

## **Flatworm Orgies: Just a Day in the Life of *Macrostomum lignano***

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Janicke T, Scharer L (2009). Determinants of mating and sperm-transfer success in a simultaneous hermaphrodite. *Journal of Evolutionary Biology* 2:405-415.

Flatworms (phylum Platyhelminthes) are hermaphrodites that participate in reciprocal mating. According to sex allocation theory, the allocation of reproductive resources to a single sex function (i.e. male or female) must show diminishing returns in order for hermaphroditism to be favored over dioecy (separate sexes). In such a case, each additional input of resources into one sex function would result in less and less of a reproductive gain, so that the animal would benefit from transferring those resources instead to the other sex function. Two of the major factors affecting these costs and benefits are the number of potential mates and the reproductive gain per mate. However, there has been little research regarding these or other factors thought to be involved in sex allocation. Janicke and Scharer (2009) studied the effects that social group size, density and morphology have on both the number of mates and on successful sperm transfer in the flatworm *Macrostomum lignano*.

To examine the effects of social group size and density on sex allocation, the researchers set up experimental mating cultures that held 2, 3, 4, 8, or 16 individuals in small and large culture containers. The body of this species is completely transparent, so that morphological characteristics could be measured non-invasively after mating. To keep track of sperm transferred during the experiment, the researchers marked focal worms with a halogenated pyrimidine that would stain the sperm at spermatogenesis. Each culture, regardless of group size, contained 1 focal worm. Worms were allowed to mate over a 24 hour period and were then fixed for later analysis. The number of mates per social group was determined by counting the number of worms that contained sperm from the focal worm. This number was a conservative estimate of the number of matings because it is possible that an act of non-reciprocal mating occurred, or that cryptic female choice took place in which the female chooses to control paternity by eliminating sperm. Sperm transfer success was estimated from the average number of sperm the focal worm transferred to each mate, as well as the average total amount of sperm transferred to all mates in its group.

The experiment showed an increase in the number of mates as social group size increased, as was predicted by Janicke and Scharer (Fig 1a). In most social groups, some worms mated with all individuals present, except in the largest (maximum of 10 mates in the groups of 16), suggesting the presence of a limit on the number of mates an individual could have. Among the morphological traits they studied, testis size was the only character that correlated significantly with number of mates and sperm-transfer success: those with larger testes obtained more mating partners, and had higher rates of successful sperm transfer (Fig. 1b). Density had no significant effect on the number of mates (Fig. 1a), which was expected by the researchers since density is not known to affect sex allocation in worms. As for sperm-transfer success, focal worms in the larger social groups had a lower mean sperm-transfer rate than those in smaller social groups (Fig. 1c), while male-biased individuals (larger testes and smaller ovaries) had a higher transfer rate. Unexpectedly, the average total number of sperm transferred ( $13.3 \pm 1.3$  sperm) did not vary significantly among social groups or densities (Fig. 1d), although male-biased individuals once again transferred a higher number of sperm. Finally, sperm transfer success was negatively related to ovary size, suggesting a tradeoff that leads to a loss in female function for gains in male function.

This study raised several interesting questions and ideas. First, sex allocation theory suggests that simultaneous hermaphroditism should involve a small average number of mates because of the high degree of competition among sperm, an expectation that was violated by the common observation of a high level of multiple matings. Second, this is the first study to show evidence of a correlation between increased social group size and sperm competition resulting from the increase in the number of mates. Furthermore, increased testis size corresponded positively to a higher amount of sperm transferred to a mate, and an increase in the number of mates. This implies that sex allocation to the male function (male-biased individuals) results in higher reproductive success as evidenced by the increased number of mates and higher sperm-transfer success rates. Third, the study demonstrated diminishing returns of investment in male function at larger group sizes, especially given the cost to female function. Other questions raised by the research include why focal worms in larger social groups transferred less sperm on average, and why the total amount of sperm did not differ

between social groups, as well as what determined the maximum number of mates an individual can have. Additional tests could be done to show the effects of sex allocation on changes in mating behavior, as well as how changes in genital morphology influence the number of mates and the success of sperm transfer.

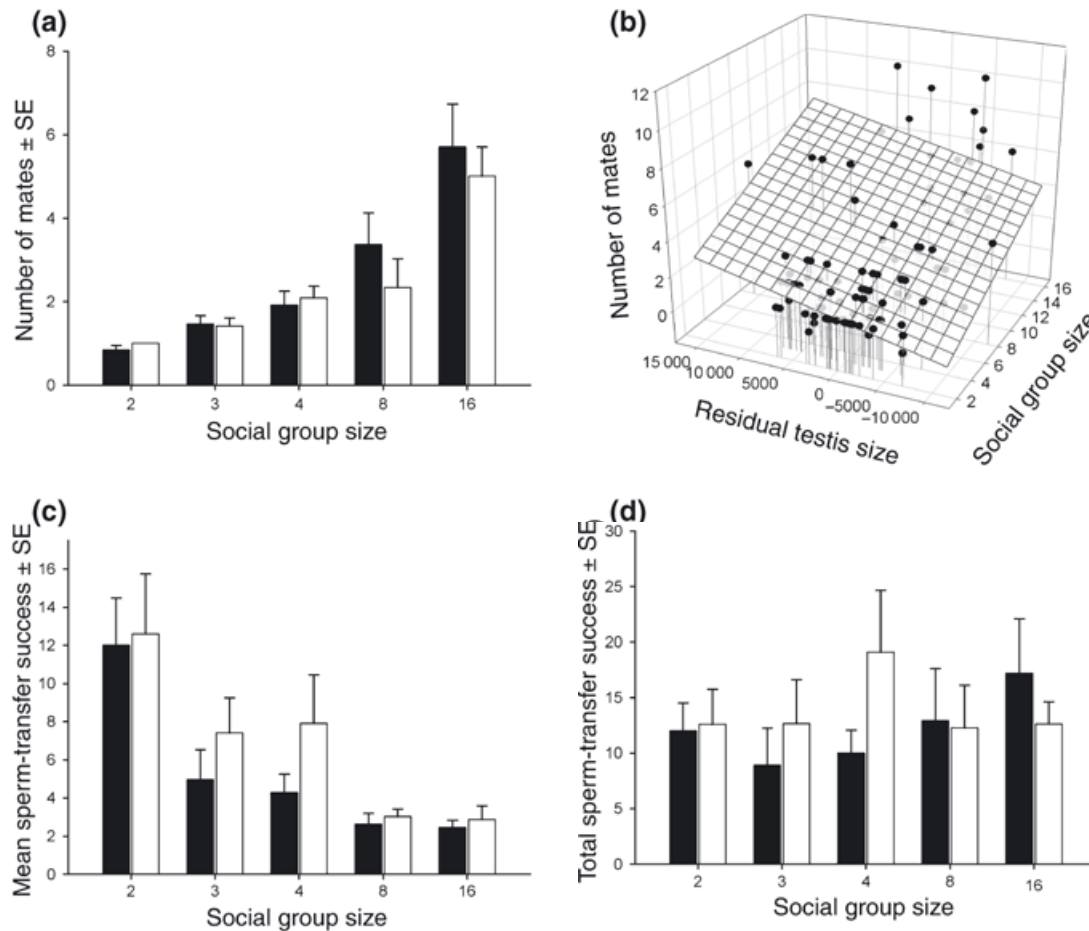


Fig. 1. Effects of social group size and density on the number of mates (a), mean sperm-transfer success (c) and total sperm-transfer success (d) and the effect of morphology of sperm donors on number of mates (b). In the bar plots filled and open bars refer to the high- and low density treatment respectively. The plane grids in the three-dimensional scatter plots represent linear regression fits of both explanatory variables. (Adapted from Janicke and Scharer 2009).