Autosomal variation for male body size and sperm competition phenotypes is uncorrelated in *Drosophila melanogaster*

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Correlations between male body size and phenotypes impacting post-copulatory sexual selection are commonly observed during the manipulation of male body size by environmental rearing conditions. Here, we control for environmental influences and test for genetic correlations between natural variation in male body size and phenotypes affecting post-copulatory sexual selection in *Drosophila melanogaster*. Dry weights of virgin males from 90 second-chromosome and 88 third-chromosome substitution lines were measured. Highly significant line effects ($p < 0.001$) documented a genetic basis to variation in male body size. No significant correlations were identified between male body size and the components of sperm competitive ability. These results suggest that natural autosomal variation for male body size has little impact on post-copulatory sexual selection. If genetic correlations exist between male body size and post-copulatory sexual selection then variation in the sex chromosomes are likely candidates, as might be expected if sexually antagonistic coevolution was responsible.

Keywords: post-copulatory sexual selection; genetic correlation; sperm competition; male body size; genetic variation

1. INTRODUCTION

Characterizing phenotypes affected by pre- and post-copulatory sexual selection has important implications for understanding the maintenance of genetic variation, sexual conflict, the evolution of mating systems, and how these factors might contribute to speciation. For example, correlations between traits can influence how selection acting on one phenotype will impact another. Correlations can arise due to environmental conditions or genetic differences among individuals. Genetic correlations may be due to variation in either the autosomes or the sex chromosomes and can lead to correlated responses to selection.

In *Drosophila melanogaster*, traits impacting pre-copulatory sexual selection are correlated with male body size. Wild males caught in copula are larger than randomly collected males (Taylor & Kekic 1988) and in population cages larger males mate with females more quickly, have greater overall mating success and

are more likely to chase smaller males (Partridge & Farquhar 1983). Environmental factors may have influenced these phenotypic correlations but strong evidence also exists for genetic correlations between male body size and pre-copulatory sexual selection (Ewing 1961; Wilkinson 1987).

There is also evidence that male body size influences post-copulatory sexual selection. Positive correlations have been observed between male body size and sperm competitive ability (Bangham *et al.* 2002; McGraw *et al.* 2007), part of which is due to differences in the size of the accessory glands (Bangham *et al.* 2002). Females also appear to remate more quickly with larger males but these females suffer reduced fertility 1 day post-mating (Pitnick 1991) and females housed with large males have reduced longevity (Friberg & Arnqvist 2003). Male body size in these studies was manipulated by varying either larval nutrient availability or larval density and thus genotypic correlations, and the potential for correlated responses to selection could not be estimated.

Here, we control for environmental influences to investigate the relationship between male body size and phenotypes affecting post-copulatory sexual selection. Using lines derived from a natural population of *D. melanogaster* we explicitly test if autosomal variation in the second or third chromosome is driving genetic correlations between these traits. We report the presence of natural genetic variation for male body size but find little evidence for genetic correlations between male body size and sperm competition phenotypes in *D. melanogaster*.

2. MATERIAL AND METHODS

We used 90 second-chromosome and 88 third-chromosome substitution lines derived from a natural population of *D. melanogaster* in State College, Pennsylvania, USA (Lazzaro *et al.* 2004; Fiumera *et al.* 2007); thus, any polymorphism we sampled is segregating in nature and potentially subject to natural selection. Chromosome substitution lines are homozygous and isogenic except that each second- or third-chromosome substitution line represents a unique second or third chromosome segregating in nature.

Previous studies assayed these lines for multiple phenotypes affecting sperm competitive ability when competing against a standard competitor strain (Fiumera *et al.* 2005, 2007). The phenotypes affecting sperm competition (both 'offence' and 'defence' measures) included the ability of males to induce fidelity in his mate (*refractoriness*), the proportion of offspring sired by the first male to mate to a doubly mated female (*P1*), the total number of offspring produced (*fecundity-defence* and *fecundity-offence*; depending upon whether the experimental male mated first or the second), the male-induced decrease in female longevity (*cost of mating*), the ability of the male to encourage a female to mate with him (*remating*), and the proportion of offspring sired by the second male to mate to a doubly mated female (*P2*). Experimental males can be either the first (defence) or second (offence) male to mate. For defence, virgin females are mated to an experimental male and 48 h later were given the opportunity to mate to a tester male. Fidelity is the proportion of females that do not remate. *Fecundity-defence* (14-day egg-laying) and *P1* (egg-laying after second mating) are calculated for doubly mated females only. For offence, the experimental male mates first and remating rate is the proportion of females that mate to the tester male and remate with the experimental male. *Fecundity-offence* and *P2* are measured similar to the defence analogues.

Here, we measure male body size and test for correlations with sperm competition phenotypes. Cultures were maintained on standard agar-dextrose-yeast media at 24°C on a 12 L:12 D cycle. For each chromosome extraction line, vials for virgin collection were set up at medium densities (approx. 75–100 larvae per vial) by allowing mated females to oviposit for 3–5 days. This was designed to mimic the conditions (including lighting, temperature, humidity) under which the sperm competition phenotypes were measured but means that densities were not perfectly

Table 1. ANOVA table for natural variation in male body size.

source	DF	SS	MS	F-value	p-value
<i>second lines</i>					
line	89	0.110383	0.001213	5.41	<0.0001
vial	4	0.089783	0.022446	100.15	<0.0001
<i>third lines</i>					
line	87	0.212271	0.00244	10.43	<0.0001
vial	4	0.002945	0.000736	3.15	0.015

controlled. Five different replicate vials (i.e. one vial set up in each of five different generations) were established for each line with all lines represented in each generation. Three virgin males were collected from each vial (over CO₂) and aged for 24 hours (± 6 hours) before being frozen at -20°C . Males were dried at 60°C in a drying oven for 24 hours before being weighed using a Mettler Toledo MX5 scale.

Analysis of variance (ANOVA) was used to test for differences in male body size across the lines and estimate least-square line means. The least-square line means from previously measured sperm competition phenotypes (*P1*, *P2*, *fecundity-offence*, *fecundity-defence*, *refractoriness*, *remating* and *cost of mating*) were taken from Fiumera *et al.* (2005, 2007). Line means were used to test for genetic correlations between male body size and sperm competition phenotypes via a Pearson correlation coefficient. The second- and third-chromosome substitution lines were analysed separately. Scoring the line means across multiple generations and not measuring body size and sperm competition phenotypes in the same individuals prevented uncontrolled environmental influences from generating spurious correlations.

3. RESULTS

Dry weights were measured from the 90 second-chromosome and 88 third-chromosome substitution lines ($n=2471$ across all 900 vials). Owing to extremely high or low density vials (determined by eye) or owing to lost or killed males, 199 measures were excluded. Highly significant line effects were detected ($p<0.001$) using ANOVA for both the second- and third-chromosome substitution lines (table 1). This demonstrates that variation in both the second and third chromosomes contributes to the genetic basis for natural variation in male body size. The average dry weight of males from the second- and third-chromosome substitution lines was 207.0 and 214.8 μg , respectively. Males from the heaviest lines weighed 319.0 and 291.0 μg , and the lightest weighed 113.0 and 158.0 μg (figure 1). There was a significant effect of vial ($p<0.015$) and our estimates of line means are based on the measures across all five vials measured in different generations. Fiumera *et al.* (2005, 2007) observed significant line effects for the sperm competition phenotypes indicating genetic variance for these traits.

Despite the highly significant line effects for body size and previously measured sperm competition phenotypes, no significant correlations (after Bonferroni correction) were identified between male body size and the measures of sperm competitive ability for either the second- or third-chromosome substitution line (table 2). Remating rate was positively correlated with male body size before correction ($p=0.028$) but failed to meet the conservative Bonferroni cut-off ($p<0.0036$). This suggests that natural genetic

Figure 1. Rank-ordered line means (and standard errors) for male body size for (a) the second-chromosome and (b) third-chromosome substitution lines.

variation for male body size has little impact on these measures of male reproductive success in post-copulatory sexual selection.

4. DISCUSSION

We observed highly significant line effects for differences in male body size in both the second- and third-chromosome substitution lines, indicating that naturally segregating polymorphisms on these autosomal chromosomes affect male body size. We then compared male body size across these lines with phenotypes affecting sperm competitive ability to explicitly test if autosomal variation is driving genetic correlations between these traits. Our results indicate that natural variation in the autosomes does not produce significant genetic correlations between male body size and traits impacting post-copulatory sexual selection (although a suggestive correlation exists with *remating rate*).

It is unlikely that the lack of genetic correlations was due to limited statistical power since strong positive correlations exist between multiple sperm competition phenotypes in these same 178 lines. For example, *P1* was positively correlated with *P2*, male-induced female fecundity and female refractoriness to remating in both sets of lines (Fiumera *et al.* 2005, 2007). Thus, we feel confident that the natural genetic variation that we sampled for male body size has little impact on these measures of male reproductive success. We think it unlikely, but cannot completely rule out the possibility that novel mutations that occurred between when we measured the sperm competition and male body size phenotypes disrupted any existing genetic correlations.

Phenotypes affecting pre- but not post-copulatory sexual selection do show genetic correlations with male body size (Ewing 1961; Wilkinson 1987) and

Table 2. Pearson correlation coefficients (*r*) and *p*-values between male body size and phenotypes impacting post-copulatory sexual selection.

	<i>P1</i>	<i>P2</i>	remating rate	fecundity (offence)	fecundity (defence)	refractoriness	cost of mating
<i>second lines</i>							
<i>r</i>	0.149	0.004	0	0.104	−0.108	−0.063	0.022
<i>p</i> -value	0.164	0.967	0.999	0.33	0.315	0.561	0.84
<i>third lines</i>							
<i>r</i>	0.185	0.054	0.234	−0.05	0.016	0.125	−0.03
<i>p</i> -value	0.084	0.616	0.028	0.646	0.881	0.246	0.782

several possible explanations exist for this disparity. Perhaps correlations between male body size and sperm competition are driven solely by environmental causes and not genetics. Flies reared under environmental conditions that produce small males sire a smaller proportion of offspring under competitive conditions (Bangham *et al.* 2002; McGraw *et al.* 2007) or when the second male (Amitin & Pitnick 2007) to mate with a doubly mated female. Small males are also less costly to mate with (Pitnick & García-González 2002; Friberg & Arnqvist 2003). Male body size may be a condition-dependent indicator of male quality (Rowe & Houle 1996) affecting pre-copulatory female mate choice but not post-copulatory female choice via sperm use (Eberhard 1996). Seminal fluid proteins may be important determinants of phenotypes affecting post-copulatory sexual selection (Clark *et al.* 1995) and male condition seems to have relatively little impact on expression of some of these genes (McGraw *et al.* 2007). If these correlations were due to environmental influences, then selection acting on phenotypes affecting post-copulatory sexual selection is unlikely to record a correlated response in male body size.

If, however, correlations are due to genetic influences, our results indicate that variation in the major autosomal chromosomes is not responsible (but we have not tested the very small fourth chromosome). Chippindale & Rice (2001) did observe a strong epistatic effect of the Y-chromosome on sperm competitive ability. Therefore, the role of variation in the sex chromosomes needs to be investigated. Lew *et al.* (2006) argued that the correlation between female preference for large males and male harm is an indicator of sexually antagonistic coevolution between the sexes. If sexual antagonism is responsible (Rice 1984) then variation in the sex chromosomes may be expected to be responsible for any genetic correlations between male body size and post-copulatory sexual selection. Previous studies, however, find that most of the genetic variation for male body size resides on the autosomes (Gockel *et al.* 2002) although this may not preclude significant correlations.

In summary, despite observing extensive natural genetic variation for both male body size and phenotypes affecting post-copulatory sexual selection we did not observe significant genetic correlations between these traits. This is in contrast to previous studies on pre-copulatory sexual selection and

suggests that the underlying genetic architectures may differ between traits impacting pre- and post-copulatory sexual selection.

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