Dispatches

Evolution: Don't Be So Butch, Dear!

Simultaneous hermaphrodites are both male and female, which could lead to conflicts between partners over optimal investment to the two sex functions. New evidence from hermaphroditic freshwater snails suggests that transferred seminal fluids affect the partner's male function.

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For many, male and female sexes imply two classes of individuals, namely males and females, but this familiar sexual system is far from universal. Most plants and a striking diversity of animals are hermaphroditic [1], uniting the two sexes in the same individual either sequentially or simultaneously. Being male and female at the same time, an individual simultaneous hermaphrodite decides economically about how it allocates its limited reproductive resources between its own male and female reproductive function [2,3], as, for instance, observed in barnacles and flatworms [4,5]. When growing up in small groups these creatures often invest little in sperm production, thereby achieving a higher egg production. And conversely, they tend to increase sperm production under more competitive conditions, often at a cost to their own female fitness. While such phenotypically plastic sex allocation permits individuals to maximize reproductive success in changing environments, it also has the potential to lead to sexual conflicts between the mating partners over this sex allocation decision [3,6,7]. A new study by Yumi Nakadera, Joris Koene and colleagues [8], published in this issue of *Current Biology*, now shows that hermaphroditic great pond snails (*Lymnaea stagnalis*) indeed transfer substances in the seminal fluid that strongly affect the male function of their partners.

First, Nakadera and colleagues [8] show that the receipt of experimentally assembled and artificially inseminated concoctions of sperm and/or seminal fluids leads to a clear effect on the number of sperm a recipient transfers as a sperm donor in a staged mating the next day. Specifically, receiving only seminal fluid reduces the number of transferred sperm to about half, while receiving only sperm had no effect compared to control inseminations with saline (when receiving both sperm and seminal fluid the results were similar but more complex). Second, the authors show that this effect is also observed in natural matings, both in terms of the amount of sperm transferred and the proportion of offspring sired. Notably, the effects materialized very quickly, affecting reciprocated matings that happened with the same individual within just 1.5 hours after initial sperm receipt. Thus, receipt of seminal fluid has rapid (hours) as well as short-term (days) effects on an individual's male function.

Arguably the most impressive achievement of the study on this non-model organism is that — in a veritable *tour de force* — the authors have identified two seminal fluid proteins that are primarily responsible for the observed reduction in the number of sperm transferred. While a role for seminal fluids and/or accessory gland products in hermaphrodite mating interactions has been suggested for a range of different animals, such as earthworms [9,10], land snails [11,12] and flatworms [13], only in this freshwater snail have substantial inroads been made into uncovering the specific substances involved in these interactions. Using HPLC the seminal fluid was fractionated into eight purified seminal fluid proteins [14] and their effects on a recipient's sperm transfer tested separately for each, again using artificial insemination. Two of these proteins, LyAcp5 and LyAcp8b (*Lymnaea* Accessory gland proteins), when injected led to a significant and substantial reduction in the amount of sperm transferred in subsequent matings. Given the limited sample sizes achieved in this screen and fairly conservative corrections for multiple testing, it would, however, be prudent not to prematurely exclude some of the other proteins from further consideration.

The current study represents a major step forward in our understanding of hermaphrodite mating interactions, by being the first to document an effect of the receipt of seminal fluid components on the male function of a hermaphrodite, and thus lending support for the intriguing suggestion that Eric Charnov [6] made over thirty years ago, namely that the partner's male function could be a target of sexual conflict. To see why, let's take the perspective of a simultaneously hermaphroditic sperm donor: once I have myself received enough sperm to fertilize my own eggs, any resources that the partner I inseminate invests in its male side (such as making and transferring sperm and other seminal components) could negatively affect my own fitness. This can occur in at least four different scenarios (Figure 1), some of which entail that hermaphrodites often mate reciprocally, i.e. often receive sperm from individuals to which they give sperm: first, my partner's male investment represents a lost opportunity, because by making many sperm it makes fewer eggs that I could fertilize (Figure 1A); second, whenever receiving (too much) sperm is costly for me and mating interactions involve reciprocity, then by trying to give my partner sperm I run a risk of receiving (too much) sperm myself (Figure 1B); third, if it makes a lot of sperm my partner is also more likely to donate sperm to others [15,16], and thus may also receive additional sperm from those, which will then compete with my sperm for my partners eggs (Figure 1C). And finally, if it makes (or transfers) a lot of sperm my partner is a stronger competitor for fertilizations of the eggs in other individuals that I also inseminate (Figure 1D). So clearly I have a range of reasons to want to steer my partner away from his male side.

But which scenario seems most likely to be relevant in *Lymnaea*? It is important to acknowledge that all scenarios assume that the altered sperm transfer (and paternity success) occurs at a cost to the sperm recipient, which, as the authors admit [8], cannot be concluded based on the current

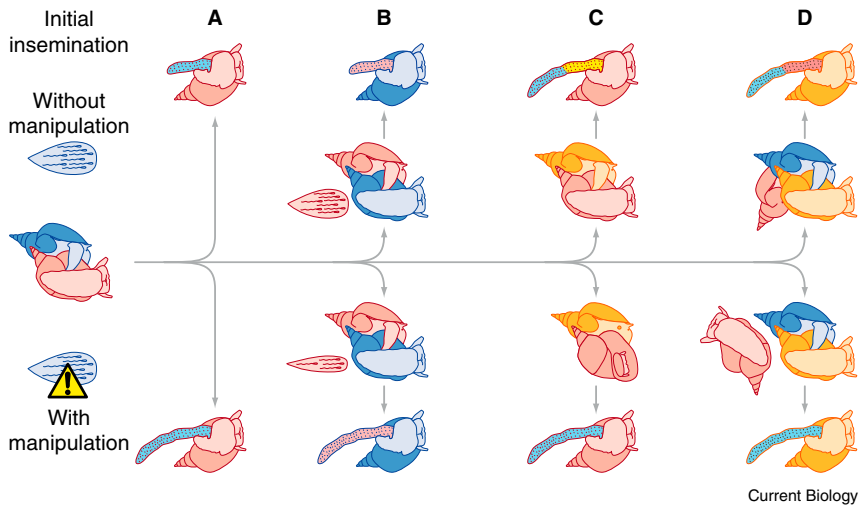

Figure 1. Four scenarios for benefits resulting from seminal fluid-mediated manipulation.

An initial insemination of the focal individual (blue) into its recipient (red) can either occur without (top) or with (bottom) the transfer of manipulating seminal fluid proteins. (A) Manipulation of the recipient's sex allocation, leading to a higher number of eggs fertilized (red lays larger clutch sired by blue); (B) reduced receipt of costly sperm/ejaculate following reciprocation, leading to a higher fecundity of the focal; (C) reduction of the recipient's sex drive protects one's own paternity in that recipient from another sperm donor (orange); and (D) reduction of the recipient's sex drive to protect one's paternity in another recipient (orange). See main text for details (illustration by Dita B. Vizoso).

data. To show fitness costs for the receipt of seminal fluids a more complete accounting of male and female fitness effects over longer time periods would be needed, which seems difficult to achieve using the current experimental paradigm. But even if we were to assume that the interaction involves conflict, it is clearly worthwhile to think about some of the conditions that would have to be fulfilled for the different scenarios to work, which may help guide future research in this system.

The first scenario (Figure 1A) requires that male and female allocations actually compete for the same investment pool — that there is a resource allocation trade-off — which in my opinion has not yet been shown convincingly for this species [3]. The observed patterns could either result from a disruption of the male function, which — assuming that this actually prevents male allocation — could lead to a higher female allocation, or alternatively, it could involve a stimulation of the female function, thus drawing resources away from the male side. Given that here only effects on the male function were assessed, these two possibilities cannot currently be distinguished, as acknowledged by the authors [8]. Moreover, it is interesting to note that one of these seminal fluid proteins, LyAcp10 (also called Ovipostatin [14]) — which here actually caused the second biggest reduction in sperm transfer (albeit not reaching statistical significance after correction for multiple testing) — has previously been shown to repress egg production as measured by the number of clutches and eggs produced [14] (although such a shift could potentially result in a higher investment per egg [17]). The data for Ovipostatin therefore suggest rather complex effects of single proteins, which may not simply function to shift allocation from the male to the female function.

The remaining three scenarios (Figure 1B–D) could be seen as just targeting the male function of the partner, and could also operate in the absence of a sex allocation trade-off. They, respectively, require that (excessive) sperm receipt is actually costly, possibly in a dose-dependent way (Figure 1B), that sperm donation often leads to sperm receipt (Figure 1 B,C), and such reciprocal sperm exchange could presumably be tested by genotyping received sperm [18] in grouped snails and looking for evidence for reciprocal sperm exchange [19]. Moreover, male allocation would need to correlate positively with male mating motivation (Figure 1C,D), and finally the local population structure would have to permit a reasonable probability for repeated interactions between mating partners (Figure 1D). Note that the plausibility of all four scenarios will be affected by: the initiation and duration of the different seminal fluid effects, the speed and flexibility of resource allocation decisions, the natural mating and reciprocation rates, and the duration and dynamics of sperm storage and sperm displacement. Estimating these interactions may require the investigation of freely mating individuals, rather than virgin or isolated snails, which may respond quite differently to (and transfer quite different amounts of) seminal fluid proteins. Moreover, as suggested by the authors [8], further research would clearly benefit from empirically informed theoretical analyses of different scenarios.

Employing artificial insemination of seminal fluid proteins will likely be difficult to scale up to more long-term experiments. An alternative paradigm for testing such effects could involve genome editing using emerging TALEN or CRISPR approaches, which permit efficient production of site-specific knock-out (and knock-in) mutations in a growing range of organisms [20]. Knock-out of specific seminal fluid proteins would allow us to study the fitness consequences over longer time periods, both with respect to the manipulated focal individual and its wild-type mating partners. Finally, analyses of the mode and site of action of the identified seminal fluid proteins may help to inform if they evidently target the male or female function of the partner, or whether they target more general life history traits.

The study by Nakadera *et al.* [8] clearly highlights that steering your partner away from its male side is a plausible strategy in simultaneous hermaphrodites. But we have to admit that we are currently far from understanding the exact context in which such manipulations have evolved. This elegant and stimulating study will help to muster the courage and resolve to find out.

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Neural Energetics: Hungry Flies Turn Down the Visual Gain

Food-deprived flies reduce the gain of a visual-motion-sensitive interneuron whilst walking, and the optomotor reflex to which it contributes, providing evidence of coupling between nutritional state, behavior and neural activity.

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Deprived of nutrients, animals experience energy shortage; under such conditions, they would benefit from promoting behaviors that lead to the acquisition of new nutrient sources whilst reducing the activity of organs and tissues that are not essential for this process, thereby saving energy. Without such a response to their internal and external environments, animals face starvation and, ultimately, death. Finding new food sources requires the activity of muscles and neurons, which are needed both to sense the environment and to co-ordinate muscles to generate the appropriate behavior.

Yet the nervous system is itself a major energy consumer; twenty percent of resting metabolism in humans is consumed by the brain, though it accounts for just 2% of the body mass, whilst in blowflies 8% of the resting metabolism is consumed by the retina alone (for example, [1–4]). Although neurons consume energy to maintain their membrane potentials even at rest, this is typically at a far lower rate than when they are active [5,6]. Numerous processes contribute to neuronal energy consumption, but experiments, computational modeling, and bottom-up budgets suggest that the generation and propagation of action potentials and synaptic transmission (including post-synaptic receptors) dominate costs, mainly because they involve the movement of ions across the cell membrane [1–7].

Reducing neural activity may save considerable amounts of energy, but a global reduction in neuronal activity would likely impede finding new food sources. Yet not all neural activity is necessary for finding food, raising the possibility that the activity of some neurons may be reduced in response to energy shortage even as the activity of other neurons is enhanced to promote food acquisition. There is evidence that food deprivation enhances the activity of peripheral gustatory and olfactory neurons and food-seeking behaviors [8,9]. There is, however, little direct evidence that the activity of specific neurons or neural circuits can be down-regulated in response to food deprivation.

In this issue of *Current Biology*, Longden *et al.* [10] demonstrate that neural activity can be down-regulated in response to food deprivation in the blowfly, *Calliphora vicina.* The authors focused on the activity of an identified interneuron in the fly brain, H2, which is excited by motion of the visual field across the compound eye from back-to-front and inhibited by motion in the opposite direction [11]. They presented visual gratings to head-fixed blowflies walking on a trackball and recorded the action potentials generated by H2 using extracellular electrodes. They characterized the temporal frequency tuning of H2 by presenting back-to-front (excitatory) gratings to well-fed flies. Walking increased the spontaneous spike rate of H2 as well as the spike rates evoked by gratings with frequencies between 10 and 25 Hz, though not at lower frequencies. Such an increase in the spontaneous activity and the gain at high temporal frequencies of motion-sensitive neurons has also been demonstrated in fruit flies [12]. When the H2 spike rate in response to a particular frequency grating was