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Heteropopulation males have a fertilization advantage during sperm competition in the yellow dung fly (*Scathophaga stercoraria*)

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Sexual conflict occurs whenever there is not strict genetic monogamy. The sexually antagonistic coevolution that potentially occurs because of this conflict involves adaptation by one sex followed by the counter-adaptation by the other, and may be thought of as an evolutionary arms-race. As a result of these cycles of antagonistic coevolution, females from one population may be less resistant to heteropopulation males, at least after short periods of allopatry, as they will not have evolved any resistance to them. We tested this prediction in yellow dung fly (*Scathophaga stercoraria*) populations from the UK and Switzerland. Males from each population mated as first and second males to females from each population, and the mean numbers of offspring sired by the last male to mate in each situation were compared. We also compared the fertility and fecundity of single females mated to males from both populations, as well as the fertility and fecundity of the F_1 crosses. Both crosses produced viable and fertile offspring and the offspring sex ratios were not skewed. However, the fecundity of F_1-cross females was greater than that of the parentals. In the sperm-competition experiment, there was a significant interaction between male and female origin influencing the proportion of offspring sired by the second male to mate, with heteropopulation males always outcompeting conpopulation males. This effect was independent of copula duration and the delay between copulations. In a separate experiment, we tested to see whether this was due to female preference for genetically dissimilar males but found no evidence for paternity biasing based on genetic similarity. Our results therefore seem to be best explained by sexually antagonistic coevolution as females appear less resistant to males with which they have not coevolved.

Keywords: sexual conflict; sperm competition; Scatophaga; yellow dung fly; heterosis; sexually antagonistic coevolution

1. INTRODUCTION

Cycles of antagonistic coevolution occur in many biological contexts (e.g. host–parasite or predator–prey coevolution). They can also take place between the sexes because of sexual conflict, which occurs whenever the evolutionary interests of males and females do not coincide (Parker 1979). This situation arises whenever there are departures from strict genetic monogamy (Rice 2000) and can lead to sexually antagonistic evolution. Several recent experimental studies provide evidence for antagonistic evolution and indicate that relaxing or increasing the degree of sexual conflict, or giving one sex an advantage in the sexual arms-race, leads to the rapid evolution of antagonistic characters (Rice 1996; Holland & Rice 1999; Hosken *et al.* 2001; Pitnick *et al.* 2001). In addition, theoretical models indicate that the evolution of conflict-related traits can be selectively favoured over a broad parameter space, even when they cause physical damage to a mate (Johnstone & Keller 2000). These outcomes are consistent with studies detecting severe damage inflicted during mating in a range of taxa (e.g. Chapman *et al.* 1995; Crudgington & Siva-Jothy 2000; Blanckenhorn *et al.* 2002).

One basic prediction of sexually antagonistic coevolution is that, at least after short periods of allopatry, males from one population will be more successful during sperm competition with females from another population because these females will have evolved resistance to their own, but not to the heteropopulation males (Rice & Holland 1997; Parker & Partridge 1998). This is because evolutionary change driven by sexual conflict can be a perpetual arms-race, where adaptations in one sex are followed by counter-adaptation in the other (Rice 1998, 2000). As a result, the genes involved should evolve continually and rapidly, and therefore populations should diverge in these portions of the genome (Rice 1998). Rice (1998) suggested that this could occur even with low levels of gene flow between populations. Work on *Drosophila melanogaster* strains supports these ideas. Yanders (1963) found that the insemination index (a measure of the rates of sperm migration to the female sperm stores) of intra- and inter-strain matings varied, with intra-strain sperm migration always slower than in inter-strain matings. Similar results were found in a more comprehensive study by DeVries (1964). However, most investigations to date have been across species or subspecies and find strong homogamy, conspecific male advantage during sperm competition (e.g. Hewitt *et al.* 1989; reviewed in Howard 1999).

It has also been suggested that hybrid infertility should evolve faster than hybrid inviability as a result of cycles of antagonistic evolution, as the loci involved should affect reproductive tissue (Rice 1998). In support of this conjecture, there is ample evidence that reproductive character

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evolution is rapid (e.g. Eberhard 1985; Aguade *et al.* 1992; Pitnick *et al.* 1999; Hellberg & Vacquier 1999; Morrow & Gage 2000). Additionally, in *Drosophila*, seminal proteins have also evolved rapidly, they negatively impact on females longevity and influence male sperm competitiveness, all of which is consistent with sexually antagonistic evolution (reviewed in Chapman 2001). Nevertheless, in practice it is often difficult to distinguish between various evolutionary mechanisms involving preference or resistance. For example, a heteropopulation male advantage could conceivably be due to female choice for genetically dissimilar males to obtain heterosis benefits for her offspring.

While most of the work on sexual conflict has been undertaken with *Drosophila* (e.g. Rice 1996; Pitnick *et al.* 2001; Civetta & Clark 2000; Knowles & Markow 2001), recent investigations with the yellow dung fly (*Scathophaga stercoraria*) indicate that similar processes occur there (reviewed in Snook 2001). The yellow dung fly is a naturally polygamous species with copulation occurring prior to each bout of oviposition (Parker 1970), and multiple mating is largely driven by the males, who are much larger than the females and can force copula (T. Tregenza, N. Wedell, D. J. Hosken and P. I. Ward, unpublished data). In addition, multiple mating is costly for female *S. stercoraria*, reducing their longevity (Hosken *et al.* 2002). Reduced longevity does not appear to be the result of physical damage, as an intensive histological study of copula found no overt signs of injury due to mating (Hosken & Ward 2000), and this cost does not appear to be balanced by fertility benefits as female dung flies store enough sperm for full fertility over at least four clutches but copulate at least once every clutch (Parker 1970). It has also been shown that flies forced to evolve under increased polyandry evolved larger reproductive organs (Hosken & Ward 2001; Hosken *et al.* 2001), although this appeared to come at a cost to the immune system (Hosken 2001). Nevertheless, males from polyandrous treatments were superior during sperm competition and polyandrous females were better able to circumvent the strong last-male sperm precedence typically found in this species, all of which are consistent with sexual-conflict predictions (Hosken *et al.* 2001).

Given the ample opportunity for sexual conflict in nature in this species (e.g. females are not genetically monogamous), together with the laboratory micro-evolutionary responses to variation in conflict, we decided to test some further predictions of sexual-conflict theory using wild populations of *S. stercoraria*. Specifically, we carried out sperm-competition experiments with flies from the UK competing against Swiss flies in females from both populations. These populations are likely to have been separated completely or to a large degree for considerable time, and show slight but significant genetic differentiation ($F_{ST} = 0.049$; $p = 0.017$; T. W. J. Garner, D. J. Hosken and W. U. Blanckenhorn, unpublished data). Based on the theory outlined earlier, we predicted that heteropopulation males would be competitively superior sires in doubly mated females. To ascertain that flies from the two populations have no gross reproductive incompatibilities, we also looked at the fertility and fecundity of females mated to single heteropopulation males, and of the F_1 'hybrids'.

2. MATERIAL AND METHODS

Mating flies from cow pats in Fehraltorf, Switzerland ($n = 40$ pairs) and eggs from Oxford, UK (approximately 120 from several cow pats) were brought to the laboratory. Swiss females were allowed to lay eggs and all resulting young (UK and Swiss) were reared, housed and mated using standard techniques (e.g. Ward & Simmons 1991). After two generations in the laboratory, a sperm-competition experiment was conducted. Males from each population competed against each other as both first and second males in females from each population. Brother–sister matings were not allowed. A female was introduced into a jar containing a smear of dung and a male and, once copulation had naturally ceased, the female was transferred to the second male. Due to the fact that copula duration can influence sperm-competition outcomes in this species (e.g. Parker & Simmons 1994; Ward 2000), durations were recorded to the nearest minute, as was the interval between them. Due to the fact that P2 (proportion of offspring sired by the second of two males to mate) is not influenced by male body size in this species (e.g. males adjust copula duration based on their size to achieve the same average P2 (Parker & Simmons 1994; Ward 2000)), male size was not measured. Females were then allowed to lay a clutch of eggs, which were subsequently reared until emergence at 18 °C. Offspring were then collected and frozen at −80 °C until the DNA was extracted. Mothers and putative fathers were also frozen for DNA extraction. Paternity was then assigned (blind) to a random sample of 12 offspring per clutch (*ca.* 20–30% of the clutch) using the PCR of microsatellite markers and examining the products using an Elchrom (SEA 2000) electrophoresis system with Spreadex gels (Garner *et al.* 2000). Parents were scored at loci sequentially until we found one or more that allowed us unequivocally to assign paternity (mean number of loci required 1.6). Paternity (P2) was then compared for each cross type using ANCOVA.

We also mated females from both populations singly to heteropopulation males and looked at their fertility and fecundity. This was to ensure that cross-population matings were fertile and gross incompatibilities (e.g. Alipaz *et al.* 2001) were not responsible for any interactions or effects in the sperm-competition experiments. We also looked at the sex ratios of these crosses, as a number of cytoplasmic incompatibility factors cause sex-ratio skews (e.g. cytoplasmic feminizers, reviewed in Hurst *et al.* (1997)). Similarly, F_1 crosses were mated with male and female parentals and their fertility was also investigated. Rice (1998) predicted rapid evolution of infertility with sexually antagonistic evolution, and hence we assessed that possibility.

In the sperm-competition experiment, it was possible that the main effect (see § 3) was due to females selecting sperm of genetically dissimilar males (i.e. preference rather than lack of resistance; cf. Olsson *et al.* 1996; Ward 2000) and potentially benefiting via a heterosis effect (see § 3). If this were the case, then we should also see some of the variance in paternity within populations explained by genetic similarity. We investigated this possibility using data from a previously published study (Hosken *et al.* 2001). In that study, flies had been maintained at relatively small population sizes for ten generations, so if genetic similarity influences paternity, it should be important and detectable in this situation (e.g. Olsson *et al.* (1996) found a strong effect in their small, inbred population), especially if heterosis (inbreeding depression) is typically due to masking (expression) of deleterious recessive alleles, as appears to be the case. We used an ANCOVA (or residuals from an ANCOVA) to remove

the effects of selection and copula duration on P2 from that dataset (see Hosken *et al.* 2001) and investigated whether or not genetic similarity of the males to a female explained any residual variance in paternity. Data were analysed in two ways. First, we used relatedness as a categorical variable (the two males in each competitive mating were classed as more or less related to the female) and then as a continuous variable. Here, residual P2 was regressed against the relatedness difference between males relative to the female (i.e. relatedness of the second male to the female minus relatedness of male one to the female). Relatedness was calculated using the KINSHIP 1.2 program (Queller & Goodnight 1989) using eight published (Garner *et al.* 2000) and three recently developed microsatellites. KINSHIP calculates pairwise similarity (relatedness) values using codominant markers and population allele frequencies. In this instance, the population was along the lines of Hosken *et al.* (2001) (cf. Reusch *et al.* 2001). Values range from zero (maximally outbred within the population = low genetic similarity) to 1 (1 = full siblings = high genetic similarity). However, by chance some zero values may be negative (K. F. Goodnight, personal communication), so we carried out our analyses using both raw values, and with negative values scored as zero. In addition, we also analysed a restricted dataset in which simulations were used to test for significant differences in genetic similarity between males relative to females. Based on simulation results, we subsequently only analysed groups where one male's genetic similarity to the female was not significantly different from zero (i.e. maximal outbreeding in our population and hence low genetic similarity) and the other's similarity was significantly equal to or greater than 0.25 (the same genetic similarity as cousins). Using this criterion, we had seven triads, and note that our final full dataset was reduced to 21 for these analyses due to the loss of one cross in a laboratory mishap.

Data were screened to check the assumptions of parametric tests before analyses, and transformed to meet them when necessary.

3. RESULTS

(a) *Single matings*

Both crosses (Swiss (S) male × UK female and UK male × S female) produced viable offspring, and the number emerging did not differ from pure Swiss matings (S × S) (mean number of offspring ± s.e.: S × S = 38.2 ± 4.7 ($n = 9$), S × UK = 38.5 ± 3.1 ($n = 27$), UK × S = 38.9 ± 3.3 ($n = 23$); $F_{2,56} = 0.007$; $p = 0.99$). In addition, neither of the crosses deviated from an even sex ratio (one-sample *t*-tests of the number of (male–female) offspring per family: both comparisons $t < 0.33$; $p > 0.74$).

The fecundity of F_1-cross females (denoted as mother's origin × father's origin) was typically greater than that of the parentals (full ANOVA: $F_{3,71} = 6.4$; $p = 0.0007$. Fisher's PLSD: S versus UK × S, $p = 0.003$; S versus S × UK, $p = 0.0002$; S versus UK, $p = 0.17$; UK versus S × UK, $p = 0.11$; UK versus UK × S, $p = 0.017$; UK × S versus S × UK, $p = 0.41$) (figure 1). This effect remained even with the body-size effects statistically removed ($p = 0.002$).

In the F_1 fertility assessment, some females had more young emerge than eggs laid (i.e. we miscounted the eggs: $n = 5$ of 98 flies). Data were therefore analysed in two ways: with more than 100% emergence set at 100%, and with the miscounted clutches excluded. Regardless of the analysis, however, the fertility of the various crosses did not statistically differ (female × male mean ± s.e.: S × (S × UK) = 0.76 ± 0.06 ($n = 23$); (S × UK) × S = 0.67 ± 0.06 ($n = 21$); UK × (UK × S) = 0.65 ± 0.07 ($n = 18$); (UK × S) × UK = 0.6 ± 0.07 ($n = 18$)) (both $F < 0.8$; both $p > 0.5$). Thus, both male and female F_1 crosses were fertile. Similarly, when compared with a large unpublished dataset of pure Swiss flies (mean hatch rate of 0.74 ± 0.026; T. Tregenza, N. Wedell, D. J. Hosken and P. I. Ward, unpublished data), the fertility of the crosses was not statistically different from females mated to conpopulation males (one-sample *t*-tests, all $p > 0.09$).

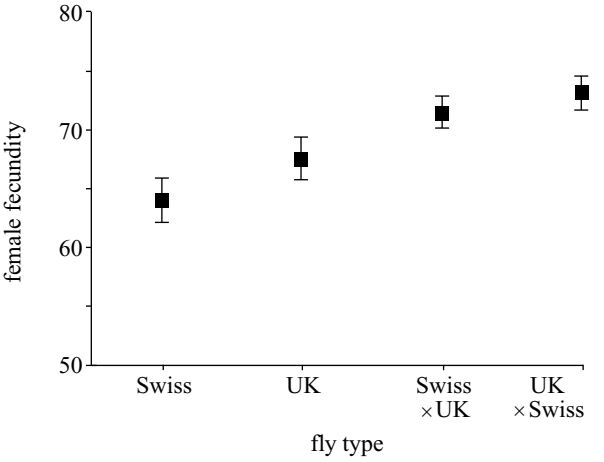

Figure 1. The mean (± s.e.) fecundity of the F_1 females (mother's origin then the father's origin) and the parentals. The F_1-cross females were more fecund than the parentals (see § 3a).

(b) *Sperm competition*

Repeated-measures ANOVA of females' two-copula durations, with flies' origin as factors, indicated that both female and male origin had a significant influence on copula duration ($F > 6.2$; $p < 0.01$), but there were no other significant effects (all $F < 2.7$; $p > 0.11$) (mean (± s.e.) copula duration in minutes: UK males, 33.5 (± 1.3) min (number in copulation 1, 22; number in copulation 2, 25); Swiss males, 38.3 (± 1.2) min (number in copulation 1, 25; number in copulation 2, 22); UK females, 33.9 (± 1.2) min (number in copulation 1, 29; number in copulation 2, 18); Swiss females, 39.4 (± 1.3) min (number in copulation 1, 18; number in copulation 2, 29)).

We also looked at the delay between the end of copula one and the beginning of copula two and its influence on the duration of copula two. When we considered all triads, there was no significant association ($F_{1,45} = 1.52$; $p = 0.22$). However, when we excluded one group with an exceptionally long delay (> 30 min = twice as long as the next longest delay), there was a marginally non-significant negative effect ($F_{1,44} = 3.80$; $p = 0.058$).

Due to the fact that we found some significant effects in these analyses and because copula duration could potentially influence paternity, analyses of paternity included copula durations (or, alternatively, the difference in durations) and the delay between copulations 1 and 2. In a full model ANCOVA with male and female origin and their interaction as factors, and delay and difference in copula duration as the covariates, only the two-way

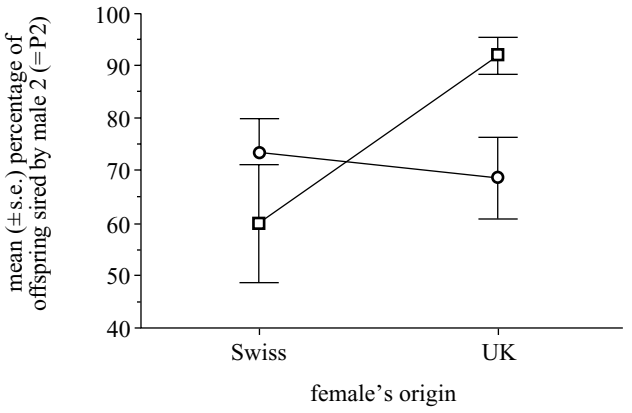

Figure 2. An interaction plot showing the proportion of the brood sired by the second male to mate in relationship to the male and female origin (Switzerland or the UK). Squares, Swiss male; circles, UK male.

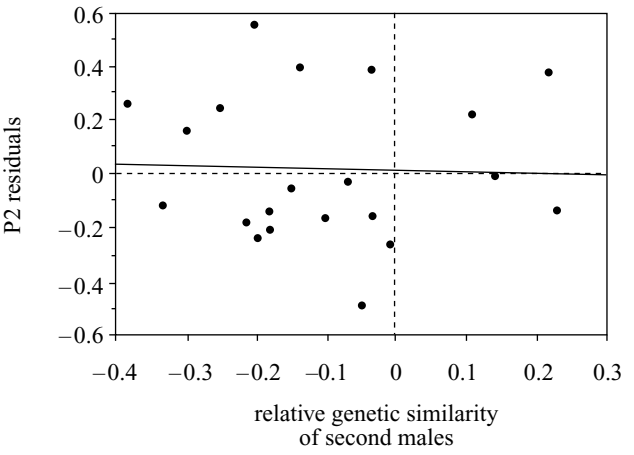

Figure 3. Regression plot of residual P2 (corrected for male and female selective regime and copula duration. See Hosken *et al.* 2001) against relative genetic similarity of male 2 (measured as the differences in relatedness of males to the female; see § 2 for further clarification).

interaction between the factors was significant (figure 2, $F_{1,41} = 6.67$; $p = 0.014$) (all other $F < 2.85$; $p > 0.09$). The same result was found when both copula durations were used as covariates and, with removal of the non-significant covariates, the interaction was even stronger ($F_{1,43} = 7.31$; $p = 0.009$).

As explained, it was possible that female preference for genetically dissimilar males rather than lack of resistance to heteropopulation males was the cause of the P2 interaction. This seems especially likely given that cryptic female choice based on PGM genotype has been detected in this species (Ward 2000). To assess this possibility, we looked for associations between P2 within-population matings from an earlier study (Hosken *et al.* 2001) and the genetic similarity of males to females. We used these data because the flies were relatively inbred and hence the likelihood of detecting an effect was enhanced (i.e. heterozygosity benefits were likely to be important because the flies were relatively inbred).

Male genetic similarity (relatedness) to females varied from 0 (−1.8) to *ca.* 0.35 (mean ± s.e., 0.1 ± 0.015), providing substantial variation that could potentially influence paternity. The mean (± s.e.) absolute difference in genetic similarity between competing males was 0.17 (± 0.02), which again indicates that, on average, substantial differences between competitors existed.

An ANCOVA using genetic similarity as a categorical variable (the two competing males were classed as being more or less similar to the female) indicated that the relative degree of similarity had no significant effect on P2 ($F_{1,16} = 1.98$; $p = 0.18$). Likewise, when we restricted our analysis to those cases where male genetic similarity differed at the 0.25 level, we found no significant similarity effect (mean P2 residuals ± s.e., more similar = 0.11 ± 0.25, less similar = 0.07 ± 0.15; $F_{1,5} = 0.02$; $p = 0.88$).

Regression analysis of P2 residuals against differences in genetic similarity found no significant associations when analysing raw data ($F_{1,19} = 0.02$; $p = 0.89$; figure 3), when corrected data were analysed (i.e. setting negatives to zero, $F_{1,19} = 0.002$; $p = 0.98$), when the data were restricted to males that significantly differed at the 0.25 level ($F_{1,5} = 0.02$; $p = 0.90$) or if multiple regressions using the similarity of male 1 and male 2 as predictors were performed ($F_{2,18} < 1.02$; $p > 0.38$).

We estimated the sample size required to have had an 80% probability of detecting a significant difference based on parameters estimated from the full ANCOVA (Zar 1999, p. 194). This analysis indicated that a sample size of about 1000 triads was necessary for there to be any chance of detecting a significant effect of genetic similarity, assuming that the MS_{error} from the ANCOVA was a reasonable estimator of population variance.

4. DISCUSSION

In contrast to many studies that show homogamy (reviewed in Howard 1999), the main result of our study was that heteropopulation males had higher fertilization success during sperm competition. That is, males that had not coevolved with females were competitively superior during sperm competition against males from the same population as females. This result is in conflict with classic female mate-choice theory, which indicates that females should prefer males with which they coevolve (Andersson 1994). However, it is consistent with either cryptic female choice for genetically dissimilar males (e.g. Olsson *et al.* 1996; Tregenza & Wedell 2002), or sexually antagonistic coevolution and female resistance to conpopulation males (Rice & Holland 1997; Parker & Partridge 1998).

We favour the latter explanation because our intrapopulation comparison revealed no evidence for any effect of genetic similarity on paternity. It could be argued that the level of similarity between males and females in this comparison was not great enough to be problematic for females (i.e. mating with first cousins has little fitness impact), and hence females did not exert a preference. This probably needs further investigation. However, in the cross-population sperm-competition experiment, females never mated with siblings. In addition, the choice for heterosis may involve (one or few) loci that are fixed for different alleles in different populations (e.g. heterosis based on a single locus; Keller & Ross 1998), hence we would not see any effect in our lines derived from a single population. However, this seems unlikely because single-locus heterosis seems extremely rare (Maynard Smith 1998),

especially compared with general heterozygosity masking recessive deleterious alleles. It may also be that the neutral markers that we used were not overly informative in terms of what females were potentially choosing. However, there is evidence that microsatellite variation predicts variation in loci coding quantitative traits (Merilä & Crnokrak 2001) and that, in at least some taxa, microsatellite variation correlates with heterosis (e.g. Coltman *et al.* 1998). In addition, and importantly, we do not expect selection to favour this form of choice in natural dung fly populations because females are unlikely to come across closely related (genetically similar) individuals in the field. In free-living flies, eggs are laid in dung pats and flies disperse from these to feed before sexual maturity is attained, males usually emerge some days after females, and after the period of post-emergence feeding, flies seek fresh dung to reproduce. With this ecology, the likelihood of meeting a related fly is probably low, and hence selection for paternity biasing based on genetic similarity is weak. In support of this conjecture, allozyme-based population genetic studies found no structuring at any level across the whole of Switzerland (Kraushaar *et al.* 2002). Nevertheless, in several *Drosophila* species, which in many ways have similar ecology to yellow dung flies (Blanckenhorn 1999), genetic similarity can influence paternity (see Markow 1997), so this explanation is not entirely satisfactory. However, the lack of association between genetic similarity and paternity supports our conclusion that lack of female resistance, rather than preference, is responsible for the male × female interaction influencing paternity. Finally, in this species cryptic female choice has been difficult to detect unequivocally in spite of concerted effort (e.g. Ward 2000 and references therein), and variation in paternity due to cryptic female choice in this species, and generally, is small (Simmons 2001), unlike the large effects that we detect here.

It therefore appears that our main result is in accordance with sexual-conflict theory which indicates that, after short periods of allopatry, males should be more successful with heteropopulation females because these females will not have evolved resistance to them in the coevolutionary struggle between the sexes. Similar results have been found in *Drosophila* (e.g. Yanders 1963) and, in the housefly, *Musca domestica*, recent evidence indicates that seminal signals and their receptors in females evolve in a sexually antagonistic manner (Andres & Arnqvist 2001). This provides a potential mechanism for our results and, for example, Clark *et al.* (1999) reported interactions between the sexes that are consistent with sexual conflict over sperm displacement mediated by seminal proteins. It is therefore plausible that female counter-adaptation to harmful male effects (e.g. via their seminal fluids) could result in a reduction in the competitiveness of ejaculates from conpopulation males relative to heteropopulation males that have different adaptations. It should, however, be emphasized that our results relate to only two populations, and we have no data on relative costs to females when mating with heteropopulation males. We are currently investigating such costs and the generality of our findings, and this work in progress should shed further light on the underlying cause of the advantage for heteropopulation males. Nonetheless, the higher P2 value when heteropopulation males are second to copulate represents a potential cost to females if it is due to sexual antagonism and greater sperm displacement because it decreases the opportunity for cryptic female choice (Ward 2000). In addition, reduced variation in a clutch is potentially costly, but the conditions favouring increased variance may be restrictive (Hosken & Blanckenhorn 1999). Our study is also one of a growing number to document an interaction between males and females influencing paternity (e.g. Wilson *et al.* 1997; Tregenza & Wedell 1998; Clark *et al.* 1999) and corroborates previous work in yellow dung flies showing clear male × female effects (Hosken *et al.* 2001).

As stated, regardless of the analysis employed, we could find no influence of genetic similarity on residual P2. Tregenza & Wedell (2000) emphasized the potential importance of genetic similarity as a source of genetic incompatibility (see also Zeh & Zeh 1996), and in other flies, genetic similarity influences paternity. For example, Markow (1982, 1997) found that female *Drosophila* mated to their brothers laid fewer eggs in spite of adequate sperm transfer, indicating an attempt to defray the costs of mating with genetically similar males by delaying oviposition. We found no such effects here, and although our males did not span the range of similarities and differences employed in some studies, we suggest that the genetic similarity values that we investigated are probably closer to the ranges encountered by females in nature.

Hybrid infertility is predicted to evolve faster than inviability under sexually antagonistic evolution (Rice 1998), but we found no evidence for sterility in the F_1 flies, in spite of the strong interaction between male and female origin. Hybrid infertility is expected to result from incompatibilities of the divergent developmental programs for gametogenesis that should occur in allopatric populations. This should then select for con-specific or -populational sperm precedence, for which there is ample evidence (e.g. Hewitt *et al.* 1989; Brown & Eady 2001; reviewed in Howard 1999). It is not clear how fast divergence sufficient to cause hybrid infertility should occur, but it seems that either insufficient divergence times or limited gene flow between the UK and Swiss flies has prevented this occurrence, although we do find slight but significant differentiation between them. In other fly studies, matings between subspecies often produce sterile hybrids (e.g. Snook 1998). Moreover, and unlike here, one study that carried out across-population competitive matings provides strong evidence for homogamy, although these populations exhibited divergence in fundamental reproductive traits, with one population's sperm being monomorphic and the other's sperm dimorphic (Joly *et al.* 1991). Nevertheless, homogamy is the pattern typically detected, and it may be the result of the predicted rapid evolution of antagonistic loci, although relatively few controlled intra-specific across-population studies have been undertaken (Howard 1999). As a result, we currently do not know whether heteropopulation males generally have an advantage during sperm competition.

The increased fecundity of F_1 females from the Swiss and UK crosses is consistent with many other studies finding hybrid vigour or heterosis (e.g. Coltman *et al.* 1998; Coulson *et al.* 1998). This effect was independent of body-size variation, and further indicates that the two populations had not greatly diverged, because hybridization often reduces offspring fitness via outbreeding

depression. However, we currently do not think the lack of homogamy relates to cryptic female choice for heterosis benefits for the reasons already outlined, but we are investigating this possibility further. We also found no fertility differences in the various crosses, and it is interesting to note that the lowest fertility came from matings between parental males and F_1-cross females. Nevertheless, the lack of significant fertility differences and even sex ratios indicate there are no overt genetic incompatibilities such as population differences in infection with cytoplasmic symbionts that are sometimes detected via skewed sex ratios (Zeh & Zeh 1996).

In conclusion, allopatric males were more competitive than sympatric males during sperm competition. This does not appear to be due to female preferences for dissimilar males, although we cannot completely rule this out, and therefore the results support a theory that indicates that sexually antagonistic evolution will cause females to evolve resistance to sympatric, but not to allopatric males. Nevertheless, these conclusions are based on only two yellow dung fly populations, and hence the generality of these findings remains unclear. Furthermore, potential fitness costs to females predicted by sexually antagonistic evolution have yet to be unequivocally demonstrated.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.