production of a normal larva . . . .

Every normal fertilized egg, therefore, as well as every cleavage-cell derived from it, must have the field of each character covered by two chromosomes—one from each parent . . . .

If, as the facts in *Brachystola* so strongly suggest, the chromosomes are persistent individuals in the sense that each bears a genetic relation to one only of the previous generation, the probability must be accepted that each represents the same qualities as its parent element. A given relative size may therefore be taken as characteristic of the physical basis of a certain definite set of qualities. But each element of the chromosome series of the spermatozoon has a morphological counterpart in that of the mature egg and from this it follows that the two cover the same field in development. When the two copulate, therefore, in synapsis (the suggestion that maternal chromosomes unite with paternal ones was first made by Montgomery, 1901) the entire chromatin basis of a certain set of qualities inherited from the two parents is localized for the first and only time in a single continuous chromatin mass; and when in the second spermatocyte division, the two parts are again separated, one goes entire to each pole contributing to the daughter-cells the corresponding group of qualities from the paternal or the maternal stock as the case may be.

There is, therefore, in *Brachystola* no qualitative division of chromosomes but only a separation of the two members of a pair which, while coexisting in a single nucleus, may be regarded as jointly controlling certain restricted portions of the development of the individual. By the light of this conception we are enabled to see an explanation of that hitherto problematical process, synapsis, in the provision which it makes that the two chromosomes representing the same specific characters shall in no case enter the nucleus of a single spermatid or mature egg.

I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing division as indicated above may constitute the physical basis of the Mendelian law of heredity. To this subject I hope soon to return in another place.

And so he did the following year, in 1903, in an even more remarkable paper, *The Chromosomes in Heredity.*

SUTTON 1903

Sutton's (1903) paper discusses the significance of what he and others were finding out about chromosomes. If one accepts his interpretation of the nature of chromosomes and their behavior in meiosis and fertilization, there is a striking resemblance between the behavior of chromosomes as determined by cytologists and the behavior of the Mendelian units. Compare, for example, Figure 12 with Figures 13 and 14. It was assumed that segregation and recombination of the hereditary units occurred during the formation of the gametes. At this same time the chromosomes were undergoing those seemingly inexplicable maneuvers of meiosis.

The basic conclusions that can be drawn from Sutton's study of the chromosomes in *Brachystola* are (as modified from his paper):

1. The diploid chromosome group consists of two morphologically similar chromosome sets. Every chromosome type is represented twice or, as we say today, chromosomes are in homologous pairs. Strong grounds exist for the belief that one set is derived from the father and one set from the mother at the time of fertilization.
2. Synapsis is the pairing of homologous chromosomes.
3. Meiosis results in a gamete receiving only one chromosome from each homologous pair.
4. The chromosomes retain their individuality throughout mitosis and meiosis in spite of great changes in appearance.
5. The distribution in meiosis of the members of each homologous pair of chromosomes is independent of that of each other pair. While each gamete receives one of each pair, *which one* is a matter of chance.

Sutton proposed the hypothesis that Mendel's results could be explained if the hereditary units were parts of chromosomes. Figure 16 shows how this is possible.

Let us assume that Mendel's *round* and *wrinkled* alleles are on one pair of homologous chromosomes, as shown in Figure 16. Let us further assume that *yellow* and *green* are on a different pair of homologous chromosomes. A cross of *round-yellow* × *wrinkled-green* will be made (as in Fig. 14).

When meiosis occurs the gametes of the *round-yellow* parent will receive one of each of the homologous chromosomes and have the genotype **RY**. The *wrinkled-green* parent will form **ry** gametes. All individuals in the F_1 will have the same genotype, namely, **RrYy**.

Meiosis in the F_1 will result in the segregation and independent assortment of the four chromosomes, each gamete receiving one or the other member of each pair. Thus one would expect four types of gametes, **RY**, **Ry**, **rY**, and **ry**. Furthermore, the meiotic divisions would have resulted in equal proportions, 25 percent, of each.

Since the four genotypic classes of gametes are produced in equal proportions, we can use a genetic checkerboard to derive the F_2 generation. The result is a 9:3:3:1 ratio.

Thus the strict parallel between the genetic and the cytological data supported Sutton's hypothesis that Mendel's units of inheritance are parts of chromosomes.

Sutton's model, as diagrammed in Figure 16, provided a formal explanation of the major Mendelian assumptions. For example, the problem of the "Purity of the Gametes" was solved if the hereditary units are parts of chromosomes. Thus, when gametes are formed in the F_1, normal meiotic divisions would prevent two homologous chromosomes from going to the same gamete. There could be no F_1 gametes with **R** and **r** or **Y** and **y**, for example.

The chromosomal movements in meiosis also account for segregation—the **R** going to one gamete and the **r** to another. If the pairs of homologous chromosomes move to the poles of the spindle independently of each other, we have also an explanation of independent assortment. Sutton did not know whether or not this was so. In this case the data of genetics helped the cytological analysis: if the hereditary units are parts of chromosomes and, if the hereditary units assort independently, the chromosomes must assort independently as well.

As noted before, the data cannot be regarded as absolute proof that genes are parts of chromosomes. Genes could be part of some other unknown cell structure that behaves in the same way as chromosomes in mitosis, meiosis, and fertilization. When a scientist is confronted with alternative hypotheses, one involving known factors and the other involving unknown factors, common sense suggests that the hypothesis involving known factors be the basis of the research program. It would be more efficient to make observations and design experiments to test the role of chromosomes in inheritance than to first search for any unknown cell structures with chromosomal-like behavior. In any event the continued testing of deductions from the

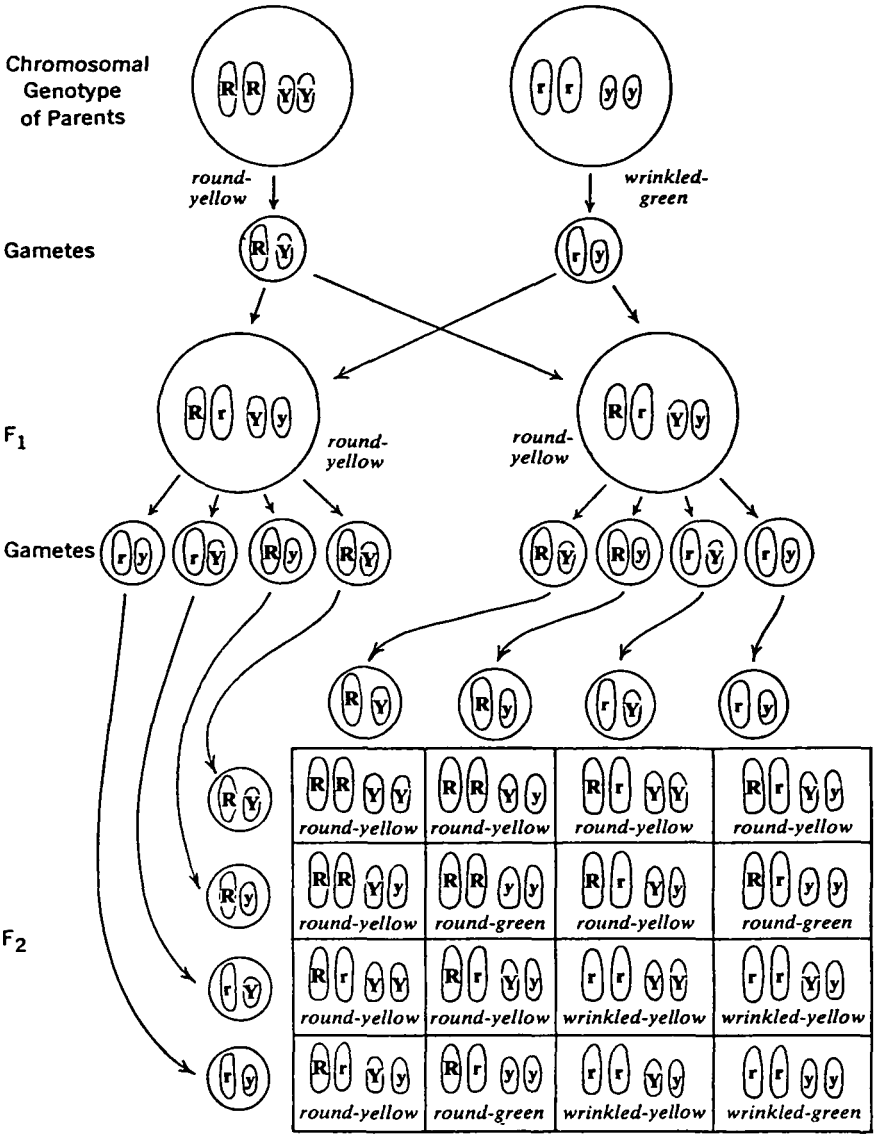


FIG. 16. The distribution of hereditary factors if they are parts of chromosomes. Compare with Figure 14.

genes-are-parts-of-chromosomes hypothesis would soon tell the experimenter whether or not a fruitful course was being followed.

The elegance of Sutton's analysis must not make us forget that it was but another step in the long and difficult research that established that the nucleus, or some part of it, is the physical basis of inheritance. Nearly 40 years had passed since Haeckel's lucky guess and nearly 20 years since the support of the hypothesis by Hertwig, Strasburger, Kölliker, and Weismann.

We may note also that by the early 1900s the number of scientists in the United States who were becoming world-class was increasing rapidly. In fact, genetics was soon to become an American science.

Deductions from Sutton's hypothesis

Sutton had formulated a hypothesis that was useful; that is, it was specific enough

to permit testable deductions. If we are to use the genes-are-part-of-chromosomes hypothesis, it will be necessary to find a parallel between all types of genetic behavior and chromosomal behavior. Any variation in chromosomal phenomena from the usual condition must be reflected in the genetic results. Similarly, if genetic ratios are obtained that cannot be explained in Mendelian terms, one must find a chromosomal basis for the deviation.

Some of the deductions have been mentioned earlier. Here is a summary. We will first assume the correctness of what Sutton had to say about chromosomes, including that each chromosome can have only one allele of a contrasting pair, and what Mendel had to say about inheritance. Thus the segregation of the different alleles, **Aa** for example, must mean that there is a segregation of the meiotic chromosomes as well. There is. Furthermore, that seemingly inexplicable fact that the gametes are "pure," that is, can have only one allele of a contrasting pair, means that only one member of a pair of homologous chromosomes can enter a gamete. Cytological observations strongly suggested that this is so. In a similar manner, the independent assortment of alleles could be accounted for by the independent assortment of chromosomes at anaphase of the second meiotic division. This, however, was only probable and would remain so until it became possible to distinguish between the members of a homologous pair of chromosomes. Sutton concludes (his italics) (1903, p. 237):

> *Thus the phenomena of germ-cell division and of heredity are seen to have the same essential features, viz., purity of units (chromosomes, characters) and the independent transmission of the same*; while as a corollary, it follows in each case that each of the two antagonistic units (chromosomes, characters) is contained by exactly half of the gametes produced.

The deductions so far mentioned could be tested because both the cytological and the genetic data were available. Sutton went on to deduce that non-Mendelian results must be expected to occur if his hypothesis was correct:

> We have seen reason, in the foregoing considerations, to believe that there is a definite relation between chromosomes and allelomorphs or unit characters but we have not before inquired whether an entire chromosome or only part of one is to be regarded as the basis of a single allelomorph. The answer must unquestionably be in favor of the latter possibility, for otherwise the number of distinct characters possessed by an individual could not exceed the number of chromosomes in the germ-products; which is undoubtedly contrary to fact. We must, therefore, assume that some chromosomes at least are related to a number of different allelomorphs. If then, the chromosomes permanently retain their individuality, it follows that all the allelomorphs represented by any one chromosome must be inherited together. On the other hand, it is not necessary to assume that all must be apparent in the organism, for here the question of dominance enters and it is not yet known that dominance is a function of an entire chromosome [would you have thought of that as a problem?]. It is conceivable that the chromosome may be divisible into smaller entities (somewhat as Weismann assumes), which represent the allelomorphs and may be dominant or recessive independently. In this way the same chromosome might at one time represent both dominant and recessive allelomorphs. (p. 240)

Thus, Sutton is deducing that there must be many genes on the same chromosome and, if there are, they must be inherited together. If inherited together there would be no possibility of independent assortment and no genetic ratios of the sort found by Mendel—and by numerous other investigators by 1903. We can deduce, therefore, that an exception to the original Mendelian ratios must occur if we find more pairs of alleles than there are pairs of homologous chromosomes.

SUTTON—WILSON—MORGAN

Sutton was 25, a student with E. B. Wilson in the Zoological Laboratory of Columbia University, when he published

the 1902 paper. He concluded that paper with,

> I take pleasure in expressing here my gratitude to Prof. E. B. Wilson for much valuable advice and assistance in the work upon *Brachystola* and in the preparation of the present paper.

As we have noted before, Wilson had long been interested in the possibility that the chromosomes were the physical basis of inheritance. Furthermore, he had a magisterial grasp of cytology and embryology, having already published the first two editions of *The Cell.* One of his closest friends was Th. Boveri, whose brilliant research had added so much to the knowledge of chromosomes and their possible participation in heredity. Wilson had come to Columbia from Bryn Mawr in 1891 and Thomas Hunt Morgan followed him from the same institution in 1904 (Crampton, 1942). The complex and synergistic interrelations of Wilson, Sutton, and Morgan were to climax during the following decade in the work with *Drosophila.*

Once again, however, we will note the extraordinary difficulty for those scientists, in this instance Wilson and Morgan, doing their normal science in the accepted paradigm of the moment, understanding a new paradigm. A young, though brilliant, scientist with a mind not saturated with a tremendous mass of competing hypotheses and confused facts, was able to see conceptual order where the giants could not.

E. B. Wilson describes how Sutton explained his hypothesis.

> I well remember when, in the early spring of 1902 [Sutton's first paper was in the December 1902 *Biological Bulletin* and the second in the April 1903 issue], Sutton first brought his main conclusions to my attention, by saying that he believed he had really discovered "why the yellow dog is yellow." I also clearly recall that at that time I did not at once fully comprehend his conception or realize its entire weight.
>
> We passed the following summer [1902] together in zoological study at the sea side, first at Beaufort, N.C., later at South Harpswell, Me., and it was only then, in the course of our many discussions, that I first saw the full sweep and the fundamental significance of his discovery. Today the cytological basis of Mendel's law, as worked out by him, forms the basis of our interpretation of many of the most intricate phenomena of heredity, including the splitting up and recombination of characters in successive generations of hybrids, the phenomena of correlation and linkage, of sex and sex-linked heredity and a vast series of kindred processes that were wholly mysterious before their solution was found through Mendel's law. Subsequent to the appearance of Sutton's papers, Boveri stated, 1904, that at the time they were published he had himself already reached the same general result. This does not, however, in the smallest degree detract from Sutton's fine achievement, which will take its place in the history of biology as one of the most important advances of our time. He made an indelible mark on scientific progress, and his name is known wherever biology is studied . . . .

During this summer Sutton had fully worked out his theory of the chromosomes in relation to Mendel's law and upon his return to New York he immediately set about the preparations for its publication. His first paper, as already stated, appeared late in 1902, the second early in the spring of the following year. These two brief papers were intended to be of a preliminary nature, a fuller presentation of his conclusions, together with a larger number of beautiful drawings, already finished at that time, being reserved for a later work which he had expected to offer as a dissertation for the Ph.D. degree at Columbia. It was a source of profound regret to us that circumstances prevented the realization of that plan and brought his cytological investigations to a close. In spite of his brilliant talents as an investigator it would perhaps be more accurate to say because of them—the career of a teacher did not tempt him. Could he have been assured of a reasonable means of support from a life devoted to pure research, he would

> not, I believe, have hesitated. But he had to make his own way in the world and from the first had a strong inclination towards the study of medicine. The combination of circumstances proved irresistible; and after a year or two spent in business he returned to Columbia, entered the Medical School, and graduated with the highest honors two years later.

Wilson's remarks are from a memorial volume published in 1917 (Sutton, 1917). After a distinguished career as a physician, Sutton died at the age of 39. In this brief life in biological research he had produced two papers that probably can stand with those of Mendel and Watson and Crick in fundamental importance and in the brilliance of the analysis.

But once convinced, Wilson became a strong advocate. Whereas before 1900, most of his work had been in developmental biology, thereafter his research was almost exclusively in chromosomal cytology.

The clarity and explanatory ability of Sutton's hypothesis did not mean that it was immediately accepted. Far from it. According to Darlington (1960) as late as the mid-1920s in England,

> Seven men might have been willing to assert their belief in the chromosome theory [of heredity] and give their reasons for it. But against this view there were seven hundred who held a contrary opinion.

The interval between the time some important concept in science becomes true beyond all reasonable doubt to the discoverer and a few cognoscenti and its acceptance by a majority in the scientific community tended to be long in the years before World War II. It is often much shorter now that there are so many more scientists working on the same problems and progress is so rapid.

BOVERI: ABNORMAL CHROMOSOMES = ABNORMAL DEVELOPMENT

It has been mentioned before that cytology at the turn of the century was largely a descriptive science. To be sure one could treat cells with various chemical reagents and differentially stain some of the cell structures. It was not practical at that time for those testing the hypothesis that the physical basis of inheritance resides in the chromosomes to proceed as follows: If the hypothesis is true, the removal of individual chromosomes should result in some change in the organism.

Nevertheless, Boveri (1902 and especially 1907) found a way to accomplish this feat. For more than a generation the eggs and embryos of echinoderms had been studied by cytologists and embryologists and it was known how to obtain their eggs and sperm artificially. Earlier investigators had observed that if concentrated sperm are used to fertilize eggs, two sperm may enter the same egg. Each sperm brings in a division center (centrioles and centrosome) that divides. Thus there are four division centers, which form a square in the egg. Spindle fibers extend from the centers not only along the sides of the square but also across the cell to centers at the opposite corners. The chromosomes are apportioned in a most abnormal manner to the first four cells that result from the first division.

Boveri realized that here was a procedure for altering the set of chromosomes that a cell receives.

The diploid number of chromosomes is 36 in the species of sea urchin that he used. They are small and apparently uniform. There was no *a priori* reason to assume that the individual chromosomes might differ from one another. Recall that Weismann had suggested that each chromosome has all of the hereditary information. Nevertheless Boveri sought to test the hypothesis that the chromosomes differed from one another and a full set of 36 would be necessary for normal development.

In a normal monospermic zygote the 36 chromosomes would replicate before first cleavage to form 72 chromosomes and these would be divided equally at the mitotic first division with 36 going to each daughter cell. Mitotic divisions throughout development would maintain this number.

Since the monoploid number of chro-

mosomes is 18, the dispermic embryo would have 54, that is, 18 each from the two sperm pronuclei and 18 from the egg pronucleus. Each chromosome would replicate before first cleavage to produce 108. The embryo would then undergo the atypical first division that results in four cells. There is no way that each of these four cells can receive the normal complement of 36 chromosomes: 108 if divided equally among the four would give each cell 27. Furthermore, examination of fixed and stained cells showed that the distribution of chromosomes among the four cells was most uneven.

Thus, if each cell must have the normal complement of 36 chromosomes for development to be normal, these dispermic eggs would be expected to develop abnormally. They did—out of 1,500 embryos, 1,499 were abnormal. (That normal one could have been experimental error.)

Boveri found that if the dispermic eggs were shaken, one of the division centers might not divide. The result would be three division centers, arranged in a triangle with spindles between. Such an embryo would divide into three cells at first division. Again the chromosomes were divided irregularly but, in this case, there would at least be a *chance* that each cell could receive a normal set of 36 chromosomes—if the total of 108 is divided by 3, the result is 36. Of 719 embryos of this sort, 58 developed normally.

According to Boveri, these data correspond fairly well with the chance expectations that each cell will receive the normal set of chromosomes and so the embryo can develop normally.

The conclusion was, therefore, that every cell in the embryo must have the normal set of 36 chromosomes if the development is to be normal. This must mean that each chromosome in the set is endowed with a specific quality in spite of the fact that morphologically all appear to be identical.

A Comparison of Sutton's Approach With Boveri's Approach

Sutton and Boveri had used entirely different methods to reach a similar conclusion: chromosomes are the physical basis of inheritance. They had not shown, of course, that chromosomes are the only bearers of hereditary information.

Sutton's hypothesis relating genes and chromosomes was made and tested without his ever seeing a gene, let alone seeing a gene as part of a chromosome. He related gene and chromosome because they behaved in an apparently identical manner in meiosis and fertilization. To be sure this was indirect evidence but the discovery of causal relations in science is often based on the parallel behavior of phenomena.

Long ago the daily cycle of tides was associated with the relative position of the moon and to a lesser degree to the relative position of the sun. The relationship of moon and tides can be checked in several ways and the hypothesis so firmly established that one can predict, with a high degree of accuracy, the tides in the future. Parallel behavior is the only practical way to study the relation of moon and tide. One cannot perform the more critical experiment of excising the moon from the solar system and noting the consequences.

Correlations need not, however, always denote a causal relation. The 28 day lunar cycle and the 28 day menstrual cycle of the human person were long suspected to be causally related but we have no convincing evidence for such a causal relation.

Boveri performed a more direct test of the relation between chromosomes and inheritance by altering the chromosomes and studying the consequences.

Which method is superior, the direct of Boveri or the correlative of Sutton? So far as supporting the hypothesis is concerned the two are about equal. Beyond that there is a large and important difference. What would be the next step in Boveri's approach? It is hard to see how deeper insights into the nature of inheritance could have been obtained with the methodology of the time. One might think of removing individual chromosomes but not only was the methodology unavailable but also there was no way of distinguishing one chromosome from another in the sea urchin.

Sutton's approach, on the other hand, was far more elegant than Boveri's. He was able to link Mendelism and cytology, which

of course Boveri could not, so closely as to suggest testable deductions. Sutton had set the stage for the culmination of classical genetics in the work of Morgan's *Drosophila* group a decade later. And, it is interesting to note, eventually the Morgan group was able to manipulate individual chromosomes by genetic methods.

The genes-are-parts-of-chromosomes hypothesis is sometimes called the Sutton-Boveri hypothesis (for example, Mayr, 1982, pp. 747–749) or even the Boveri-Sutton hypothesis. This is astonishing when one considers the relative contributions of the two in 1902–1903. Boveri only hinted. Sutton worked out the hypothesis and its implications brilliantly. One suspects that Boveri is listed as a co-equal more because of who he was than for what he said. And he was, indeed, a brilliant scientist with a long record of fundamental discoveries.

Sutton and, to a lesser degree, Boveri were not the only ones, in the first two years after Mendel's work became generally known, to suspect that cytology was to provide the mechanism for Mendelian inheritance. E. B. Wilson (1924), who was surely in a position to know, wrote:

> A possible connection between the Mendelian disjunction and the reduction division was suggested nearly at the same time by several observers, including Strasburger, Correns, Guyer, and Cannon. It was, however, Sutton (1902–3) who first clearly set forth in all its significance the cytological explanation of the Mendelian phenomena that is offered by the behavior of the chromosomes, and thus initiated the remarkable movement in this direction that followed. (p. 9)

That is the same Correns who was one of the first to appreciate Mendel's work. The case of W. A. Cannon is especially interesting. He was also a student at Columbia University but, whereas Sutton was in the Department of Zoology, Cannon was in the Botany Department. Cannon was studying the cytology of cotton hybrids and observed the reduction division and saw a possible relation to Mendelian inheritance.

This hypothesis was "hot property" and the question of priority was sure to arise. The two students asked Wilson to publish a short paper announcing what they had done. He did in 1902.

> Since two investigators, both students in this University, have been led in different ways to recognize this clue or explanation, I have, at their suggestion and with their approval, prepared this brief note in order to place their independent conclusions in proper relation to each other and call attention to the general interest of the subject.

Cannon's first paper appeared in December 1902—as had Sutton's. In 1903 two additional papers appeared.

Once again an important concept was "in the air." When Mendelism emerged in 1900, cytology was ready:

> Montgomery (1901), without knowledge of Mendel's fundamental law of segregation, brought together almost all of the essential data for its explanation, though he did not bring them into specific relation with the genetic phenomena. (Wilson, 1924, pp. 8–9).

Then Sutton went on to make a small evidential step and a giant conceptual contribution. But, as we have already seen, not everyone was listening.

Now is the time to return to Bateson and the breeders and observe the rapid expansion, amplification, and extension of Mendelism. Thereafter we will return to the cytologists and see how they dealt with those extra chromosomes ("accessory", "X") that Montgomery, Sutton, and others had reported.

DEFINING SOME GENETIC TERMS

In the year 1902 still another publication of fundamental importance appeared—the first of the *Reports to the Evolution Committee of the Royal Society.* This one was by Bateson and Miss Saunders (1902). In 1897 they began a series of crosses of different varieties of plants and animals. Their initial intent was to learn more about continuous and discontinuous inheritance as well as the phenomenon of "prepotence," which would later be known as dominance. At that time they thought that,

> From what had been hitherto ascertained regarding the phenomena of heredity, the inference could scarcely be avoided that no universal law obtains, but that by studying various specific cases distinct specific laws may be detected. (p. 3)

So much for Darwin, Nägeli, Weismann, and Galton! Before Bateson and Saunders published their results they realized that "the whole problem of heredity has undergone a complete revolution" (p. 4) and they were able to use the Mendelian paradigm to account for their results.

Bateson (in Bateson and Saunders, 1902) provided us with some of the basic terminology for Mendelian genetics:

> This purity of the germ-cells, and their inability to transmit both of the antagonistic characters, is the central fact proved by Mendel's work. We thus reach the conception of unit-characters existing in antagonistic pairs. Such characters we propose to call *allelomorphs,* and the zygote formed by the union of a pair of opposite allelomorphic gametes, we shall call a *heterozygote.* Similarly, the zygote formed by the union of gametes having similar allelomorphs, may be spoken of as a *homozygote.* (p. 126)

In time "allelomorph" was shortened to "allele."

At this point I will explain how I plan to use "gene," "locus," and "allele." Those with long experience in teaching genetics in introductory courses know how difficult these terms may be for students. The problem, however, is less with the competence of students and more with the inexact way geneticists use these terms. And in our time, the more we learn of the molecular basis of a gene locus, the fuzzier the gene becomes. For the moment I will ignore the present and discuss genes as they existed in the Golden Years of Classical Genetics, when they were those little round beads on a string—well, not quite.

Much of the trouble comes from the frequent use of allele and gene as synonyms. I will try not to do that but, since many of my friends are geneticists, I may slip into that error. A *gene* will be a portion of a chromosome that produces an indivisible effect (the atoms of heredity!), which must be detectable, of course (or we would never know of its existence). The position that the gene occupies on a chromosome will be its *locus. Alleles* will be the different detectable variations of that gene. Every gene must have at least two alleles—otherwise we would not know of its existence. A gene reveals its existence when it *mutates* in such a manner that the new mutant allele has a detectable effect.

VARIATIONS IN MENDELIAN RATIOS

The results of crossing varieties of peas—dominance and recessiveness, segregation, independent assortment with the consequences that one observed a 3:1 ratio in the F_2 of a monohybrid cross and a 9:3:3:1 ratio in the F_2 of a dihybrid cross—exhibited a high degree of uniformity and thus raised the question of the universality of these findings.

The development of this topic provides a fine opportunity for students to become involved in suggesting hypotheses and using actual data to test their hypotheses. Toward that end the following suggestions are offered.

Experience suggests that it is best to start with very simple crosses and work up to some that cannot be solved until more information is provided. Thus, students might be asked to write out schemes similar to Figures 13, 14, and 16, using the general symbols **A**, **a**, **B**, and **b** for the following situations:

1. Scheme and expectations for a simple monohybrid cross.
2. Scheme and expectations for a simple dihybrid cross.
3. Scheme and expectations should the genotype **Ab** not have the appearance of the *A-type* individual but rather of something else.
4. Scheme and expectations should a single or double dose of **A** plus a single or double dose of **B** result in a phenotype unlike either the *A-type* or *B-type* individuals. The expectations can be revealed by checking the genetic checkerboard for a dihybrid cross and scoring the expected phenotypes of the various genotypes.

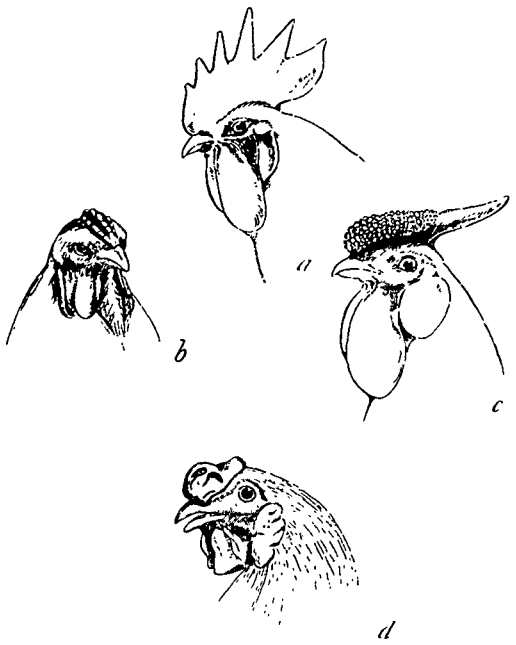


FIG. 17. Comb shape in chickens. *a* is single comb. *b* is rose. *c* is pea. *d* is walnut. (Morgan, 1919)

These four possibilities may be regarded as hypotheses. The student's problem will then be to determine which hypothesis best explains the following data.

In the first report to the Evolution Committee, Bateson and Saunders (1902) described numerous crosses, many of them begun before they knew of Mendel's work. Miss Saunders described her experiments with wild species of the genus *Lychnis,* the campion. Some of the species are *hairy* and others are *glabrous,* that is, without hairs.

a. Crosses of *hairy* × *glabrous* produced an F_1 consisting of 1006 *hairy* and 0 *glabrous.*

b. When the F_1 individuals were crossed they produced an F_2 consisting of 408 *hairy* and 126 *glabrous.*

c. When an F_1 individual was crossed with a pure breeding *hairy,* the offspring consisted of 41 *hairy* and 0 *glabrous.*

d. When an F_1 individual was crossed with a pure breeding *glabrous,* the offspring were 447 *hairy* and 433 *glabrous.*

The students should be able to deduce the possible genotypes of individuals in a, b, c, and d and, finally, summarize all the information into a diagrammatic hypothesis to explain the results.

In the same publication Bateson reported his early experiments with chickens. He studied many sorts of characteristics, including the shape of the combs that were typical of the various breeds (Fig. 17). One type was called *pea* and another *single.*

a. When *pea* was crossed with *single,* all of the F_1 had *pea* combs.

b. When the F_1 were crossed, the offspring were 332 with *pea* combs and 110 were *single.*

Again, the students should suggest the genotypes and be able to offer a genetic diagram of the crosses.

In their second report (Bateson *et al.,* 1905), Miss Saunders reported on many crosses with plants of the genus *Salvia* (mints). True breeding strains with pink and white flowers were used.

a. When *pink* is crossed with *white,* all of the F_1 are *violet.*

b. In a cross of the F_1 plants, one cross produced 59 *violet,* 25 *pink,* and 34 *white.* In another cross, there were 225 *violet,* 92 *pink,* and 114 *white.*

This situation should prove easy for your students.

Bateson reported additional experiments on the inheritance of comb shape in chickens. *Rose* comb when crossed with *single* gave all *rose* in the F_1.

a. When the F_1's were crossed with one another, the F_2 gave 221 *rose* to 83 *single.*

b. When an F_1 was crossed with a *single,* the offspring were 449 *rose* and 469 *single.*

Thus, both *rose* and *pea* are dominant to *single.* But how could there be 3 alleles: *single, rose,* and *pea*? The plot really thickened with a cross of pure-breeding *pea* with pure-breeding *rose.* The F_1 generation was uniform but all had a type of comb not seen in either parent. It was *walnut,* a comb shape that was known in other breeds (Fig. 17).

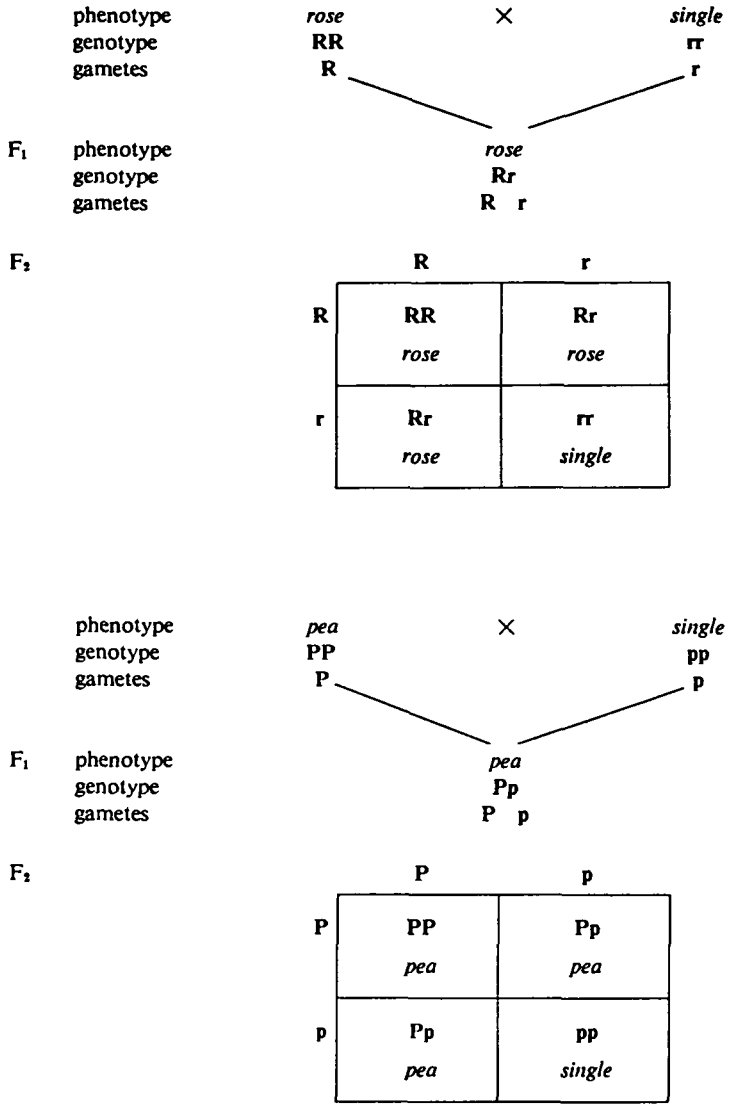


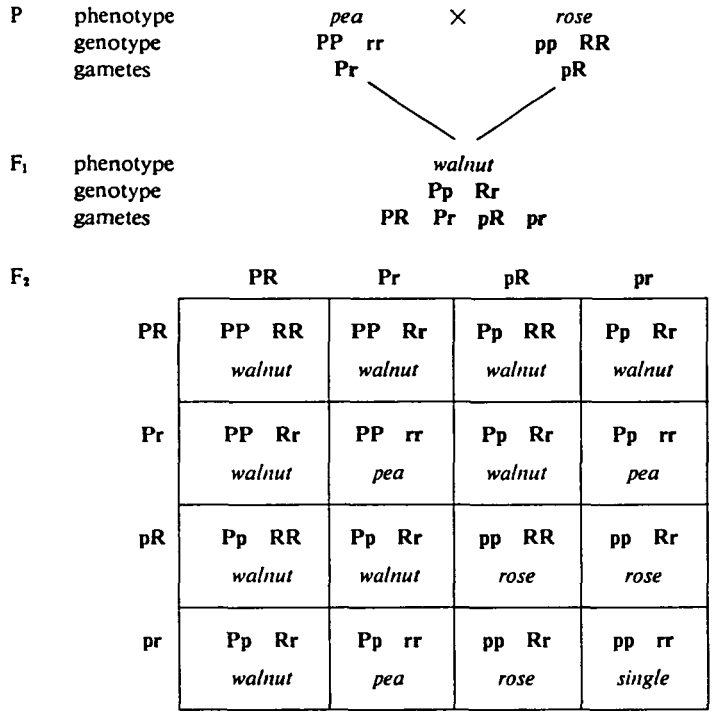
FIG. 18. Genetic diagram for crosses of chickens with different types of combs.

c. When the F_1's were crossed, the F_2 consisted of 99 *walnut,* 26 *rose,* 38 *pea,* and 16 *single.*

d. When the F_1 was crossed with *single,* the offspring were 139 *walnut,* 142 *rose,* 112 *pea,* and 141 *single.*

These crosses involving comb shape may prove a real puzzle for many students but, if they have considered the hypothetical situations suggested, they will realize that the ratio is approximately 9:3:3:1 in c, meaning that two pairs of alleles are involved. These alleles are abiding by the Mendelian rules of dominance, segregation, and independent assortment. They are puzzling only because both pairs are affecting the same character—comb shape. Hopefully your students will identify the situation as that of scheme 4 and shown in Figures 18 and 19.

The following crosses represent still another variation on Mendelian ratios and it will probably be too difficult for most students. It may be useful, however, for



F_2 ratio: 9 *walnut;* 3 *rose;* 3 *pea;* 1 *single.*

FIG. 19. Diagram of a cross of *pea comb* × *rose comb* chickens showing the production of *walnut.*

the better and more highly motivated students to give it a try.

Bateson and others (1906) had done numerous crosses with confusing results with both sweet peas (*Lathyrus*) and stocks (*Matthiola*).

a. When two different varieties with *white* flowers were crossed, all of the F_1 plants were *colored.*

b. When the F_1's were crossed it appeared, at first, that there were equal numbers of *white* and *colored.* Continued study showed, however, the ratio to be 9 colored to 7 white.

Figure 20 is Bateson's diagram of the F_2 results. He and his co-workers concluded that the white flower color of the two original *white* varieties was not controlled by the same genotype. One was shown as **CCrr** and the other as **ccRR**. Color requires at least one **C** and one **R**. The F_1 would all be **CcRr**, and hence colored.

These few examples of genetic crosses indicate the sorts of problems that can be given to students to solve. There are numerous others, of course. The standard textbooks of genetics have many examples: Ayala and Kiger (1984), Strickberger (1985), Suzuki, Griffiths, and Lewontin (1981). Many of the problems in these books give only the ratios observed, not the actual numbers of individuals in each phenotypic class. It will be more valuable if the students deal with the real data because they can reach an important conclusion, but when ratios alone are given that important conclusion has already been reached. If the problems range in difficulty from simple to challenging, there will be something for all. We tend to neglect the better students so it is important that there be an opportunity for them to be challenged as well.

It will be especially valuable if the students can be given an opportunity to determine, statistically, how well the actual data conform to expectations.

More problems will be given when *Drosophila* is discussed. As a concluding prob-

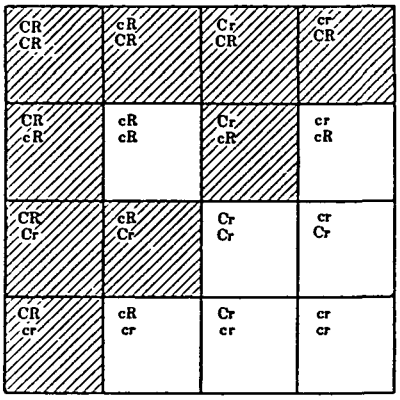


FIG. 1.—Diagram showing the nature of the ratio 9 : 7 in F_2. The character, colour for example, appears only when C and R meet. Each square is a zygote, and the lettering shows its gametic composition. The hatched squares represent coloured plants; the plain are whites.

FIG. 20. The genotypes and phenotypes of the F_2 of a cross of two different types of plants with *white* flowers. (Bateson, Saunders, and Punnett, 1906)

lem at this stage, extract from your students an answer to this: if we wish to determine the genotype for an individual with one or more different pairs of alleles, what cross will provide the most information? It is hoped that some of the students will realize that when the individual of unknown genotype is crossed to a pure recessive, the phenotypic classes of the offspring will be identical to the genotypic classes of the unknown individual's gametes. And, if we know that, it is simple to determine the genotype of the unknown individual. Thus, if we wished to determine the genotype of a *round-yellow* pea plant of unknown parentage, we could cross it with a *wrinkled-green* one. Such a cross to the pure recessive is known as a *test cross*, a term suggested by Bridges (1934). If the offspring are ¼ *round-yellow*, ¼ *round-green*, ¼ *wrinkled-yellow*, and ¼ *wrinkled-green*, the gametes of the unknown individual must have been **RY**, **Ry**, **rY**, and **ry**. Thus the unknown would have been **RrYy**.

This survey of the genetics of the first few years of the 20th century makes us acutely aware of the complexity and the confusion that existed. Those who wished the world to be as outlined for Mendel's peas were soon to find that it was not. This does not mean that the original Mendelian story was "wrong." It means only that it was incomplete and was being replaced with a deeper understanding of the nature of inheritance.

Not one of the original Mendelian rules was found to be correct for all cases. It can be argued that the remarkable progress of genetics was based on an attitude that might seem "unscientific." That is, from the time that Mendel's work became known, it was clear that the original Mendelian hypothesis did not apply to all organisms. Nevertheless, the "true believers" ignored the exceptions and slowly found what could be explained in the original Mendelian terms. As they came to know more and more about breeding experiments in different species, it became possible to expand theory to accommodate the new data. It proved possible to understand more and more of the exceptions.

It was eventually found that some of the most intractable problems had a chromosomal basis. One such problem had to do with those puzzling "accessory" or "X" chromosomes, so we should now check on what the cytologists were doing in the first few years of the 20th century.

MONTGOMERY 1901: BEGINNING TO PUT IT TOGETHER

One of the most influential cytological studies at the turn of the century was a detailed investigation of spermatogenesis and oogenesis in a variety of Hemiptera by

Montgomery (1901). The importance of the paper lies in the rich variety of material described and the fact that, in many instances, he marshalled evidence that would enable others to make important breakthroughs in theory. Both Sutton and Wilson found much of importance in Montgomery's observations and interpretations.

At the time when none of the following hypotheses were widely accepted, Montgomery interpreted his data as suggesting that chromosomes are permanent cell structures; that they exist in homologous pairs consisting of one originally inherited from the mother and the other from the father; that synapsis consists of the coming together of these homologous chromosomes; that in meiosis each spermatid receives one chromosome of each type. He described accessory chromosomes but failed to relate them to the sex determination.

The species of Hemiptera are ideal from several points of view. The chromosomes are not overly numerous, they often differ from one another structurally, and the species are easily collected. One of the most important features, however, is the organization of the testes. The immature cells are at one end and, as one passes through the organ, the various stages in spermatogenesis occur in sequence, ending with mature sperm. In a single testis, therefore, one can study the entire process and be sure of the order of the various stages.

Montgomery starts by listing the problems of interest, such as,

> the significance of the changes in the synapsis stage, the significance of the chromatin nucleoli, the reason for a reduction division, the significance of the sequence of the stages of the germinal cycle, and the question as to why different species have different numbers of chromosomes . . . .
>
> It is impossible to answer these problems by an examination of a single species, and accordingly there are presented here the results of a comparative study of the spermatogenesis of some forty-two species of Hemiptera heteroptera, belonging to twelve different families. This comparative study has brought to light certain wholly unexpected phenomena, and none less anticipated than the discovery of four species with an uneven normal number of chromosomes [these are the sex chromosomes]; this discovery has furnished facts for explaining how the chromosomal numbers may change with the evolution of the species, and how the chromatin nucleoli may have originated. And only such a comparative study could furnish facts to show that in the synapsis stage bivalent chromosomes are formed by the union of paternal with maternal chromosomes—*i.e.*, that this is the stage of conjugation of the chromosomes. The comparative method in Cytology cannot be overestimated, [t]hough of course careful detailed examinations should be carried on at the same time. For a single object is rarely capable of serving as the basis of explanations of all the problems; an investigation of a number of forms always shows that some are more favorable than others for answering certain questions, and then there is the chance that a wholly unexpected discovery may be made that may have great significance. So the plea is made here for the comparative method in Cytology . . . . (pp. 154–155)

Montgomery's remarks emphasize a very important principle of scientific investigation: more often than not one seeks specific sorts of evidence rather than considering all the evidence in an even-handed manner. If the chromosomes of one species of Hemiptera exhibited a peculiar behavior, why use this to support a hypothesis rather than those 41 species that do not? "Unscientific" as this procedure may appear, we will find that the great success of genetics was a consequence of emphasizing data that conformed to Mendel's hypothesis and ignoring that which did not. In time the exceptions came to be understood and incorporated into genetic theory.

One might liken the conceptual development of genetics to the formation of a crystal in a super-saturated solution. The

ions in solution are the unorganized facts about chromosomes, breeding data, and much of biology. A tiny crystal, the working hypothesis, begins to form and gradually all those randomly distributed ions become incorporated in an organized whole.

Montgomery was 28 when his classic paper was published. He was almost the same age as Sutton. Both died before they were 40.

THE DISCOVERY OF SEX CHROMOSOMES

As Montgomery had suggested in his 1901 paper, just quoted, it is important to study a variety of organisms since some may show variations in the behavior of their chromosomes and this will provide data not otherwise available and conclusions not otherwise possible. The accessory chromosomes turned out to be a case in point. In fact, it was by studying their behavior that the critical evidence that genes are parts of chromosomes was eventually obtained.

Recall the reason that Boveri experimented with polyspermy in sea urchins. His system provided a mechanism for allocating abnormal groups of chromosomes to the cells of the early embryo. As a consequence, the embryos died and the hypothesis that a set of normal chromosomes is necessary for normal development was supported.

Nevertheless, this was not a fruitful type of experimentation. There was no means of recognizing individual chromosomes, of relating specific chromosomes to specific phenotypes, or of controlling which chromosomes entered which cell.

As it so often happened, nature was doing the required experiment all along. And as it so often happens, it took a considerable length of time for cytologists to realize that was so.

In 1891 H. Henking published his observations on the behavior of chromosomes in spermatogenesis in the bug, *Pyrrhocoris* (Fig. 21). This species has a diploid number of 23 chromosomes—11 pairs plus an extra one, which he called the "X." At synapsis the 11 homologous pairs formed 11 tetrads. But the behavior of the **X** was different. Having no homologue it could not synapse but it replicated to form a dyad-like structure. At the beginning of meiosis, therefore, the cell would have 11 tetrads plus the **X** in the form of a dyad. At the first meiotic division the 11 tetrads separated, a dyad from each going to each daughter cell. The **X**-dyad, however, went entirely to one pole of the spindle and, hence, was included in only one of the daughter cells.

At the second meiotic division of the cell with only the 11 dyads, separation of the dyads was observed and one chromosome of each went to each daughter cell. The cell with the 11 dyads plus the **X**-dyad divided and one chromosome of each of the 11 dyads went to opposite poles of the spindle. The **X**-dyad divided also and each of the daughter cells received one **X** chromosome.

Thus, of the four cells produced by meiosis, two would have 11 chromosomes and two would have 11 chromosomes plus an **X**. Therefore two types of sperm would be formed, one type with an **X** and the other without.

Henking reported what he had seen and left it at that. Thereafter other observations were made on species that had these peculiar chromosomes. They were noticed either because they stained differently from the other chromosomes, or they might move to the poles of the spindle earlier or later than the other chromosomes, or they lacked a mate for synapsis, or they were distributed to only half of the sperm. The vast majority of the observations were made on males since, for technical reasons, spermatogenesis was easier to study than oogenesis.

MCCLUNG 1901: THE X DETERMINES MALENESS

In 1901 the American cytologist, C. E. McClung, suggested that the **X** chromosome was in some way connected with the determination of sex.

> Being convinced from the behavior in the spermatogonia and the first spermatocytes of the primary importance of the accessory chromosome, and attracted

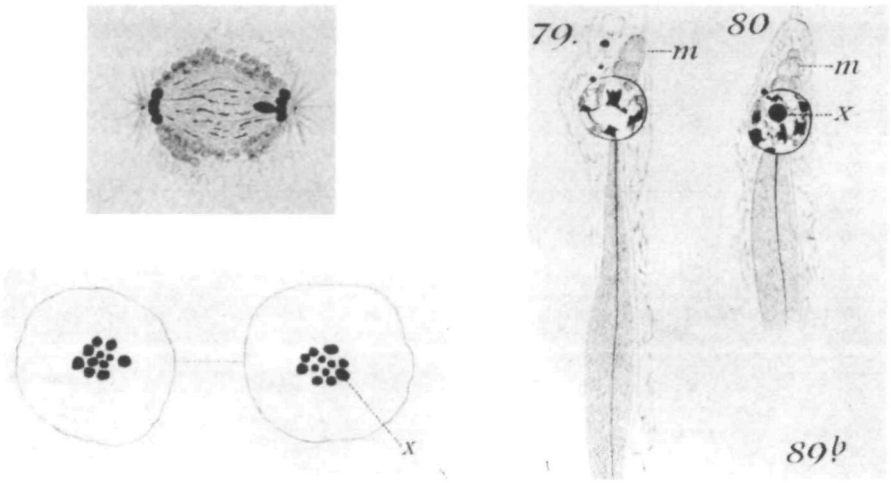


FIG. 21. Meiosis in *Pyrrhocoris*. The upper left figure is of a spermatocyte in telophase of the second meiotic division. The **X** chromosome, lagging behind the rest, is going to the pole at the right. The resulting daughter cells are shown at the lower left figure with the **X** in the right cell only. Two sorts of sperm will be formed, as shown in the rightmost figure—one with an **X** and one without. (Henking, 1891)

by the unusual method of its participation in the spermatocyte mitoses [*i.e.*, meiosis], I sought an explanation that would be commensurate with the importance of these facts. Upon the assumption that there is a qualitative difference between the various chromosomes of the nucleus, it would necessarily follow that there are formed two kinds of spermatozoa which, by fertilization of the egg, would produce individuals qualitatively different. Since the number of each of these varieties of spermatozoa is the same, it would happen that there would be an approximately equal number of these two kinds of offspring. We know that the only quality which separates the members of a species into these two groups is that of sex. I therefore came to the conclusion that the accessory chromosome is the element which determines that the germ cells of the embryo shall continue their development past the slightly modified egg cell into the highly specialized spermatozoon.

It would not be desirable in a preliminary paper of this character to extend it by a detail of the discussion by which the problem was considered. Suffice it to say that by this assumption it is possible to reconcile the results of many empirical theories which have proved measurably true upon the general ground that the egg is placed in a delicate adjustment with its environment, and in response to this, is able to attract that form of spermatozoon which will produce an individual of the sex most desirable to the welfare of the species. The power of selection which pertains to the female organism is thus logically carried to the female element.

Numerous objections to this theory received consideration, but the proof in support of it seemed to overbalance them largely, and I was finally induced to commit myself to its support. I trust that the element here discussed will attract the attention which I am convinced it deserves and can only hope that my investigations will aid in bringing it to the notice of a larger circle of investigators than that now acquainted with it.

This hypothesis *was* noticed since it provided an explanation for those odd chromosomes that were being found in more and more species. Montgomery (1901) had observed several cases. Sutton (1902) had described the same condition for *Brachystola* and wrote,

> thus we seem to find a confirmation of McClung's suggestion that the accessory chromosome is in some way concerned with the determination of sex. (p. 36)

At first it was believed that the accessory chromosomes were extra and restricted to males. Sutton had reported that the chromosomes of ovarian cells resembled those in the testis except for the lack of the accessory. Subsequently it was discovered that the female of *Brachystola,* far from lacking the accessory chromosome, has two.

Thus McClung had proposed a fruitful hypothesis—if we exclude that extraordinary suggestion in paragraph two of the quotation that the ovum can choose which type of sperm will enter, and do it for the welfare of the species.

WILSON 1905–1912: SEX CHROMOSOMES

By the time McClung proposed that the accessory or **X** chromosomes were somehow involved in sex determination, they had been observed in a variety of species. Since this was a most important hypothesis, many species of both animals and plants were studied to see to what extent the hypothesis was supported.

During the first decade of the 20th century the study of sex chromosomes exhibited a pattern not uncommon in science. An important hypothesis, presumably of wide applicability, is proposed—although on inadequate evidence. This was McClung's (1901) suggestion that the accessory chromosome might determine maleness. This initial suggestion was followed by a period of active research. There emerged conflicting observations and it was clear that the original suggestion that males have an extra chromosome did not hold for all species. There were also conflicting conclusions. Some investigators failed to find accessory chromosomes. Those who did suggested a variety of hypotheses to account for them. Some believed them to be degenerating chromosomes, others believed them to be a special type of nucleolus, and still others thought that McClung was probably correct.

The final stage in this scenario is when one or a few individuals, careful of their supporting data and cautious in their conclusions, bring conceptual order to the subject being investigated. And, again, as so often happens, two or more individuals, working independently, reach essentially the same conclusion at the same time. E. B. Wilson was the person mainly responsible for solving the riddle of the accessory chromosomes but the announcement of his discovery coincided with a report reaching similar conclusions by Nellie M. Stevens.

Wilson (1905*c*) begins as follows:

> Material procured during the past summer demonstrates with great clearness that the sexes of Hemiptera show constant and characteristic differences in the chromosome groups, which are of such a nature as to leave no doubt that a definite connection of some kind between the chromosomes and the determination of sex exists in these animals. These differences are of two types. In one of these, the cells of the female possess one more chromosome than those of the male; in the other, both sexes possess the same number of chromosomes, but one of the chromosomes in the male is much smaller than the corresponding one in the female (which is in agreement with the observations of Stevens on the beetle *Tenebrio*). These types may conveniently be designated as A and B respectively. [Subsequently A was to be called **XX** female–**XO** male and B was to become **XX** female–**XY** male.] . . . .
>
> These facts admit, I believe, of but one interpretation. Since all of the chromosomes in the female (oogonia) may be symmetrically paired, there can be no doubt that synapsis in this sex gives rise to the reduced number of symmetrical bivalents, and that consequently all of the eggs receive the same number of chromosomes. This number . . . is the same as that present in those spermatozoa that contain the 'accessory' chromosomes. It is evident that both forms of spermatozoa are functional, and that in type A females are produced from eggs fertilized by spermatozoa that contain the 'accessory' chromosome, while males are produced from eggs fertilized by sper-

matozoa that lack this chromosome (the reverse of the conjecture made by McClung).

The situation in type B species was essentially the same, except that one class of sperm contained the **X** and the other the **Y**.

Stevens (1905) summarized her discovery as follows:

> From the standpoint of sex determination, we have in *Tenebrio molitor* the most interesting of the forms considered in this paper. In both somatic and germ cells of the two sexes there is a difference not in the number of chromatin elements, but in the size of one, which is very small in the male and of the same size as the other 19 in the female. The egg nuclei of the female must be alike so far as number and size of the chromosomes are concerned, while it is absolutely certain that the spermatids are of two equal classes as to chromatin content of the nucleus—one half of them have the 9 large chromosomes and 1 small one, while the other half have 10 large ones. Since the male somatic cells have 19 large and 1 small chromosome, while the female somatic cells have 20 large ones, it seems certain that an egg fertilized by a spermatozoon which contains the small chromosome must produce a male, while the one fertilized by a spermatozoon containing 10 chromosomes of equal size must produce a female. (p. 18)

Neither Wilson's nor Stevens' reports mention the great difficulty in studying chromosomes. In *Tenebrio*, for example, all the autosomes are identical in appearance—and very small. The male differs by having that one small chromosome—and many observers might have missed it. Figure 22, from Stevens' paper, shows the type of illustration that was usual in cytological reports of the time. Tissue sections would be searched for cells that showed the full set of chromosomes. Her 207 shows an ovarian follicle cell with the 20 large chromosomes. In 208a and 208b, part of the chromosomes were in one section and the rest in the adjacent section. The diploid set of chromosomes of the male is shown in 169 and 170. Number 196 shows the monoploid number in spermatids with the 9 large and the 1 small chromosome and 197 shows those with the 10 large chromosomes.

Considering the difficulty in working with such material, it is not surprising that most problems in cytology had a shaky beginning.

Wilson's most important contributions are contained in eight long papers, *Studies on Chromosomes I—VIII*, published between 1905 and 1912. His own observations, together with those of others, revealed a complexity not imagined by McClung and Sutton. In most groups of animals the female has a pair of homologous **X** chromosomes and is designated as **XX**. The males of various species, on the other hand, vary considerably. Some have only a single **X** and are designated as **XO**—the "**O**" indicating the absence of a chromosome (Fig. 23).

In other species the males may have two chromosomes, one like the **X** of the female and the other, usually differing in size or shape, called the **Y**. Thus these males are designated **XY**. With respect to the sex chromosomes, the males in this case produce two sorts of sperm, **X**-carrying sperm and **Y**-carrying sperm and thus are heterogametic. The females produce a single type of ovum and hence are homogametic. [It was found later that both human beings and *Drosophila* are of the **XX** female and **XY** male type.]

These two patterns of sex chromosomes, while the ones most commonly encountered, do not exhaust the range of possibilities. Some species may have multiple sex chromosomes. In birds and Lepidoptera the females are heterogametic and the males homogametic for the sex chromosomes.

These are some of the conclusions that can be drawn from the numerous studies of Wilson, Stevens, and others.

1. The sex of an offspring is determined at the time of fertilization.

2. The sex of an individual will be irreversible if it is based solely on the sex chromosomes—unless we can alter the chromosomes.

3. If meiosis is normal and fertilization

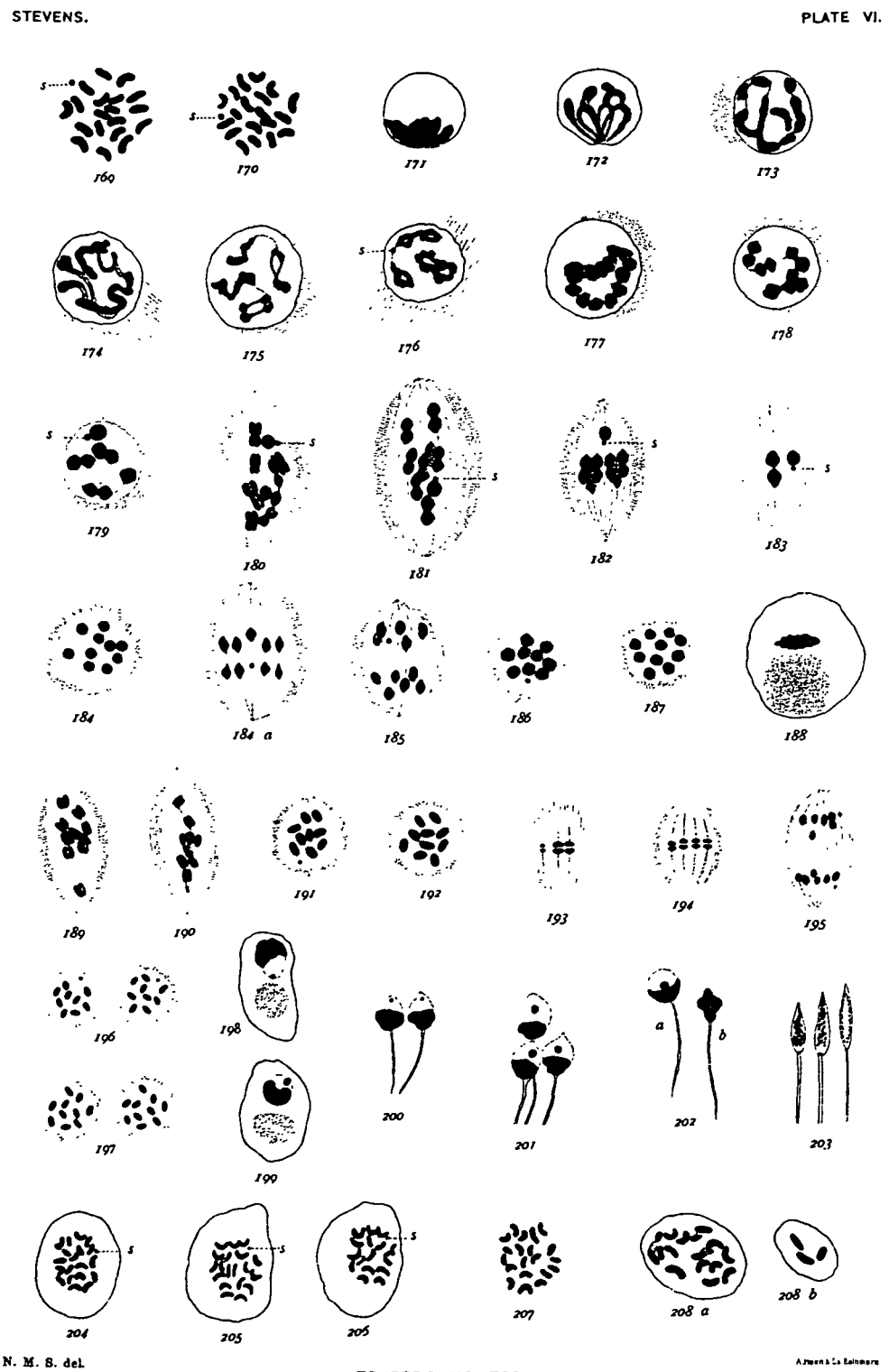


FIG. 22. The chromosomes of *Tenebrio*. (N. Stevens, 1905)

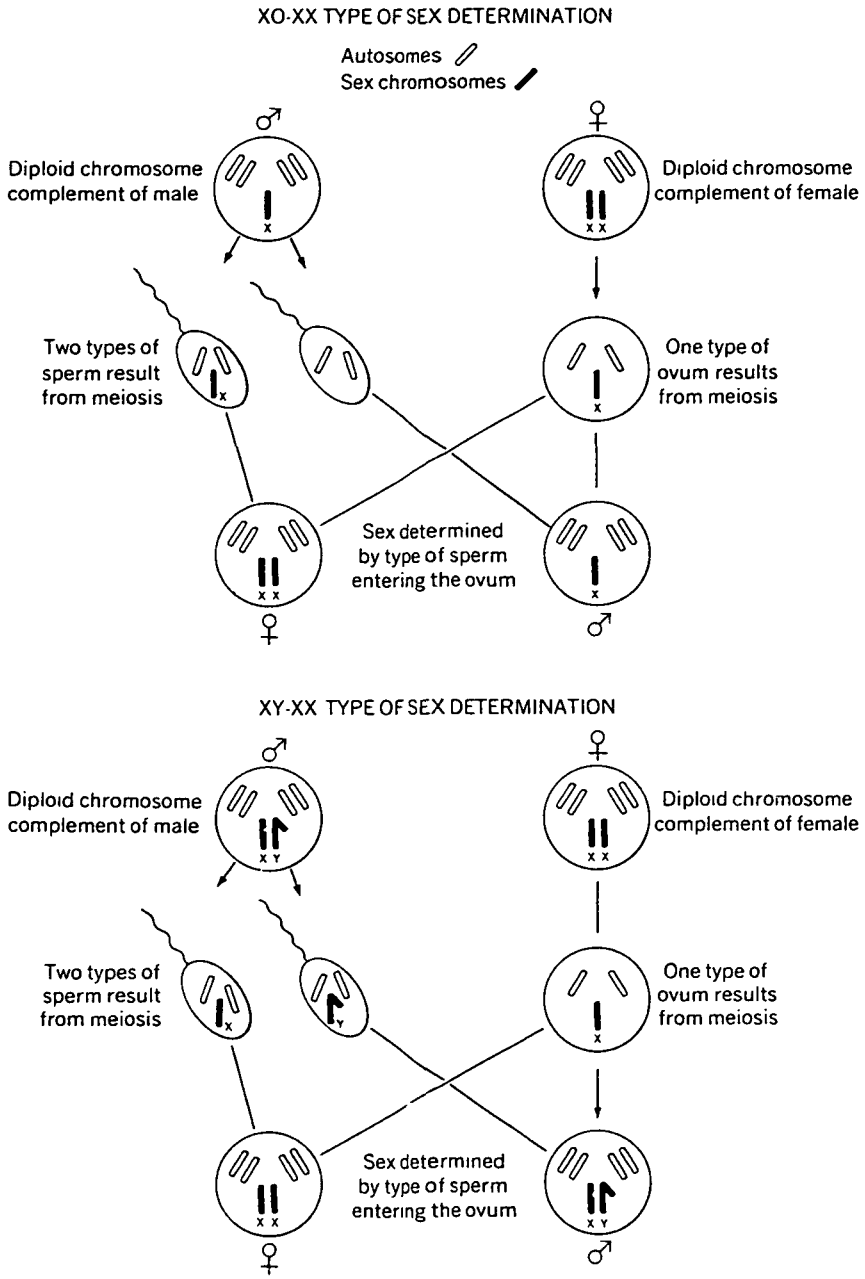


FIG. 23.

is random, the two sexes should be produced in approximately equal numbers.

4. The relation between sex and chromosomes, firmly established by 1910, is additional evidence supporting Sutton's hypothesis that chromosomes are the basis of inheritance.

1910: POSSIBLE CONCLUSIONS ABOUT THE PHYSICAL BASIS OF INHERITANCE

On 13 December 1910, Wilson finished writing *Studies on Chromosomes VII* (1911). A few months earlier his colleague, Thomas Hunt Morgan, had published a brief note on *Sex Limited Inheritance in Drosophila*

(1910*a*). Morgan's paper described the first of the experiments that were to be taken by almost all biologists as the "final proof" that genes are parts of chromosomes. After our survey of the work of Montgomery, Sutton, Boveri, McClung, and Wilson one might have suspected that no "final proof" was needed—the case was already convincing. But that was not so. Wilson, always cautious, wrote:

> Studies on the chromosomes have steadily accumulated evidence that in the distribution of these bodies we see a mechanism that *may* be competent to explain some of the most complicated of the phenomena that are brought to light by the study of heredity. New and direct evidence that the chromosomes are in fact concerned with determination has been produced by recent experimental studies, notably by those of Herbst ('09) and Baltzer ('10) on hybrid sea-urchin eggs. But the interest of the chromosomes for the study of heredity is not lessened, as some writers have seemed to imply, if we take the view—it is in one sense almost self-evident—that they are not the exclusive factors of determination. Through their study we may gain an insight into the operation of heredity that is none the less real if the chromosomes be no more than one necessary link in a complicated chain of factors. From any point of view it is indeed remarkable that so complex a series of phenomena as is displayed, for example, in sex-limited heredity [*i.e.*, Morgan's just-published research] can be shown to run parallel to the distribution of definite structural elements, whose combinations and recombinations can in some measure actually be followed with the microscope. Until a better explanation of this parallelism is forthcoming we may be allowed to hold fast to the hypothesis, directly supported by so many other data, that it is due to a direct causal relation between these structural elements and the process of development. (p. 106)

"This parallelism" allows deductions to be made, as noted before. Here is one related to sex chromosomes: *If genes are parts of the sex chromosomes, one must expect the inheritance of these genes to follow the inheritance of the sex chromosomes.*

Consider for example the case of a gene of the **X** chromosome of a species with **XX** females and **XY** males (Fig. 23). The distribution of these chromosomes is such that a male offspring can receive his **X** only from his mother (if he also received an **X** from his father, he would be a daughter). Daughters on the other hand receive an **X** from each parent. In a similar manner, any genes of the **Y** chromosome are transmitted exclusively through males.

Following a list of references, we shall begin the test of that deduction.

REFERENCES TO MENDELISM AND CYTOLOGY, 1900–1910

The decade between the rediscovery of Mendel's paper and the beginning of the work of the Morgan school with *Drosophila* saw great activity. There are two essentially independent lines of research, namely breeding and cytology—with a few tentative attempts to unite them. The literature is enormous and the following serves only as an introduction.

General works. Bateson (1894, 1900*a*, 1900*b*, 1902, 1906, 1908, *1909, 1913*b*, 1914, 1916, 1920, 1926, 1928), Bateson *et al.* (1902, 1905, 1906, 1908, 1911), Boveri (1902, 1907), Cannon (1902, 1903*a*, 1903*b*), Castle (1903, 1911), Chubb (1910–1911), Conklin (1908), O. F. Cook (1907), Correns (1900), Crew (1965, 1966), Darbishire (1911), Davenport (1901, 1907), Galton (1889, 1897), Henking (1891), Hurst (1925), Johannsen (1911), Lock (*1906), McClung (1901), Mendel (1865, 1902), Mitchell (1910–1911), Montgomery (1901), Moore (1972*a*,), Morgan (1903, 1909, 1913), Pearson (1898), Punnett (1911), Schrader (1928), Sharp (1934), Spillman (1902), Sutton (1902, 1903), Tschermak (1900), Vernon (1903), de Vries (1900, 1901–1903, 1906, 1909–1910, 1919), Weldon (1902), Wheldale *et al.* (1909), E. B. Wilson (1902, 1903, 1905*a*, 1905*b*, 1905*c*, 1906, 1909*a*, 1909*b*, 1909*c*, 1910, 1911, 1912, *1914, 1924, *1925), Wright (1966).

History, biography, and anthologies. G. E.

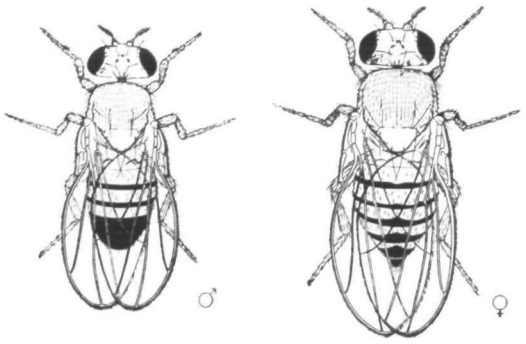


FIG. 24. Male (left) and female (right) of *Drosophila melanogaster*. (Morgan, 1919)

Allen (1966*a*, 1966*b*, 1969, 1974*a*, 1974*b*, 1976, 1978, 1979, 1985), Babcock (1949–1951), Baltzer (1964, 1967), B. Bateson (1928), Bennett (1965), Boyes (1966), Brink (1967), Carlson (1967*a*, 1967*b*), Castle (1951), Coleman (1965, 1970*a*, 1970*b*, 1971), Conklin (1913), Crampton (1942), Crew (1968), Darlington (1960, 1969), Dodson (1955), Dorsey (1944), Dunn (*1951, *1965*a*, 1965*b*, 1969), East (1922, 1923), Eichling (1942), Fantini (1985), Fisher (1936), Fong (1969), Gabriel and Fogel (1955), Gasking (1959), Genetics (1950), Glass (1947, 1953, 1959*a*), Hughes (1959), Iltis (1932, 1947, 1951), Kalmus (1983), Krizenecky (1965), McKusick (1960, 1976), Mayr (1973, *1982), Moore (1972*a*, 1972*b*, 1983), Morgan (1926*b*, 1940), Muller (1943), Nardone (1968), Olby (*1966, 1979), Oppenheimer (1970), Orel (1968, 1984), Orel and Varva (1968), Pearson (1924), Peters (1959), Pollister (1974), Provine (*1971), Punnett (1950), Robinson (1979), Root-Bernstein (1983), Rosenberg (1976), Stern and Sherwood (*1966), Stomps (1954), Stubbe (*1972), Sturtevant (*1965*a*, 1965*b*), Sutton (1917), Tschermak (1951), Voeller (1968), Weir (1968), Wilkie (1962), Winge (1958), Zirkle (1951*a*, 1964, 1968*a*, 1968*b*).

A FLY WITH WHITE EYES

The most famous fly in the history of science is a male fruit fly with the name *Drosophila melanogaster* (Fig. 24). This individual became famous because it had white eyes instead of the normal red ones, but most importantly because it happened to appear in Room 613 of Schermerhorn Hall at Columbia University in the spring of 1910. This was the "Fly Room," the laboratory of Thomas Hunt Morgan and a remarkable group of young students. Down the hall was the laboratory of E. B. Wilson, who was finishing up his series—*Studies on Chromosomes.*

That fly had chosen the proper time and place to spin out its short life and achieve immortality.

Morgan (1910*a*) tells the story:

> In a pedigree culture of *Drosophila* which had been running for nearly a year through a considerable number of generations a male appeared with white eyes. The normal flies have brilliant red eyes.
>
> The white-eyed male, bred to his red-eyed sisters, produced 1,237 red-eyed offspring, (F_1), and 3 white-eyed males. The occurrence of these three white-eyed males (F_1) (due evidently to further sporting) will, in the present communication, be ignored.
>
> The F_1 hybrids, inbred, produced:
>
> 2,459 red-eyed females,
> 1,011 red-eyed males,
> 798 white-eyed males.

(If time permits, this might pose an interesting problem for the class to consider—overnight, perhaps.)

> *No white-eyed females appeared.* The new character showed itself therefore to be sex limited in the sense that it was transmitted only to the grandsons. But that the character is not incompatible with femaleness is shown by the following experiment.
>
> The white-eyed male (mutant) was later crossed with some of his daughters (F_1), and produced:
>
> 129 red-eyed females,
> 132 red-eyed males,
> 88 white-eyed females,
> 86 white-eyed males.
>
> The results show that the new character, white-eyes, can be carried over to the females by a suitable cross, and is in consequence in this sense not limited to one

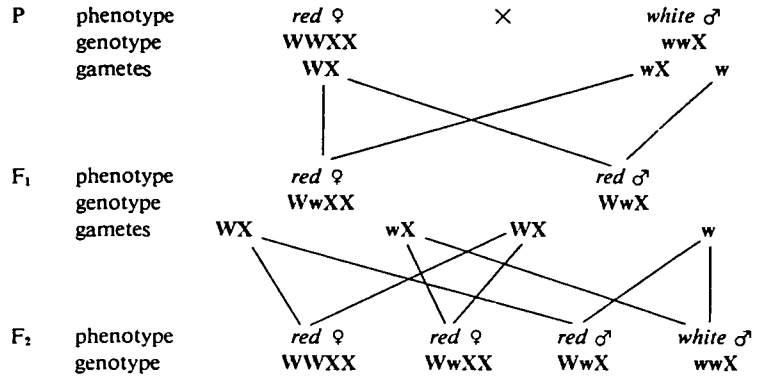


FIG. 25. Morgan's first hypothesis to explain the inheritance of *white eyes.*

sex. It will be noted that the four classes of individuals occur *roughly* in equal numbers (25 per cent.).

What was one to conclude? The original cross of the *white-eyed* male with the *red-eyed* females gave an F_2 ratio of 4.3 to 1. This might be accepted as a 3 to 1 ratio since it seemed clear that the *white-eyed* flies were less viable than their *red-eyed* sibs (as shown in the later cross of the F_1 daughters with the *white-eyed* male).

But to interpret this as a typical 3 to 1 F_2 ratio was spurious. White eye color was not evenly distributed among females and males as it should be in a normal Mendelian cross. In the F_2 of the original cross there were no *white-eyed* females. This association of inheritance with sex hinted that a critical test of Sutton's hypothesis might be in the making. Back to Morgan:

MORGAN'S FIRST HYPOTHESIS

An Hypothesis to Account for the Results.— The results just described can be accounted for by the following hypothesis. Assume that all of the spermatozoa of the white-eyed male carry the "factor" for white eyes "W"; that half of the spermatozoa carry a sex factor "X" the other half lack it, *i.e.,* the male is heterozygous for sex. Thus the symbol for the male is "WWX," and for his two kinds of spermatozoa WX—W.

Assume that all of the eggs of the red-eyed female carry the red-eyed "factor" R; and that all of the eggs (after reduction) carry one X, each, the symbol for the red-eyed female will be therefore RRXX and that for her eggs will be RX—RX.

It is of the greatest interest to note how Morgan indicated the genotype of both adults and gametes. He recognized both genetic "factors" *and* chromosomes as though they were independent phenomena. His symbolism of "R" for the allele for red-eyes and "W" for the allele for white was eventually replaced by the Mendelian scheme for using upper and lower case symbols for dominant and recessive alleles so, for clarity, I will alter Morgan's original notation and use **w** for the allele for *white eyes* and **W** for the allele for *red eyes.* Another point to be noted is that the male was assumed to have only one **X** chromosome, that is, to be of an **XO** type of male. Subsequently it was realized that the *Drosophila* male has a **Y** chromosome as well.

Figure 25 uses Morgan's hypothesis to explain the results of the first cross of the *white-eyed* male with a red-eyed female. The scheme fits the data, that is, the F_1 is predicted to consist only of *red-eyed* daughters and *red-eyed* sons. Continuing to the F_2, the hypothesis predicts that all of the females will have *red eyes* and that half of the sons will have *red eyes* and half will have *white eyes.*

Not surprisingly the hypothesis predicts what was found. After all, the observations were made before the hypothesis was formulated and there would be no reason to

propose a hypothesis that failed to account for the data already at hand.

But the hypothesis explained the data only with one important qualification. Note the F_1 individuals. When gametes are formed by the female, half are shown with the **W** going with an **X** and half with the **w** going with an **X**. However, the hypothesis demanded a very different situation for the F_1 male. The male is shown as **WwX**. One should expect, therefore, four classes of gametes: **WX**, **wX**, **W** (or **WO**), and **w** (or **wO**). Morgan recognized only two classes of sperm: **WX** and **w**. He explains:

> It is necessary to assume . . . that when the two classes of spermatozoa are formed in the F_1 red male (WwX), W and X go together—otherwise the results will not follow (with the symbolism here used). This all-important point can not be fully discussed in this communication.

TESTING THE FIRST HYPOTHESIS

The value of a hypothesis is not only to explain the data at hand but also to predict what will happen in new situations. Morgan undertook four tests of his hypothesis.

1. If the genotype of the *white* males is **wwX** and of the *white* females **wwXX**, their offspring should consist of *white* males and *white* females only. The diagrammatic representation of this cross in terms of Morgan's hypothesis is shown in Figure 26. The cross was made and the results were according to predictions.

2. The F_2 *red-eyed* females in the first cross (Fig. 25), were predicted to be of two genotypes, **WWXX** and **WwXX**, even though all were identical in appearance. If several of these females were crossed individually with *white-eyed* males, one would expect two results as shown in Figure 26. Approximately half of the crosses should result in all of the offspring having *red* eyes and the other half should produce four phenotypes among the offspring. These crosses were made and the predicted results were observed.

3. The genotype of the F_1 female of the original cross (Fig. 25) was predicted to be **WwXX**. If so, the cross of such a female with a *white-eyed* male should give the same results as shown in Test 2b of Figure 26. Again the cross was made and the predicted outcome was observed.

4. The hypothesis requires that the original F_1 males (Fig. 25) be **WwX**. If such a male is crossed with a *white-eyed* female, the prediction would be for *red-eyed* females and *white-eyed* males, as shown in Figure 26. The crosses were made and the prediction verified. Once again, however, the hypothesis required an unusual type of meiosis in the **WwX** males: the **W** factor would always be with the **X**, to form **WX** sperm; there could be no **wX** sperm.

TRUE, BEYOND ALL REASONABLE DOUBT?

Well, maybe. Few new hypotheses in the early days of genetics, apart from Mendel's, were tested so thoroughly as this one. Nearly all of Morgan's first hypothesis was based on well-substantiated genetic principles: dominance and recessiveness, segregation, and the behavior of sex chromosomes. His four deductions were explicit and critical. In every case the experiments to test the deductions provided data that verified the predictions.

To be sure there was that qualification about spermatogenesis in **WwX** males but by 1910 his colleague Wilson, and other cytologists, were reporting all sorts of strange behavior of chromosomes in meiosis. There was no *a priori* reason to exclude the hypothesis of the association of **W**, but never **w**, with the **X** in males.

Morgan reported another discovery that was difficult to explain:

> A most surprising fact appeared when a white-eyed female was paired to a wild, red-eyed male, *i.e.*, to an individual of an unrelated stock. The anticipation was that wild males and females alike carry the factor for red eyes, but the experiments showed that all wild males are heterozygous for red eyes, and that all the wild females are homozygous. Thus when the white-eyed female is crossed with a wild red-eyed male, all of the female offspring are red-eyed, and all of the male offspring white-eyed.

These data presented a difficulty. If all males in natural populations are hetero-

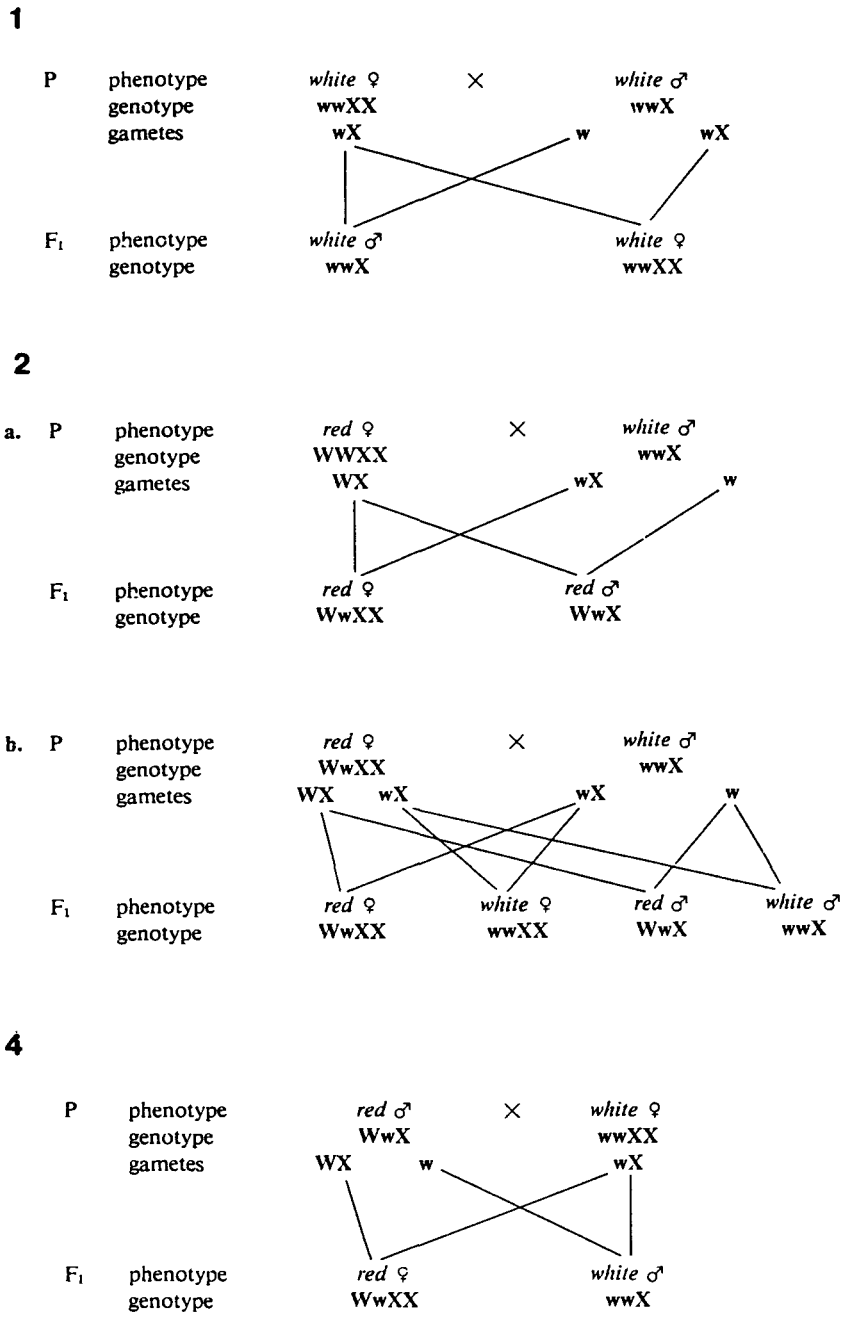


FIG. 26. Tests of Morgan's first hypothesis to explain the inheritance of *white eyes*.

zygous for these eye color alleles, one would expect numerous *white-eyed* flies to be present in wild populations and in cultures. Yet Morgan had been raising *Drosophila* for many months and had observed no such thing.

As yet I have found no evidence that white-eyed sports occur in such num-

bers. Selective fertilization may be involved in the answer to this question.

There are many interesting points about this famous paper that started the line of experimentation that revolutionized genetics. The most puzzling is why Morgan failed to realize that the data could be explained more simply by assuming that the alleles for eye color were *parts* of the **X** chromosome. Instead he treated the situation almost as a dihybrid cross. To be sure in 1910 he was still most suspicious of the Suttonian hypothesis but would he not have discussed the data with his colleague Wilson? To be sure the paper had been written in haste. G. E. Allen (1978, p. 153) estimates that the *white-eyed* male was discovered about January of 1910. Then the experiments were done. The paper was finished 7 July 1910 after Morgan had gone to Woods Hole, and was published in the 22 July 1910 issue of *Science*.

Of considerable pedagogical interest is the fact that the paper is written in a form that corresponds to the popular view of "The Scientific Method." First there are the observations of some natural phenomenon, in this case the crosses involving the strange new fly with the white eyes. Then a hypothesis is formulated. Finally deductions are made from the hypothesis and these are tested. The tests are assumed to have supported the hypothesis so the scientist goes on to the next problems. These steps are rarely mentioned in published reports, even though something like the "scientific method" is happening in the mind of the investigator. Morgan's 1910*a* paper is unusual in that these steps are explicitly stated in the published report.

MORGAN'S SECOND HYPOTHESIS

It took Morgan only a few months to realize that his first hypothesis to explain sex-limited inheritance of eye color was fundamentally flawed. Several additional mutations were found and these were inherited in the same manner as the *white-eyed* allele. The results were "first announced in a public lecture given in the Marine Biological Laboratory at Woods Hole, Mass., July 7, 1911" (Morgan, 1911*a*, p. 365). The new hypothesis was simplicity itself: instead of thinking of the sex-limited alleles as being associated with the **X** chromosomes (the first hypothesis) why not think of them as part of the **X** chromosome?

> The experiments on Drosophila have led me to two principal conclusions:
>
> First, *that sex-limited inheritance is explicable on the assumption that one of the material factors of a sex-limited character is carried by the same chromosomes that carry the material factor for femaleness.*
>
> Second, *that the 'association' of certain characters in inheritance is due to the proximity in the chromosomes of the chemical substances (factors) that are essential for the production of those characters.* (Morgan, 1911*a*, p. 365)

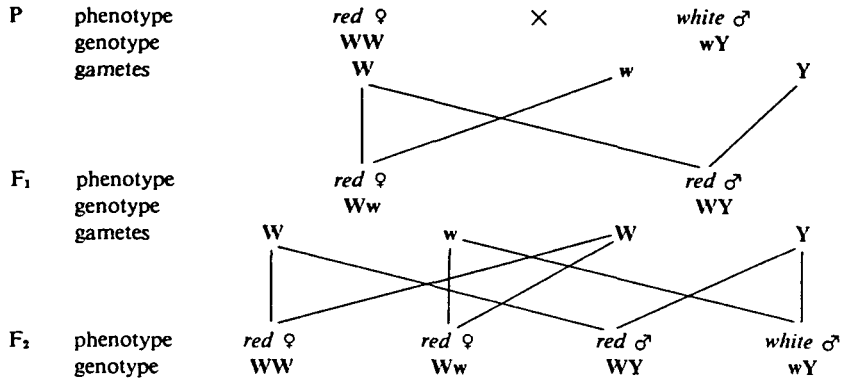
Therefore, if one assumes that the allele for *white eyes* and the dominant allele for *red eyes* are parts of the **X** chromosome, the results of all the crosses correspond to what would be expected from the distribution of the **X** chromosome in meiosis and fertilization. It would then be unnecessary to invoke subsidiary assumptions, such as the **w** allele not being able to associate with the **X** in meiosis of the **WwX** males or that all wild males must be heterozygous.

Morgan's second hypothesis has withstood every conceivable test and it can be accepted as true beyond all reasonable doubt. Figure 27 shows how it explains the inheritance of *white eyes*. This figure also shows a **Y** chromosome because it was soon realized that the male *Drosophila* is **XY**, not **XO**. The data indicated that the **Y** chromosome did not have an allele at the locus for *white eyes* and, as we now know, it has only a very few active gene loci of any sort.

THE NON-OBVIOUSNESS OF THE "OBVIOUS"

Once again we find an example of the "obvious" not being obvious at all. More often than not, things become obvious after the fact. One is reminded of the oft-quoted remark of Thomas Henry Huxley when the concept of natural selection became clear to him:

> My reflection, when I first made myself



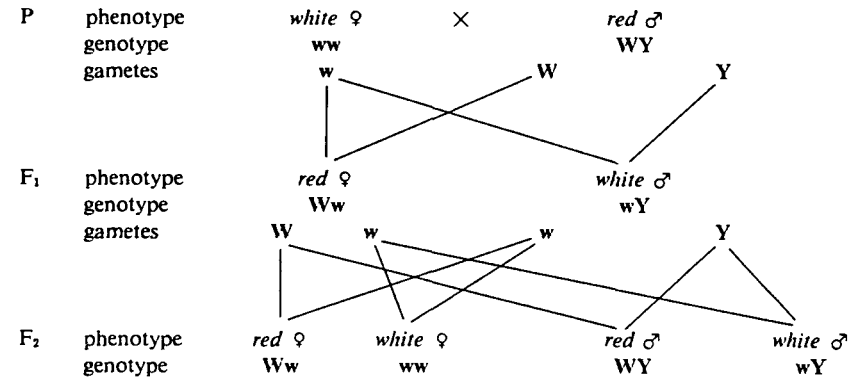


FIG. 27. Morgan's second hypothesis to explain the inheritance of *white eyes*.

> master of the central idea of the 'Origin' was, 'How extremely stupid not to have thought of that'. (Huxley, 1868, p. 197)

Morgan was in the Zoology Department where a short seven years earlier Sutton had maintained that genes must be parts of chromosomes. His colleague E. B. Wilson had continued to work within the Suttonian paradigm. However, Morgan had not accepted the chromosomes as the physical basis of inheritance and was not to do so until his own experiments convinced him. In fact, he had a poor opinion of the explanations being used by geneticists to account for the data of inheritance. In January 1909, the year before his first paper on the *white-eyed* fly, he had this to say in a lecture to the American Breeders' Association:

> In the modern interpretation of Mendelism, facts are being transformed into factors at a rapid rate. If one factor will not explain the facts, then two are invoked; if two prove insufficient, three will sometimes work out. The superior jugglery sometimes necessary to account for the results may blind us, if taken too naively, to the common-place that the results are often so excellently "explained" because the explanation was invented to explain them. We work backwards from the facts to the factors,

> and then, presto! explain the facts by the very factors that we invented to account for them . . . . I cannot but fear that we are rapidly developing a sort of Mendelian ritual by which to explain the extraordinary facts of alternative inheritance. (p. 365)

Such was the opinion of the one who, in a few short years, was to be recognized as the Giant of Genetics of our century—and who surely will be the last giant of genetics working above the molecular level.

Morgan, together with many others, was still troubled in 1909 by the notion of the "purity of the gametes." He continues in his lecture to the American Breeders' Association:

> I should like to point out certain implications in the current assumption that the factors (sometimes referred to as the actual characters themselves—unit-characters, not infrequently) are dissociated in the germ-cells of the hybrids into their allelomorphs. For instance a tall pea crossed with a dwarf pea produces in the first generation a tall hybrid. Such tall peas inbred produce three tall peas to one dwarf. Such are the surprising facts. Mendel pointed out that the numerical results could be explained if we assume that the hybrid peas produce germ-cells of two kinds, tall-producing and dwarf-producing. The simplicity of the explanation, its wide applicability and what I may call its intrinsic probability will recommend his interpretation to all who have worked with such problems of heredity. Out of this assumption the modern factor hypothesis has emerged. The tallness of the tall pea is said to be due to a tall-factor; the dwarfness of the dwarf-pea, to be a dwarfness factor. When they meet in the hybrid, the tall-factor gets the upper hand. So far we do little more than restate Mendel's view. But when we turn to the germ-cells of the hybrid we go a step further. We assume that the tall-factor and the dwarf-factor retire into separate cells after having lived together through countless generations of cells without having produced any influence on each other. We have come to look upon them as entities that show a curious antagonism, so that when the occasion presents itself, they turn their backs on each other and go their several ways. Here it seems to me is the point where we are in danger of over-looking other possibilities that may equally well give us the two kinds of germ-cells that the Mendelian explanation calls for. (pp. 365–366)

Morgan then proposed a vague alternative mechanism that reveals his basic training as an embryologist. He detects an element of preformation (a red flag to the embryologists) in the hypotheses of the geneticists. His proposed hypothesis involved "alternative states of stability," "local conditions," "changes in equilibrium," and interactions between homologous chromosomes. This seems out of character for Morgan—always one to insist on experimentation as the proper way to understanding. He was rejecting a hypothesis that was far more amenable to experimental verification in favor of one serviceble only for speculation.

But Morgan did not have an entirely closed mind. After rejecting the hypothesis, well into 1909, that the segregation of alleles in Mendelian crosses could be explained by the segregation of chromosomes in meiosis, he became the strongest proponent of the hypothesis that Mendelism finds its explanation in the behavior of chromosomes.

THE "FLY ROOM"

In the decade of the 1910s a medium-sized room in the Zoology Department of Columbia University, occupied by Morgan and his students, became the center of genetics. In 1911 Morgan (1866–1945) was 45 years old. He had come to Columbia in 1904 as a world-class embryologist. Throughout this formative decade he had three close associates, who began as his students and remained as co-workers: Sturtevant, Bridges, and Muller. Alfred Henry Sturtevant (1891–1970) was to receive his Ph.D. in 1914 for using linkage data to construct the first genetic map of chromosomes. Calvin B. Bridges (1889–1938) received his Ph.D. in 1916 for a classic

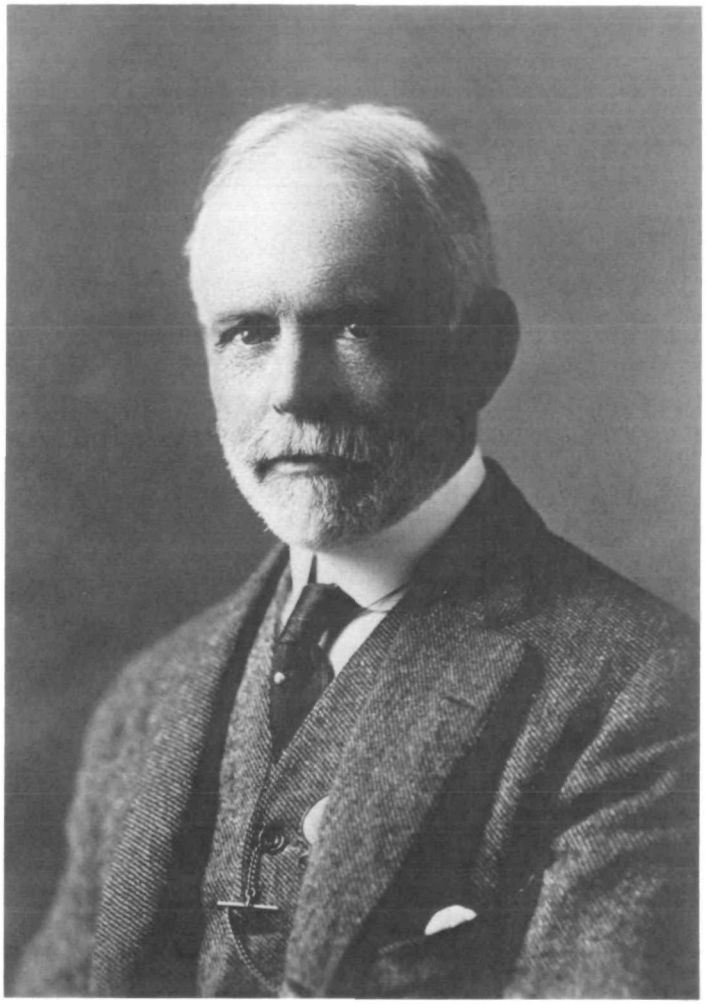


FIG. 28. Edmund Beecher Wilson.

paper on non-disjunction, widely regarded as the final and conclusive proof that genes are parts of chromosomes. Herman J. Muller (1890–1967) received his degree in 1915 for a definitive study of crossing-over. Biologists came from all over the world to visit or to do research in the Fly Room. The room and some of those associated with it are shown in Figures 28–31.

The basis for all these discoveries was the fruit fly, *Drosophila melanogaster.* It appears to be an immigrant from the Old World and as a "domestic species" is frequently found in homes, stores, and garbage dumps—wherever there is fresh fruit. It has also spread into more natural habitats and in some areas is the most abundant species of its genus.

Morgan began to use *Drosophila* because he was unable to obtain funds for experiments on mammals. *Drosophila* could be raised in large numbers on inexpensive food, at first bananas, in small milk bottles, which Morgan apparently appropriated from those brought to his home by the local milkman. A few other laboratories were using *Drosophila* at the same time (G. E. Allen, 1975*a*) and there has been much speculation about where Morgan obtained the stocks of those famous flies. There is no reason to believe that there was a single source. When I was a student at Columbia

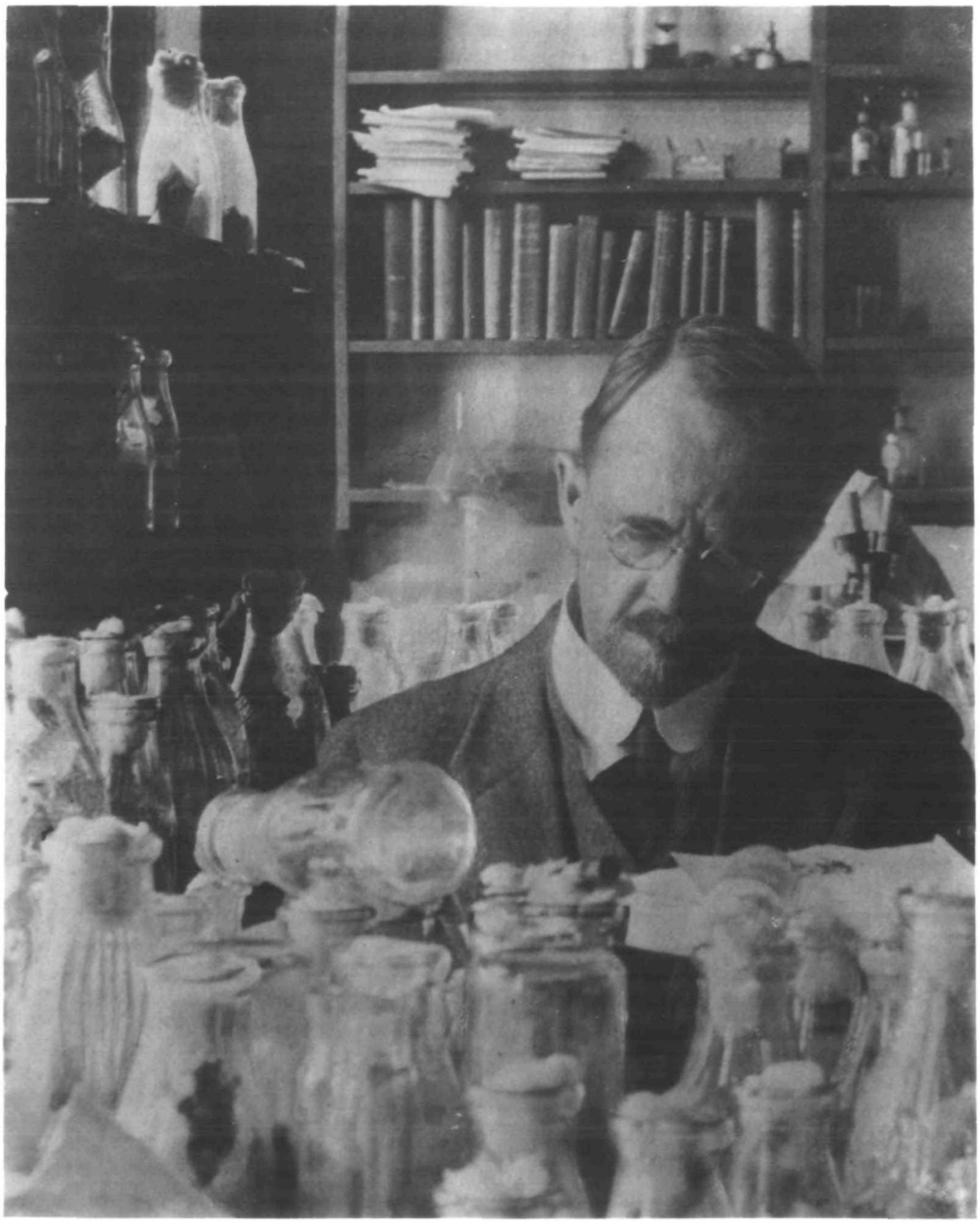


FIG. 29. Thomas Hunt Morgan in the Fly Room. Taken about 1917 by Calvin Bridges.

in the 1930s, the source was remembered as a pineapple on the window sill outside of Morgan's laboratory. The discovery of the *white-eyed* male was credited to Calvin Bridges. He was at the time a Columbia College undergraduate, hired to wash the dirty fly bottles. Just before washing one he noticed a fly with *white eyes.* Shine and Wrobel (1976, ch. 5) have a nice discussion of the possible origin of that *white-eyed* male but they are unable to reach any definitive conclusion.

FIG. 30. Calvin Bridges in the Fly Room, about 1926.

Morgan did not begin work with *Drosophila* in the hope of extending Mendelism to that small insect. Instead, he was more interested in problems of evolution, and especially that argument of long duration—continuous *vs.* discontinuous variation. He was especially interested in testing the mutation hypothesis of de Vries (1901–1903, 1906) and realized that a species with a short generation time, easily cultured, and with numerous offspring would serve his purpose.

The interval between Morgan's first paper (1910*a*) on the *white-eyed* fly and Bridges' paper of 1916 on non-disjunction saw the foundations of *Drosophila* genetics laid and the conceptual basis essentially completed—all in the Fly Room. This was to supply the critical evidence that, beyond all reasonable doubt, the Suttonian paradigm could be accepted as true. From 1916 until 1953 most work in genetics was the normal science that fleshed out the conceptual framework of the paradigm. Sturtevant (1965*a*) gives a lively account of those brave days. See also G. E. Allen (1978), Carlson (1981), and Shine and Wrobel (1976).

We will now discuss some of the monuments that emerged from the Fly Room.

LINKED GENES

Calvin Bridges (Fig. 30) is remembered as the person in the Fly Room with the sharpest eyes for detecting new mutants. The group soon had dozens for use in their experiments. One might ask, "Why study so many?" Once it had been established that the Mendelian scheme worked for alleles on the autosomes and, with modification, for alleles on the **X**, why pile confirmation upon confirmation? The answer was simple: the mutant alleles could be used as probes to gain more information about the physical basis of inheritance, *i.e.*, the relation of genes to chromosomes, the location of genes, the preparation of genetic maps of the chromosomes, and various alterations of the structure of the chromosomes themselves.

FIG. 31. The corner of the Fly Room where the fly food was made.

When Sutton started it all in 1903, he argued that there must be more pairs of alleles than there were pairs of homologous chromosomes.

> We must, therefore, assume that some chromosomes at least are related to a number of different allelomorphs. If then, the chromosomes permanently retain their individuality, it follows that all the allelomorphs represented by any one chromosome must be inherited together. (p. 240)

One cannot read the papers of this extraordinary young scientist without being in awe of the brilliance of his analysis. Note the proviso: the different alleles must be inherited together *if* the chromosomes retain their individuality. One suspects that when the peculiar ratios that were labelled "coupling" and "repulsion" were discovered, he would have recognized that they must somehow be associated with the presence of different genes in the same chromosome. And when coupling was not complete, he may well have realized that a mechanism must be sought to account for the observation that the chromosomes do not always retain their individuality.

It was obvious to those geneticists who accepted the Suttonian hypothesis, therefore, that the original Mendelian scheme could not account for the results when two or more pairs of different alleles were parts of the same pair of homologous chromosomes.

COUPLING AND REPULSION

Bateson, Saunders, and Punnett (1906, pp. 8–11) had not accepted Sutton's hypothesis and they had great difficulty in explaining some of their crosses that failed to give the usual Mendelian ratios. They noted that

> Early in the revival of breeding experiments, attention was called, especially by Correns, to the phenomenon of coupling between characters. Complete coupling has so far been most commonly met with among characters of similar physiological nature . . . . Examples of *partial* cou-

pling have not hitherto been adequately studied.

They then gave an example. In one of the experiments with sweet peas involving two pairs of alleles, a ratio of 7:1:1:7 was observed in the F_2.

In sweet peas *blue* (**B**) flower color is dominant to *red* (**b**). *Long* (**L**) pollen grain is dominant to *round* (**l**). When a *blue-long* was crossed with a *red-round,* all of the F_1 individuals were *blue-long.* Nothing surprising so far. In the F_2 one observed the usual ratio of 3:1 so far as *blue vs. red* and *long vs. round* are concerned. Since this is a cross involving two pairs of alleles, the normal Mendelian expectation would be 9 *blue-long*: 3 *blue-round*: 3 *red-long*: 1 *red-round.* Instead, the observed phenotypic ratios were roughly 7:1:1:7 in the order of the phenotypes just listed—hardly Mendelian.

Figure 32A shows the cross. The F_1 *blue-long* individual should have produced the four types of gametes as shown. In order to be sure that this was the case a test cross was made. That is, the F_1 individuals were crossed with the pure recessive as shown in Figure 32B. The expected results are indicated—25 percent each of the four phenotypes. But this is what happened:

	Expected	Actual
blue-long	25%	43.7%
blue-round	25%	6.3%
red-long	25%	6.3%
red-round	25%	43.7%

Although these are not the results expected from a dihybrid Mendelian cross, there must be some rule at work since, when the experiments were repeated, Bateson and his associates always observed the same results. The two rules seemed to be:

1. The most common phenotypes are those of the original parents and they are in the same frequencies—43.7 percent each (data given above for the Fig. 32B cross and 32C).

2. The two recombinant classes, *blue-round* and *red-long* are much less frequent than expected but their frequencies are equals, namely, 6.3 percent.

Somehow the alleles of the original parents are "coupled" in some manner and are more frequent than expected. Yet coupling is not complete and the alleles of the original parents may be "repulsed" and produce the recombinant classes.

This tendency for the different alleles to be coupled was confirmed by the cross of *blue-round* × *red-long* individuals (Fig. 32C). If these peas were playing by the Mendelian rules, one would expect this cross to be exactly the same as that of Figure 32A. The only difference is that the two pairs of alleles are distributed differently between the parents. The F_1 individuals have the same phenotypes (*blue-long*) and the same genotypes (**BbLl**).

When one of the Figure 32C F_1 individuals is crossed with the pure recessive, one would expect the same results as in the Figure 32B cross—after all the two F_1 individuals have the same genotype and phenotype. The results were dramatically different. The *blue-long* and *red-round,* which each had a frequency of 43.7 percent in the Figure 32B cross, have now dropped to 6.3 percent. The two other phenotypic classes have increased from 6.3 percent to 43.7 percent.

Bateson, Saunders, and Punnett were unable to provide a satisfactory explanation for these crosses. They could conclude only that, in crosses of this sort, the alleles of the parents were coupled. Coupling was not complete and in a small fraction of the gametes there was a repulsion of the two different alleles. Such an explanation, however, does no more than describe what in fact happens.

More and more examples of coupling and repulsion continued to be reported and no more than a formal explanation was possible. In the year that Morgan was to propose a satisfactory hypothesis Bateson and de Vilmorin (1911) wrote as follows (I have changed the genotypic symbols to those now used):

> If **A**, **a** and **B**, **b** are two allelomorphic pairs subject to coupling and repulsion, the factors **A** and **B** will repel each other in the gametogenesis of the double heterozygote resulting from the union **AAbb** × **aaBB**, but will be coupled in the gametogenesis of the double heterozygote resulting from the union **AABB** × **aabb** . . . .

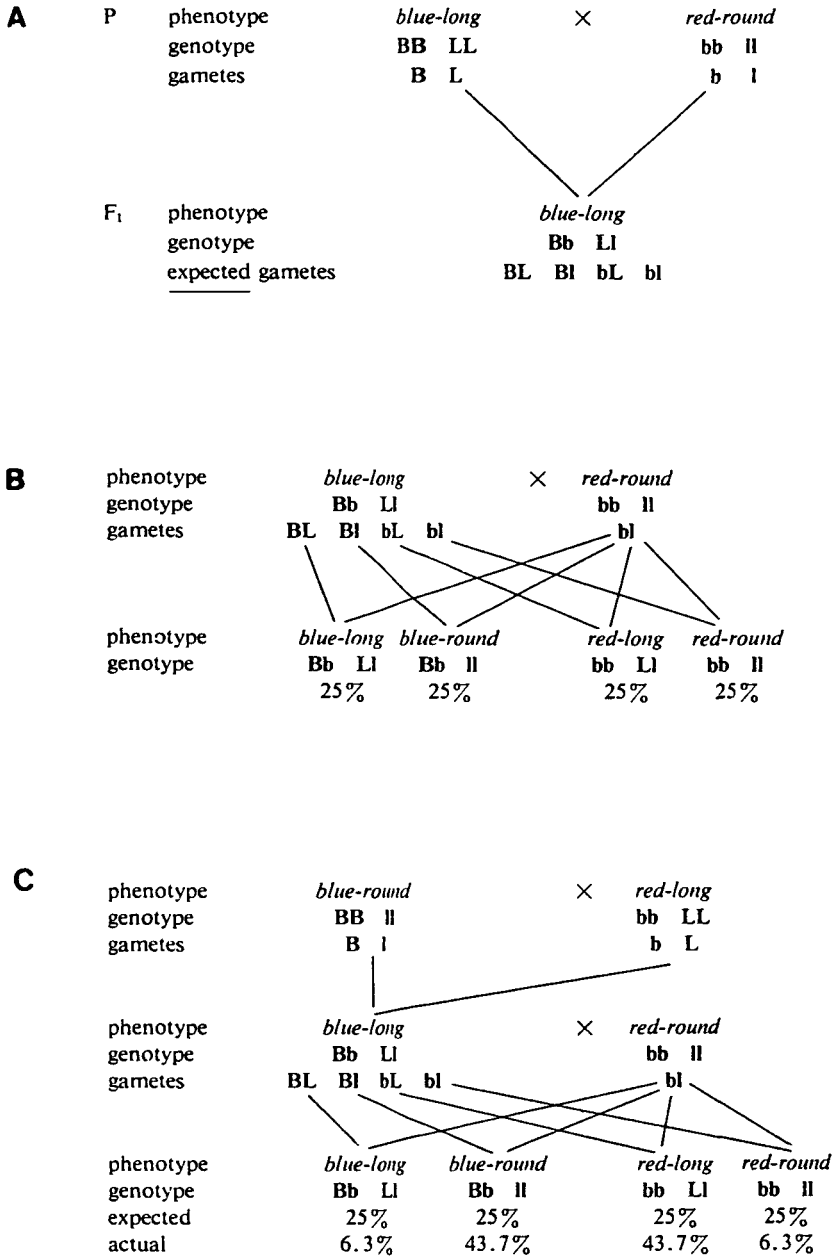


FIG. 32. Experiments with sweet peas. A shows the first cross and the gametes of the F_1 if there was independent assortment. B shows how the F_1 should have behaved if there was independent assortment. The expected frequencies, 25 percent for each, were not observed. Instead there were 43.7 percent each of *blue-long* and *red-round* and 6.3 percent each of *blue-round* and *red-long*. C is the reciprocal of A.

We have as yet no probable surmise to offer as to the essential nature of this distinction, and all that can be said is that in these special cases the distribution of the characters in the heterozygote is affected by the distribution in the original pure parents.

Bateson and his colleagues were not aficionados of Sutton's hypothesis but, as we

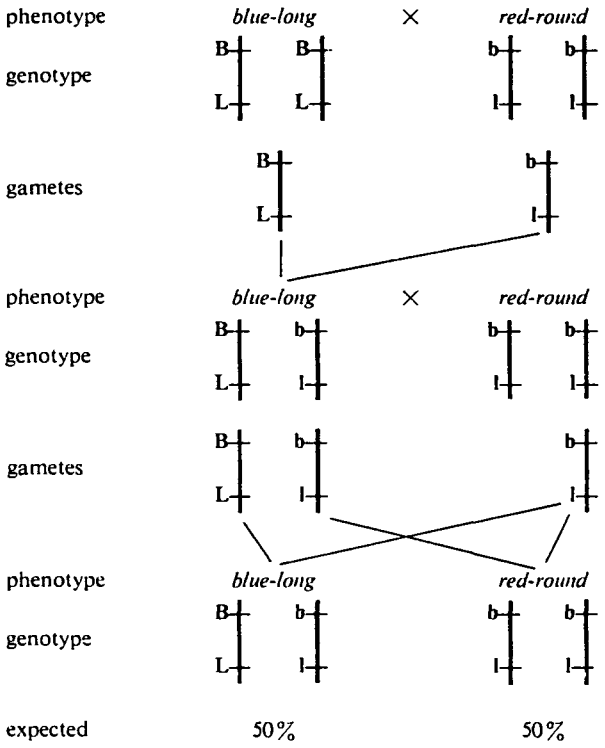


FIG. 33. An hypothesis to explain the cross of Figure 32A and 32B assuming complete linkage.

will see, unmodified Suttonism was no answer.

After seeing these results, and the admission of failure to offer an explanatory hypothesis, the faint hearted might have considered abandoning Mendelism as a broadly applicable hypothesis. Nevertheless, Mendel's rules did apply to many other crosses, even including the *blue vs. red* and *long vs. round* alleles when considered separately. Furthermore, the fact that constant, though mysterious, frequencies were observed suggested that there must be some constant and discoverable cause for them. Note also that in Figure 32B and 32C the alleles of the original parents were coupled. An orderly, though non-Mendelian, process appears to be at work. Could this be an example of Sutton's prediction for the behavior of different alleles that were part of the same chromosome?

WILL SUTTON'S HYPOTHESIS EXPLAIN COUPLING AND REPULSION?

We will assume that the loci for the alleles **B** and **L** are on the same chromosome and the *blue-long* parent in the cross of Figure 32A is homozygous for them as shown. We will assume that the other parent is homozygous for **b** and **l**. Figure 33 offers an explanation, such as Sutton might have proposed for the cross of Figure 32A and 32B.

When the F_1 *blue-long* individual is crossed with *red-round* the predicted offspring are of only two phenotypic classes: *blue-long* and *red-round*. However, that is not what was observed. Recall that the actual results were 43.7 percent for each of these two classes; in addition, 6.3 percent were *blue-round* and 6.3 percent were *red-long*. There is no possibility, however, of either of these less frequent classes appearing in Sutton's model as shown in Figure 33.

So the answer to the question at the head of this section appears to be "No." Nevertheless a modification of Sutton's hypothesis would eventually provide the answer.

In the buzzing activity of the Fly Room, Morgan and his associates were discovering dozens of new mutant flies. These new

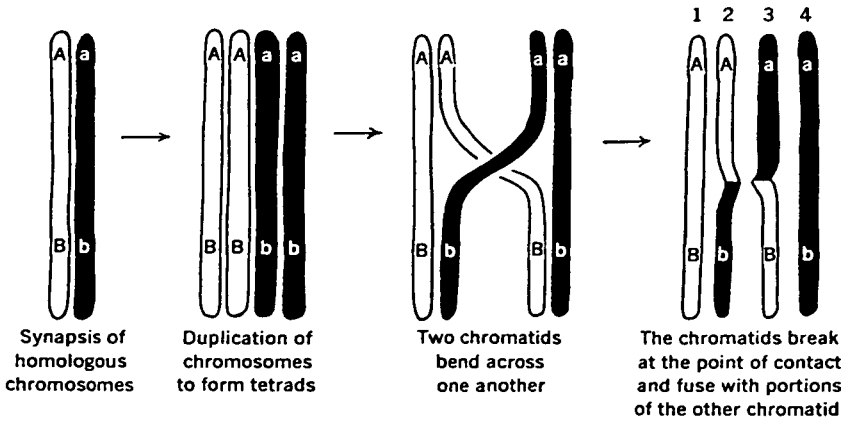


FIG. 34. Janssens' hypothesis of crossing-over.

mutants were tested in crosses with other mutants. In many instances the dihybrid crosses gave the normal Mendelian 9:3:3:1 ratio in the F_2, which indicated that the two pairs of alleles were assorting independently.

Very quickly, however, problems arose; they were not unexpected. The reason was that, since *Drosophila melanogaster* has only four pairs of chromosomes, independent assortment of genes would be impossible, at the very latest, by the time the fifth mutant was discovered—there would be no remaining chromosome not already occupied by one of the previously discovered mutants. And, of course, independent assortment is possible only when the different genes are parts of different chromosomes.

Coupling and repulsion of the sort so puzzling to Bateson and his associates were observed in many of the *Drosophila* crosses. Sutton's hypothesis could explain the coupling but not the repulsion. Yet Morgan was convinced of the correctness of Sutton's hypothesis that genes are parts of chromosomes, so he assumed that there must be some mechanism whereby parts of chromosomes, with their alleles, could be exchanged.

Here was a case where the genetic data demanded a cytological explanation. One could imagine many ways that parts of chromosomes could be shuffled to provide an explanation for repulsion of different alleles. In fact, a cytological phenomenon had been described recently that might be the answer.

JANSSENS AND THE CHIASMATYPE THEORY

In 1909—the year before the birth of that *white-eyed* fly—the cytologist F. A. Janssens (1863–1924) had described a chromosomal phenomenon that Morgan required for his hypothesis.

The phenomenon that Janssens described is a meiotic event, now called crossing-over (Fig. 34). During synapsis the homologous chromosomes come close together with their long axes parallel. Both chromosomes replicate and a tetrad of four chromatids is formed. This much can be observed.

Next according to Janssens, there is considerable coiling of the chromatids around one another at this time and in some cases two of the chromatids break at the corresponding place on each. The broken chromatids rejoin in such a way that a section of one chromatid is now joined with a section of the other. As a result "new" chromatids are produced that are mosaics of segments of the original ones. The breaking and rejoining could not be seen so this event was but a hypothesis.

Janssens' Chiasmatype Theory is a case, not too infrequent in science, where the hypothesis turns out to be correct even though the supporting data were probably

erroneous (McClung's hypothesis for the relation of the chromosomes and sex is an example).

The evidential basis for Janssens' hypothesis left much to be desired; nevertheless it was the only acceptable way to explain the data. This is E. B. Wilson's appraisal in 1925 (see also E. B. Wilson and Morgan, 1920):

> The basis for a more adequate cytological interpretation of crossing-over was first provided by Janssens' theory of the *chiasmatype* ('09) as elaborated by Morgan and his co-workers. Unfortunately this ingenious theory, though it may be correct in principle, still rests upon an inadequate cytological basis; it was, indeed, founded originally upon what now seems to have been a misinterpretation of certain cytological appearances. (p. 954)

MORGAN'S EXPLANATION FOR COUPLING AND REPULSION

In a one page paper Morgan (1911*b*) proposed a new hypothesis that, having been tested repeatedly, can be accepted today as true beyond all reasonable doubts. He started the analysis by noting that exceptions to the 9:3:3:1 ratio were being observed more frequently but that Bateson's hypothesis of coupling and repulsion was not a satisfactory explanation.

> In place of attractions, repulsions and orders of precedence, and the elaborate systems of coupling, I venture to suggest a comparatively simple explanation based on results of inheritance of eye color, body color, wing mutations and the sex factor for femaleness in *Drosophila.* If the materials that represent these factors are contained in the chromosomes, and if those factors that "couple" be near together in a linear series, then when the parental pairs (in the heterozygote) conjugate [*i.e.*, synapse] like regions will stand opposed. There is good evidence to support the view that during the strepsinema stage [when the tetrad begins to separate] homologous chromosomes twist around each other, but when the chromosomes separate (split) the split is in a single plane, as maintained by Janssens. In consequence, the original material will, for short distances, be more likely to fall on the same side of the split, while remoter regions will be as likely to fall on the same side as the last, as on the opposite side. In consequence, we find coupling in certain characters, and little or no evidence at all of coupling in other characters; the difference depending on the linear distance apart of the chromosomal materials that represent the factors. Such an explanation will account for all of the many phenomena that I have observed and will explain equally, I think, the other cases so far described. The results are a simple mechanical result of the location of the materials in the chromosomes, and the method of union of homologous chromosomes, and the proportions that result are not so much the expression of a numerical system [as Bateson proposed] as of the relative location of the factors in the chromosomes. *Instead of random segregation in Mendel's sense we find "associations of factors" that are located near together in the chromosomes. Cytology furnishes the mechanism that the experimental evidence demands.*

The term linkage was introduced for cases where different genes are parts of the same chromosome. Crossing-over, which occurs in meiosis, consists of the homologous chromosomes coming together at synapsis, replicating, then breaking, and finally the chromatids rejoining in new ways that result in altered associations of genes.

Thus, can we give credit to Thomas Hunt Morgan for having established that those puzzling exceptions to simple Mendelian inheritance are a consequence of the fact that genes are parts of the same chromosome and at times they are reshuffled by crossing-over during meiosis?

In truth, we can do nothing of the kind. All that could be concluded was that linkage *could* be an explanation of coupling and that crossing-over *could* be an explanation of repulsion. We credit Morgan with these important insights because later research showed that his hypothesis was correct.

This is a common pattern in the progress of our understanding of the phenomena of the natural world. The great hypotheses of the great men are those, among many competing hypotheses, that are eventually established by the work of numerous scientists as true beyond all reasonable doubt.

Morgan's realization that Janssens' hypothesis of chromatids breaking and rejoining in new combinations could explain the data was not readily accepted by other workers. It was impossible to observe directly such breakage and fusion. Sturtevant (1959) recalls why Janssens' hypothesis was so appealing:

> The cytological evidence was not conclusive, and the idea was not generally accepted—although it was becoming clear that only in some such way as this could the chromosomal interpretation of Mendelian inheritance be saved. (p. 294)

There was a way, however, to test the hypothesis that linkage is a consequence of different genes being parts of the same chromosome.

LINKAGE GROUPS AND CHROMOSOME PAIRS

By 1911 there was no longer any doubt that in diploid organisms the chromosomes are in homologous pairs, with the exception of the sex chromosomes where there might be deviations from this general rule. As noted before, Sutton (1903) had pointed out that "all the allelomorphs represented by any one chromosome must be inherited together." That means that the number of these groups of alleles inherited together cannot exceed the number of pairs of homologous chromosomes. Thus it would be possible to test this hypothesis in *Drosophila.*

Drosophila melanogaster has four pairs of chromosomes—three pairs of autosomes and a pair of sex chromosomes. In mitotic metaphase there are two pairs of long bent autosomes and one pair of tiny dot-shaped autosomes (Fig. 35). In females the two **X** chromosomes are rods of medium length and in males there is one **X** and a hook-shaped **Y**.

In the early months of experimentation, the Morgan group quickly found that a number of different genes were linked and that their pattern of inheritance suggested strongly that they were part of the **X** chromosome (Morgan, 1911*a*). Soon two other linkage groups were found (Morgan and Lynch, 1912; Sturtevant, 1913*c*). It was assumed that these were associated with the two pairs of long autosomes. So there were three linkage groups but four pairs of chromosomes. The discrepancy could have been due to the small size of the pair of dot autosomes—possibly consisting of only a few undiscovered genes or perhaps they are without any gene loci. The latter seemed to be the case for the **Y** chromosome.

Eventually one mutant fly was discovered and, when crossed with flies with mutant alleles of the three known linkage groups, there was independent assortment (Muller, 1914). It was highly probable, therefore, that this new mutant gene was part of the dot-shaped autosomes. Eventually other genes were discovered to belong to this fourth linkage group.

By 1915 the Morgan group had worked out the inheritance of 85 genes. These fell into four linkage groups as shown in Figure 35, which also shows a diagram of the metaphase chromosomes. The parallelism between the number of chromosomes, as determined by cytological examination, and the number of linkage groups, as determined by genetic experiments, was strong evidence not only that genes are parts of chromosomes but also that those that are parts of the same chromosome will be inherited together.

The data of Figure 35 are instructive in another way. Notice that many different genes affect the same character: 13 influence eye color; 33 modify the wings in some manner; 10 affect the color of the body.

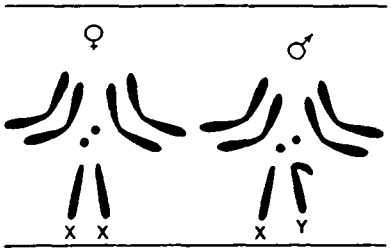
What, then, determines the normal red eye color? The answer is that the wild type alleles of all these 13 eye color genes, together with many discovered later and others yet to be discovered, act together to produce the normal wild-type red eyes. If an individual is homozygous for the mutant allele of any one of these genes, the

GROUP I

Name	Region Affected
Abnormal	Abdomen
Bar	Eye
Bifid	Venation
Bow	Wing
Cherry	Eye color
Chrome	Body color
Cleft	Venation
Club	Wing
Depressed	Wing
Dotted	Thorax
Eosin	Eye color
Facet	Ommatidia
Forked	Spines
Furrowed	Eye
Fused	Venation
Green	Body color
Jaunty	Wing
Lemon	Body color
Lethals, 13	Die
Miniature	Wing
Notch	Venation
Reduplicated	Eye color
Ruby	Legs
Rudimentary	Wings
Sable	Body color
Shifted	Venation
Short	Wing
Skee	Wing
Spoon	Wing
Spot	Body color
Tan	Antenna
Truncate	Wing
Vermilion	Eye color
White	Eye color
Yellow	Body color

GROUP IV

Name	Region Affected
Bent	Wing
Eyeless	Eye



GROUP II

Name	Region Affected
Antlered	Wing
Apterous	Wing
Arc	Wing
Balloon	Venation
Black	Body color
Blistered	Wing
Comma	Thorax mark
Confluent	Venation
Cream II	Eye color
Curved	Wing
Dachs	Legs
Extra vein	Venation
Fringed	Wing
Jaunty	Wing
Limited	Abdominal band
Little crossover	II chromosome
Morula	Ommatidia
Olive	Body color
Plexus	Venation
Purple	Eye color
Speck	Thorax mark
Strap	Wing
Streak	Pattern
Trefoil	Pattern
Truncate	Wing
Vestigial	Wing

GROUP III

Name	Region Affected
Band	Pattern
Beaded	Wing
Cream III	Eye color
Deformed	Eye
Dwarf	Size of body
Ebony	Body color
Giant	Size of body
Kidney	Eye
Low crossing over	III chromosome
Maroon	Eye color
Peach	Eye color
Pink	Eye color
Rough	Eye
Safranin	Eye color
Sepia	Eye color
Sooty	Body color
Spineless	Spines
Spread	Wing
Trident	Pattern
Truncate intensf.	Wing
Whitehead	Pattern
White ocelli	Simple eye

FIG. 35. The linkage groups of *Drosophila melanogaster* as known in 1915. The 85 genes fell into 4 linkage groups. The diagram at the lower left shows the chromosomes in somatic cells. (Morgan, 1915)

eye color will not be red but, instead, white, or peach, or sepia, etc. We should think of the normal red eye color as the end product of a series of gene actions. If any of these actions is altered, the eye color will be different.

It is important to realize also that there is more to the compound eye of an insect than its color. There are many other genes that influence the morphology of the eyes—some drastically as in the case of *eyeless* in the fourth linkage group or the *bar eye* mutant of the **X** chromosome.

The mutant alleles were named for their most viable effects—the *white-eyed* allele produces white eyes. When the *white-eyed* flies were carefully examined, however, it was found that the pigmentation of the ocelli and some of the internal organs were affected as well. This is not an unusual case—many genes are pleiotropic, that is, they affect more than one structure or process. Some geneticists in the early days even went so far as to suspect that every gene affects, at least in some small way, all aspects of structure and function of the body.

THE CYTOLOGICAL PROOF OF CROSSING-OVER

It was essential that Morgan's hypothesis of crossing-over as a mechanism to explain the recombination of linked genes be thoroughly tested, and if validation was not possible, that it be replaced. The hypothesis had been proposed to account for some exceptions to simple Mendelian inheritance; so the fact that the data verified the hypothesis could not be accepted as support for the correctness of the hypothesis.

The critical proof would be cytological evidence for the actual breaking and recombination of homologous chromatids. Such events were assumed to take place in meiosis during synapsis and tetrad formation. At that time the chromosomes were exceedingly difficult to study. The problem was further exacerbated by the fact that homologous chromosomes are identical in appearance, so even if crossing-over had occurred during synapsis, the chromatids would give no visible evidence that they had broken and recombined.

Ask your students to consider this difficulty and to suggest how an experiment might be designed to provide critical evidence. They might suggest that, since evidence cannot be gained when the homologous chromosomes are identical, it will be necessary to find a species with dissimilar homologous chromosomes or to make them different by experimental means.

In the 1910s there was no obvious way of making chromosomes different and the *Drosophila* group accepted the hypothesis of crossing-over because it continued to explain their data. In fact it was almost 20 years before Curt Stern (1931) was able to provide cytological proof of crossing-over.

By the time Stern began his work, *Drosophila* geneticists had a large number of stocks of mutant flies, including numerous ones with chromosomal abnormalities. Some of these had appeared "spontaneously" in the stocks in the Fly Room but others had appeared in cultures of flies that had been exposed to radium or X-rays.

There was a remarkably cooperative spirit among the *Drosophila* geneticists and they exchanged stocks with their fellow scientists in various institutions within the United States and throughout the world. For years the Morgan group, first at Columbia University and after 1928 when they moved to the California Institute of Technology, kept hundreds of stocks for their own use and for any geneticist requesting them. Subsequently a large collection was maintained at the University of Texas, and currently there is a National Drosophila Species Resource Center, supported by the National Science Foundation, at Bowling Green State University in Kentucky.

Stern constructed stocks that provided the test material that he needed—flies with structurally and genetically different homologous chromosomes. The females used had structurally and genetically different **X** chromosomes (Fig. 36 is a simplified representation of one of Stern's critical experiments). One of the **X** chromosomes was in two portions: one portion behaved as an independent chromosome and the other was attached to one of the tiny fourth chromosomes. The other **X** had a piece of a **Y** attached to it. These struc-

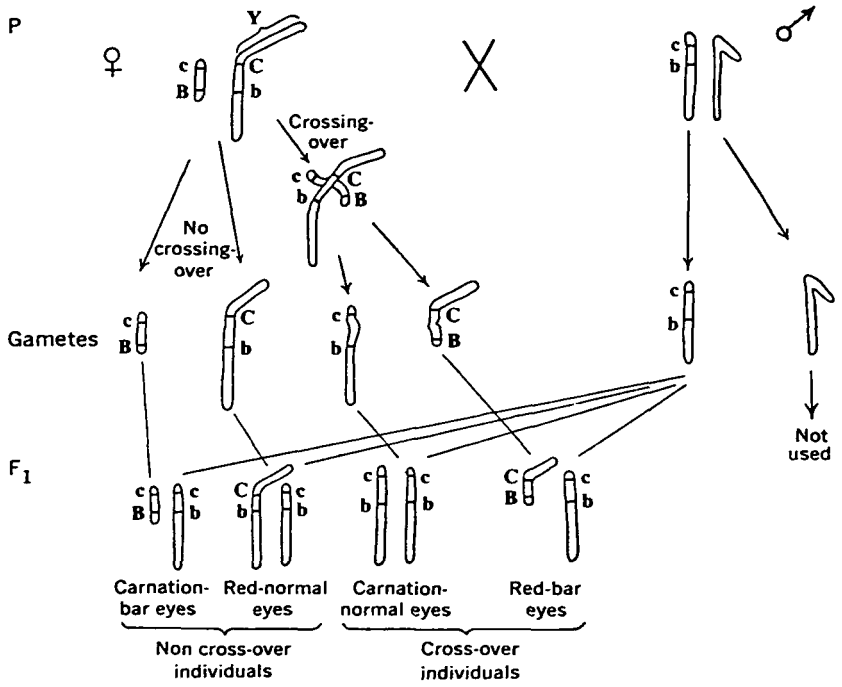


FIG. 36. Stern's cytogenetic proof of crossing-over.

tural differences were so great that it was possible to identify the chromosomes in fixed and stained cells.

The two **X** chromosomes carried genetic markers as well. One part of the divided **X** carried the recessive allele *carnation* (**c**), which when homozygous produces eyes of a dark ruby color, and the dominant allele *bar* eyes (**B**), which reduces the normal nearly round eyes to a narrow band. The **X** with the piece of the **Y** carried the *wild-type* alleles **C** and **b**, which produce *red* eyes and *normal-shaped* eyes.

During meiosis in the female there will be crossing-over between the two loci in some instances but not in others. As a consequence four types of eggs will be produced. Each of these is unique both genetically and structurally. When such a female is crossed with the double recessive, a *carnation normal-eyed* male, the alleles of each of the gametes of the female will be expressed.

The critical evidence was provided by the F_1 females, which would be in four phenotypic classes. Furthermore, each phenotypic class would have predictably different chromosomes. Stern had set up the cross in such a manner that the crossover individuals with *carnation normal-shaped* eyes would have two long **X** chromosomes. The other crossover class would have eyes that were both *red* and *bar*. Their chromosomes would show one long **X** and one **X** with the piece of the **Y**. The other two phenotypic classes, the non-crossovers, would also have unique chromosomal configurations.

Stern checked the chromosomes of nearly 400 of the females of all four classes and found that the phenotypes corresponded to the predicted cytological configurations. This was an elegant demonstration that Morgan's hypothesis of cytological crossing-over as the basis for genetic crossing-over was indeed correct.

And history was repeating itself. In a paper published a few weeks earlier Harriet Creighton and Barbara McClintock (1931) demonstrated that crossing-over has a cytological basis in corn (*Zea mays*). Their basic method was the same as that used by

Stern for *Drosophila*. They had developed strains of corn with genetically and cytologically different 9th chromosomes. Their evidence was the presence of predicted cytological configurations in the plants with the different phenotypes.

MAPPING THE CHROMOSOMES

Much of *Drosophila* genetics was anticipated in Morgan's first full length paper (1911*a*). It is interesting to note that, although nearly 50 pages in length, there are no references, just two footnotes mentioning earlier expressions of his ideas. One anticipation suggested was the possibility of preparing a genetic map of *Drosophila* genes. In this paper, dealing with the first mutant genes found on the **X**, Morgan noted that the data showed

> the necessity of assuming some . . . localization [of the genes] amongst some of the substances resident in the same chromosome. (p. 403)

He noted that Janssens' hypothesis would seem to require that

> the chromosomal materials that represent the factors of heredity are placed linearly along the chromosome and in corresponding linear series in each pair of homologous chromosomes. (p. 404)

Morgan noted also that for genes on the same chromosomes the

> associations will be more or less common according to the nearness of the associated factors in the chromosome. (p. 404)

In a very short paper that same year (1911*b*) Morgan is more definite:

> we find coupling in certain characters, and little or no evidence at all of coupling in other characters; the difference depending on the linear distance apart of the chromosomal materials that represent the factors.

These quotations stress two principal hypotheses: genes are localized in definite places in the chromosome, and they are in a linear order. If one assumes that crossing-over can occur at any place along a chromosome, the chance of one occurring in any one segment will depend on the length of that segment—the longer the segment, the greater the probability that crossing-over will occur somewhere within it. The discovery that the percentage of recombination ("repulsion") between any two genes on the same chromosome was constant suggested that some discoverable mechanism was in operation. Bateson and his associates had also observed this in their crosses.

The first genetic map was produced by Sturtevant (1913*a*) as his Ph.D. thesis. He used five mutants and the corresponding wild type alleles that are on the **X** chromosome: *yellow* body (**y**), *white* eyes (**w**), *vermillion* eyes (**v**), *miniature* wings (**m**), and *rudimentary* wings (**r**).

Sturtevant accepted Morgan's hypothesis for the position of genes in chromosomes and made the following deduction:

> It would seem, if this hypothesis be correct, that the proportion of 'cross-overs' could be used as an index of the distance between any two factors. Then by determining the distances (in the above sense) between A and B and between B and C, one should be able to predict AC. For, if proportion of cross-overs really represents distance, AC must be approximately, either AB plus BC, or AB minus BC, and not any intermediate value. (p. 45)

Sturtevant then began the experiments in which he crossed flies with mutant alleles on the **X** with wild-type flies. The F_1 females were then crossed with males carrying the recessive alleles. (F_1 males were not used since Morgan had discovered earlier that crossing-over does not occur in them.) The offspring were then scored as to whether recombination of the alleles being followed had occurred. Such recombination would indicate that a cross-over had occurred between the loci.

The percentage of crossovers between **y** and **v** was found to be 32.2 and between **y** and **m** to be 35.5 percent. Morgan's hypothesis would suggest that **v** would be slightly closer to **y** than **m** would be. But what could be concluded about the relative positions of **m** and **v**? Sturtevant's predic-

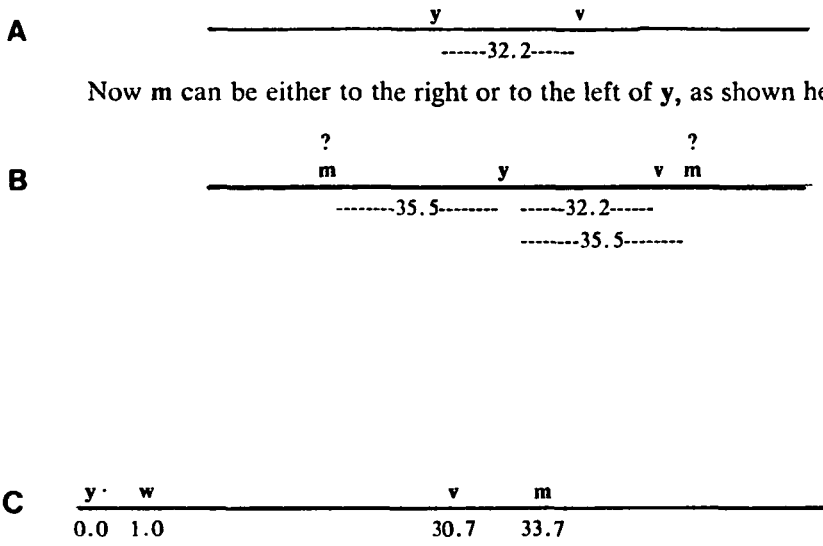


FIG. 37. Sturtevant's method for determining the linear order of gene loci. A shows y and v separated by a distance equal to the percentage of crossing-over between them. B shows the percentage of crossing-over between y and m and the impossibility of knowing whether m is on the side with v or not. C shows the relative position of five loci on the X chromosome.

tion was that the distance between **m** and **v** must be either 67.7 (35.5 + 32.2) or 3.2 (35.5 − 32.2).

Presenting this argument in an illustration, such as Figure 37, may not be equal to a thousand words, but it is surely equal to a few hundred. We will represent the chromosome as a line and assume that its hereditary materials are linear in their arrangement. Morgan had no doubt adopted such a hypothesis because he was initially influenced by the shape of chromosomes—long threads, especially in prophase.

Figure 37A represents the data for crossing-over between **y** and **v**. The amount was 32.2 percent and, in drawing the figure, the two genes were put 32.2 mm apart. The second experiment showed that the percentage of crossing-over between **y** and **m** was 35.5. Now we have two choices since we have already put two points on our chromosome-line: **m** can be either on the same side as **v** or it can be on the other side of **y** (Fig. 37B).

If we assume the working hypothesis to be correct, in one case **v** and **m** would be very close to one another—only 3.2 units apart. In the other they would be 67.7 (35.5 + 32.2) units apart. The precise form of the hypothesis allowed a critical deduction and test to be made—measure the amount of crossing-over between **v** and **m**. Sturtevant did the experiment and found the value to be 3 percent. This indicated that **v** and **m** were close together and therefore on the same side of the chromosome relative to **y**. The close correspondence between the actual and expected results was strong support for the correctness of the hypothesis.

Sturtevant made similar crosses with the *white* eyes (**w**) and *rudimentary* wings (**r**) alleles and constructed the first genetic map, which is redrawn in Figure 37C. The **y** locus was taken as the starting point and the other gene loci placed at a distance equal to the percentage of crossing-over between adjacent loci.

A puzzle was soon encountered. Repeated experiments showed that the apparent distance between loci depended on how the genetic map was constructed. The map of Figure 37C shows the distance between **y** and **r** to be 57.6. This is the sum of the individual values for crossovers between **y** and **w**, **w** and **v**, etc. If the percentage of crossing-over was determined by actual

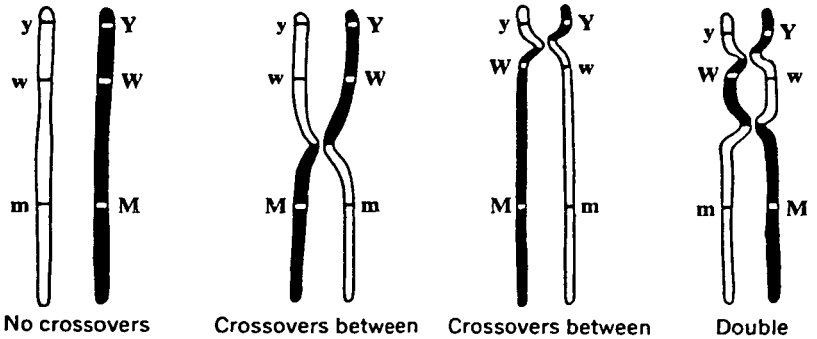


FIG. 38. Sturtevant's experiment. See text for details.

experiment using the two genes **y** and **r**, the value was found to be less than that predicted.

It would be interesting to ask students to suggest explanatory hypotheses to account for this puzzle. If they have trouble proposing hypotheses, mention that Sturtevant's own hypothesis was that double crossing-over might have occurred. This would put the alleles back in the same chromosome. The result would be an apparent lack of crossing-over between the two loci when, in fact, there had been two. Then ask the students how one could possibly test such a notion.

The answer is that the test experiment must involve at least three loci. In one experiment Sturtevant crossed individuals that had **y**, **w**, and **m** on one **X**, and **Y**, **W**, and **M** on the other with males with the three recessive alleles. He raised 10,495 offspring and by checking the phenotypes could test whether or not crossovers had occurred (Fig. 38). Thus, if there were no crossovers between **y** and **m**, the offspring would either have *yellow* bodies, *white* eyes, and *miniature* wings or be *wild-type*—in approximately equal numbers. He found this to be true of 6,972 flies, which were of these two phenotypes. If crossing-over occurred between the **w** and **m** loci, the flies would be either *yellow-bodied, white-eyed,* with *wild-type* wings or *wild-type* body and eye color but with *miniature* wings. Flies of these two kinds numbered 3,454. Crossing-over between the **y** and **w** loci would give flies that had either *yellow* bodies and both *wild-type* eye color and wings, or *wild-type* body color with *white* eyes and *miniature* wings. Only 60 flies were of these two kinds indicating that **y** and **w** are close to one another.

And finally there were 9 individuals, in the total of 10,495 that had *yellow* bodies, *wild-type* red eyes, and *miniature* wings or *white* eyes and *wild-type* body color and wings. These could be accounted for on the basis of double crossing-over, as shown in Figure 38.

It should be clear, therefore, that a minimum of three genes is necessary to detect double crossovers. If only the **y** and **m** loci had been used, any double crossing-over between them would have gone undetected since **y** and **m** would have resumed their original positions (Fig. 38, rightmost case). Because of the occurrence of double crossing-over, the workers in the Fly Room made their genetic maps by summing the data from crosses involving loci close together, not those that were far apart.

Is the genetic map, constructed in this manner, an accurate reflection of the positions of the genes on the chromosomes? Sturtevant has this to say (the genetic symbols have been updated):

> Of course there is no knowing whether or not these distances as drawn represent the actual relative spacial distances apart of the factors. Thus the distance **wv** may in reality be shorter than the distance **yw** but what we do know is that a break is far more likely to come between **w** and

> v than between y and w. Hence, either wv is a long space, or else it is for some reason a weak one. The point I wish to make here is that we have no means of knowing that the chromosomes are of uniform strength, and if there are strong or weak places, then that will prevent our diagram from representing actual relative distances—but, I think, will not detract from its value as a diagram. (p. 49)

Sturtevant reaches the following conclusion in his Ph.D. thesis:

> These results are explained on the basis of Morgan's application of Janssens' chiasmatype hypothesis to associative inheritance. They form a new argument in favor of the chromosome theory of inheritance, since they strongly indicate that the factors investigated are arranged in a linear series, at least mathematically.

An aside on presenting this topic to students. First-year students do not normally think in terms of the position of genes in chromosomes so the method used by Sturtevant to map the genes might present some difficulties. Nevertheless here, as with many problems in genetics, a more familiar metaphor may help the students to understand the problem as well as involve their reasoning powers. What I am about to propose will require more time for covering the topic but one can argue it may be better for the student to take that extra time and to understand the topic.

Assume that we have some data relative to a trip on Interstate 80 from New York to San Francisco and we wish to use these data to locate some of the cities *en route* and to find the distance between them. Assume also that the data given will be the only data available. Our first facts will be the distances, in kilometers, between these cities:

Chicago and New York	1,290
Chicago and Salt Lake City	2,592
Chicago and San Francisco	3,450
Chicago and Cleveland	540
Chicago and Cheyenne	1,536
Chicago and Omaha	740

Now ask "What is the distance from New York to San Francisco?" Hopefully some student will respond with 4,740, the sum of 1,290 and 3,450. Point out that the answer given assumes that we know the relative positions of Chicago, San Francisco, and New York. If we did not know that, it would be equally probable that the distance between New York and San Francisco is 2,160 kilometers (3,450 minus 1,290) if New York was between Chicago and San Francisco.

The students should then be asked to specify what additional sorts of information are required for the cities mentioned to be placed in their relative positions. This can be done, of course, without knowing whether San Francisco is north, south, east, or west of Chicago.

Now back to chromosomal distances. You may recall that in his address to the American Breeders Association, Morgan blasted the Mendelians for suggesting all sorts of unproven mechanisms to explain the exceptions to the original Mendelian rules. The same criticism was now to be turned on the Morgan group and it was to continue for some years: Was it permissible to imagine the chromosomes doing all these wonderful things, such as single and double crossing-over, when there is not the slightest cytological evidence for such events?

That was a difficult criticism to answer in the 1910s but the basic fact remained—as more and more data were accumulated they were found to "make sense" on the basis of the hypotheses being developed by the *Drosophila* group. They were providing a conceptual scheme that accounted for more and more of the phenomena of genetics. This, in itself, makes it more probable that the conceptual scheme is correct.

CAN TWO STRUCTURES OCCUPY THE SAME SPACE?

Here is another puzzle that will be profitable for your students to consider. When Sturtevant (1913*a*) conducted the experiments that led to the construction of the genetic map of the **X** chromosome, he identified the locus of the mutant *eosin*,

which when homozygous produces eyes colored like the cytological stain of that name. He also found that, so far as he could see, *eosin* and *white* gave the same crossover percentages relative to adjacent loci. How could that be? This problem was studied by Morgan (1912*a*, 1914*a*). By 1915 the answer seemed clear (Morgan, Sturtevant, Muller, and Bridges, 1915).

> The "distance" between them [white and yellow] is 1 unit, which means that crossing over takes place about once in a hundred times. Eosin eye color gives the same crossing over frequency with yellow. White eye color gives with miniature wings about 33 percent crossing over. Eosin gives the same value with miniature. White gives 44 per cent. of crossing over with bar eye. Eosin has the same value. Similar relations hold for all the characters of the first [linkage] group. (p. 156)

What is one to make of this? If crossover data can be used to determine the relative positions of genes in a chromosome, the information just given would seem to indicate that *eosin* and *white* occupy the same locus. Can this be? Some alert students may suggest that *eosin* and *white* may be so close together that one would have to count hundreds of thousands of flies in order to detect any crossing-over.

Next ask students what is the accepted explanation for a 3:1 Mendelian ratio.

Morgan, Sturtevant, Muller, and Bridges continue,

> 1. If a white-eyed male of Drosophila is mated to a red-eyed female, the F_2 ratio of 3 reds to 1 white is explained by Mendel's law, on the basis that the factor for red is the allelomorph of the factor for white.
>
> 2. If an eosin-eyed male is mated to a red-eyed female, the F_2 ratio of 3 reds to 1 eosin is also explained if eosin and red are allelomorphs.
>
> 3. If the same white-eyed male is bred to an eosin-eyed female, the F_2 ratio of 3 eosins to 1 white is again explained by making eosin and white allelomorphs. (p. 155)

Your students may be able to discover the concept of "multiple alleles" for themselves. If not, this is how the Morgan group explained the data.

> This example indicates that the conception of allelomorphs should not be limited to two different factors that occupy identical loci in homologous chromosomes, but that there may be three, as above, or even more different factors that stand in such a relation to each other. Since they lie in identical loci they are mutually exclusive, and therefore no more than two can occur in the same animal at the same time. On *a priori* grounds also it is reasonable to suppose that a factor could change in more than one way, and thus give rise to multiple allelomorphs . . . .
>
> On the chromosome hypothesis the explanation of this relation is apparent. A mutant factor is located at a definite point in a particular chromosome; its normal allelomorph is supposed to occupy a corresponding position (locus) in the homologous chromosome. If another mutation occurs at the same place, the new factor must act as an allelomorph to the first mutant; as well as to the "parent" normal allelomorph. (pp. 155–157)

As the years went by many more mutants were discovered that mapped at the same *white* locus. This is no longer an isolated case. Multiple alleles of the same gene are a common genetic phenomenon.

It should be emphasized, once again, how new insights into the mechanisms of heredity were obtained as the body of information about this one species increased. It was far more profitable for that very active group in the Fly Room to have concentrated their efforts on one species than for them to have studied the genetics of a dozen. With the extensive library of mutant alleles available as early as 1915, all sorts of questions could be asked and there was a good chance of obtaining acceptable answers. Years later the fact that *E. coli* received such concentrated attention meant that its biology was to become the best known of any species.

Consider the Ph.D. thesis of Calvin Bridges as an example of how the genome could be manipulated to supply critical answers to critical questions.

THE FINAL PROOF

During the last two decades of the 19th century, the hypothesis that the factors responsible for inheritance, whatever they might be, were associated with chromosomes was held by only a few prominent cytologists. That hypothesis was given new life by Sutton in 1903. In the 1910s the investigations on *Drosophila* by Morgan, Sturtevant, Bridges, and Muller made it increasingly probable that genes are parts of chromosomes, yet Bateson and many others remained totally unconvinced. It is Calvin Bridges who is credited with having provided the "final proof" for that hypothesis.

Now that Sutton's hypothesis is totally accepted, it is hard to understand the reluctance of geneticists in the years between 1903 and 1916 to "see the light." To be sure that "light" is brighter today than it was in the first two decades of the 20th century. Probably an important factor in their reluctance was the extraordinary rapidity with which astonishing data and concepts emerged from the Fly Room. In those decades the biological sciences moved slowly and it may be that even the most capable geneticists at other institutions had trouble digesting the data and coping with the concepts. Then too, the terminology and symbolism of the papers describing the *Drosophila* crosses were difficult to understand. Even today when one looks at the papers published by the *Drosophila* group between 1910 and 1920, it is difficult to know, without considerable study, what was done.

Another reason for resistance to *Drosophila* genetics seems to have been emotional. When I was at Columbia during the 1930s, memory of the Morgan group was still strong (Morgan, Sturtevant, and Bridges had moved to Cal Tech in 1928). I was told by those who had been at Columbia during the late 1910s and early 1920s that the *Drosophila* group was regarded as being rather abrasive by some biologists. This may well have been so. Scientists working in fields making rapid progress may be impatient and condescending to those doing their Kuhnian normal science at a slower pace. In the opinion of those Columbia zoologists in a position to know, a large factor in the acceptance of the work on *Drosophila* was due to E. B. Wilson. Though one of the giants in the biological sciences of the time, he was a gentle person, warmly regarded, and his opinions were highly respected. I never heard of an "enemy" of E. B. Wilson. According to their story, it was Wilson's firm belief in the correctness of the *Drosophila* work that accelerated its acceptance by the biological community as a whole.

CALVIN BRIDGES 1916

In presenting Bridges' experiments to students, it is important that the normal inheritance of sex chromosomes be thoroughly understood. Figure 39 provides a review. The chromosomes of the female are shown in large letters and those of the males in small letters. The **x** chromosome of the male is transmitted only to his daughters and his **y** only to his sons. The **X** chromosomes of the female are transmitted to both sons and daughters. Thus a daughter receives one **X** from her mother and one **x** from her father. The son receives his **X** from his mother and his **y** from his father.

Again, with all that intense work in the Fly Room, innumerable new mutants were discovered. One strain behaved in a most unusual manner. In a cross of a *white-eyed* female and a *red-eyed* male one would expect in the F_1 generation daughters with *red* eyes and sons with *white* eyes, nothing more (Fig. 27). However, Bridges found that there were some *white-eyed* daughters and some *red-eyed* sons. There was no way this could have occurred, given normal inheritance of the sex chromosomes. Those daughters with the *white* eyes could not have received the **X** of the father, since it carried the dominant allele for *red* eyes and its influence would have prevailed. Therefore these exceptional daughters must have inherited their sex-linked genes from the mother alone. The exceptional *red-eyed* sons demanded a similar explanation. Since their **X** chromosome could normally come only

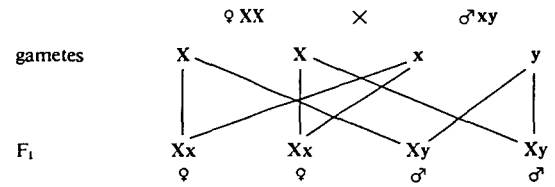


FIG. 39. The normal inheritance of sex chromosomes in *Drosophila melanogaster*. Female chromosomes in large letters and male chromosomes in small letters.

from the mother, and both of her **X**s carried the allele for *white* eyes, the **X** of the *red-eyed* male must have come from his *red-eyed* father.

What a mess. After four years of things seeming to work, it must have been most puzzling to encounter this strain of flies. But the point had been reached when the *Drosophila* group could be confident enough to treasure exceptions, since a phenomenon not explicable by the existing paradigm might, with further analysis, provide deeper understandings. But in order to save the paradigm, Bridges had to concoct a truly bizarre hypothesis to explain the data. Yet that bizarre hypothesis was capable of being tested.

The hypothesis Bridges proposed was that the female parent with the exceptional offspring had not only two **X** chromosomes but also one **Y**. During meiosis in this hypothetical **XXY** female, four classes of gametes were imagined to be produced: **X**, **XX**, **XY**, and **Y**. (A normal female would have produced only one class of gametes so far as the sex chromosomes are concerned—**X**.) There was no way of predicting the percentages of these gametes but experiments showed that 46 percent were **X**, 46 percent were **XY**, 4 percent were **XX**, and 4 percent were **Y**.

These **XXY** flies were called non-disjunction females. The term refers to the fact that in some of the ova there is no segregation, or disjunction, of the two **X** chromosomes and both remain in the ovum (a similar number would have gone into a polar body but, of course, polar bodies leave no progeny). In normal meiosis, one **X** would pass to the second polar body and the other **X** remain in the ovum.

One of the critical crosses made by Bridges is shown in Figure 40. It must have taken considerable courage to postulate this seemingly preposterous hypothesis yet, if one were to continue to maintain that genes are parts of chromosomes, some such hypothesis was required.

The fact of key importance in Bridges' hypothesis is that it could be tested, and hence its degree of preposterousness estimated. These are the main deductions:

1. If the hypothesis is true, we would expect 50 percent of the daughters to be non-disjunctional females (classes 1 and 7 of Fig. 40; the percentages shown in the figure are for all the flies so when females alone are being considered, the values should be doubled). All of the *white-eyed* females (class 7) should be non-disjunctional. The vast majority of the females were predicted to have *red* eyes (classes 1 and 2). These could not be distinguished by their phenotypes but, if used in genetic experiments, half would be normal (class 2) and half non-disjunctional (class 1). Bridges made the crosses and found that this deduction was true.

2. If the hypothesis is true, we would expect the exceptional males (class 4), that is, those males that inherited their **X** chromosome from their fathers, not to transmit the power of producing exceptional offspring in later generations. They were predicted to behave like normal males. They were tested and this was found to be true.

3. If the hypothesis is true, we would expect 46 percent of the males to be **XXY**. These would be expected to produce four types of sperm: **X**, **YY**, **XY**, and **Y**. Such a male crossed to a normal female should produce no exceptional offspring, that is, males inheriting their sex-linked characteristics only from the father and females inheriting theirs only from their mothers. However, every **XY** sperm entering a nor-

P	Non-disjunctional white eye ♀ XXY	×	Normal red eye ♂ XY
Gametes	XY (46%); X (46%) XX (4%); Y (4%)		X (50%) Y (50%)

F_1	XY (46%)	X (46%)	XX (4%)	Y (4%)
	1 XXY 23% Red eye ♀ Would show non-disjunctional behavior if crossed.	2 XX 23% Red eye ♀ Normal chromosome behavior.	3 XXX 2% Triploid X. ♀ Usually dies.	4 XY 2% Red eye ♂ The X has come from the father and the Y from the mother. This is the reverse of the normal situation.
X 50%				
	5 XYY 23% White eye ♂ With extra Y chromosome.	6 XY 23% White eye ♂ With normal chromosome behavior.	7 XXY 2% White eye ♀ Would show non-disjunctional behavior if crossed.	8 YY 2% Dies
Y 50%				

FIG. 40. Bridges' experiment with non-disjunction females.

mal egg with its single **X** would produce an **XXY** daughter. She would be non-disjunctional. This was tested and the predictions verified. That last short sentence gives no notion of the huge amount of work involved in this test as well as the others.

4. If the hypothesis is true, we would expect that 50 percent of the daughters (classes 1 and 7) would be **XXY**. This deduction was tested by making slides of the chromosomes of many of the females. Figure 41 shows what was found. Approximately half of the females (*a*) had the normal chromosome set with two **X**s. The other half (*b*) had the normal autosomes but two **X**s and one **Y**.

These were demanding deductions and elegant tests. Young Bridges concluded

> there can be no doubt that the complete parallelism between the unique behavior of the chromosomes and the behavior of the sex-linked genes and sex in this case means that the sex-linked genes are located in and borne by the X-chromosomes.

That is a brave, though properly restricted statement. The only thing the experiments had shown was that, at the time they were conducted, it was true beyond all reasonable doubt that the *white* and *red* alleles were parts of the **X** chromosomes in the strain of *Drosophila melanogaster* used in the experiments.

What, then, is the basis for claiming that these experiments were the final proof that genes are parts of chromosomes—implying that this is true for all genes in all species at all times? Had this been the first genetic experiment done with any organism, Bridges' conclusion just quoted would have been as much as could be said. But it was not the first. In the sixteen years since 1900, an enormous amount of genetic information had accumulated. Many species of animals and plants, each far from being unique, showed a pattern of inheritance that appeared to be based on simple rules. In fact, there was an underlying uniformity of genetic systems among the species in contrast to the vast differences in their structure and physiology.

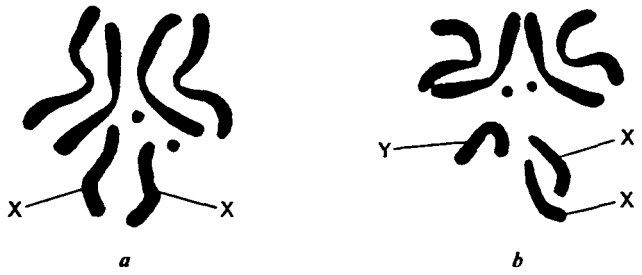


FIG. 41. Bridges' drawings of the chromosomes of female offspring in the cross shown in Figure 40. Approximately half of the females checked had the normal chromosomal complement shown in *a*. These would have been class 2. The remaining females (classes 1 and 7) were **XXY**. (C. Bridges, 1916)

Bridges was adding to the Theory of Genetics—to the entire body of data, hypotheses, and conclusions about inheritance. He was not challenging some well established paradigm. He was supporting that paradigm with a truly elegant proof.

Thus it was reasonable to extend the conclusion based on non-disjunction experiments to the other genes of *Drosophila melanogaster* and to genes of other species and conclude that in all species genes are parts of chromosomes.

Bridges' final proof in 1916 that genes are parts of chromosomes, which started on page 1 of volume 1 of the new journal *Genetics,* was a culmination of a series of investigations at Columbia University begun by Sutton in a laboratory on the same floor in Schermerhorn Hall. Sutton had stressed the parallelism of changes in chromosomes and Mendelian factors as indicating that the factors are probably associated with chromosomes. Wilson had corrected McClung's original misinterpretation of sex chromosomes and had gone on to produce his classic *Studies of Chromosomes.* At frequent intervals he published cautious updates on what was known of inheritance. Morgan, more interested in evolution than in heredity, had bred *Drosophila* to see if he would find similar abundant mutations, with striking phenotypic effects, that de Vries had reported for *Oenothera.* He did not find those de Vriesian mutations but, after many months, he did find the *white-eyed* male. Two Columbia undergraduates, Sturtevant and Bridges, began to work in Morgan's lab and soon Muller joined them. From then on the fate of classical genetics was sealed.

One can only speculate what the history of genetics might have been had those talented individuals not been in the same place and within the same decade—and after 1909 all working on that one tiny species, except for Wilson who was doing cytological work that was basic to genetic conclusions.

Now to another Columbia legend, which involves Bateson's visit to the Fly Room in 1921. One of the main events was a demonstration by Bridges of the chromosomal preparations from the non-disjunction experiments. Bateson, who knew next to nothing about cytology, is said to have gone from microscope to microscope dropping ashes from his pipe over everything. Eventually he announced that he was convinced that genes were parts of chromosomes. However, so the story goes, Bateson went to the AAAS meetings in Toronto and largely rescinded his acceptance of the chromosomal theory of heredity. G. E. Allen (1978, pp. 275–276) has a more complete and probably a more accurate account of Bateson's visit to Columbia.

In truth Bateson (1922) was most generous in his lecture in Toronto:

> We have turned still another bend in the track and behind the gametes we see the chromosomes. For the doubts—which I trust may be pardoned in one who has never seen the marvels of cytology, save as through a glass darkly—can not as regards the main thesis of the Drosoph-

ila workers, be any longer maintained. The arguments of Morgan and his colleagues, and especially the demonstration of Bridges, must allay all skepticism as to the direct association of particular chromosomes with particular features of the zygote. The transferable characters borne by the gametes have been successfully referred to the visible details of nuclear configuration.

The traces of order in variation and heredity which so lately seemed paradoxical curiosities have led step by step to this beautiful discovery. I come at this Christmas Season to lay my respectful homage before the stars that have arisen in the west.

THE MALE—MORE OR LESS?

These experiments on *Drosophila* plus those on many other species showed that the sex of an individual is determined by the sex chromosomes it receives when the ovum and sperm combine at fertilization. (We now know this is not true for all species.) Your students may have concluded that the full explanation of sex determination is at hand when, as in *Drosophila* and *Homo sapiens,* the zygote contains either **XX** or **XY**. But did any of your students inquire further? Is a female a female because she as two **X** chromosomes or because she has no **Y**? Is a male a male because he has a **Y** or because has has only one **X**? Or is sex determination the consequence of more complex phenomena?

If students are asked to suggest how such hypotheses could be tested, their proposals might not come readily. How can one juggle the chromosomes in ways that would provide tests of deductions? After learning about Bridges' experiments with **XXY** females, students might suspect that *Drosophila* could provide the material for answers.

Two bits of evidence have already been given that would provide a clue. The first is that in some species males are **XO**, that is they have only a single sex chromosome. The second datum is that, for the most part, the **Y** chromosome of *Drosophila* is genetically inert. Thus, one could argue that in the course of evolution the **Y** has become progressively less important, and finally in some species it has been totally eliminated.

Therefore, the hypothesis that males are males because they have only one **X** and females are females because they have two, has some support.

This hypothesis was strengthened by some remarkable flies that appeared in the Fly Room—they were female on one side of the body and male on the other. Similar individuals, known as gynandromorphs, had been observed in other species. Detailed analysis had not been done however, and the underlying cause remained unknown.

Males and females in *Drosophila* differ externally in several ways. The males have groups of bristles, the sex combs, on the forelegs and the posterior portion of the abdomen is solid black, whereas it is barred in the females. The genitalia of the two sexes differ considerably. In addition, males are smaller than females.

Cytological study indicated that these gynandromorphs began as normal **XX** females but, through some cytological accident at the very beginning of development, one of the **X** chromosomes was lost from a cell in part of the embryo. The descendents of this cell would have only a single **X** and, hence, have the genotype of a male. As a consequence, some individuals developed that were male on one side of the body and female on the other. The male side had the sex combs and the solid black posterior abdomen. The difference in body size resulted in this gynandromorph having a bent body—the larger female side bent the body considerably, making the male side concave. The genitalia were typically male on one side and abnormal on the female side.

Various sorts of gynandromorphs were observed depending on the time in development when the **X** chromosome was eliminated and the region of the embryo where it occurred. Not all were bilateral. The most interesting were those of known pedigree where the two **X** chromosomes had different alleles. One spectacular class of bilateral gynandromorphs were those with the

allele for *red* eyes on one **X** and the allele for *white* eyes on the other. The result was an individual with a *red* eye on one side and a *white* eye on the other.

The hypothesis that an individual *Drosophila* was male or female depending on the number of **X** chromosomes in its cells was made highly probable by these observations. Through some accident of development there had been a juggling of the chromosomes and an important test of deductions had become possible.

The work of Bridges on non-disjunction (1921, 1939) showed that accidental events were producing even more striking chromosomal variations. As a consequence it became feasible to test in new ways the relation of the number of **X** chromosomes to the sex of the individual.

As we have seen, Bridges' **XXY** fly (Fig. 41) was a structurally normal and fertile female. She was among the first of many individuals discovered in the Fly Room that had abnormal chromosomes. After careful study Bridges gradually came to believe that sex was not determined solely by the number of **X** chromosomes (his data suggested little role for the **Y**) but by some relation between the **X**s and the autosomes. The following is a simplified version of his hypothesis.

Recall that a *Drosophila melanogaster* female has three pairs of autosomes and two **X**s (Fig. 35). We will use the term "autosomal set" and the letter **A** to apply to the monoploid group of autosomes—one of each homologous pair. The normal female, therefore, will have two sets of autosomes and a pair of **X**s. The ratio of **X**s to sets of autosomes will be $2\mathbf{X}/2\mathbf{A} = 1.0$. The male will have a single **X** and two sets of autosomes. His ratio will be $1\mathbf{X}/2\mathbf{A} = 0.5$.

A female was discovered that proved to be triploid—three of each kind of chromosome. What would that extra **X** do? A superfemale? Not at all. She was normal, and on the scheme just described, she would be $3\mathbf{X}/3\mathbf{A} = 1$.

It seemed, therefore, that the ratios 1.0 = female and 0.5 = male was the rule. Were other combinations possible?

Once a fertile triploid female was available, the possibility of creating chromosomal havoc was at hand. Such a female crossed with a diploid male would produce various sorts of abnormal chromosomal combinations. If any of these new combinations were fertile they could be used in crosses to further perturbate the chromosomal system.

Some of the various combinations are shown in Figure 42. So long as the number of **X** chromosomes equals the number of autosomal sets, the ratio is 1.0 and the fly is a female. If a fly has two **X** chromosomes but 4 sets of autosomes then the ratio is 0.5 and its sex is male. Thus **XX** = female is true only if there are also two sets of autosomes.

But what would happen if the ratio were between 0.5 and 1.0? The extraordinary thing is that such a question could be asked and answered. The answer was that such flies are intermediate in their sex characteristics. They are called intersexes.

It was possible also to increase the ratio to values higher than 1.0 by having more **X** chromosomes than autosome sets. These flies, often called superfemales, tended to have the female characteristics exaggerated.

The combinations shown in Figure 42, plus others, were obtained and Bridges recognized a consistent pattern. Sex, far from being determined by genes of the "sex" chromosomes alone, is the result of interactions between genes on the **X**s and genes on the autosomes. The autosomal genes have a net male-forming tendency and the **X** chromosomes a net female-forming tendency. In a normal male the genes of the two autosomal sets overbalance the genes of the single **X**. In the normal female the double dose of genes provided by the two **X**s overbalance the genes on the autosomes.

Seemingly *Drosophila* genes and chromosomes could be altered to answer even the most difficult questions.

THE ORIGIN OF NEW MUTANTS

De Vries' (1901–1903, 1909–1910) report of frequent appearance of mutants in *Oenothera* stimulated many geneticists and evolutionists to search for them in other organisms. As noted earlier, Morgan

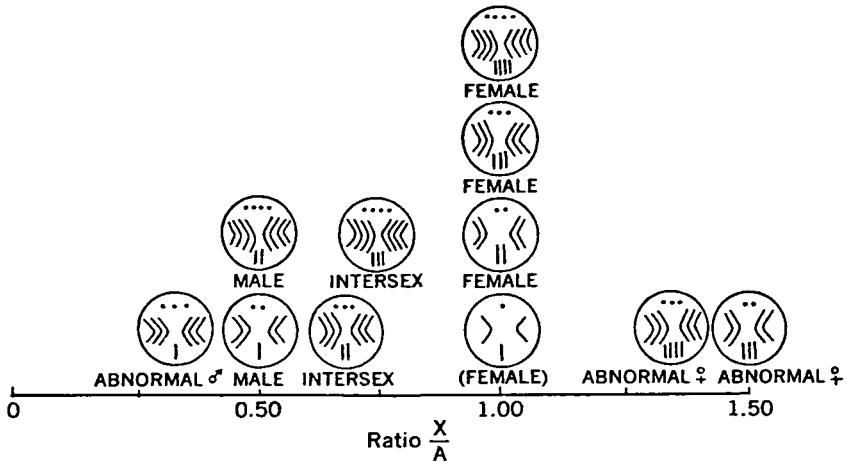


FIG. 42. The various combinations of **X** chromosomes and autosomes obtained by Bridges and others. The lowest circle at ratio 1.00 is a monoploid female. Bridges did not observe such an individual but some diploid flies were discovered to have monoploid areas in their bodies. If such areas happened to include sex structures, they were of the female type.

had started breeding *Drosophila* with that purpose in mind.

If one searches for mutants of *Drosophila*, or any other organism, merely by keeping a culture going and examining the individuals of each new generation, new mutants are exceedingly rare. *Drosophila* with *white eyes* or *vestigial wings* are encountered only when thousands of individuals are examined. Morgan wrote in 1914*a*:

> In fact, our experience with *Drosophila* has given us the impression that mutations are rare events, although the actual number of our mutations is now quite large.

There are two main reasons for this: the rarity of mutation at any locus and the recessiveness of nearly all mutant alleles. As a consequence nearly all mutant alleles are carried in the heterozygous state and masked by the dominant wild-type alleles. Your students will come to understand the problem if you ask them to devise an experiment for detecting mutant alleles. We will assume that a single recessive allele occurs in one heterozygous individual in a population of 1,000 flies. How would your students go about discovering it? They will soon conclude that a very large number of crosses would have to be made and a very much larger number of individuals have to be checked.

During the first decade of the 20th century, organisms were treated in various ways in the hope of increasing the rate of mutation. Morgan injected various chemical substances into different species of insects with the hope of obtaining mutants (G. E. Allen, 1978, p. 148). Later he exposed *Drosophila* to radium. The idea for this may have come from his Columbia colleague, James Howard McGregor, who was one of the first to test the effect of radium radiations on living organisms—he used frog gametes and embryos. Later Muller's work demonstrated that X-rays could indeed induce mutations.

The fact that some of Morgan's first cultures of *Drosophila* had been exposed to radiations makes it remotely possible that some of the mutants first discovered were radiation-induced. Morgan (1914*a*), however, did not believe that radiations had been the cause and subsequent experiments using radium and X-rays seemed not to produce mutations. Morgan (1914*b*) also wondered if etherization of the flies could cause mutations but could not find that it did.

E. B. Lewis (personal communication) believes that it is most unlikely that the swarm of mutants encountered in the Fly Room was radiation-induced. One reason is the very low dosages of radiations that would have been available to Morgan.

Lewis suspects the cause was hybrid dysgenesis(Lewin, 1985, pp. 626–627)brought about by crossing numerous different strains of *Drosophila melanogaster* caught in the wild. If this explanation is true it means that the advent of *Drosophila* genetics was a highly improbable event. If Morgan had used only a single culture, whether from Lutz, Castle, Payne, or caught personally, hybrid dysgenesis would not have occurred and there would have been no swarm of mutants.

Nevertheless once that original *white-eyed* male had been discovered, other mutant alleles were found. In just a few years the number had risen to 85 (Fig. 35). The unusual ability of Calvin Bridges to spot variations from the normal wild type played a large role in their discovery. But all in the Fly Room were active and successful in discovering mutant alleles. Sturtevant discovered many new mutant alleles even though he was color-blind. A near astronomical number of flies was examined and one suspects that the dedication, focus, and discipline of those working in the Fly Room were the major reasons that so much was discovered in such a short time.

INDUCED MUTATIONS

The nature and causes of the mutation process were of great interest not only to geneticists but to evolutionists as well. Were the sorts of inherited changes being studied in the Fly Room the basis of the variability required for Darwinian evolution? No one thought that mutational changes would be of such magnitude that a cytological examination of chromosomes would reveal them. But if the physical nature of the change could not be detected, possibly the process of mutation itself could be studied. That might become feasible if mutations could be produced by experimental means.

None of the early experiments to induce genetic changes was conclusive because of the difficulty of distinguishing induced mutations from spontaneous ones and because of the inadequate design of the experiments. Mutants appeared in stocks not exposed to the putative mutagenic agents. Their appearance could not be correlated with any known cause so they were termed "spontaneous mutations." They were rare. In experiments attempting to produce mutations by physical or chemical means, mutations occurred but also rarely. Thus, if we expose *Drosophila* to radium in the hope of producing mutations, and if a mutant fly appears in the F_1 or F_2 or later generations, we cannot be sure whether it is spontaneous or induced.

Since new mutant genes appear infrequently and nearly all are recessive, their detection poses a problem—as your students will have realized if they tackled the problem suggested at the beginning of this section. Assume, for example, that one autosomal gene in a sperm nucleus mutates. If this sperm then enters an egg—a highly improbable event in itself—the new individual will have one mutated allele from the father and the normal, and surely dominant, allele from the mother. The observer checking the offspring for new mutants will not recognize that this one fly in the group has a new mutant allele since it will be in the heterozygous state.

Appropriate crosses could have been made to produce the desired individuals homozygous for the mutant allele if there was some way of identifying the original heterozygote. Since there was no way of knowing this, the alternative would have been to make innumerable crosses in the hope of including that one heterozygote. This procedure was impractical for those interested in obtaining quantitative data on the production of new mutants.

MULLER'S **ClB** METHOD

H. J. Muller (1927) was the first person to give a practical solution for this problem. He devised an ingenious experiment that provided a simple, yet accurate, measure of mutation rate. He wished to compare the spontaneous mutation rate with the rate after exposing *Drosophila* to X-rays.

Muller developed a special strain of flies, known as **ClB**, that would enable him to measure the rate of mutation to a lethal state of any of the **X** chromosome genes.

A **ClB** female has one of her **X** chro-

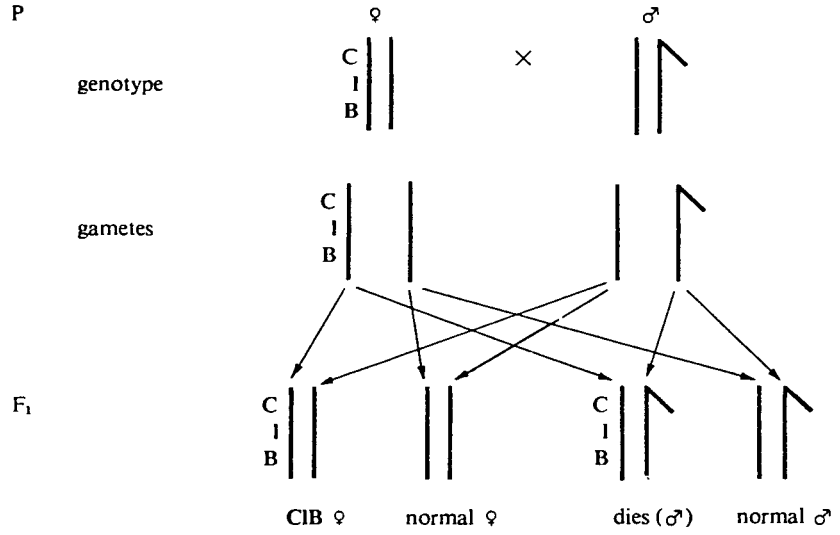


FIG. 43. Muller's **ClB** method. See text for details.

mosomes with an inversion designated **C**, a recessive lethal allele **l**, and the dominant *bar-eyed* allele **B**. The loci of both **B** and **l** are within the inverted region **C**.

An inversion is a region of the chromosome that has been reversed. If the normal order of hypothetical loci is **abcdefg**, a chromosome with loci in the order **abedcfg** would contain an inversion. Inversions are formed when a chromosome breaks in two places, in this case between **b** and **c** and **e** and **f**, followed by the rotation of the central piece and its fusion with the broken ends of the original chromosome. In this case the section **cde** rotates 180° and fuses with **b** and **f**.

By 1915 *Drosophila* workers were observing that some stocks showed very much reduced crossing-over between specific loci. Since crossover percentages were so important in locating the gene loci, this deviation was of concern. The cause was unknown but, since it was inherited, it could be studied. The "thing" causing the reduction in crossing-over was called a crossover suppressor. Sturtevant suspected that it might be an inversion, and in 1926 presented genetic data making that hypothesis probable. It was assumed that crossing-over was suppressed because in meiosis the two homologous chromosomes could not synapse in the region of the chromosomes where one homologue would have the normal sequence of loci and the other would have the sequence reversed (Fig. 46).

Muller had constructed his **ClB** stock with **B** and **l** within the **C** inversion knowing that they would remain linked, since no crossing-over would occur to separate them. The dominant **B** allele had the sole purpose of serving as a ready means of recognizing females heterozygous for the **ClB** chromosome. Homozygous females, with their two **ClB** chromosomes, would die, since there would be no normal allele to counteract the effects of **l**. Any male inheriting a **ClB** chromosome would also die, since there is no allele on the **Y** to counteract the effect of **l**.

Figure 43 shows what happens when a female heterozygous for a **ClB** chromosome is crossed with a wild-type male. Half of the daughters will be normal, and half will have *bar eyes* and hence carry a **ClB** chromosome. The sons inheriting the **ClB** chromosome will die because they have an unopposed **l** allele. The sex ratio, therefore, will be 2 females : 1 male.

Lethal genes on the **X** of *Drosophila* were first recognized by Morgan (1912*b*) when he encountered a stock that gave this 2:1 sex ratio. This was found to be due to a

lethal allele carried by the females in the heterozygous state. Ask your students if it would be possible for homozygous females to be formed even if they would die. They may find it impossible to cross a heterozygous **ClB** female with a (deceased) **ClB** male.

At the time Muller was doing these experiments it was well known that many separate gene loci can mutate in such a way as to cause death. These lethal genes were nearly always recessive. Since many different loci can mutate to the lethal state, the chance of getting some one lethal mutation is greater than the chance of getting a specific mutation at a specific locus. Thus, if we studied the rate of mutations to the lethal condition on the **X** chromosome, we would be measuring the sum of the rates for *all* loci that could change in such a way as to lead to death in the male offspring. The number of such loci would be large but unknown.

The **ClB** stock allowed Muller to measure the frequency that some locus on the unmarked **X** will carry a lethal allele. First he wished to determine the normal rate of such mutations. With that information as a baseline, he could then try the effects of putative mutagenic agents—such as X-rays.

Figure 44 shows the experiment. What is being measured is the frequency with which a mutational change to the lethal condition has occurred in some one of the alleles on the **X** of the P generation male. The * indicates the presence of such a mutation.

This **X** of the male will be transmitted to the daughters. Thus the **ClB** daughters will have one **X** with the new lethal mutation and one with **ClB**. At this point it will almost surely be necessary to explain to some students why this female does not die—after all, she does have a lethal in each **X**. It is hard for some students to understand that in this case the lethal in one chromosome is not at the same locus as in the other and that each lethal allele has a normal allele on the other homologous chromosome. The confusion arises since both mutants have the same name—"lethal."

The F_1 **ClB** females are now crossed with normal males, as shown in the bottom of Figure 44. The daughters will be of two classes. Half will be **ClB** and the other half normal, though carriers for the new lethal. There will be no males. Half of the males would have died because they received the **ClB** chromosome and the other half because they received the **X** with the new lethal mutant.

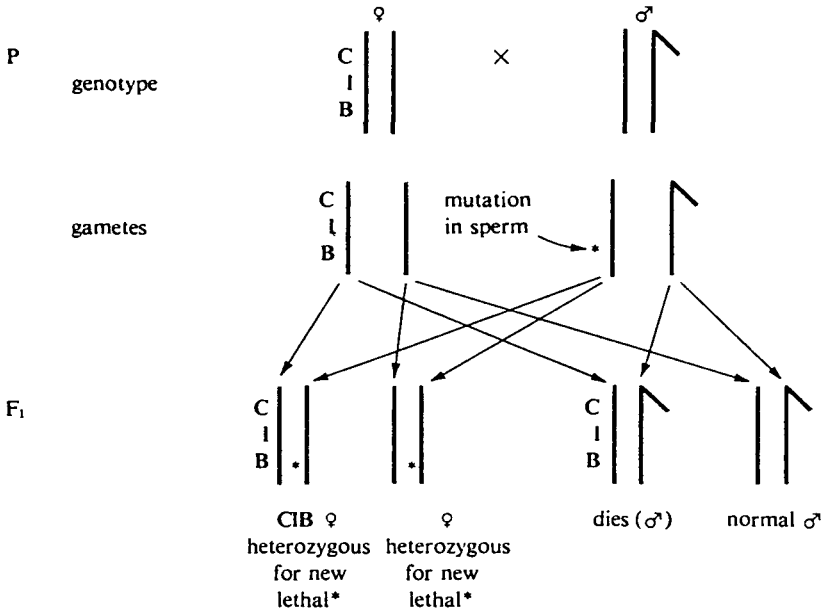
Although *Drosophila* is a small fly, it is possible to distinguish males from females with the unaided eye. Thus, Muller could check his culture tubes rapidly to see whether or not males were present. It was then possible to ask "What is the frequency with which *some* locus on the **X** chromosome mutates to a lethal allele?"

The percentage was suspected to be very small, so thousands of crosses had to be made. Muller found that approximately one cross in a thousand, 0.1 percent, gave only females. This is the spontaneous mutation rate. Once again, this is not the rate for a single gene but for all the genes on the **X** that can mutate to a lethal state.

Although Morgan and other workers a decade earlier had concluded that X-rays would not induce mutations, Muller now found that they did. If males were exposed to about 4,000 r-units of X-rays, approximately one cross in ten gave only females—a mutation rate 100 times the spontaneous mutation rate.

Not only were these data inherently important but Muller had shown that X-rays were a convenient means of inducing mutations—not all of which were lethal, of course. In fact it was found that X-rays would induce not only gene mutations but also cause inversions, translocations (the transfer of a portion of one chromosome to another chromosome—as in Stern's experiment of Fig. 36), or deficiencies (elimination of a section of a chromosome). The chromosomes and genes of *Drosophila* could now be modified in complex ways that would allow geneticists to answer many questions not otherwise possible.

Apart from the importance of the data and the conclusions, we should not lose sight of the fact that Muller's **ClB** method was extraordinarily ingenious. He had constructed the genome of the **ClB** flies in such



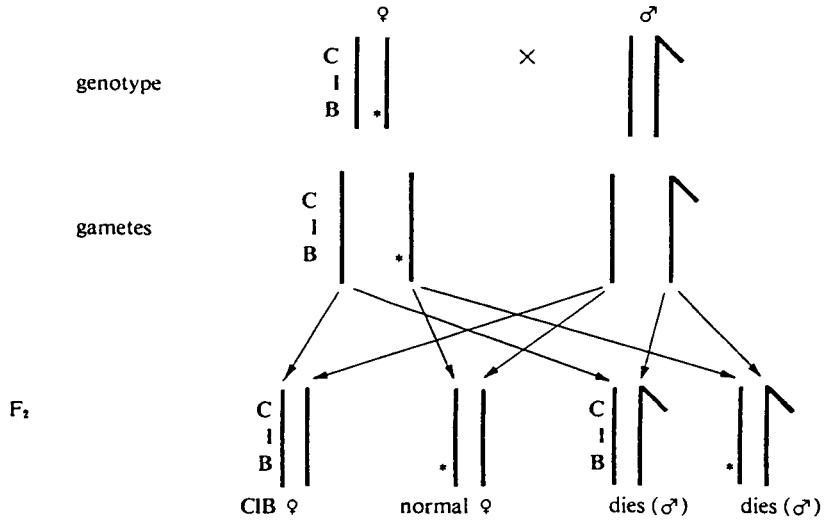


FIG. 44. Muller's **CIB** method used to detect lethal mutations. See text for details.

a way that he could measure the occurrence of a very infrequent phenomenon. In many instances, *Drosophila* could be molded to the needs of an experiment—at least in the hands of a genius. Once he could measure the spontaneous mutation rate accurately, he could then determine the mutagenicity of various external conditions. This was the beginning of a line of experimentation that is so very important to us today—the detection of environmental hazard, such as radiations and toxic

chemicals, that induce mutations. We now live in such a hazardous environment that monitoring it is now a common public health practice.

SALIVARY GLAND CHROMOSOMES

The concepts of genetics were developed mainly on data derived from breeding experiments, with an occasional assist from cytology. Cytology provided gross confirmations: the number of homologous chromosome pairs equal to the number of linkage groups; chromosomes correlated with the sex of the individual; chromosomal behavior adequate to explain segregation and independent assortment. The usefulness of cytology was severely restricted, however, because available techniques were not sufficient to reveal the fine structure of chromosomes. If genes are in a linear order, it would be extremely useful to be able to recognize linear differentiations of the chromosomes.

Until the 1930s, however, the standard cytological techniques showed the chromosomes, with few exceptions as uniformly-staining structures, with no differentiations that could be associated with genes. The working hypothesis of geneticists was that genes were probably proteins. If so, it would be impossible to observe them, even with the most powerful compound microscopes, since protein molecules would be below the limits of resolution of these instruments. Geneticists became resigned to investigating their invisible genes, just as chemists study their invisible molecules and the physicists their invisible sub-atomic particles by indirect means.

To be sure some rather sophisticated methods for tagging chromosomes had developed for *Drosophila, Zea mays,* and a few other species. Stern had made homologous chromosomes different in *Drosophila* as Creighton and McClintock had done for corn. Dobzhansky had used radiations to break chromosomes in order to make a crude-genetic map of the second chromosome (1930) and to demonstrate translocations (1929), both in *Drosophila.* None of these methods, however, had the precision that was so highly desirable.

But more was needed than these gross changes. The *Drosophila* workers were postulating all sorts of chromosomal rearrangements to explain aberrations in their genetic results. As noted earlier, cases of reduced or eliminated crossing-over were blamed on inversions. The *bar eye* allele was suspected to be a duplication of one of the loci responsible for normal eyes. Other aberrant genetic results were assumed to be consequences of the translocation of gene loci from one chromosome to another.

These explanations for chromosomal rearrangements were brilliant hypotheses to solve difficult problems yet, except in the case of large translocations, they could not be confirmed by cytological means. This lack of confirmation made the claims of geneticists "just too much" for some biologists. Was the *Drosophila* group erecting a reliable edifice of science or were they constructing a house of cards? The answer depended on whom you asked.

And for nearly half a century a splendid method for answering these questions had been available but its usefulness not apparent. In 1881 Balbiani had described the strange structure of the nuclei in salivary glands and Malpighian tubules in larvae of the fly *Chironomus.* The chromosomes were visible in non-dividing cells. They were very large and appeared to be fused as a continuous spireme all twisted and jumbled together in the nucleus. They were cross banded. Thereafter similar descriptions were published for other Diptera (Painter, 1934*a*).

In contrast with Mendel's paper, Balbiani's was well known. E. B. Wilson (1900, p. 36) wrote:

> The most striking case of this kind [the chromatin of resting nucleus forming a continuous spireme] occurs in the salivary glands of dipterous larvae (*Chironomus*) where, as described by Balbiani, the chromatin has the form of a single convoluted thread, composed of transverse discs and terminating at each end in a large nucleolus.

Balbiani's figure was reproduced by Wilson. The discs were also called chromo-

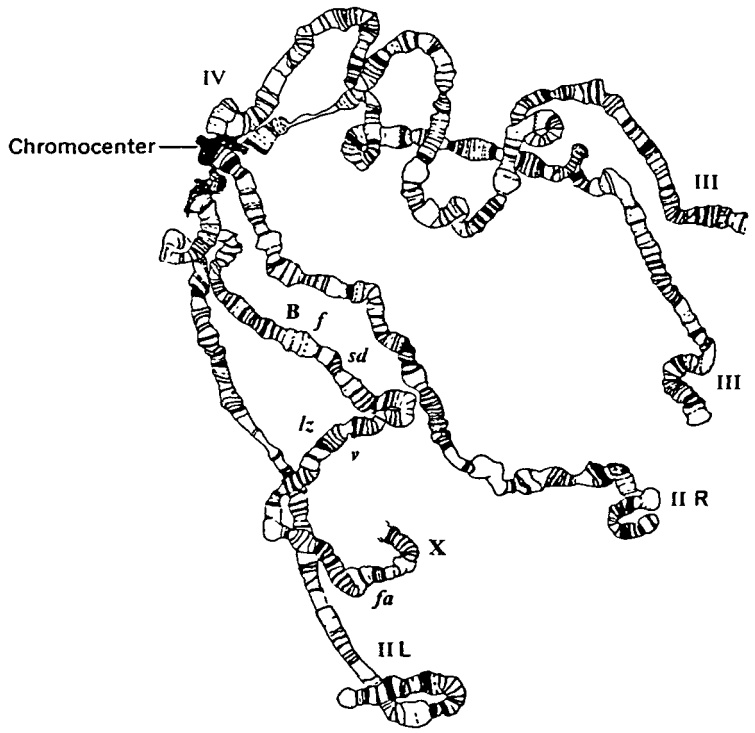


FIG. 45. Painter's first illustration of the salivary gland chromosomes of *Drosophila melanogaster*. The chromosomes are attached to the chromocenter, the X and the fourth by their ends. The two large autosomes are attached by their middles and, hence, have two arms (labelled L and R for left and right, in terms of the linkage maps). The letters show the provisional locations of some of the genes. (Painter, 1934*b*)

meres and were thought to divide in prophase (Sharp, 1934, p. 141, who also reproduces Balbiani's figure).

The possible importance of these banded chromosomes may have been missed because they were thought to be part of a continuous spireme and not individual chromosomes.

In 1933 Heitz and Bauer studied these giant banded chromosomes in another dipteran, *Bibio*, and reported that the chromosomes were not in a continuous spireme but consisted of the haploid number of elements. They therefore concluded that each salivary gland chromosome consisted of the two homologues fused together. When the cells were squashed chromosomes were spread out and could be studied. Of the greatest importance was their report that the banding pattern appeared to be specific for the regions of the chromosomes. That meant that the individual chromosomes could be identified by their characteristic banding pattern.

Later that same year Painter (1933) published a preliminary paper describing the similar huge salivary gland chromosomes of *Drosophila melanogaster*. They were at least 100 times as long as metaphase chromosomes. The homologous chromosomes were fused and the line of separation was difficult to see.

Once again, we have a case of simultaneous and independent discovery, for Painter (1934*a*) wrote,

> As I was in the midst of my first year's work, an article appeared by Heitz and Baur [=Bauer] dealing with the salivary chromosomes of *Bibio hortulanus*.

Painter had discovered the salivary gland chromosomes for himself; he was not aware

of the extensive literature on the subject (1934*b*) until after his work was well under way.

Painter followed his first report with a series of papers describing in great detail the structure of the four pairs of homologous chromosomes (1934*a*, 1934*b*, 1934*c*, 1935) and determined which salivary gland chromosome corresponded with the four linkage groups (Fig. 45). Figure 46 is a photograph of some of the chromosomes of *Drosophila pseudoobscura* and *Drosophila persimilis*.

Your students may wish to speculate on how Painter was able to associate specific linkage groups with those cytological zebras of Figures 45 and 46. Some may suggest that if a specific inversion, for example the **C** inversion used in Muller's **ClB** stock, is known from the genetic data to be on the **X**, then the salivary gland chromosome that has an inverted section of bands would probably be the **X**. In the case of the **ClB** individuals, one of the **X** chromosomes would have one sequence of bands and the other would have a reversed sequence, since only heterozygous individuals are viable. Confirmation would come when other inversions ("crossover suppressors") of known linkage groups could be associated with the specific salivary gland chromosomes.

Painter was even able to determine the approximate position of gene loci.

> There are three general ways of determining the position of gene loci. Simple mutual translocations or inversions in which we know genetically between what genes the break or breaks have occurred; short deletions where we know what genes are missing; and a study of a series of breaks all falling between the same two gene loci.

Figure 45 shows the location of a few **X** chromosome genes.

This method was pure gold and many geneticists realized it at once. Th. Dobzhanksy told me this story long ago. Painter's first paper was reported at a seminar at Cal Tech and immediately thereafter Bridges came rushing into Dobzhansky's lab and said "Dobzhansky where are the salivary glands?" Bridges started work immediately and prepared maps of the *melanogaster* salivary gland chromosomes (1935, 1938, and 1939 with his son P. N. Bridges). Dobzhansky himself was to use the salivary gland chromosomes of *Drosophila pseudoobscura* (Fig. 46) and other wild species to obtain the basic data for his classic series *Genetics of Natural Populations* (reprinted in Lewontin, Moore, Provine, and Wallace, 1981). The salivary gland chromosomes attracted considerable attention. See, for example, Koltzoff (1934), Muller (1935), and Ris and Crouse (1945).

The bands on the salivary glands provided a critical cytological test for the many deductions the *Drosophila* workers had made on the basis of genetic data alone. Where they had invoked a reversed order of the genes to explain the absence of crossing-over, the bands on the salivary gland chromosomes were reversed. When their data suggested that a portion of one chromosome had become attached to another, the bands were found to be translocated. From some strange data they deduced that a small portion of a chromosome must have disappeared. The salivary gland chromosomes then revealed a few missing bands. The *bar eye* allele was suspected to be caused by the duplication of a small portion of the **X** chromosome. Figure 47 shows this to be true.

The *Drosophila* group had the last laugh—they had demanded a seemingly endless list of chromosomal aberrations if they were to explain their genetic data and the bands on the salivary gland chromosomes confirmed their hypotheses.

WHERE ARE THE GENES?

Now that specific and minute portions of *Drosophila* chromosomes could be defined, there was great interest in discovering, if possible, the place occupied by the genes. Were the bands the genes? Was the non-staining area between the bands genetically inert?

Attempts to localize the positions of genes were based mainly on studies of small deletions. These could be produced in large numbers with X-rays. It was not possible,

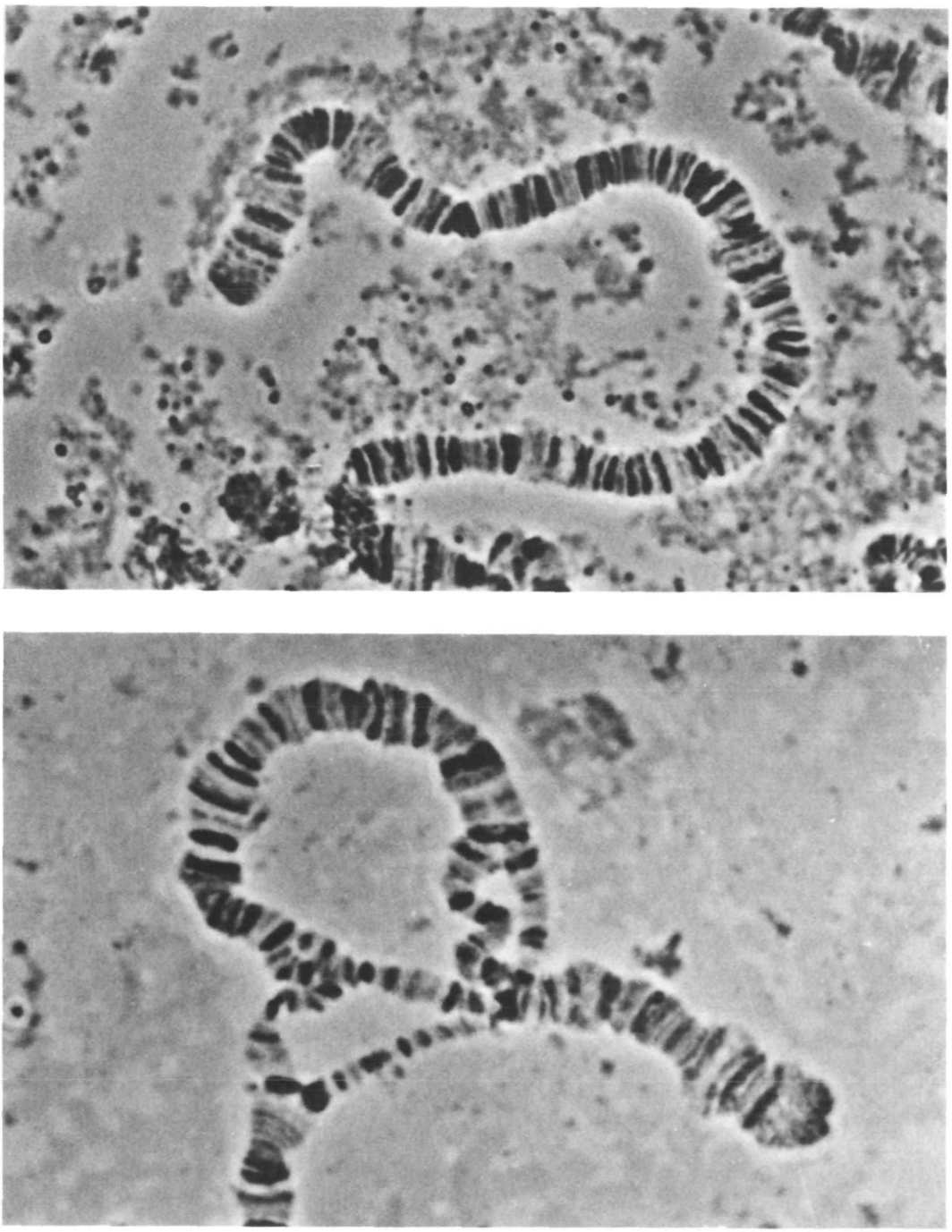


FIG. 46. Photographs of salivary gland chromosomes. The upper one is the so-called Klamath gene arrangement in *Drosophila persimilis*. The lower figure shows the area of the inversion of an individual heterozygous for the Pikes Peak and Standard gene arrangements in *Drosophila pseudoobscura*. The chromosomes at the bottom left are fused but slightly above the inversion begins—it can be seen that the bands do not correspond and pairing is not possible. One of the inverted sections makes a twist and can pair, as in the topmost section. Farther on the two homologues are separate but at the bottom right their loci are the same and pairing is possible again. (Photographs by Betty C. Moore)

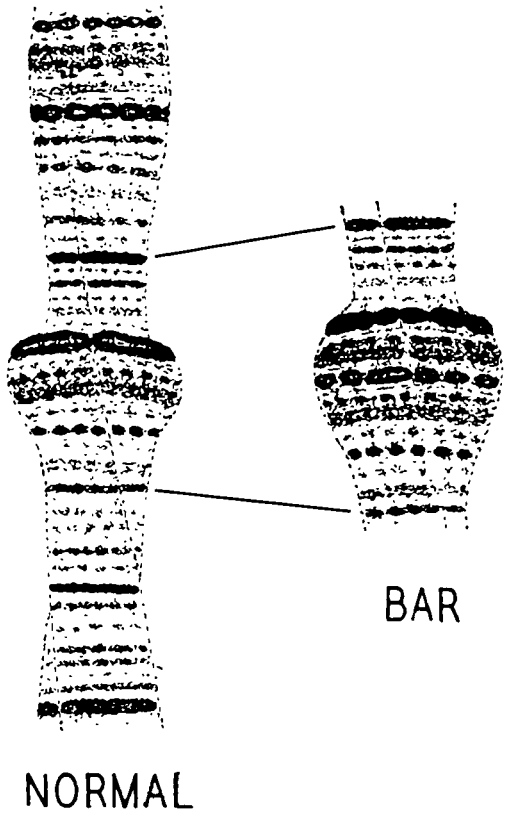


FIG. 47. Salivary gland chromosomes showing the area of *bar* in the **X** chromosome. The lines show the corresponding bands on the two chromosomes. (Bridges, *Science* 83:210, 1936)

of course, to induce deletions at specific places in chromosomes so the procedure was to irradiate many flies and examine their offspring in the hope of obtaining deletions in the desired region of the chromosome. A very large amount of work was required but for a motivated and dedicated geneticist it was worth it.

The following example is from the work of Demerec and Hoover (1936). They studied stocks with three deficiencies near one end of the **X** chromosome. Most deficiencies, except when very small, are lethal when homozygous but they can be carried in the heterozygous state.

Deficiencies have a special genetic effect as will be seen from the following consideration. Assume that a fly is heterozygous for a deficiency that includes the locus of gene **A**. That means that the allele at the **A** locus on the normal chromosome will determine the phenotype, since there is nothing on the chromosome with the deficiency to counteract its effect. This situation is similar to that in *Drosophila* males where, with the **Y** having almost no genes, whatever is on the **X** will determine the phenotype.

Demerec and Hoover determined the precise bands that were absent in the three deletions. They selected three mutant alleles, **y** (*yellow*—a body color mutant), **ac** (*achaete*—removes some bristles), and **sc** (*scute*—removes other bristles), which previous study had shown to be almost at the end of the chromosome.

The crosses were made in such a way that the flies studied would have one entire chromosome with **y**, **ac**, and **sc** and the other with one of the deficiencies but no mutant alleles. The experiments are diagrammed in Figure 48.

The first deficiency removed the 4 bands at the tip of the chromosome. The flies were *wild-type* indicating that the loci of the genes being used were not in the first 4 bands. The next deficiency removed the 8 terminal bands. The flies were *yellow* and *achaete,* showing that the loci for those alleles were in the terminal 8 bands. However, the first deficiency showed they were not in the first 4 bands. Therefore the *yellow* locus and the *achaete* locus must be in the regions of bands 4–8. The third deficiency removed the terminal 10 bands and this time the flies were *yellow, achaete,* and *scute.* Therefore, the locus for *scute* must be in the region covered by bands 8–10.

Using this method *Drosophila* workers were able to determine the approximate loci for many genes. No locus was found in the interband regions and, in a few cases, it was possible to place a locus in a small region having a single band. These observations suggested the tentative hypothesis that the bands or some portion of them are the gene loci.

If this hypothesis is true, a tentative estimate for the number of genes in *Drosophila melanogaster* could be obtained by counting the bands. This is a tricky business because the number of bands depends to some

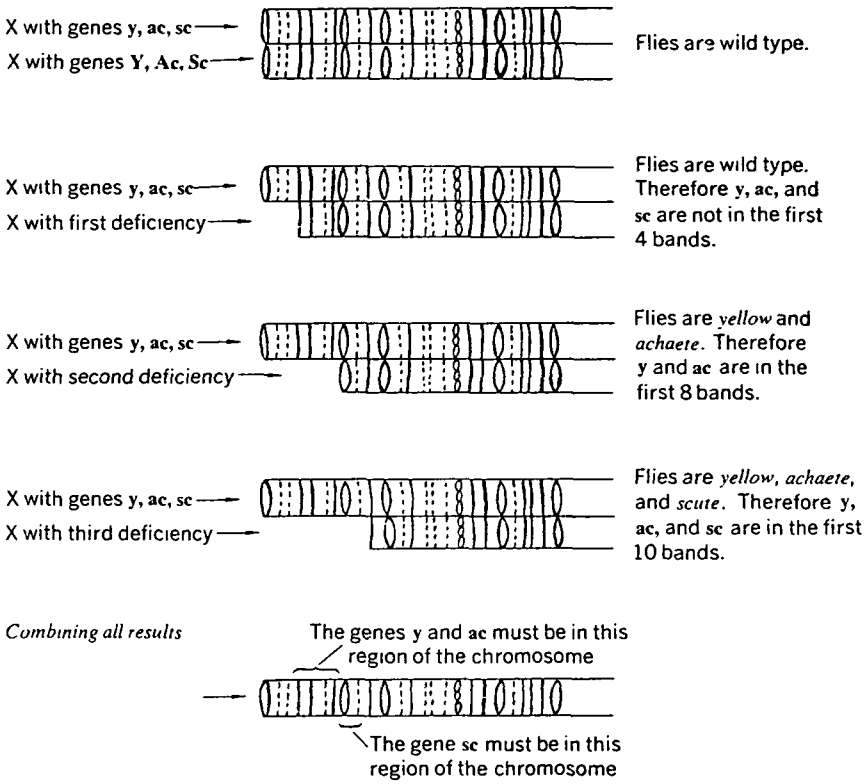


FIG. 48. The experiments of Demerec and Hoover to localize the genes of the X chromosome. See text for details.

extent on how the chromosomes are stained—they vary from bands that stain heavily to others so indistinct as to be at the limit of resolution. Nevertheless, there seemed to be about 5,000 bands so that was taken as the tentative minimum number of genes.

Once the genes were located on the salivary gland chromosomes, a comparison of the chromosomal maps could be made with the genetic maps. Bridges made an especially careful study and his comparison is shown in Figure 49. The resemblance is close. Although this is what geneticists expected, still it was astonishing. The comparison is being made here between one set of data based on the phenotypes of offspring of genetic crosses and another set of data based on the cytological descriptions. The two sets of data are about as different as one can imagine. And, once again, it was Calvin Bridges who did so much to validate the hypothesis.

The genetic data support the hypothesis that the genes are arranged in linear order and in a certain sequence. The cytological data support the same hypothesis. Once again, genetics and cytology were found to be mutually supportive and that fact in itself proved the hypothesis as true beyond all reasonable doubt.

This ability to check the findings in one field with findings in a completely different field is one of the most powerful techniques available to scientists. Two sorts of genetic data or two sorts of cytological data supporting the hypothesis of the linear order of genes are not as convincing as one set of genetic data in concordance with one set of cytological data.

The Suttonian paradigm, having guided the most gifted cytologists and geneticists for three decades, was now losing its ability to suggest new and exciting research problems. This was not because it was wrong but because it was so right. Its well-estab-

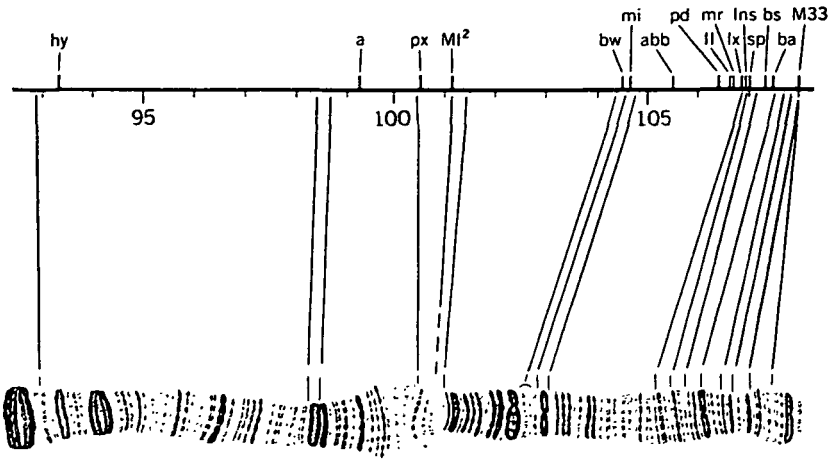


FIG. 49. Corresponding points in the salivary chromosome and the linkage map for the tip of the second chromosome of *Drosophila melanogaster*. (Modified from Bridges, 1937)

lished principles were found to be of great importance in experimental medicine and experimental agriculture. The experimental and theoretical challenges lay elsewhere and, after a summing-up and a pedagogical diversion, we shall see what they were.

THE CONCEPTUAL FOUNDATION OF CLASSICAL GENETICS

After thousands of crosses had been made and millions of offspring classified, geneticists of the late 1930s had the satisfying feeling that the big questions that had been asked for centuries had acceptable answers. When genetically unknown species were studied for the first time the rules of Mendel, Sutton, and Morgan accounted for the data.

The science of genetics had reached an acceptable level of maturity—it could predict the outcome of experiments. Genetics was the first branch of biology to reach this level of conceptual adequacy. It was inevitable that this be so. Even though it deals with the most basic problems of biology, it is the least complex part of biological science. The genotype must be simpler than the phenotype, since what is basic is less complex than what is derived. The genetic code is essentially universal in the realm of life, while the structure and functions of organisms take their myriad forms.

If one asks what had been accomplished in genetics and cytology the answer is that it was nothing less than discovering the rules governing the transmission of genes from parent to offspring. These rules, seemingly universal, held for plants, animals, and microorganisms. What are they?

Now follows a list of the major concepts of classical, or transmission, genetics.

1. The basic morphology, physiology, and molecular biology of an individual are determined by its inheritance, acting in a defined environment.

2. Although an individual's material inheritance is small in quantity, it contains all the genetic information in its genes necessary for the development of an organism like its parents.

3. Genes are parts of chromosomes. (Later research was to show that some genetic information is contained in mitochondria, plastids, viruses, and some virus-like bodies.)

4. Each gene usually occupies a definite site, its locus, in the chromosome. Understandable exceptions to this concept—inversions and translocations—were known and examples of the movement of genetic material from chromosome to chromosome have increased with time.

5. Each chromosome has many genes, except for a few cases like the Y of *Dro-*

sophila, and the genes are arranged in a linear order.

6. The somatic cells contain two of each kind of chromosome, that is, they are in homologous pairs. That means that every gene locus is represented twice. There are some well-known exceptions. In some species, bees for example, queens and workers are diploid females and the drones are monoploid males. Sex chromosomes are another exception where **XO** and **XY** males have only single copies of sex-linked genes. Also some of the cells in some tissues of some animals may be polyploid, as in our livers.

7. During each mitotic cycle the genes are duplicated from the chemical substances in the cell. Cellular duplication involves a prior genic duplication.

8. Although genes are characterized by great stability through time, possibly replicating a million times in many generations before any heritable change, a mutation, occur. Thus genes are capable of existing in several states known as alleles.

9. Genes can be transferred from one homologous chromosome to another by cytological crossing-over. This is a normal part of meiosis but there are a few exceptions, such as the male of *Drosophila melanogaster,* where crossing-over does not occur in genetically active regions.

10. The meiotic process ensures that each gamete receives one chromosome of each homologous pair. *Which* of the two homologues it receives is a matter of chance. Thus the gametes will receive one or the other of each gene pair (segregation). Each homologue, with the genes it contains, will be distributed to half of the gametes. **XO** males are an obvious exception.

11. In the formation of gametes, the segregation of the chromosomes of one homologous pair, with the genes it contains, has no effect on the segregation of the other pairs of homologous chromosomes with their genes.

12. Fertilization consists of the random union of ova and sperm, each with one chromosome of every homologous pair. Therefore, the zygote receives one chromosome of each homologous pair from the mother and one from the father. Again, sex chromosomes introduce an understandable exception.

13. When two different alleles of the same locus are present, the individual is heterozygous for that gene. The allele with the greater phenotypic effect is known as the dominant, and the other as the recessive. In most cases the heterozygote appears to be identical with individuals homozygous for the dominant allele. Less frequently the heterozygotes are intermediate.

14. Finally, genes must produce their effects through the production of chemical substances, which in turn control the biochemical reactions of the cell. In the 1930s this was little more than a tentative hypothesis but no other alternative seemed possible. Some geneticists suggested that the major function of genes is to produce specific enzymes, which in turn control the life of the cell.

These 14 propositions account for most of the phenomena of classical, or transmission, genetics. They formed a satisfying conceptual whole. But this was not enough. The inquisitive human mind is more stimulated by what is unknown than by what is known. One knew with great precision how the genes for eye color were inherited but essentially nothing of the structure of those genes or their mode of action. One could sense that would be the concern of the next major paradigm of genetics—carrying the analysis to the level of cells and molecules.

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tin, Moore, Provine, and Wallace (1981), Moore (1972*a*, 1972*b*), Morgan (1909, 1910*a*, 1910*b*, 1911*a*, 1911*b*, 1912*a*, 1912*b*, 1913, 1914*a*, 1914*b*, 1914*c*, 1915, 1917, 1919, *1922, *1926*a*, 1932, 1935), Morgan and Bridges (1919), Morgan, Bridges, and Sturtevant (*1925), Morgan and Lynch (1912), Morgan, Sturtevant, Muller, and Bridges (*1915), Muller (1914, 1916, 1922, 1927, 1928, 1929, 1940, 1947*a*, *1947*b*), Painter (1933, 1934*a*, 1934*b*, 1934*c*, 1935), Plough (1941), Ris and Crouse (1945), Stern (1931), Stevens (1905), Sturtevant (1913*a*, 1913*b*, 1913*c*, 1917, 1920, 1925, 1926, 1948), Sturtevant and Beadle (*1939), Whitehouse (*1965), E. B. Wilson (1914), and E. B. Wilson and Morgan (1920).

References more of historical interest. G. E. Allen (1966*a*, 1966*b*, 1969, 1974*a*, 1974*b*, 1975*a*, 1975*b*, *1978, 1979, 1983, 1985), Blakeslee (1936), Carlson (*1981), Cook (1937), Crew (1966), Dunn (*1951, *1965*a*, 1965*b*), Gilbert (1978), Glass (1963), Haldane (1938), Hall (1969), Hayes and Burnham (1959), Komai (1967), Mayr (*1982), Moore (1983), Morgan (1926*b*, 1939, 1940, 1941, 1942), Muller (1943), Payne (n.d.), Robinson (1979), Shine and Wrobel (1976), Stern (1970), and Sturtevant (1959, 1965*a*).

GENETIC PROBLEMS INVOLVING *DROSOPHILA*

If students are asked to solve genetic problems using *Drosophila*, they will come to realize very quickly what they understand and do not understand about genetics. *Drosophila* can be used for the more basic crosses of the monohybrid and dihybrid variety similar to those suggested earlier. Here are a few examples and the standard textbooks of genetics will have many more.

In *Drosophila vestigial-wings* (**v**) is an autosomal recessive to the wild-type *long-wings* (**V**). *Sepia-eye* color (**s**) is an autosomal recessive to the wild-type *red-eye* (**S**).

1. Describe the genotype and phenotype of the F_1 and F_2 of a cross of a *vestigial* female and a *sepia* male.

2. Describe the genotype and phenotype of the F_1 and F_2 of a cross of a *vestigial* male and a *sepia* female.

3. A normal-appearing male fly, of unknown genotype, is crossed with a female homozygous for both *sepia* and *vestigial*. The offspring were: ¼ *sepia-vestigial*, ¼ *red-vestigial*, ¼ *sepia-long*, and ¼ *red-long*. What was the genotype and phenotype of the male parent?

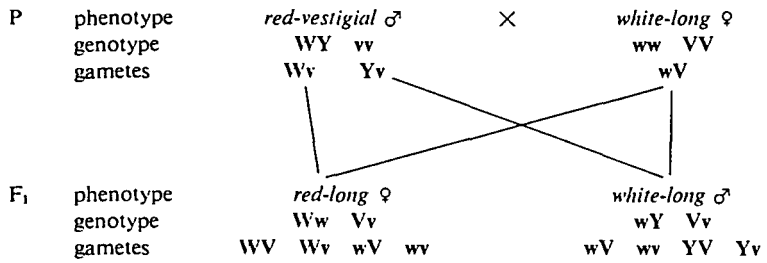
Problems of the sort given in 3 are especially valuable. If students have difficulties solving them the following hint should help. First, the problem should be solved for one pair of alleles at a time. A cross involving dominant and recessive autosomal alleles can have four possible outcomes. All of the offspring could have the phenotype of the recessive, which would mean that both parents had the recessive genotype. Or the offspring could all be of the dominant phenotype, which would mean that one parent must have been homozygous for the dominant phenotype and the other parent could have been either homozygous for the dominant or recessive allele or heterozygous. The third possibility would be a 3:1 ratio, indicating that both parents were heterozygotes. And finally, there could be a 1:1 ratio, meaning that one parent was a homozygous recessive and the other a heterozygote. One cannot tell, in the case of autosomal genes, which parent is which.

In problem 3, half are *sepia* and half are *red*. Therefore, one parent must have been **Ss** and the other **ss**. Since we know that the female parent was **ss**, the male must have been **Ss**. In addition, half of the offspring are *vestigial* and half are *long*. So one of the parents, the female, must have been **vv** and so the male parent must have been **Vv**.

4. In an F_1 the following offspring were obtained: ⅜ *red-long*, ⅜ *red-vestigial*, ⅛ *sepia-long*, and ⅛ *sepia-vestigial*. What were the genotypes and phenotypes of the parents?

5. A female with *sepia-eyes* and *long-wings* is crossed with a male of unknown parentage. The offspring were: ¾ *red-long* and ¼ *red-vestigial*. What was the genotype of the male?

Crosses involving autosomal and sex-linked genes represent a somewhat higher level of difficulty. The following cross is an example.



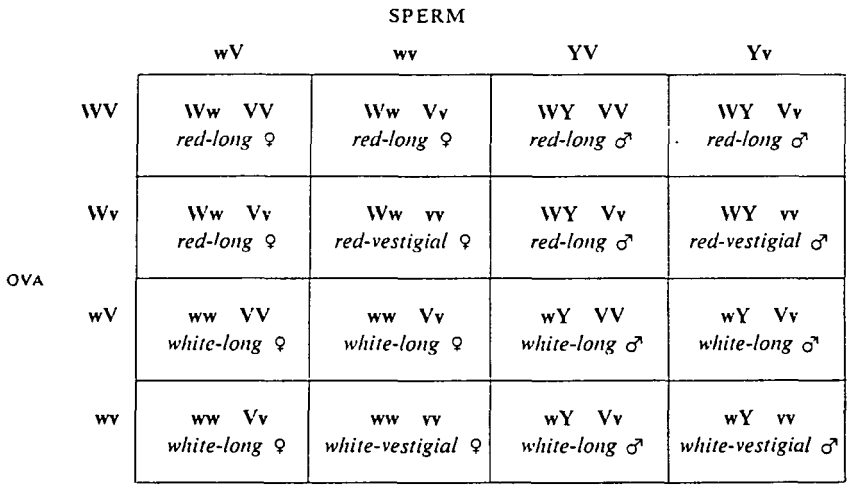


FIG. 50. Diagram of a cross involving an autosomal and a sex-linked gene.

White-eyes is a sex-linked recessive to the wild-type *red-eyes*. The mutant *vestigial-wing* is an autosomal recessive to the wild-type *long-wing*. Figure 50 diagrams a cross of a *red-vestigial* male with a *white-long* female. The F_2 ratios, which can be derived from the genetic checkerboard, will be new to your students. They are:

Females: ⅜ *red-long*, ⅜ *white-long*, ⅛ *red-vestigial*, ⅛ *white-vestigial*.
Males: the same.

If this seems confusing to your students, have them determine the genotypes and phenotypes for the sex-linked alleles and the autosomal alleles separately. Ask them why the F_2 does not show the usual 9:3:3:1 ratio, since the two pairs of alleles are on different chromosomes and should we not expect independent assortment? Some students may realize that the problem here is that the **Y** chromosome does not have the eye color locus. Therefore, instead of the P generation having a total of 4 alleles in the two parents, there are only 3. If the **Y** had an active locus there would have been a total of 4 alleles, and the F_2 ratio would have been 9:3:3:1.

Then have the students do the reverse cross, *white-long* male × *red-vestigial* female, to see if the results are the same.

Once the students have mastered problems of this sort, it will be challenging for them to start with the offspring and see what can be said about the parents. Here are some examples.

6. The following offspring were observed in an F_1:

Males: ½ *white-vestigial*, ½ *white-long*.
Females: ½ *red-vestigial*, ½ *red-long*.

What are the genotypes and phenotypes of the parents?

This type of problem should be solved for the autosomal alleles first using the method described in problem 3. Then the inheritance of the sex chromosome alleles should be solved, remembering the following relations:

a. The daughters receive one **X** from the father and one from the mother (see Fig. 39 for a reminder).

b. The sons receive their **X** chromosome only from the mother. Therefore, the phenotypes of the males will indicate what the genotype of the mother must have been. Using the *white-eyes* and *red-eyes* alleles as an example, if all the sons have *red-eyes*, the mother must have been homozygous for *red-eyes*, **WW**. If all of the sons have *white-eyes*, the mother must have been homozygous for *white-eyes* **ww**. And lastly, if half of the sons have *white-eyes* and half have *red-eyes*, the mother must have had both alleles and have been a *red-eyed* heterozygous, **Ww**.

One can tell a good deal, but not all, about the parents in problem 6. The phenotype of the autosomal alleles shows a 1:1 ratio, which means one of the parents was **Vv** and the other **vv**. There is no way of telling which is which for autosomal genes.

All of the males have *white-eyes*, so the mother must have been **ww**. Now we have to determine what the eye color of the father must have been. Since we have established that the mother was homozygous for *white-eyes*, all of her daughters would have received an **X** from her with **w**. Yet we are told that all of her daughters are *red-eyed*. Therefore, the daughters' other **X**, which comes from the father, must have carried the allele for red, **W**. We conclude that the father was **WY**.

Therefore the cross was either **wwvv** female × **WYVv** male or **wwVv** female × **WYvv** male.

Here are some additional problems involving the same genes.

7. These offspring were obtained:

Males: ¼ *white-long*, ¼ *white-vestigial*, ¼ *red-long*, ¼ *red-vestigial*.
Females: ½ *red-long*, ½ *red-vestigial*.

What were the genotypes and phenotypes of the parents?

8. These were the F_1 of another cross:

Males: ⅜ *white-long*, ⅜ *red-long*, ⅛ *white-vestigial*, ⅛ *red-vestigial*.
Females: ⅜ *white-long*, ⅜ *red-long*, ⅛ *white-vestigial*, ⅛ *red-vestigial*.

What were the genotypes and phenotypes of the parents?

9. In still another cross these were the F_1:

Males: ⅜ *white-long*, ⅜ *red-long*, ⅛ *white-vestigial*, ⅛ *red-vestigial*.
Females: ¾ *red-long*, ¼ *red-vestigial*.

What were the genotypes and phenotypes of the parents?

A final type of problem will involve linked genes and, hence, crossing-over. This may appeal to the more interested students.

In *Drosophila black-body* color (**b**) is recessive to *gray-body* (**B**). The locus is on the same chromosome as the *vestigial-wing* (**v**) and *long-wing* (**V**) used in the preceding problems. Bridges and Brehme (1944) place the locus of *black* at 48.5 and *vestigial* at 67, both on Chromosome 2. The difference, 16.5 units, means that crossing-over occurs in that percentage of the cases.

These crossover values, which are the basis of the genetic map, are determined experimentally. Therefore, students cannot determine the percentages of genotypes and phenotypes in the F_1 and F_2 without being told the crossover percentages.

10. Describe the F_1 and F_2 of a cross of a *black-vestigial* female and a *gray-long* male. For simplicity we will assume that the two loci are 17 units apart in the same chromosome.

Figure 51 develops the answer, line by

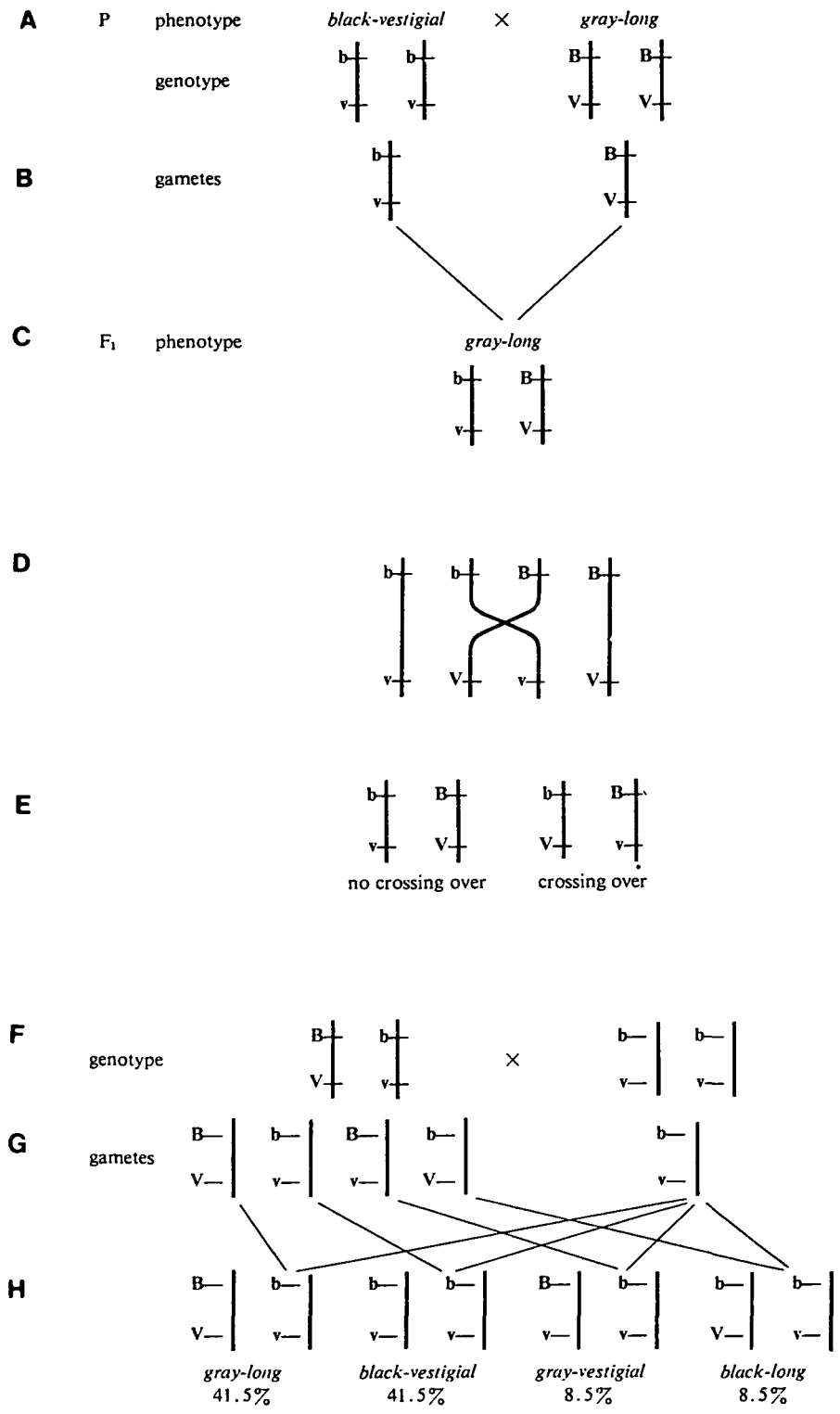


FIG. 51. Diagram of a cross involving two linked genes.

line. Line A shows the phenotype and genotype of the parents. The vertical line connecting the alleles represents a chromosome.

Line B shows the gametes produced by each parent. There is no crossing-over in the male so his sperm will be **BV**. There is crossing-over in females but since the two chromosomes are identical for the loci being followed, it will not be detected.

Line C shows the F_1 individuals—all of the same genotype and phenotype. In order to measure the amount of crossing-over, we use F_1 *females* only for further study.

Line D shows what will happen during meiosis in the F_1 female. Crossing-over occurs after the homologous chromosomes have synapsed and replicated to form 4 chromatids. The mechanics of crossing-over are such that, at any one locus, only 2 of the 4 strands will cross-over. This means that there will be 2 chromatids that do not cross-over at this locus and 2 that do. The products are shown in E. It should be obvious to your students that the chromatids resulting from crossing-over will be in equal numbers. In addition the 2 non-crossover chromatids will also be in equal numbers.

Since we know that crossing-over accounts for 17 percent of the chromatids, 8.5 percent will be **bV** and 8.5 percent will be **Bv**. The 83 percent of the non-crossover chromatids will be divided equally between **bv** and **BV**—41.5 percent of each.

In line F we take one of the F_1 females (from line C) and do a test-cross, that is, cross her with a male homozygous for both pairs of recessive alleles. In experiments involving crossing-over the female being tested is always crossed with a male homozygous for all recessive alleles. Should any of your students be flagellants, they could try to solve a variant of problem 10 by crossing two F_1 individuals to obtain an F_2.

Line G shows the gametes of the female, as we derived them in lines D and E, and the single class of sperm of the male.

Ova and sperm combine at random and the percentages of the genotypes and phenotypes of the F_2 are shown in line H.

11. In *Drosophila, ebony* (**e**) and *stripe* (**s**) are autosomal recessive genes and are parts of the same chromosome. *Ebony* makes the color of the body a shiny black. *Stripe* produces a dark line on the thorax. There is 8 percent crossing-over between them. Describe the offspring of a female heterozygous for both genes (her father was *ebony-stripe*) with an *ebony-stripe* male.

Problems of this sort are simple to make up—check any standard genetics textbook for genetic maps showing crossover distances between loci.

THE CHANGING PARADIGM

By the end of the 1930s there were no big questions remaining in transmission genetics so the emphasis switched to the difficult questions of "What do genes do?" and "What is the chemical nature of genes?" Of course there had been interest in these questions from the turn of the century but, with the techniques available, there was little possibility of obtaining any detailed answers. None of the routine technology of today such as electron microscopes, radioactive isotopes, computers, chromatography, and unbelievably sophisticated analytical instruments was available. There was no NSF, no overhead, and little external source of support for research. Laboratory assistants and post-docs were scarce. Teaching and research were accepted as of equal importance in the operations of the great universities so less time was available for research. E. B. Wilson was able to accomplish incredible research and scientific publication while carrying a teaching load that would astonish most cutting-edge biologists today.

Then, too, the fields concerned with gene structure and function—cell biology and biochemistry—had not advanced to the stage where such questions could be answered in a definitive manner.

But one ingredient for scientific discovery, in fact the *sine qua non*, was not lacking. That was brains. By the time more sophisticated techniques became available, students of the gene had established in a general way that genes control the metabolic activities of cells and that the hereditary material was probably nucleic acid. The stage was set for Watson and Crick to formalize in 1953 the next paradigm of

genetics—which shortly became the central paradigm of the biological sciences.

WHAT DO GENES DO?

Nevertheless the crude probes available before 1953 made possible important discoveries in gene function. Among the probes were those developed for studying enzymes. During the first half of the 20th century one of the most vigorous fields of cell biology and biochemistry was the study of enzymes. Enzymes were viewed as one of the major factors making life possible. The sorts of reactions that were known or suspected to occur in cells simply could not take place without these organic catalysts.

In one of those strange episodes in the history of ideas, genes and enzymes were first linked at a time when very little was known about either.

An English physician, Archibald E. Garrod (1857–1936), had a patient, a baby, with a rare disease—alkaptonuria. It was so named because the urine of patients has alkapton bodies, which consist largely of homogentisic acid. That substance becomes dark red or black when oxidized. A clue to the patient's problem was stains on its diapers (or, since the baby was British, its nappys).

Garrod knew that the baby's parents were first cousins and he wondered if alkaptonuria might be an inherited disease. In 1902 (!) he consulted Bateson, who suggested that the disease might be due to recessive alleles.

Garrod (1908*a*, 1908*b*; Harris, 1963) spoke of alkaptonuria and similar ailments as "inborn errors of metabolism." Bateson continued to be interested and wrote in 1913*a* (p. 233):

> Alkaptonuria must be regarded as due to the absence of a certain ferment which has the power of decomposing the substance alkapton. In a normal body that substance is not present in the urine, because it has been broken up by the responsible ferment; but when the organism is deficient in the power to produce that ferment, then the alkapton is excreted undecomposed and the urine is coloured by it.

The hypothesis, then, is "one allelomorph, one ferment." Thirty years later, with the terminology brought up to date, this was to become one of the most important hypotheses guiding genetic research.

Neither Garrod nor alkaptonuria is mentioned in any of the books written by the Morgan school in the years of active discovery. Even if Morgan knew of Garrod's hypothesis he may have ignored it. Morgan was so pro experimental science and anti all else—including non-experimental science—that he would have viewed Garrod's hypothesis as useless, for he had written:

> It is the perogative of science, in comparison with the speculative procedures of philosophy and metaphysics, to cherish those theories that can be given an experimental verification and to disregard the rest, not because they are wrong, but because they are useless.

Sturtevant in his history (1965*a*, p. 134) notes,

> There are other examples of a widespread failure to appreciate first-rate discoveries in genetics, and it is perhaps worthwhile to examine some of these briefly. Perhaps the most remarkable examples are the work of . . . and of Garrod on biochemical genetics . . . .
>
> Garrod was concerned with biochemical processes, and few geneticists were well enough grounded in biochemistry to be willing to make the moderate effort required to understand what he was talking about.

But possibly an important part of the answer lies elsewhere. When research programs were developing rapidly and productively, as they were for the *Drosophila* workers, there is little stimulus to look for new things to do. It was not until the 1930s, with transmission genetics satisfactorily explained, that geneticists began an intensive study of the sorts of problems that interested Garrod.

METABOLIC PATHWAYS IN CELLS

George W. Beadle (born 1903), Edward L. Tatum (1909–1975), and Boris Ephrussi (1901–1979) were leaders in the quest for information on how genes act. By the late

1930s there was considerable information about cell metabolism. That fundamental reaction of all life,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

had been resolved into several dozen separate reactions, each controlled by a specific enzyme.

The elucidation of this one metabolic pathway had required the efforts of many scientists for many years. One of the major problems was the speed of the reactions, often requiring a fraction of a second. How was one to study a reaction that would be over before the investigator knew it had started? The standard way was to use chemical substances ("enzyme poisons") that would block the action of a specific enzyme. The result would be that the substrate for that enzyme would then accumulate in the cell and could possibly be detected and identified.

Assume, for example, that one metabolic pathway in cells involves molecule A being changed into molecule B and then B into molecule C and then down the alphabet to molecule Z. We will assume that the change from A to B is controlled by enzyme A-ase and from B to C by B-ase and from Y to Z by Y-ase. All we know at first is that the cell changes molecule A to molecule Z. That is, the conversion may be accomplished by a single enzyme in a single reaction.

One of the first enzyme poisons we try is cyanide. We observe that no Z is formed and, instead, a previously undetected molecule, M, is found. What can we conclude? Can we say that the cell converts A to Z in two steps: A is converted to M and then M to Z? That may have been said a few generations earlier but, as the complexity of intracellular metabolism came to be understood in the 1930s, the conclusion would be no more than "there are at least two intermediary steps from A to Z."

Other poisons could be tried, and with time more and more could be learned about normal metabolism by throwing these chemical wrenches into the biochemical gears of the cell.

Some early studies of Beadle and Ephrussi on the way that eye color genes of *Drosophila* produce their effects had indicated that gene action might be mediated by enzymes. Enough was discovered to suggest that the hypothesis "one gene, one enzyme" might be a fruitful approach. The biochemistry of *Drosophila* proved to be too complex to test that hypothesis and for the first time that noble animal let a geneticist down.

So a long-standing experimental technique was invoked: if the experiment cannot be done with one organism, search for another one that is suitable. By this time Beadle was at the California Institute of Technology with Morgan. Before Morgan left Columbia, Bernard Dodge of the New York Botanical Garden gave him a culture of the red bread mold, *Neurospora crassa*, in the belief that it might be of use in genetic experiments. Morgan never used *Neurospora* but it was still being cultured in his laboratory when Beadle and Tatum sought an organism for their research.

NEUROSPORA CRASSA

Beadle and Tatum (1941) reasoned that lethal mutations change alleles so that they are incapable of producing an enzyme essential for the life of the organism. Thus they intended to induce lethal mutations with radiations and to study their biochemical effects. This might appear to your students to be a considerable problem since, if the lethal kills the individual, there would not appear to be much to investigate. But Beadle and Tatum solved that problem in what was surely one of the most innovative and productive lines of experimentation in the late 1930s and 1940s. Others must have thought so too because Beadle and Tatum shared a Nobel Prize for this work.

For reasons that will shortly become apparent they first had to determine exactly the minimum variety of molecules required for normal growth—the minimal medium. The menu was surprisingly simple: air, water, inorganic salts, sucrose, and the vitamin biotin. *Neurospora* is, of course, composed of innumerable organic compounds, all interacting as the life of that organism. Yet from those few raw materials it is able to synthesize all of the amino acids, proteins, fats, carbohydrates, nucleic

acids, vitamins, and other substances of its body.

As an example of the many experiments done by Beadle and Tatum, we will discuss those concerned with the synthesis of the amino acid arginine. The working hypothesis was that specific genes control the production of specific enzymes that catalyze the reactions that lead to the formation of arginine. Presumably these genes could mutate to allelic forms that would either be unable to make the enzyme or not be able to make it in sufficient quantity. Since arginine is essential for the life of *Neurospora,* such mutations would be lethal.

Beadle and Tatum then devised a method for the production of these lethal mutations, for identifying them as related to the synthesis of arginine, and for maintaining them in culture in order to work out the metabolic pathway of arginine synthesis. This may sound impossible, especially when we realize that for most of its life cycle *Neurospora* is monoploid and hence any lethal mutations could not be carried as heterozygotes.

This was their game plan. First, X-rays were used to induce mutations. They assumed that all sorts of mutations would be produced, but by chance some might be involved with the production of arginine. When we remember how rare any specific mutation would be, the chance of obtaining the desired mutations would be exceedingly small.

Spores from the irradiated *Neurospora* were then placed on the minimal growth medium. Most of them grew, showing that whatever mutations may have occurred none was so serious as to prevent the *Neurospora* from synthesizing all of its substance from the few chemicals in the minimal medium. Other spores did not germinate, and among these might be some biochemical mutants that could not produce the enzymes necessary for normal growth and development. And somewhere among them might be genes involved in the synthesis of arginine. How could one find them? The spores were not germinating, so they were for practical purposes "dead."

The solution of this apparently insolvable difficulty was elegant in its simplicity and effectiveness. If the spores could not grow because they could not synthesize their own arginine, why not give it to them? And that is precisely what Beadle and Tatum did. Again most of the spores did not grow but a precious few did. Among these precious few might be mutants of genes involved in arginine synthesis.

The next, and critical, step in the analysis was to make sure that whatever was wrong with the spores was inherited. It could not be concluded that, just because the otherwise "lethal" spores could grow on arginine, that a mutational event was the cause.

The life cycle of *Neurospora* makes it ideal for some sorts of genetic analysis. The colonies are monoploid for nearly their entire life. There are two mating types, *A* and *a,* which cannot be distinguished except by their mating behavior. If colonies of *A* and *a* are grown together, parts of each will fuse and *A* nuclei will unite ("fertilize") with *a* nuclei to form diploid zygotes. Meiosis occurs immediately and 4 monoploid spores are formed. These divide, by mitosis, to produce 8 monoploid spores. These 8 spores are enclosed in an elongate spore sac (ascus). They are arranged in the sac in a linear order that reflects the two meiotic divisions and the single mitosis. The spore sacs can be opened under a microscope and the individual spores removed and placed in culture media. Thus one can obtain all of the products of meiosis of a single zygote.

The presumed mutant strains were crossed to normal strains. Meiosis occurred immediately afterwards and monoploid spores were formed. These were then isolated. Half were found to grow on the minimal medium and half only if arginine was added. These results were consistent with the hypothesis that the wild-type *Neurospora* had a gene **A**, which was necessary for the synthesis of arginine. The radiation treatment had caused a mutation of **A** to **a** and **a** was unable to play some essential role in arginine synthesis.

The experimental procedure appeared to be working and numerous genetic strains were isolated that required arginine for

growth. Were all the genetic strains alike or had different genes mutated to alleles that could not synthesize arginine? Can your students suggest how one could go about answering that question?

There were two possible answers:

First, all of the mutant strains could be due to changes at a single gene locus.

Second, many different loci could have mutated. In this case one would suspect that many genes are involved in arginine synthesis: $\mathbf{A}_1$, $\mathbf{A}_2$, $\mathbf{A}_3$, $\mathbf{A}_x$, etc. Any one of these could have mutated to $\mathbf{a}_1$, $\mathbf{a}_2$, etc. In all these mutants the same phenotype would be observed—inability to grow on minimal medium without arginine.

Crosses could test the alternatives. If a single locus is involved, a cross of two strains would produce spores unable to grow without arginine. Alternatively, if different loci are involved, some of the spores will grow as wild-type colonies for the following reason. Assume that different genes are involved and we are crossing $\mathbf{a}_1 \times \mathbf{a}_2$. If a mutation had occurred at only one locus in each strain, which is overwhelmingly probable (why?), the mutated strain would have a normal allele at the other locus. Thus, mutant strain $\mathbf{a}_1$ would be expected to have $\mathbf{A}_2$. Strain $\mathbf{a}_2$ would be expected to have $\mathbf{A}_1$. Thus a cross of $\mathbf{a}_1\mathbf{A}_2 \times \mathbf{A}_1\mathbf{a}_2$ would produce diploid zygotes with a genotype $\mathbf{A}_1\mathbf{a}_1\ \mathbf{A}_2\mathbf{a}_2$. Meiosis then occurs and the monoploid spores are produced. If the two loci are on different chromosomes the isolated spores should give these results:

- ¼ should be $\mathbf{A}_1\mathbf{A}_2$ and grow on minimal medium.
- ¼ should be $\mathbf{A}_1\mathbf{a}_2$ and will require arginine since $\mathbf{a}_2$ cannot function.
- ¼ should be $\mathbf{a}_1\mathbf{A}_2$ and require arginine since $\mathbf{a}_1$ is not functioning.
- ¼ should be $\mathbf{a}_1\mathbf{a}_2$ and require arginine since neither allele can function.

If the loci are on the same chromosome, the frequency of the four genotypes will depend on the amount of crossing-over.

Early on in the experiments, Beadle and Tatum discovered seven genetically different mutants, each requiring supplemental arginine if it was to grow normally. Various interpretations of the data were possible but Beadle and Tatum preferred the hypothesis that the synthesis of arginine required that at least seven normal genes be present—each producing an essential enzyme. When any one of these genes mutated in such a way that its specific enzyme could not be produced, the synthesis of arginine was blocked. There was no reason to believe, of course, that there are only seven steps in the synthesis of arginine in *Neurospora*. We can conclude only that seven was the minimum number.

It was possible to extend the analysis by taking advantage of what was already known about the synthesis of arginine. In 1932 the biochemist Hans A. Krebs had discovered that in some vertebrate cells arginine is formed from citrulline, citrulline from ornithine, and ornithine from an unknown precursor. A specific enzyme is required for each transformation.

If *Neurospora* has a similar metabolic pathway, one should be able to determine how the seven mutant strains are involved. This could be done by seeing which, if any, of the seven would grow if either citrulline or ornithine was used to replace arginine. Your students should be able to predict what conclusions could be drawn if a mutant strain, normally requiring supplemental arginine, would grow if citrulline was subsituted or if ornithine was substituted.

Many experiments were done. Four of the mutant strains would grow if either ornithine, citrulline, or arginine was added. This suggested that these four mutants were involved in reactions before the ornithine stage. If ornithine was added, the remaining enzymatic steps, being normal, could carry the reactions to arginine.

Two of the strains would not grow if only ornithine was added but they would grow if either citrulline or arginine was added. In these cases the block was between ornithine and citrulline. Since two genetically different strains were both blocked between ornithine and citrulline, it is reasonable to conclude that there are at least two steps between these molecules.

Finally, one strain was found that would

grow only if arginine was added. This suggests that some enzyme between citrulline and arginine was deficient or defective.

Thus, Beadle and Tatum were able to conclude that, for *Neurospora* to synthesize arginine, a minimum of seven enzyme-controlled reactions are required and a minimum of seven kinds of molecules are involved. Two of these are known: ornithine and citrulline.

The hypothesis that a function of genes is to control the production of specific enzymes was supported. One could not conclude that this is the only thing genes do. Beadle and Tatum had designed their experiments solely to detect enzymes involved in metabolic pathways.

Much as Sutton had linked cytology and genetics in the early 1900s, Beadle and Tatum effectively linked genetics and biochemistry in the early 1940s. Their type of experimentation was used immediately by numerous other investigators on other molds, yeasts, and bacteria. This approach led directly to the molecular biology of today.

While all this was going on still another attempt to study genetics at the molecular level was underway. This was a line of investigation that began in the 1920s and ultimately led to the positive identification of the gene as DNA. That will be our final topic, bringing us to the formulation of the current paradigm of genetics by Watson and Crick in 1953.

THE SUBSTANCE OF INHERITANCE

The dynamics of scientific discovery elude us to this day. There is no way of predicting the who?, the what?, and the where? Important discoveries are nearly always made by scientists active in the field. The breakthrough may be made by an outstanding scientist or by a novice. Neither Mendel, Sutton, Morgan, Watson nor Crick was a leader in the field of inheritance to which each made such notable contributions. The revolution in biology that followed from Watson and Crick (1953*a*, 1953*b*) was due in part to scientists from other fields (mainly physics) deciding that the problems in biology were more exciting than their own (Fleming, 1968; Judson, 1979). Many prominent molecular geneticists of today remember being made aware of new possibilities for genetic research by a slender book written by Schrödinger (1945), himself a physicist.

It could be that it is easier for those not steeped in the data and traditions of a field to see problems and solutions clearly than for those fully engaged in their Kuhnian normal science. As Hanson says (1965, p. 30):

> Physical science is not just a systematic exposure of the senses to the world; it is also a way of thinking about the world, a way of forming conceptions. The paradigm observer is not the man who sees and reports what all normal observers see and report, but the man who sees in familiar objects what no one else has seen before.

Some important discoveries are the outcome of deliberate attempts to find answers to specific questions. In other cases discovery is more of an accident. The elegant experiments of Beadle and Tatum are examples of experiments planned to test a specific hypothesis. The road to DNA was not nearly so straight. The zero milestone cannot be identified but we can start in 1928 with some observations in another field that were to lead, a quarter of a century later, to the description of the chemical structure of DNA.

TRANSFORMATION IN PNEUMOCOCCUS

Pneumonia in human beings and many other mammals is caused by the pneumococcus bacterium (properly known as *Diplococcus pneumoniae*). As in many disease-causing microorganisms, there are numerous genetic strains. These are called Type I, Type II, etc. The specificity is based on the chemical composition of the bacterium's polysaccharide coat. The strains are identified immunologically. If they are injected into rabbits, antibodies are formed against the polysaccharide antigens.

If capsulated cells are grown on culture plates, they form colonies that are *smooth* and shiny. Some of the colonies may have

a different appearance—they are *rough.* These changes were observed long before the cause was known—the change from *smooth* to *rough* is the result of a gene mutation. There was considerable medical interest in this phenomenon because the *smooth* cells cause pneumonia but the *rough* mutant does not. It was discovered that the *smooth* cells have the polysaccharide capsules but the *rough* cells do not.

The road to DNA begins in 1928 with F. Griffith, a Medical Officer with the British Ministry of Health. His publications give no evidence of an interest in genetics; he was a medical bacteriologist concerned with diseases of human beings. He knew that if he injected mice with capsulated Type II *smooth* (capsulated) cells, they would die. Type II *rough* (non-capsulated) cells would not cause the death of his mice. However, heat-killed *smooth* cells did not kill the mice. Therefore, it was not the polysaccharide coat that was the cause of death.

The next experiment is the crucial one for us. Griffith gave four mice a double injection of Type II cells: living *rough* cells plus dead *smooth* cells. Survival was expected, since the *rough* cells are not pathogenic and the pathogenic *smooth* cells had been killed. Nevertheless, all four mice died after five days. Type II *smooth* cells were found in their blood. Thirty control mice injected only with living *rough* cells remained healthy.

This was an unbelievable result—but the experiment was repeated and confirmed. It appeared that the ability to synthesize a capsule had been transferred from the dead capsulated cells to the living non-capsulated cells. Any geneticist of 1928 who might have known of these experiments would have shuddered and rededicated himself to *Drosophila melanogaster.*

During those years geneticists ignored microorganisms almost entirely and microbiologists ignored genetics. It was not suspected by either group that microorganisms possessed a genetic system remotely similar to that of higher organisms. Joshua Lederberg, who as a young student worked in the Zoology Department at Columbia University and who was to find that "adaptation" in bacteria is a mutational event, was far in the future.

A later generation of geneticists might have suspected that a mutation from *rough* to *smooth* had occurred but another experiment by Griffith showed this not to be so. This time the living and the dead cells were of different Types. The living cells without capsules (*rough*) were Type II and the killed cells with capsules (*smooth*) were Type I. Eight mice were injected and two died. Their blood was found to contain virulent capsulated cells of Type I. Somehow the Type II non-capsulated cells had been transformed to Type I. This was not a transitory change. They were cultured and thereafter remained Type I. The change was permanent, and hence in a broad sense genetic. In today's terms we also might suspect the transformation to virulence to be due to mutation. But this second experiment rules out that possibility since, had the living Type II cells mutated from capsule-less to capsulated, they would still have been Type II. However, the capsulated cells were like the dead cells, Type I.

This line of research was taken up by many bacteriologists, including M. H. Dawson and Oswald T. Avery of the Rockefeller Institute in New York. They became convinced that transformation must be due to some chemical substance and it was reasonable to suspect the polysaccharide of the capsule. Nevertheless that proved not to be so. Alloway, another member of the Rockefeller group, summed up the problem in 1932 as follows (with my paraphrasing):

> The polysaccharide when added in chemically purified form, has not been found effective in causing transformation of non-capsulated organisms derived from *Diplococcus* of one Type into capsulated forms of the other Type. When non-capsulated cells change into the capsulated form they always acquire the property of producing the specific capsular substance. The immunological specificity of the encapsulated cell depends upon the chemical constitution of the particular polysaccharide in the

capsule. The synthesis of this specific polysaccharide is a function peculiar to cells with capsules. However, since the non-capsulated cells under suitable conditions have been found to develop again the capacity of elaborating the specific material, it appears in them this function is potentially present, but that it remains latent until activated by specific environmental conditions. The fact that a non-capsulated strain derived from one Type of *Diplococcus,* under the conditions defined in this paper, may be caused to acquire the specific characters of the capsulated forms of a Type other than that from which it was originally derived, implies that the activating stimulus is of a specific nature.

There is nothing in this quotation, or in the writings of other bacteriologists of the period, to suggest that transformation might be a genetic phenomenon. It seemed more probable that some sort of physiological modification had occurred. Many bacteriologists at the time suspected that some sort of Lamarckian evolution was responsible for this phenomenon known as "adaptation." It was much later that it was found that mutation and selection would account for the phenomena observed.

DNA Is the Transforming Substance

But if "the activating stimulus is of a specific nature," hard work and luck might discover what it is. It was found that the transforming principle could be extracted from capsulated cells and that transformation could occur *in vitro*—no need that mice be used. After a decade Avery, MacLeod, and McCarty (1944) reported that they had purified the transforming substance and that it was almost certainly DNA. The overall elemental composition of the transforming principle agreed closely with that of DNA. The molecular weight was judged to be about 500,000. The substance was highly active—one part in 600 million was effective. Treatment with trypsin and chymotrypsin left activity intact indicating that it was not protein. Ribonuclease, which denatures RNA, was also without effect. However, a then available crude deoxyribonuclease destroyed the activity of the purified transforming substance.

What does this all mean? This is how Avery, MacLeod, and McCarty interpreted their experiments (see also McCarty, 1985):

> Various hypotheses have been advanced in explanation of the nature of the changes induced. In his original description of the phenomenon Griffith suggested that the dead bacteria in the inoculum might furnish some specific protein that serves as a 'pabulum' and enables the [non-capsulated] form to manufacture a capsular carbohydrate.
>
> More recently the phenomenon has been interpreted from a genetic point of view. The inducing substance has been likened to a gene, and the capsular antigen which is produced in response to it has been regarded as a gene product. In discussing the phenomenon of transformation Dobzhansky has stated that "If this transformation is described as a genetic mutation—and it is difficult to avoid so describing it—we are dealing with authentic cases of induction of specific mutations by specific treatments . . . ."
>
> It is, of course, possible that the biological activity of the substance described is not an inherent property of the nucleic acid but is due to minute amounts of some other substance adsorbed to it or so intimately associated with it as to escape detection. If, however, the biologically active substance isolated in highly purified form as the sodium salt of deoxyribonucleic acid actually proves to be the transforming principle, as the available evidence strongly suggests, then nucleic acids of this type must be regarded not merely as structurally important [at the time biochemists could not discover any function for the nucleic acids] but as functionally active in determining the biochemical activities and specific characteristics of [the bacterial]

cells. Assuming that the sodium deoxyribonucleate and the active principle are one and the same substance, then the transformation described represents a change that is chemically induced and specifically directed by a known chemical compound. If the results of the present study on the chemical nature of the transforming principle are confirmed, then nucleic acids must be regarded as possessing biological specificity the chemical basis of which is as yet undetermined.

Was DNA only an inducing agent or was it something else? Most geneticists would probably have agreed with Dobzhansky that DNA could not be the genetic material. The evidence was fairly convincing. Enough was known about DNA to realize that it was a rather simple molecule—composed of a few bases, a simple sugar, and phosphate. Presumably an extremely complex substance would be required to control the life of cells. Proteins were a far more likely candidate than DNA to be the gene. They could be huge and were composed of a number of amino acids about equal to the number of letters in our alphabet. Just as the combinations of a few letters can give us the uncounted numbers of words in the languages of the world, that same number of amino acids should be adequate to supply all the genetic variation required.

CORE OR COAT?

The answer came in less than a decade: DNA *is* the gene, not a mutagenic agent. One of the more important experiments was done in 1952 by A. D. Hershey and Martha Chase. By that time much more sophisticated experimentation was possible. In large part as a result of the work on the atom bomb in World War II many sorts of radioactive substances had been produced that could be used to study intracellular reactions. Methods were developed for culturing many different sorts of microorganisms and, for many reasons, they were becoming the favorite experimental organisms for geneticists. There was also very much more research being done. The extraordinary contributions of scientists to the war effort were recognized in Washington and the work of scientists began to be supported on a lavish scale. It was estimated that in the 1950s the number of active scientists was equal to all the scientists who had ever lived. Big Science was national policy and a national activity.

Hershey and Chase took advantage of the peculiar life cycle of bacteriophage to ascertain whether or not DNA contains the information for that organism. Bacteriophages, or phages, are incapable of an independent life. They are parasites of bacteria, upon which they depend for their own reproduction.

If the bacterium *Escherichia coli* is infected with a phage called T_2, the bacterium is killed in about 20 minutes. Before entrance of the phage, the bacterial cell was synthesizing its own specific molecules: bacterial proteins, bacterial nucleic acids, and so on. The phage changes all this. It assumes control of the bacterial synthetic machinery and diverts it to producing phage molecules instead of *E. coli* molecules. About 100 phages are made in about 20 minutes. The bacterium bursts and liberates the phages. They can then enter (they must if they are to live and reproduce) other bacterial cells and repeat the process.

There are many kinds of phages that maintain their genetic identity and other specific characteristics. Structurally they are simple, being composed of a protein coat and a DNA core. The protein of the phage coat is chemically very different from the DNA core. The coat contains sulfur but little or no phosphorus. The reverse is true for DNA. Radioactive isotopes of both phosphorus and sulfur were available to Hershey and Chase.

The experiment was as follows. One group of bacteria was grown in a medium with ^{32}P, which became incorporated in the bacterial molecules. Later, phages were introduced. When the bacteria then began to synthesize new phages, the latter's DNA became tagged with the ^{32}P. The protein coat would have little or no label.

In a parallel experiment bacteria were grown in a medium containing ^{35}S. This became incorporated in some of the bac-

terial proteins. Later phages were introduced and in this case the protein coats of the phages became labelled with ^{35}S.

These two sorts of phages, one labelled for the protein coat and the other for the DNA, were then used in separate experiments. They were introduced into cultures of bacteria and Hershey and Chase found that the labelled DNA entered the bacterial cells. The labelled protein remained on the outside. These observations, together with others, suggested that the phage attaches itself to the cell wall of the bacterium and injects its DNA core, the coat remaining on the outside.

The phages in both experiments reproduced and destroyed the bacterial cells. The experiments had shown that the entire genetic information on "how to make phage" is contained in the phage DNA.

The work surveyed in this chapter, together with a very much larger amount going on at the same time, leads to this tremendous thought: the once mysterious gene, which though invisible could be mapped and followed through the generations with precision, is revealed as an identifiable molecule—DNA. Just as E. B. Wilson had said in 1895.

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THE END

This essay, as part of the symposium *Science as a Way of Knowing—Genetics,* has sought to provide a background for the papers by the symposium speakers and to provide materials for teachers using the science as a way of knowing approach. For the most part, the speakers will be dealing with events that occurred after 1953. These events have been so staggering in their importance and different in their problems and procedures that we must recognize that a new paradigm now guides the investigators.

The old paradigm of the Chromosomal Theory of Heredity, or transmission genetics, held the attention of geneticists to the mid-1930s but by then it was so well established that geneticists sought new challenges. It was during the 1930s and 1940s the groundwork was laid for an attack at the molecular level on what genes are and what they do. Molecular genetics is very different from classical genetics, which is the concern of this essay.

And that raises a difficult problem for what should be taught in the first-year biology course in the colleges and universities when the time available is severely limited. Can Mendel, Sutton, and Morgan hold the attention of students who live in a world where genetic engineering is about to perform its miracles? Should students be taught about these classical experiments and concepts?

I think they should and there is no need

for an either/or structuring of the curriculum. The basic argument of the *Science as a Way of Knowing* approach is that students are best served if they are provided with the conceptual framework of the field. Full appreciation of the events of today is possible only if that conceptual framework is understood.

There is a practical matter also. Few students in first-year courses have the background necessary to understand the tremendously sophisticated experiments and data of modern molecular genetics. In many instances they may be able to *memorize* the material but I am talking about something else—*understanding*. Classical genetics, on the other hand, is approachable to a considerable degree by students in first-year courses. They really can understand the questions, the data, and the reasons for the conclusions. This is another of our goals—having students understand how science works.

Nevertheless we serve our students poorly if we leave them ignorant of the general results and especially the implications of the science of the day. My recommendation, therefore, is to emphasize classical genetics and then discuss the main conclusions of molecular genetics, stressing its implications for better health and better food. And, most certainly, there should be consideration of some of the more difficult ethical questions that are being raised by molecular genetics.

Remember also that everything does not have to be included in a first-year course. Something of importance and interest should be left for the more advanced courses. Biologists, alone among scientists, seem to believe that all the cream has to come that first year. It really does not.

My suggestions may not have much appeal for some university scientists for according to Sydney Brenner (*Nature* 317: 209, 1985):

> For most young molecular biologists, the history of their subject is divided into two epochs: the last two years and everything else before that. The present and very recent past are perceived in sharp detail but the rest is swathed in a legendary mist where Crick, Watson, Mendel, Darwin—perhaps even Aristotle—coexist as uneasy contemporaries.

Too bad, if so. We have to do better for our students.

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