Evolutionarily dynamic sperm

Evolutionary biologists really got interested in sperm only after the realization that if more than one male inseminates a female, their sperm will be in competition[1]. At last, we had a plausible explanation for the absurdly large numbers of sperm found in most species, and an entirely new field sprang up examining how sperm competition occurs and how males have adapted to it. Now, nearly 30 years later, we have progressed from concentrating on interactions between males to considering the roles of both sexes, their conflicting interests and the evolutionary consequences of everything that goes on between copulation and fertilization[2]. The 5th Biology of Spermatozoa meeting* echoed the field's broadening horizons and its fundamental role in understanding sexual selection.

Instead of regarding females as passive receptacles for sperm, the dominant paradigm is now to regard interactions between the sexes as an ongoing conflict. Males attempt to inseminate as many mates as possible, even if this has costs for females, and females attempt to avoid these costs and to control fertilization of their eggs. A classic example of a male trait detrimental to females is the ejaculate of *Drosophila melanogaster*, which contains toxins that reduce female lifespan but increase sperm storage and delay female remating[3]. Tracey Chapman (University College, London, UK) and collaborators are now identifying specific chemicals involved in male manipulation of females. To achieve this, they use mutant males that lack particular ejaculate proteins and separate their effects from those of the sperm themselves by using spermless irradiated males. This reveals that males who lack one glycoprotein, the evocatively named 'Acp36DE', are less able to displace the sperm of previous males and have their own sperm stored by the female. The magnitude of this disadvantage depends on whether sperm are transferred, indicating that both accessory proteins and sperm themselves are involved in sperm competition and female manipulation.

A more drastic male tactic is employed by bed bugs (*Cimex lectularius*). In this species, males pierce the female body wall and inject sperm directly into her body cavity from where it migrates to the ovaries. Alastair Stutt (University of Sheffield, UK) has found, as might be expected, that this makes mating costly, with females exposed to more matings having substantially reduced lifespans.

Of course, females are not passive victims of aggressive male strategies – sexual conflict drives coevolution between males and females. This process can be seen in action in multigenerational lines of *D. melanogaster*, in which one pair of lines has females kept with four males (polyandry), and the other pair has females kept with a single male (monandry)[4]. Brett Holland (University of California, Lafayette, USA) described how, after 32 generations, males from the monandrous line are more benign when mated to test females and also court less readily. Similarly, monandrous-line females suffered more when mated to a test male than polyandrous females, indicating that they had lost some of their resistance to toxic effects of male ejaculates. Both results indicate that within a few generations both sexes adapt to a reduction in sperm competition.

Holland has passed his selected lines on to Scott Pitnick (University of Syracuse, NY, USA), who is investigating differences in sperm competitive ability between the lines. Although this work is ongoing, it is already apparent that males from the polyandrous lines are more successful when their sperm are in competition with those of a previous male than are males from the monandrous line. The reduction in sperm-competition ability in monandrous males suggests that success in sperm competition has costs, either directly or through reducing female fecundity.

The generality of costs of sperm competition to both sexes found in *D. melanogaster* could be investigated by comparing patterns of male–female interactions across populations or species of other taxa. However, it was pointed out by Geoff Parker (University of Liverpool, UK) that even rare polyandry (as tends to be found in apparently monogamous species) theoretically is predicted to create similar selection pressures on males as rampant polyandry[5]. This suggests that differences between completely monandrous and polyandrous selection lines might not be seen between populations differing in their degree of polyandry in natural situations.

As well as adapting to male sperm-competitive tactics, there is growing evidence that females exercise considerable control over inseminated sperm, and might even actively choose to fertilize their eggs with sperm from particular males[6]. An example of such behaviour has been studied by Tommaso Pizzari (University of Sheffield), who showed that female feral chickens can not only physically eject inseminated sperm, but that they do so more frequently when inseminated by a lower ranking male. However, chickens might be out-done by female dungflies, which are equipped with multiple sperm storage organs from which they release sperm to fertilize their eggs. Paul Ward (University of Zurich, Switzerland) found that flies reared from eggs collected from shady or sunny areas of a cowpat differ in the frequency of different forms of the enzyme phosphoglucomutase (PGM). Because PGM differences are associated with differences in the effect of temperature on larval growth, Ward suggests that multiply mated females can selectively use sperm from males differing in PGM genotype according to whether an egg is being laid in a sunny or shady position[7].

With growing interest in the effects of selection on sperm characteristics has come recognition of the need to examine their genetics and heritability. Ted Morrow (University of Liverpool) examined 11 species from humans to insects and found significant differences in sperm length between males in all cases. This supports the consensus that sperm characteristics are controlled by the paternal genotype, rather than by sperm themselves, because haploid expression would predict high variation within the ejaculate of a single male. Using field crickets (*Gryllus bimaculatus*), Morrow demonstrated that sperm length can be artificially selected and that much variability may be carried on the X chromosome. If this is the case, males with long sperm cannot pass the trait to sons, but have daughters who pass the trait to their own sons. So, if sperm length comes under directional selection, this has to act through the success of grandsons.

Although the benefits of having sperm of a particular length are not clear, having more mobile sperm is likely to be advantageous. David Froman (Oregon State University, Corvalis, USA) has found that male chickens differ in sperm mobility independent of differences in body mass or overall sperm production. Again, these differences are heritable, with much higher measured heritability through females (the heterogametic sex in birds) than through males. This difference does not appear to be due to nongenetic maternal effects, suggesting that variation in sperm mobility reflects variation in mitochondria (which are inherited only through eggs). This is intriguing because Tim Birkhead's work (University of Sheffield) on the

*Biology of Spermatozoa, University of Sheffield, UK, 28–31 October 1999.

 0169-5347/00/$ – see front matter PII: S0169-5347(99)01798-X

same species indicates that sperm traits might be under directional selection. Females inseminated with a mixture of sperm from two males differing only in sperm mobility had more of their eggs fertilized by the male with higher mobility sperm[8]. If mobility differences are due to mitochondria, then selection will not be able to act on them, because even if males with particular mitochondrial genotypes are more successful in fertilizing eggs, none of their offspring will inherit this characteristic. Therefore, mitochondrial inheritance provides a potential explanation for the maintenance of variation in a directionally selected trait.

Overall, the meeting clearly demonstrated that our current understanding of the role of postcopulatory processes is just the tip of the iceberg. To have any hope of understanding the extravagant world of sexual selection, we must consider the world of the sperm.

Acknowledgements

Thanks to Tim Birkhead and Harry Moore for organizing the meeting and to Nina Wedell, Paul Ward, Alastair Stutt, Scott Pitnick, Ted Morrow, Matt Gage, Dave Froman, Tracey Chapman and Tim Birkhead for helpful comments on this article. The meeting was supported by the Association for the Study of Animal Behaviour and the Wellcome Foundation.

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Testosterone and maternal effects – integrating mechanisms and function

One hundred and twenty-eight years after Darwin first described the two processes of sexual selection – male–male competition and female choice – the latter component continues to cause controversy. The main debate lies in those cases, such as lekking species, where females apparently obtain nothing but semen from the male with which they copulate. In such species, males contribute no paternal care, and thus it is assumed usually that females obtain only indirect or genetic benefits from their choice of partner. Consequently, female choosiness in return for no obvious benefit is referred to as the 'lek paradox'[1]. Several different types of genetic benefit are plausible[2], but the one that has attracted the most interest is the idea of viability genes. This 'good genes' hypothesis has been difficult to test, but there is now some evidence for it because several studies have reported increased viability of offspring fathered by attractive males[3]. Researchers testing the good genes hypothesis have had to be careful to avoid the possible confounding influence of maternal effects. For example, over ten years ago, it was shown that within pairs of zebra finches (*Taeniopygia guttata*), females paired to attractive males invested more in offspring than females paired to less attractive males[4]; this raised the possibility that any increase in the viability of offspring fathered by attractive males could be due either to a maternal effect (increased investment by the mother), to the father's 'good genes' or, more probably, to both (given that females are expected only to invest more if they increase their fitness). Several studies looking for good gene effects in birds have attempted to control for maternal effects; for example, by hatching eggs in incubators[5], but, of course, this does not preclude the possibility of differential maternal investment before egg-laying. A new study of zebra finches by Gil *et al.*[6] has shown that one maternal effect in particular – the amount of testosterone deposited in eggs – could have a profound influence on a female's offspring, in a way that varies according to the attractiveness of her mate.

Gil *et al.* found that in captivity female zebra finches deposit relatively more of the androgens testosterone and 5α-dihydrotestosterone into their eggs when paired to an attractive male. They were able to demonstrate this in a particularly elegant manner, because the attractiveness of male zebra finches (Fig. 1) can be manipulated by the addition of colour-rings – red rings render males more attractive and green rings reduce male attractiveness[7]. Gil *et al.* looked at androgens in the eggs of females paired to the same males wearing red or green rings in different clutches. Females put significantly more androgens into their eggs when their male social partner wore red rings compared with when the same male wore green rings.

In canaries, *Serinus canaria*, a higher level of androgens in the egg gives chicks of both sexes a head start in several ways – in the nest they beg more and grow faster, and they have higher social rank once fledged[8,9]. Gil *et al.* assume that similar advantages would accrue in zebra finches hatching from high androgen eggs.

With these advantages, why don't females put similar amounts of androgens into all eggs regardless of their partner's apparent quality? Gil *et al.* suggest that androgens might be costly, either to the mother or to her offspring; for example, by suppressing the immune system. They propose also that only offspring fathered by genuinely high quality males would be able to withstand the high concentration of androgens. There are other potential costs not mentioned by Gil *et al.*; for example, although increased growth might provide short-term benefits, other studies of zebra finches indicate that it can result in reduced subsequent survival[10].

These remarkable results raise several other questions. The zebra finch is a socially monogamous species, but (in common with many other passerine birds) also engages in extra-pair copulations (EPCs), which result in extra-pair paternity

  PII: S0169-5347(99)01803-0