

# Meiotic drive alters sperm competitive ability in stalk-eyed flies

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Meiotic drive results when sperm carrying a driving chromosome preferentially survive development. Meiotic drive should therefore influence sperm competition because drive males produce fewer sperm than non-drive males. Whether meiotic drive also influences the competitive ability of sperm after ejaculation is unknown. Here we report the results from reciprocal crosses that are designed for estimating the sperm precedence of male stalk-eyed flies (*Cyrtodiopsis whitei*) with or without X-linked meiotic drive. We find that nearly half of all sex-ratio males, as compared with 14% of non-sex-ratio males, fail to produce young in a reciprocal cross. Furthermore, the proportion of progeny sired by a sex-ratio male in a female jointly inseminated by a non-sex-ratio male was less than expected from the number of sperm transferred. These effects are not due to differential sperm storage by females because, after a single mating with a sex-ratio male, all females stored sperm and because two sex-ratio males share paternity after jointly mating with a female. In addition to demonstrating a new mechanism of sperm competition, these results provide insight into the maintenance of sex-ratio polymorphisms. Sex-ratio males have less than one-half the fertility of non-sex-ratio males, as is required in order for frequency-dependent selection on males to produce a stable sex-ratio polymorphism.

**Keywords:** X chromosome; sex-ratio dynamics; sperm precedence; cryptic female choice

## 1. INTRODUCTION

Variation in sperm precedence, i.e. the proportion of offspring sired by a male in a multiply inseminated female, is widespread (Simmons & Siva-Jothy 1998; Clark *et al.* 1999) but not well understood. In many cases sperm precedence is determined by the amount of sperm males transfer to females (cf. Simmons & Siva-Jothy 1998). The male who transfers the most sperm fertilizes more eggs as a consequence of dilution or displacement. Less often, sperm precedence has been related to differential fertilization success among sperm. For example, sperm competitive ability is influenced by size in nematodes (LaMunyon & Ward 1998) and by the presence of an intracellular parasite in flour beetles (Wade & Chang 1995). In other cases, sperm appear to be selected by females in order to reduce inbreeding with close relatives or outbreeding with incompatible genotypes. Unrelated males fertilize more eggs than related males in sand lizards (Olsson *et al.* 1996), an ascidian (Bishop *et al.* 1996), two species of cactophilic *Drosophila* (Markow 1997) and decorated field crickets (Stockley 1999). Sperm competition studies involving conspecific and heterospecific matings have provided evidence for conspecific sperm precedence in grasshoppers (Hewitt *et al.* 1989; Bella *et al.* 1992), ground crickets (Gregory & Howard 1994), flour beetles (Wade *et al.* 1994) and *Drosophila* (Price 1997; Price *et al.* 2000).

Stable polymorphisms for meiotic drive have been found in a number of species (Carvalho & Vaz 1999) and may provide an under-appreciated cause of variation in sperm precedence. Meiotic drive can be viewed as the result of sperm competition within a male (Haig & Bergstrom 1995), because the development of sperm lacking a drive chromosome is disrupted after meiosis (Policansky & Ellison 1970; Montchamp-Moreau & Joly

1997; Wilkinson & Sanchez 2001). Consequently, drive-carrying males produce fewer functional sperm than non-drive males and should experience sperm precedence that is proportional to the amount of drive sperm stored by multiply inseminated females. Studies on *Drosophila pseudoobscura* (Beckenbach 1981; Wu 1983) have confirmed that drive males have reduced fertility when mated to non-virgin females. Meiotic drive also reduces pollen competitive ability in a dioecious plant (Taylor *et al.* 1999), but the effect of drive on the competitive ability of sperm has not been previously examined for any animal system. Here we address this question using the stalk-eyed fly *Cyrtodiopsis whitei*, which is a sexually dimorphic species with sex chromosome meiotic drive (Presgraves *et al.* 1997).

In order to determine whether males with a sex-ratio X chromosome differ from non-sex-ratio males in sperm competition, we used two populations of *C. whitei* and conducted paired matings to single females in reciprocal order. We use progeny counts from sex-ratio and non-sex-ratio males for predicting the precedence of sex-ratio sperm in multiply inseminated females and estimating sperm competitive ability independent of effects due to sperm number. In addition, we test whether females are exhibiting cryptic choice by selectively rejecting spermatophores or storing sperm. Our results suggest a new mechanism for sperm competition and shed light on the evolutionary dynamics of sex-ratio polymorphisms.

## 2. MATERIAL AND METHODS

### (a) Fly stocks

We used two populations of *C. whitei* that exhibit heritable differences in body colour, but are otherwise morphologically indistinguishable. Our laboratory populations are derived from flies that were collected near Ulu Gombak, Malaysia (350 m elevation) (3°19' N, 101°43' E) and near Chiang Mai, Thailand

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(1600 m elevation) (19°9'N, 98°7'E) in January 1996. Subsequent to capture, we have kept flies from each population in a large Plexiglas cage (40 cm × 40 cm × 40 cm) at  $25 \pm 2^\circ\text{C}$  with at least 75% relative humidity on a 12 L:12 D cycle. The flies feed and oviposit on a medium of ground yellow corn treated with 10% methylparaben as a mould inhibitor. Newly eclosed flies are returned to each cage as necessary in order to keep populations above 100 individuals. The flies used in the reciprocal crosses described below were cultured at low density in order to minimize variation in eye span (David *et al.* 1998), which is known to influence pre-copulatory mate choice (Wilkinson *et al.* 1998a).

### (b) Sex-ratio genotyping

In order to assess the presence or absence of the sex-ratio chromosome in males from Gombak and Chiang Mai, males were allowed to mate with virgin females and then the sex-ratio of their progeny was scored. We obtained progeny from a total of 98 males (53 Gombak and 45 Chiang Mai males) after aspirating a male and one to five virgin Gombak females into small, ventilated plastic cages (12 cm × 16 cm × 13.5 cm) lined with moist cotton and blotting paper. Females were allowed to mate and oviposit for at least 4 weeks. Males and females were at least 4 weeks past eclosion and, therefore, sexually mature (De la Motte & Burkhardt 1983) when mated. We tested the progeny sex-ratios for deviation from 1:1 using  $\chi^2$ -tests that were corrected for continuity. Males with a significantly ( $\alpha = 0.05$ ) female-biased progeny sex-ratio were designated as carrying a sex-ratio X chromosome. Otherwise they were assumed to have a non-sex-ratio X chromosome.

### (c) Sperm precedence

In order to separate the effects of mating order and population of origin on sperm precedence from those of meiotic drive, we conducted four sets of reciprocal matings. Each reciprocal mating involved two females from the same population and two males from different populations. One was mated first to a Gombak male and 24 h later to a Chiang Mai male for each pair of females, while the other was mated to the same males in reverse order. We tested four sex-ratio genotype pairs between populations:  $ST_G-ST_C$ ,  $SR_G-ST_C$ ,  $ST_G-SR_C$ , and  $SR_G-SR_C$  (where SR denotes sex-ratio populations, ST denotes non-sex-ratio populations and the subscripts denote the population of origin). We isolated females with food in small plastic cages overnight (9 cm × 11 cm × 9 cm) and then introduced a male by aspiration between the hours of 16.00 and 19.00, which corresponds to the dusk peak of mating activity (Lorch *et al.* 1993). Females that did not mate within 3 h were replaced. Only eight (six with non-sex-ratio males and two with sex-ratio males) out of 120 females tested needed replacement. The females were transferred to larger plastic cages (12 cm × 16 cm × 13.5 cm) after two successful copulations and allowed to oviposit on corn medium until pupal production ceased or the female died.

In order to compare sperm defence ( $P_1$ ) and offence ( $P_2$ ), we report the proportion of offspring sired by the Gombak male when mating first ( $P_1$ ) versus when mating second ( $P_2$ ). The sperm precedence for the Chiang Mai male is therefore  $1 - P$ . We assigned paternity to each offspring using the heritable variation in body colour as assessed at ×10 magnification. Chiang Mai flies have dark yellow legs, Gombak flies have brown legs and hybrid offspring have legs of an intermediate dark orange colour.

We used the angular transformation on proportions prior to analysis of variance (ANOVA). We report mean ± s.e. values

using untransformed data in order to indicate the magnitude and direction of effects for all analyses. We attempted 56 reciprocal crosses, but could not compare sperm precedence in 20 pairs. Comparisons were not possible in 12 pairs because one of the two males produced too few progeny to score his sex-ratio genotype and in another eight pairs because one of the females failed to produce any progeny. The remaining 36 crosses included 10  $ST_G-ST_C$ , 14  $SR_G-ST_C$ , 5  $ST_G-SR_C$  and 7  $SR_G-SR_C$  male pairs and used 36 females from each population.

### (d) Cryptic female choice

In *C. whitei* sperm are transferred to a female inside envelopes of male accessory gland secretions called spermatophores (Kotrba 1996). In this species sperm move from the spermatophore up a pair of ducts to one of three spermathecae where they are stored. Since females can eject spermatophores before they are empty (Kotrba 1996), we examined the possibility that females might exhibit cryptic choice by preferentially storing sperm according to the sex-ratio genotype. We allowed virgin males (19 Gombak and 15 Chiang Mai) to mate once with each of two virgin Gombak females with at least 24 h between matings. One female was anaesthetized at  $-20^\circ\text{C}$  for 1–3 min immediately after copulation termination. Her internal reproductive tract was then removed and placed in a small drop of phosphate buffer solution (pH = 7.4) on a glass slide. The slides were examined at ×200 magnification with differential interference contrast optics and scored for the presence or absence of a spermatophore within the reproductive tract. The other female was anaesthetized and dissected 1 h after copulation. Her spermathecae were removed and crushed under a cover-slip in order to release sperm and observe their motility.

Because the females in the cryptic choice experiment were not allowed to produce progeny, the presence or absence of a sex-ratio X chromosome in their mates was not determined by progeny sex-ratios. Male *C. whitei* sex-ratios can be accurately quantified by the presence of non-elongated sperm within spermatocyst bundles in the testes (Wilkinson & Sanchez 2001). Therefore, we removed testes and scored males with greater than 20% non-elongated sperm bundles as sex-ratio males.

## 3. RESULTS

### (a) Sex-ratio distortion

The bimodal distributions of the male progeny sex-ratios (figure 1) indicated that a sex-ratio X chromosome was present and segregating in both populations. Sex ratios were obtained from  $72.2 \pm 5.6$  progeny per mated male. ANOVA confirmed that the arcsine square-root proportion of male progeny differed dramatically between sex-ratio and non-sex-ratio males ( $F_{1,94} = 147.2$  and  $p < 0.0001$ ). In addition, the progeny sex-ratios differed between populations ( $F_{1,94} = 5.74$  and  $p = 0.019$ ) and there was a significant interaction between population and male genotype ( $F_{1,94} = 6.90$  and  $p = 0.0101$ ) that was caused by a higher proportion of male progeny among Chiang Mai sex-ratio males than Gombak sex-ratio males. Sixteen Chiang Mai sex-ratio males produced  $0.211 \pm 0.030$  male progeny as compared with  $0.117 \pm 0.023$  male progeny from 31 Gombak sex-ratio males. These results suggest that sex-ratio males from the two populations differ in their production of fertilization-competent Y-bearing sperm.

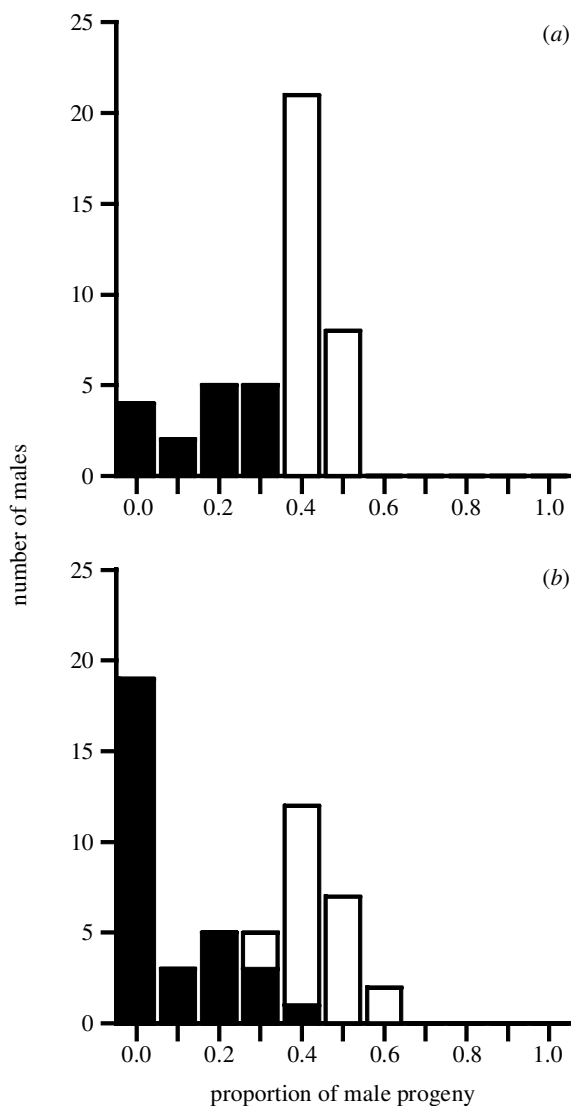


Figure 1. The progeny sex ratio distributions of males used in the sperm precedence experiment from (a) the Chiang Mai population and (b) the Gombak population. Filled bars indicate sex-ratio males, i.e. sex ratios that deviate significantly from 1:1.

### (b) Sperm precedence

Genetic incompatibilities between populations could cause differences in embryo survival that might contribute to variation in sperm precedence (Gilchrist & Partridge 1995). We tested this possibility by using contingency tests for comparing the presence or absence of progeny against the type of population cross (within or between) and male pair genotype for all fertile males tested. This analysis revealed no evidence of any differences in embryo survival. Crosses involving flies from the same population were as likely to produce offspring as crosses involving flies from different populations ( $\chi^2 = 1.49$  and  $p = 0.22$ ).

By contrast, a contingency test between the sex-ratio genotype of a male and his production of offspring in the reciprocal cross showed that sex-ratio males were much less likely to produce offspring after a single mating than non-sex-ratio males ( $\chi^2 = 14.3$  and  $p = 0.0002$ ). Only 13.7% of the 51 non-sex-ratio males tested failed to father

young as compared with 48.9% of the 47 sex-ratio males tested. This pattern was present in both populations (Chiang Mai,  $\chi^2 = 4.76$ ,  $p = 0.032$  and  $n = 45$  and Gombak,  $\chi^2 = 7.2$ ,  $p = 0.007$  and  $n = 53$ ). Thus, sex-ratio males have lower fertility than non-sex-ratio males.

We tested for differences in sperm precedence between drive and non-drive males using repeated-measures ANOVA in which the populations of origin of the female and male pair genotypes were included as main effects and mating order was the repeated measure. This analysis revealed that sperm precedence was strongly influenced by the male pair genotype but not by the female population, mating order or any interaction between these factors (table 1). Sex-ratio males competing against non-sex-ratio males sired significantly fewer offspring than any other combination of male genotypes tested (figure 2). The absence of a mating order effect is illustrated by the similarity between  $P_1$  and  $P_2$  for all male genotype combinations (figure 2).

In order to determine whether the decrease in sperm precedence observed for sex-ratio sperm exceeds expectations based on differences in the number of sperm transferred to a female, we compared the number of progeny produced by the 72 males in the sperm precedence comparison using a three-way ANOVA. This analysis revealed no significant effects of male population ( $F_{1,64} = 2.63$  and  $p = 0.11$ ), a weak effect of female population ( $F_{1,64} = 4.30$  and  $p = 0.042$ ), a large difference between male genotypes ( $F_{1,64} = 7.18$  and  $p = 0.009$ ) and no significant interactions ( $p > 0.5$  in all cases). Gombak females produced fewer offspring ( $16.8 \pm 3.7$ ) than Chiang Mai females ( $24.9 \pm 3.5$ ) while sex-ratio males produced approximately half as many offspring ( $13.1 \pm 3.0$ ) as non-sex-ratio males ( $27.4 \pm 3.7$ ).

Assuming that the difference in progeny produced by sex-ratio and non-sex-ratio males reflects the difference in the number of fertilizable sperm each male genotype transfers to a female, then a sex-ratio male should father 32% of the offspring after sex-ratio and non-sex-ratio males jointly inseminate a female, assuming that sperm mix at random and have equal competitive ability. Random sperm mixing is consistent with the lack of sperm precedence we observed in sex-ratio–sex-ratio and non-sex-ratio–non-sex-ratio genotype pairs, as well as with results from a previous study (Lorch *et al.* 1993). By contrast, we found that sex-ratio males from both populations sired far fewer offspring than expected. Chiang Mai sex-ratio males sired  $13.1 \pm 4.9\%$  offspring, while Gombak sex-ratio males sired  $6.4 \pm 1.7\%$  offspring from a female also mated to a non-sex-ratio male from the other population. Thus, the sex-ratio sperm's competitive ability decreased inversely with the intensity of drive in each population.

### (c) Cryptic female choice

Differential fertility between male genotypes in the reciprocal cross could have been caused by females that either reject spermatophores from sex-ratio males or selectively store and use non-sex-ratio sperm rather than sex-ratio sperm. We found no support for either of these hypotheses. Spermatophores were present in all 34 females examined immediately after mating, thereby indicating that sex-ratio and non-sex-ratio males do not differ

Table 1. Repeated-measures ANOVA on the arcsine square-root transform of sperm precedence by Gombak males mating first ( $P_1$ ) versus second ( $P_2$ ).

(\*\*\* $p < 0.0001$ .)

source	d.f.	mean squares	$F$
male pair genotype	3	3.698	39.82***
female population	1	0.049	0.59
male genotype $\times$ female population	3	0.197	2.39
subject	28	0.083	—
order ( $P_1$ versus $P_2$ )	1	0.063	1.13
order $\times$ male genotype	3	0.040	0.73
order $\times$ female population	1	0.110	1.99
order $\times$ male genotype $\times$ population	3	0.043	0.77
order $\times$ subject	26	0.055	—

in their ability for transferring sperm. Furthermore, females did not selectively store sperm. Seven out of seven females mated to sex-ratio males stored sperm from Gombak males and seven out of seven stored sperm from Chiang Mai males. Eleven out of 12 females mated to non-sex-ratio males stored sperm from a Gombak male and eight out of eight stored sperm from a Chiang Mai male. Motile sperm were present in all three spermathecae in every case where a female stored sperm. Hence, sperm storage was not related to male genotype.

#### 4. DISCUSSION

The results presented here demonstrate that sperm from males carrying a driving X chromosome are less successful at fertilizing eggs in multiply inseminated females than sperm from males that lack a driving X chromosome. Nearly half of all the sex-ratio males we tested failed to produce young in a reciprocal cross despite having produced progeny when mated with females alone. Furthermore, the proportion of progeny sired by a sex-ratio male in a female jointly inseminated by a non-sex-ratio male is much less than expected if fertilization success depends solely on the number of fertilization-competent sperm transferred. That sex-ratio males transfer fertilization-competent sperm in single matings was confirmed by inspection of the females, all of which had sperm in their three spermathecae after a single mating to a sex-ratio male and by our observation of equal shared paternity when two sex-ratio males jointly mate with one female.

Our data allow us to eliminate several possible mechanisms that might cause this difference in sperm precedence. Precedence of non-sex-ratio over sex-ratio sperm cannot be caused by sperm displacement, as has been recently documented in *Drosophila* (Price *et al.* 1999, 2000), because the effect is independent of mating order. Differential survival of embryos involving crosses within and between populations, for example (Price 1997), is also unlikely to be a factor as we found no difference in the fertility of those crosses and no interaction between the populations of origin of males and females on the number of progeny produced. We can also rule out two possible mechanisms for cryptic female choice. Sperm was both

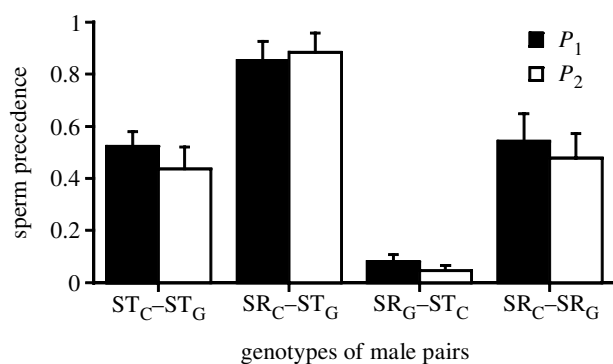


Figure 2. Mean  $\pm$  s.e. proportions of the progeny sired by the Gombak male for each of the four possible sex-ratio genotype combinations of male pairs. SR indicates a male that produces a significantly female-biased sex ratio. The sex ratios of non-sex-ratio males do not differ from 1:1. The subscripts denote the populations: G, Gombak; C, Chiang Mai. The sperm precedences when mating first versus second are indicated by  $P_1$  and  $P_2$ , respectively.

transferred to and stored by females independent of male genotype. We found no evidence of non-random sperm storage as has been described, for example, in dung flies (Ward 2000) and *Drosophila* (Price *et al.* 2001).

Thus, we conclude that sperm competitive ability differs between male sex-ratio genotypes. Hypothetically, either female- or male-mediated mechanisms could account for this result. Females would have to discriminate sperm by type in order for female choice to occur in the absence of differential sperm storage. This mechanism predicts lower fertility of sex-ratio males as compared with non-sex-ratio males even in the absence of non-sex-ratio sperm. Accordingly, when single male *C. whitei* are allowed to remate with females continuously, sex-ratio males produce 72% as many progeny per week as non-sex-ratio males (Wilkinson & Sanchez 2001). However, this difference could be due to sex-ratio males producing fewer sperm than non-sex-ratio males. Invoking sperm selection by females is therefore unnecessary in explaining the fertility difference between sex-ratio and non-sex-ratio males.

Alternatively, sex-ratio sperm could be incapacitated by the seminal fluid from non-sex-ratio males. Recent results from *Drosophila* suggest that this mechanism is plausible. Experiments using spermless males (Price *et al.* 1999) and heterospecific males (Price *et al.* 2000) have shown that seminal fluid can cause incapacitation of stored sperm. Furthermore, the presence or absence of the accessory gland protein Acp36DE can influence whether a female stores the sperm of subsequent mates (Chapman *et al.* 2000).

Our results indicate that the meiotic drive system has deleterious effects on both the X- and Y-bearing sperm of sex-ratio males. The presence of male progeny from sex-ratio males in both populations indicates that some Y-bearing sperm remain capable of fertilization. Consequently, some X-bearing sperm must fail to become fertilization competent in order to account for the twofold difference in progeny production we observed between sex-ratio and non-sex-ratio males. Cytological studies are consistent with this conclusion as the number of sperm with recognizable developmental

abnormalities typically underestimates the degree of sex-ratio distortion in several species of *Drosophila* (Hauschteck-Jungen & Maurer 1976; Montchamp-Moreau & Joly 1997) as well as in *C. whitei* (Wilkinson & Sanchez 2001). Thus, it appears that sperm competitive ability within multiply inseminated females is impaired progressively as sperm competition within a male intensifies meiotic drive.

This result has direct implications for the possibility that a meiotic drive polymorphism may be maintained by frequency-dependent selection in males. Frequency dependence is expected because sex-ratio males' fertility should decrease as drive increases the number of females and, therefore, the frequency of male remating in a population (Jaenike 1996). Carvalho & Vaz (1999) argued that this mechanism is unlikely to provide a general explanation for the stability of sex-ratio polymorphisms because it assumes that the fertility of sex-ratio males is less than half that of non-sex-ratio males. If only the Y-bearing sperm of sex-ratio males are dysfunctional, their fertility should not decrease below half that of non-sex-ratio males. However, our sperm precedence results show that the paternity loss for sex-ratio males is much more than one-half that of non-sex-ratio males. This result cannot be due to sperm depletion because males were mated only twice within 24 h, a rate which is not sufficient for causing depletion (Lorch *et al.* 1993). More frequent matings by males would be expected to deplete sperm stores and would augment the effect of sperm competition described here.

In addition to influencing the stability of a meiotic drive polymorphism, variation in sperm competitive ability is expected to affect female mating behaviour (Haig & Bergstrom 1995; Zeh & Zeh 1996). Selection should act on females in order to avoid mating with sex-ratio males and minimize the possibility of producing a biased sex-ratio. The male eye span in stalk-eyed flies provides a cue that females apparently use for reducing the frequency with which they mate with sex-ratio males (Wilkinson *et al.* 1998*b*). However, the condition-dependent expression of the eye span (David *et al.* 2000) may reduce the reliability of the eye span as a signal of sex-ratio genotype. Given the differences in sperm competitive ability described here, the increased frequency of female remating in *C. whitei* as compared to its congeners (Wilkinson *et al.* 1998*a*), provides an additional mechanism for avoiding undesirable sex-ratio sperm. Sperm precedence studies on other species with a sex-ratio polymorphism are warranted in order to determine whether these patterns are widespread.

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## REFERENCES

Beckenbach, A. T. 1981 Multiple mating and the 'sex-ratio' trait in *Drosophila pseudoobscura*. *Evolution* **35**, 275–281.

- Bella, J. L., Butlin, R. K., Ferris, C. & Hewitt, G. M. 1992 Asymmetrical homogamy and unequal sex ratio from reciprocal mating-order crosses between *Chorthippus parallelus* subspecies. *Heredity* **68**, 345–352.
- Bishop, J. D. D., Jones, C. S. & Noble, L. R. 1996 Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. II. Investigation of male mating success using RAPD markers. *Proc. R. Soc. Lond. B* **263**, 401–407.
- Carvalho, A. B. & Vaz, S. C. 1999 Are *Drosophila* SR chromosomes always balanced? *Heredity* **83**, 221–228.
- Chapman, T., Neubaum, D. M., Wolfner, M. F. & Partridge, L. 2000 The role of male accessory gland protein Acp36DE in sperm competition in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* **267**, 1097–1105. (DOI 10.1098/rspb.2000.1114.)
- Clark, A. G., Begun, D. J. & Prout, T. 1999 Female–male interactions in *Drosophila* sperm competition. *Science* **283**, 217–220.
- David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A. & Fowler, K. 1998 Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. *Proc. R. Soc. Lond. B* **265**, 2211–2216. (DOI 10.1098/rspb.1998.0561.)
- David, P., Bjorksten, T., Fowler, K. & Pomiankowski, A. 2000 Condition-dependent signalling of genetic variation in stalk-eyed flies. *Nature* **406**, 186–188.
- De la Motte, I. & Burkhardt, D. 1983 Portrait of an Asian stalk-eyed fly. *Naturwissenschaften* **70**, 451–461.
- Gilchrist, A. S. & Partridge, L. 1995 Male identity and sperm displacement in *Drosophila melanogaster*. *J. Insect Physiol.* **41**, 1087–1092.
- Gregory, P. G. & Howard, D. J. 1994 A postinsemination barrier to fertilization isolates two closely related ground crickets. *Evolution* **48**, 705–710.
- Haig, D. & Bergstrom, C. T. 1995 Multiple mating, sperm competition and meiotic drive. *J. Evol. Biol.* **8**, 265–282.
- Hauschteck-Jungen, E. & Maurer, B. 1976 Sperm dysfunction in sex ratio males of *Drosophila subobscura*. *Genetica* **46**, 459–477.
- Hewitt, G. M., Mason, P. & Nichols, R. A. 1989 Sperm precedence and homogamy across a hybrid zone in the Alpine grasshopper *Podisma pedestris*. *Heredity* **62**, 343–353.
- Jaenike, J. 1996 Sex-ratio meiotic drive in the *Drosophila quinaria* group. *Am. Nat.* **148**, 237–254.
- Kotrba, M. 1996 Sperm transfer by spermatophore in Diptera: new results from the Diopsidae. *Zool. J. Linn. Soc.* **117**, 305–323.
- LaMunyon, C. W. & Ward, S. 1998 Larger sperm outcompete smaller sperm in the nematode *Caenorhabditis elegans*. *Proc. R. Soc. Lond. B* **265**, 1997–2002. (DOI 10.1098/rspb.1998.0531.)
- Lorch, P., Wilkinson, G. S. & Reillo, P. R. 1993 Copulation duration and sperm precedence in the Malaysian stalk-eyed fly, *Cyrtodiopsis whitei* (Diptera: Diopsidae). *Behav. Ecol. Sociobiol.* **32**, 303–311.
- Markow, T. A. 1997 Assortative fertilization in *Drosophila*. *Proc. Natl Acad. Sci. USA* **94**, 7756–7760.
- Montchamp-Moreau, C. & Joly, D. 1997 Abnormal spermiogenesis is associated with the X-linked sex-ratio trait in *Drosophila simulans*. *Heredity* **79**, 24–30.
- Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelstöm, H. 1996 Sperm selection by females. *Nature* **383**, 585.
- Policansky, D. & Ellison, J. 1970 'Sex ratio' in *Drosophila pseudoobscura*: spermiogenic failure. *Science* **169**, 888–889.
- Presgraves, D. C., Severence, E. & Wilkinson, G. S. 1997 Sex chromosome meiotic drive in stalk-eyed flies. *Genetics* **147**, 1169–1180.
- Price, C. S. C. 1997 Conspecific sperm precedence in *Drosophila*. *Nature* **388**, 663–666.
- Price, C. S. C., Dyer, K. A. & Coyne, J. A. 1999 Sperm competition between *Drosophila* males involves both displacement and incapacitation. *Nature* **400**, 449–452.

- Price, C. S. C., Kim, C. H., Posluszny, J. & Coyne, J. A. 2000 Mechanisms of conspecific sperm precedence in *Drosophila*. *Evolution* **54**, 2028–2037.
- Price, C. S. C., Kim, C. H., Gronlund, C. J. & Coyne, J. A. 2001 Cryptic reproductive isolation in the *Drosophila simulans* species complex. *Evolution* **55**, 81–92.
- Simmons, L. W. & Siva-Jothy, M. T. 1998 Sperm competition in insects: mechanisms and the potential for selection. In *Sperm competition and sexual selection* (ed. T. R. Birkhead & A. P. Møller), pp. 341–434. New York: Academic Press.
- Stockley, P. 1999 Sperm selection and genetic incompatibility: does relatedness of mates affect male success in sperm competition? *Proc. R. Soc. Lond. B* **266**, 1663–1669. (DOI 10.1098/rspb.1999.0829.)
- Taylor, D. R., Saur, M. J. & Adams, E. 1999 Pollen performance and sex-ratio evolution in a dioecious plant. *Evolution* **53**, 1028–1036.
- Wade, M. J. & Chang, N. W. 1995 Increased male fertility in *Tribolium confusum* beetles after infection with the intracellular parasite *Wolbachia*. *Nature* **373**, 72–74.
- Wade, M. J., Patterson, H., Chang, N. & Johnson, N. A. 1994 Postcopulatory, prezygotic isolation in flour beetles. *Heredity* **72**, 163–167.
- Ward, P. I. 2000 Cryptic female choice in the yellow dung fly *Scathophaga stercoraria* (L.). *Evolution* **54**, 1680–1686.
- Wilkinson, G. S. & Sanchez, M. I. 2001 Sperm development, age and sex chromosome meiotic drive in the stalk-eyed fly, *Cyrtodiopsis whitei*. *Heredity* **87**, 17–24.
- Wilkinson, G. S., Kahler, H. & Baker, R. H. 1998a Evolution of female mating preferences in stalk-eyed flies. *Behav. Ecol.* **9**, 525–533.
- Wilkinson, G. S., Presgraves, D. C. & Crymes, L. 1998b Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. *Nature* **391**, 276–278.
- Wu, C.-I. 1983 Virility deficiency and the sex-ratio trait in *Drosophila pseudoobscura*. II. Multiple mating and overall virility selection. *Genetics* **105**, 663–679.
- Zeh, J. A. & Zeh, D. W. 1996 The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proc. R. Soc. Lond. B* **263**, 1711–1717.