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Does segregating variation in sexual or microhabitat preferences lead to non-random mating within a population of *Drosophila melanogaster*?

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Variation in female choice for mates has implications for the maintenance of genetic variation and the evolution of male traits. Yet, estimates of population-level variation in male mating success owing to female genotype are rare. Here, we used a panel of recombinant inbred lines to estimate the strength of selection at many genetic loci in a single generation and attempt to assess differences between females with respect to the males they mated with. We performed selection assays in a complex environment to allow differences in habitat or social group preference to be expressed. We detected directional selection at loci across the genome, but are unable to provide support for differential male success because of variation in female genotype.

Keywords: *D. melanogaster*; selection; mate choice; pyrosequencing; recombinant inbred lines

1. INTRODUCTION

Even within a species, males vary greatly in their type and degree of sexual signal (Van Doorn & Weissing 2004), and genetic variation in signalling traits is high (Hine *et al.* 2004). Why there should be such variance, when directional selection depletes genetic variation, is one of the most debated topics in the sexual selection literature (Rowe & Houle 1996). One major hypothesis regarding the maintenance of signal-trait variation is condition dependence. Abundant, novel deleterious mutations will decrease male vigour and result in reduced signalling ability, constantly generating new variation (e.g. Tomkins *et al.* 2004). Another set of hypotheses posit that there is no single direction of selection because females vary in their choices, thus variance in male signals is maintained (Krupa & Sih 1993; Coleman *et al.* 2004). Another form of mate choice might be indirect mate choice mediated by environmental preferences (Edelaar *et al.* 2008). This may occur when different genotypes prefer distinct habitats or groups (Stamps *et al.* 2005), generating differential mate choice via encounter rates (Krupa & Sih 1993; Berlocher & Feder 2002).

While the strength and directionality of sexual selection in *Drosophila* have been measured from the population to the species level, estimates of variation in female preference at the population level are rare (but see Ritchie *et al.* 2005; Cabral *et al.* 2008; McGuigan *et al.* 2008). Here, we estimate the strength of selection at many genetic loci in a single generation. We release a mix of recombinant inbred line (RIL) males into a room and allow females to choose among them to mate. We then measure marker allele frequency changes between the male parents and their offspring to estimate the effects of selection. We vary the female 'chooser' genotypes to see whether we can detect differences between females in male success. We perform the mating assays in a complex room-sized environment in order to allow differences in habitat or social group preference to be expressed.

2. MATERIAL AND METHODS

(a) *Flies*

For the female genotypes, we used two isogenized lines (lines 145 and 83) that were collected from an orchard in 1998 (Winters, CA) (Yang & Nuzhdin 2003). Males originated from 214 RILs that were constructed by crossing two Winters CA isogenic lines (Bergland *et al.* 2008). In RILs, each line represents a unique combination of haplotypes, and they are used to detect associations between genetic loci and traits of interest. Winters lines were used because they have been found to have significant overall gene-expression differences (e.g. Wayne *et al.* 2007), and extensive genotypic differences in behavioural traits such as male aggression and mating success (Cabral *et al.* 2008). Extensive variation in homogeneous contexts suggests that these lines might demonstrate differences in an environment designed to increase opportunities for behavioural variation (discussed subsequently). Flies were maintained under constant environmental conditions 12 L : 12 D; 25°C throughout the experiments. Virgin females and males were collected and held in same-sex, low-density vials prior to being released into the rooms. At the same time, males and females were collected for genotyping (discussed subsequently).

(b) *Room assays*

Three humidified, temperature-controlled rooms were used, and each female line was trialled once in each room. Room assays were performed in two blocks. Each room was set up similar to the protocol in the experiments of Stamps *et al.* (2005), with nine patches and a wire frame for flies to use as a perch (figure 1). Each patch had 12 banana sections, covered in gauze to facilitate egg collection and spread with yeast paste, distributed around the perches. We released three males from each of 210 RILs into the rooms 2 h before relative dusk and allowed them to settle into their chosen habitats. In Stamps *et al.* (2005), conducted on different lines derived from the same population, extensive genotypic differences in habitat use were found. Flies evidently can and do respond differently to the environmental context provided. In each trial, we then released approximately 400 virgin females from one genotype and allowed them to choose mates and lay eggs. More males than females were released in order to increase competition between males and maximize our chances of detecting genotypic effects on female choice.

Eggs were collected from the bananas at three time points throughout the experiment at 12 h intervals beginning the morning after release, and the bananas were replaced with fresh bananas after each collection. All eggs were frozen until genotyping.

(c) *DNA extraction and genotyping*

Egg DNA was collected by removing the eggs from the gauze and homogenizing them. Egg and adult DNA extractions were performed using standard phenol–chloroform methodology. The single nucleotide polymorphism (SNP) markers used in this study are described in Bergland *et al.* (2008). A subset of 48 SNPs was genotyped in the parents and in the egg offspring samples considered in this experiment (table S1, electronic supplementary material). Marker information, primers, amplicon size and sequence are detailed in

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Figure 1. Picture of a microhabitat-diverse corner of one room (left), with several wire perches as well as banana food patches (right, top and bottom).

table S1 of the electronic supplementary material. Pyrosequencing was performed using the PSQ96 Pyrosequencer System and the Pyro Gold Reagents (Pyrosequencing, Biotage, VA, USA), according to the manufacturer's instructions. Out of 48 SNPs tested, only 29 with unequivocal interpretation were retained for our analysis. Detailed DNA methodologies are available on request from A.G.

The reactions were performed at the Veterinary Genetics Laboratory (University of California at Davis, Davis, CA, USA). The pyrograms were visualized, and the alleles were quantified using the SNP Software AQ (Pyrosequencing, Biotage).

To verify the significance of our results, we first scaled allele frequency changes to normalize the errors by dividing by $p(1-p)$, where p is the predicted frequency of the focal allele (Fisher 1925). We permuted the corrected errors among trials 1000 times, and on each permuted dataset, performed t-tests as above and counted the number of significant allele frequency changes, at $p < 0.05$.

3. RESULTS AND DISCUSSION

To test for differential male performance, including mating success, we performed a t-test comparing the difference between predicted allele frequencies (assuming that all males contributed equally to the next generation) and the measured allele frequencies. Six alleles differed significantly from their predicted frequency, more than the three we would expect assuming a false discovery rate of 0.05 for 29 tests. Likewise, of 1000 randomly permuted datasets, we determined we should only expect to see three significant allele frequency changes by chance at the $p < 0.05$ level. Only one of the permuted datasets showed as many as six significant results. We interpret these results as evidence of variation in premating male performance, or in mating success, as there is no evidence of a change in male allele contribution across time periods within trials (table S2, electronic supplementary material) as would be expected for differential success in sperm competition (Wolfner 1997).

During the construction of RILs, there may have been cryptic natural selection that resulted in the strong deviations in marker frequency from 0.5 found among the RILs (see Bergland *et al.* (2008) for details). This gives us the opportunity to compare selection during RIL generation and within our room assays. We compared the deviation in allele frequencies among the RILs with allele frequency shifts in our room assay (table 1) using Pearson's correlation and found no significant relationship (d.f. $= 27$, $t = 0.8981$, $p = 0.3771$). This suggests that selection during inbreeding may have been in part against alleles, which were less adaptive in laboratory vials (Hoffmann *et al.* 2001) but not in the more complex environment of the rooms. Alternatively, alleles under opposing selection in the rooms and vials perhaps had female-limited deleterious effects—selection in our experiment was only acting on male genotypes.

There is abundant evidence of interpopulation variation in female choice in a wide variety of taxa. This kind of selection results from variation in signal traits and also results from genetic differences in habitat or group preferences (Krupa & Sih 1993; Schluter 2001; Rundle & Nosil 2005). Selection on sexual signals and preferences, and habitat choice, is known to rapidly lead to assortative mating between populations. Variation allowing the evolution of these traits must exist at some scale within species and populations (Hendry *et al.* 2007; Rice & Pfennig 2007), but measures of the magnitude of variation in female preference differences within populations are scarce. We attempted a first measure of this variation.

If allele frequency shifts in our trials depended on the female genotype, this would be a strong indication that male behavioural diversity is facilitated by the variation in female preferences. We compared the allele frequency change between female treatments and found that there were only two differences with $p < 0.05$ (type III ANOVA, R package 'car'). Two p-values less than 0.05, out of 29 trials, is approximately what would be expected by chance (expected: $n = 29$, $29 \times 0.05 = 1.45$; d.f. $= 1$, $\chi^2 = 1.58$, $p = 0.209$). It may naively seem unsurprising that we found no evidence for opposing selection, given the limited number of genotypes we employed. However, we were attempting to measure differential selection on 29 male loci, each representing a large number of linked genes, as a result of any differences between the female genotypes, so that we were sampling among a very large number of potential genetic interactions

Table 1. Significance of the difference in frequencies of male allele transmission to the next generation overall (directional selection) and the significance of the difference of selection between female genotypes (divergent selection). (Values significant at the 0.05 level are highlighted in bold.)

		directional selection					divergent selection		
			focal allele						
cytological position	gene name	n = trials	initial (%)	(%) change	t-value	p-value	n = samples	χ^2-value	p-value
3F	CG2904	3	42.8	2.5	0.81	0.462	5	1.49	0.222
7F	intergenic	6	36.6	5.6	4.69	**0.005**	13	8.43	**0.004**
10B	CG18361	6	56.6	−1.2	−1.11	0.317	13	1.51	0.219
15D	intergenic	4	28.6	−4.0	−0.95	0.414	6	0	0.995
22B1	*cpb*	6	36.7	−2.7	−1.88	0.118	14	0.14	0.712
28A4–6	*Rapgap1*	6	52.4	−4.8	−0.57	0.595	15	3.74	0.053
35C5–D1	*gft*	4	42.1	−2.3	−0.22	0.839	8	3.77	0.052
35E1	*beat-Ic*	5	21.0	−10.1	−2.57	0.062	8	2.23	0.135
41F2–3	*TpnC4*	6	55.4	−2.7	−2.27	0.072	15	3.69	0.055
42C5–6	CG3409	6	52.2	−5.7	−6.68	**0.001**	15	1.24	0.266
43A4–B1	*Dscam*	6	40.5	−0.9	−0.25	0.810	12	0	0.959
47A11–13	*lola*	6	4.6	0.6	1.76	0.138	14	0.38	0.537
47F11	*Drip*	6	54.1	−1.5	−0.72	0.503	13	0.03	0.863
52B3–5	*fus*	6	10.7	−2.8	−3.24	**0.023**	14	2.96	0.085
56C4	*cora*	6	28.9	−0.8	−0.23	0.825	12	2.99	0.084
56F4	CG8654	6	77.7	−4.5	−2.49	0.055	13	0.02	0.883
62B4	*Spec*	6	36.8	5.5	2.11	0.088	15	0.14	0.711
64E12–F2	*vn*	6	60.9	−0.5	0.32	0.802	15	0.30	0.585
67D2	CG12524	5	52.5	−4.7	−2.69	0.055	11	1.63	0.201
68B4–C1	*scyl*	6	75.3	−5.7	−3.73	**0.014**	14	2.17	0.141
69A2	*Adk1*	6	14.9	1.8	2.03	0.098	15	0	0.998
73B1–4	*Abl*	3	76.8	−1.4	−1.01	0.372	5	0.08	0.780
75A10–B6	*Eip75B*	6	80.5	−0.2	−0.11	0.915	14	5.87	**0.015**
83A2	*Or83b*	3	16.5	−2.8	−1.10	0.323	4	0.10	0.747
86B1	*jumu*	6	64.7	1.7	0.97	0.376	13	0.80	0.370
89B4–6	*Sb*	6	45.8	3.3	1.04	0.346	11	0.96	0.327
89E2	*abd-A*	6	59.7	3.7	2.92	**0.033**	15	0.09	0.768
98D6–7	CG10011	6	53.8	−7.6	−3.14	**0.026**	15	0	0.990
98F1	CG33203	4	49.9	3.4	1.12	0.344	5	0.07	0.792

between males and females which might affect male mating.

Given that lines derived from the Winters population, including the lines we assay here, harbour extensive gene-expression variation (Wayne *et al.* 2007), and genetic variation for mating, aggressive behaviours (Cabral *et al.* 2008), habitat use (Stamps *et al.* 2005) and reproductive traits (Bergland *et al.* 2008), we might expect that some of that variation might be relevant to variation between females in mate choice. This is particularly true, given that traits under nonlinear (e.g. balancing or diversifying) selection are expected to harbour a relatively large amount of standing additive genetic variation (Harris *et al.* 2008). Surprisingly, this variation resulted in detectable variation in male mating success between female genotypes.

4. CONCLUSION

It is interesting to estimate what the lower bounds of variation in male success owing to female genotype might be within a population. Given our results, it seems possible that allelic interactions between sexes, with large, differential effects on male mating success, are not common between genotypes, within even a genetically and behaviourally diverse population. Extensive further work needs to be carried out to evaluate the nature of the variation that exists at within population scales, as it relates to the evolution of sexual selection differences between populations.

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