arrive at a similar time, after the saccade has been completed.

To conclude, we agree that eye movements should be thought of as an essential part of active vision, a form of 'interrogation' [3], not merely a nuisance by-product of motor acts. But it is also clear that there must exist neural mechanisms to amalgamate these movements with perceptual processes. Tantalizing progress of how this occurs has been made over the past few years, identifying many transient changes in spatio-temporal tuning that create a local and very rapid spatiotopicity. Exactly how this transient spatiotopicity interacts with other spatiotopic mechanisms to provide stability will be one of the main challenges for future research.

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Sperm Competition: Discrimination Isn't Always Bad

Observing sperm in competition has been limited by our ability to discriminate between males' sperm. Recent work has overcome this obstacle, while another study reports on seminal fluid with very specific spermicidal activity, suggesting discrimination is easy for some.

Kensuke Okada and David J. Hosken*

It was Geoff Parker who first realised that competition between males did not cease at mating [1] and while it took some years for the rest of us to appreciate the depth of Parker's insights, there is now widespread awareness of the importance of sperm competition, and of post-copulatory sexual selection in general. Sperm competition selects on many traits, including primary sexual characters previously viewed as being unaffected by sexual selection, and the most thoroughly studied of these is testis size. Testis size variation has been investigated across and within species, and almost without exception, the higher the risk of sperm competition, the greater the investment in testes [2,3], much as Parker predicted. As phenotypic responses to selection through sperm competition have become clearer, investigations of post-copulatory male–male competition have increasingly focussed on mechanisms, and this is where two recent papers make their impact [4,5].

Inferring mechanism often involved employing mathematical models to test potential explanations for patterns of paternity-sharing, and Parker's pet insect, the yellow dung fly, has been particularly well studied using this approach. In yellow dung flies, males mating last typically have a fertilization advantage, but this advantage can be eliminated by forcing males to stop copulating before they otherwise would [6]. This, and other evidence, suggested males were displacing rival sperm from storage with their own ejaculates, a notion supported by models [7]. Two investigations that attempted to observe sperm movement within females largely confirmed this, but also corrected some erroneous detail of precisely how displacement occurred [8,9]. These two studies were important because they showed that observation of ejaculates within females is the best way to understand sperm competition mechanisms, but both were very low-tech, which limited the inferences that could be drawn from them.

Studies of another sperm competition model, *Drosophila melanogaster*, had also directly observed sperm within females, but because of the genetic tools available for *Drosophila*, they could employ transgenic males that produced sperm with fluorescent tails [10,11]. Using labelled sperm greatly increased our ability to observe interactions between rival ejaculates inside the female, and while these studies seemed to confirm previous inferences about sperm competition mechanisms in these flies, direct assessment of sperm behaviour, number and position within females was very difficult because the tagged sperm-tails fluoresced so much. Additionally, transgenic males often produced far fewer sperm than non-transgenic males, which compromised their utility. As a result, many questions remained unanswered, such as how many sperm were stored and where, and do the different female sperm-stores have different functions? Partly as a result of these ambiguities, debate continued over the precise mechanisms involved in generating the second male fertilization advantage observed in *D. melanogaster*.

Now, work by the Pitnick lab [4] using more specific labelling of sperm, has finally clarified precisely what occurs inside female *D. melanogaster* when they mate with two males [4].

This exciting new study exploits novel transgenes expressing fluorescent red or green labels attached to sperm-specific chromosomal proteins. These tags are easy to visualise in the heads of mature sperm, allowing sperm to be tracked within females (Figure 1). Furthermore, because there are two labels, red and green, interactions between the competing sperm can be clearly assessed. The stunning videos the authors produced show just how dynamic sperm are within the female reproductive tract. This is especially startling because *D. melanogaster* sperm are long — about 1.9 mm, approximately 30 times longer than human sperm. Theoretically, substantial drag in the low Reynolds number environment in which sperm operate should limit their movement speed. In spite of this, however, the sperm move extremely rapidly and can change directions seemingly effortlessly. This is all extremely surprising and again shows just how important direct observation can be.

Armed with their differentially labelled sperm, the researchers first ensured that the labelled sperm were functional — they were — and that transgenic males had normal fertility —they did over the first 10 or so days of female egg laying after a copulation [4]. They next addressed a range of questions that have been the subject of substantial debate. By observing sperm movement within females at different times after a second copulation, they were able to convincingly show that displacement of a first male's sperm occurs in a manner similar to that inferred for the dung flies discussed above — the first male's sperm are moved from storage back into the uterus (*bursa copulatrix*) where they are diluted by the second male ejaculate before movement back to storage. This dilution of the first male's sperm provides the second male with a fertilization advantage. Importantly, the number of sperm in the second male's ejaculate was positively associated with the proportion of rival sperm displaced from the female sperm stores. Thus, there is selection for increased sperm number in an ejaculate as theory generally predicts, and we now have a very good picture of the temporal sequences of sperm transfer and movement in the female. The authors were also able to unequivocally show that females eject sperm from their reproductive tracts. This had previously been inferred by indirect methods [12], but now the ejected sperm could actually be observed. Sperm ejection occurs very frequently, in more than 80% of females, and the ejected mass is the ejaculate left in the uterus once sperm storage is complete. Furthermore, sperm stored in the ventral receptacle and not the spermathecae are used in fertilization in the 72 hours after second matings, and a male's paternity share is proportional to the relative number of his sperm stored in the ventral receptacle. Thus, sperm competition from the fertilizing set — the group of sperm available to fertilize ova, i.e. those in the ventral receptacle — conforms to a 'fair raffle'. This means, a male's chance of winning (fertilizing eggs) is determined by the number of tickets in the raffle (sperm).

The Pitnick group then investigated one of the more contentious claims to be made about sperm competition mechanisms in *Drosophila*. It had previously been suggested that males are somehow able to damage or incapacitate rival sperm [11], although this was subsequently contested [12]. While evidence suggesting that incapacitation did not occur seemed fairly conclusive, the assessment was based on whether or not sperm were dead, and could not rule out more subtle mechanisms of incapacitation, such as reducing the motility of rival sperm. The new work [4] found that female re-mating had no detrimental effects on the motility of stored sperm, all of which indicates that sperm incapacitation does not occur in *D. melanogaster*. Has the idea of sperm incapacitation finally been shot and laid to rest? Actually no, as another study [5] just published indicates that seminal fluid can be spermicidal, the ultimate in sperm incapacitation, and with a specificity that is unexpected in the extreme.

The authors of this new study [5] investigated sperm survival in the presence of seminal fluid in monandrous and polyandrous ants and bees. They found that a male's seminal fluid enhanced the survival of his own sperm. However, sperm of rival males were preferentially killed by seminal-fluid, but this effect only occurred in polyandrous species.

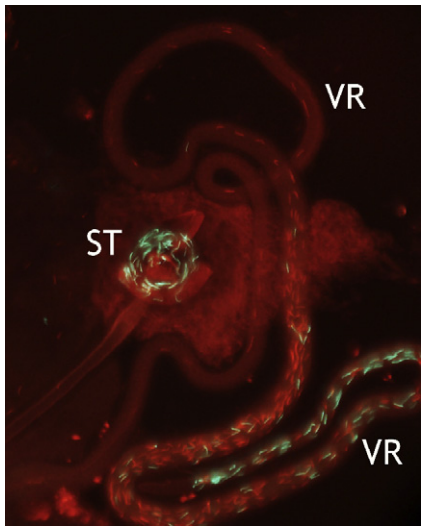

Figure 1. Fluorescing sperm.

Part of the reproductive tract of a female *Drosophila melanogaster* after copulating with two males with differentially labelled sperm. The long tubular structure (labelled VR) is the ventral receptacle, the primary sperm store [4], the other main structure is another sperm store, a spermatheca (ST), which seems to be a longer-term storage unit. The rod-like red and green structures within the two sperm stores are the differently labelled sperm heads. The image clearly shows how easy it is to differentiate between the ejaculates of two males. (Image courtesy of S. Pitnick.)

That is, killing of non-self sperm only took place in those species where the ejaculate of one male would come into contact with a rival ejaculate and hence selection could favour killing. Furthermore, this enhanced killing occurred when the rival was related (a brother), or when the rival was unrelated to the seminal fluid donor, and the addition of self-seminal fluid did not counter the deadly effect of non-self fluid. That seminal fluid enhances self-sperm survival is unremarkable [13], but that there is such specificity in spermicide is utterly so. How can seminal fluid recognize its associated sperm? The need to be able to target only rival and not self-sperm has been a major objection to the whole concept of sperm incapacitation. How could the required specificity be generated, and especially with the degree of discrimination reported? At present we simply do not know, but the fact that the effects were reported for polyandrous bees as well as ants suggests multiple evolutionary origins for the effect.

Spermicide has been reported in other insects [14], but this seems to be the result of male–female conflict rather than male–male competition. Killing of sperm by females (and other anti-sperm activity such as sperm digestion) does not require the level of sophistication reported in the bee and ant study, but would nevertheless provide females with a mechanism to restrain selfish male fertilization interests. There is evidence of sexual conflict over sperm-killing in the ant and bee study too because fluid from the female sperm-store prevents killing in one spermicidal ant species [5]. Sophisticated killing of rival sperm like that reported is a neat way to enhance self-fitness when sperm compete numerically, and various means of damaging rival sperm have been proposed in the past (e.g. the 'kamikaze sperm' hypothesis). However, these claims have usually floundered on closer examination [15], as seen with the coshing of incapacitation in the *Drosophila* study [4]. Nonetheless, as one study slams the lid on sperm incapacitation [4], another resurrects it in a most remarkable way [5]. Spermicidal specificity like that identified [5] could also limit the exploitation of rival ejaculates. Exploitation of rivals has recently been proposed as a way of reducing the costs of dealing with female reproductive-tract hostility to sperm [13], but spermicidal rival semen could eliminate this possibility. The bee and ant work is also noteworthy because the effects documented are found in so many species, but the occurrence of such highly targeted spermicide outside the Hymenoptera remains to be demonstrated. The study of sperm competition has now matured into a broad and diverse field, and it seems the road to further enlightenment is via fluorescently labelled sperm.

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Cell Polarity: Lateral Perspectives

The outer and inner (lateral) plasma membranes of the outermost cell layer in plants provide selective barriers to the environment. Recent studies provide perspectives on how asymmetric protein localization is established at lateral membranes.

Markus Grebe

Flowering plants display an obvious asymmetric organization along their shoot–root axis. At the sub-cellular level, such asymmetries are reflected as polar protein localization at plasma membranes facing the shoot (apical membranes) or the root (basal membranes). While our understanding of the mechanisms underlying formation of apical–basal cell polarity has improved considerably [1], nothing is known about how polarity of membranes facing the plant surface (referred to as *outer lateral*, *peripheral* or *distal* membranes), or of membranes oriented towards the centre of the root and shoot (called *inner lateral*, *central* or *proximal* membranes), is established (Figure 1). Yet, the outer membrane of the surface tissue layer (epidermis) fulfils important functions. It provides a barrier for selective uptake of nutrients, extrusion of toxic compounds, and is the first membrane that encounters abiotic and biotic stresses. Consequently, outer lateral membrane polarity may be crucial to plant survival under challenging conditions. For example, the essential nutrient boron needs to be taken up from the soil, but it is toxic at high concentrations. Here, polar localization of the *Arabidopsis* boron transporter BOR4 at the outer lateral membrane of the root epidermis comes into play because BOR4 confers boron efflux at high concentrations [2]. Consistent with the view that proteins required for defence against penetrating pathogens may act at the outer lateral membrane, the PENETRATION3 (PEN3) protein fused to green-fluorescent protein (GFP) (PEN3–GFP) localizes to the epidermal plasma membrane [3], specifically, at the outer lateral membrane of root epidermal cells [4]. With regard to the shoot–root axis, the apical membrane is marked by the PIN-FORMED2 (PIN2) protein [5], while the basal membrane can be visualized by