

Positive genetic correlation between female preference and offspring fitness

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In many species, females display preferences for extreme male signal traits, but it has not been determined if such preferences evolve as a consequence of females gaining genetic benefits from exercising choice. If females prefer extreme male traits because they indicate male genetic quality that will enhance the fitness of offspring, a genetic correlation will evolve between female preference genes and genes that confer offspring fitness. We show that females of *Drosophila serrata* prefer extreme male cuticular hydrocarbon (CHC) blends, and that this preference affects offspring fitness. Female preference is positively genetically correlated with offspring fitness, indicating that females have gained genetic benefits from their choice of males. Despite male CHCs experiencing strong sexual selection, the genes underlying attractive CHCs also conferred lower offspring fitness, suggesting a balance between sexual selection and natural selection may have been reached in this population.

Keywords: female preference; genetic correlation; male genetic quality; sexual selection

1. INTRODUCTION

A key controversy involving sexual selection is whether the evolution of female preference for extreme male signal traits is the result of an underlying female preference for male genetic quality (Fisher 1930; Trivers 1972; Zahavi 1975; Kirkpatrick & Ryan 1991). If females choose males on the basis of genetic quality, they may be able to enhance the fitness of their offspring, relative to females that mate at random. Females may be able to distinguish between males varying in genetic quality directly, or they may use other male traits such as signals or indicators of genetic quality (Fisher 1930; Maynard Smith 1991). Genetic correlations between male signal traits and offspring fitness indicate that some male traits may be reliable indicators of male genetic quality (Norris 1993; Møller 1994; Petrie 1994; Hasselquist *et al.* 1996).

Although females may sometimes be able to use male signal traits to distinguish between males of varying genetic quality, this does not demonstrate that female preference for male traits has evolved as a consequence of females gaining genetic benefits from exercising choice. If females gain genetic benefits from choice, then linkage disequilibrium will develop between preference genes and genes contributing to variation in fitness (Iwasa *et al.* 1991; Kirkpatrick & Barton 1997). A genetic correlation between female preference and fitness is therefore a direct consequence of the continual action of female preference for male genetic quality. This genetic evidence for the process of good genes' sexual selection has not, to our knowledge, been estimated in any population.

Here, we estimate the genetic correlation between female preference and offspring fitness in a population of *Drosophila serrata*. We chose *D. serrata* as the subject of this quantitative genetic study for two reasons. First, the mechanism of mate recognition in the *D. serrata* complex is well understood. Species within the *D. serrata* complex

use cuticular hydrocarbons (CHCs) for mate recognition (Blows & Allan 1998), and CHCs have been shown to be important for mate recognition under field conditions, and to respond to selection under laboratory conditions (Higgin *et al.* 2000). Second, females may use male CHCs to indicate male genetic quality in this species complex. The manipulation of natural and sexual selection in a previous evolutionary experiment (Blows 2002) demonstrated that the interaction between these two evolutionary forces was critical in determining how mate recognition evolved over 20 generations. Male CHCs rapidly responded to sexual selection in the absence of natural selection, as a consequence of female choice. However, when natural and sexual selection were allowed to operate together, male CHCs became more exaggerated than under sexual selection in isolation, suggesting that females gained genetic benefits from their choice of males.

We present the results of two experiments. First, a female choice experiment was conducted to determine whether females of *D. serrata* display a preference for extreme male CHCs, from which we estimated the sexual selection fitness function, and linear selection gradient, for the attractive male CHC blend. Second, we determined if female choice affected offspring fitness within a quantitative genetic experiment, which enabled the estimation of the genetic relationships between female preference, male attractiveness and offspring fitness within a single experimental design.

2. MATERIAL AND METHODS

(a) Female choice experiment

Mate choice tests were conducted using individuals from the Forster population of *D. serrata* that has been previously described (Higgin *et al.* 2000). Virgin six-day-old *D. serrata* females were individually placed in vials with two virgin *D. serrata* males, in a total of 123 such mate choice tests. Females were watched, and when a male successfully mounted a female and had achieved intromission, the chosen and rejected males were removed from the vial and immediately killed and prepared

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for analysis on the gas chromatograph (Blows & Allan 1998). Although this experimental design does not exclude male competition, its influence on our genetic analysis of preference may be largely ruled out for two reasons. First, females of *D. serrata* exercise a degree of cryptic female choice during courtship and have evolved a number of mechanisms to control the success of copulations (Hoikkala & Crossley 2000). Second, although males sometimes fight each other (Hoikkala *et al.* 2000), if a female's preferred male is not available to her as a result of male competition or male courtship activity, this can only contribute environmental variance to our analysis (i.e. a female's genotype is unlikely to affect the outcome of male competition). CHC profile of each male consisted of the relative abundances (peak areas) of nine individual CHCs. This compositional dataset was transformed to logcontrasts (Aitchison 1986), as previously described for CHC data (Blows & Allan 1998), before using MANOVA to test for differences in CHC profile between chosen and rejected males. The same log contrasts were used in the subsequent canonical discriminant analysis to generate the canonical variate that best distinguished between chosen and rejected males.

(b) Genetic experiment

We tested whether female choice for male CHCs affected offspring fitness within a half-sibling genetic design. From the Forster population of *D. serrata*, 66 virgin males were each mated to three virgin females to obtain 198 families. Two virgin five-day-old daughters from each family were each placed in a vial with five virgin males randomly selected from the Forster stock. After a mating occurred, the chosen male and one of the rejected males were placed in separate vials with two virgin females randomly selected from the Forster stock and allowed to mate. After 24 hours, the two females were separated into individual vials and left to lay for 3 days. Offspring fitness (productivity) was measured as the average weight of all the progeny in grams that were produced by the two females inseminated by each male, resulting in measures of offspring fitness for 371 chosen and 376 rejected males. Offspring fitness measured in this way encompasses offspring viability, one of the most important fitness components that may be indicated by male sexual displays (Kokko *et al.* 2002), but does not include how successful offspring may be in gaining mates, which we measure independently below. Female preference for male genetic quality was calculated as the difference between the offspring fitness conferred by the males chosen and rejected by each daughter. This design ensured that only the genetic basis of choice was included in our analysis of female preference, as daughters did not produce the progeny scored, and daughters were not able to invest differentially in the males they chose and rejected.

To measure offspring fitness conferred by sons, two four-day-old virgin sons from each family were each placed in a vial with two virgin females randomly selected from the Forster stock. After 24 hours, the two stock females were separated into individual vials and left to lay for 3 days, and productivity was measured in the same way as for the daughter side of the experiment, resulting in measures of offspring fitness for 355 sons. In addition, sons had their CHC phenotypes assayed on the gas chromatograph to measure male attractiveness. The canonical variate from the mate choice experiment that distinguished between chosen and rejected males represented male attractiveness to females. The equation for the canonical variate was therefore applied to the CHC phenotypes of sons so that a score for attractiveness could be allocated to each son.

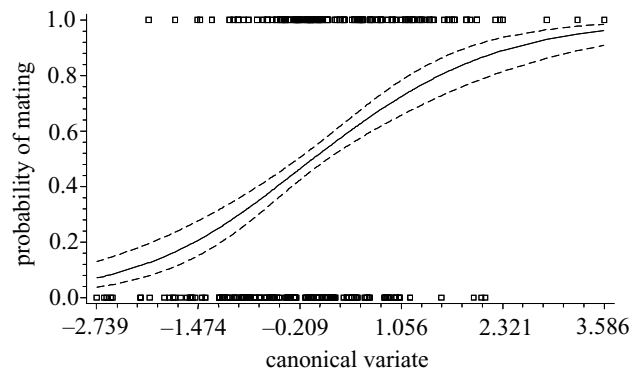


Figure 1. Sexual selection fitness function of male CHCs preferred by females. The probability of a male mating is displayed as a function of the value of the CHC canonical variate for each male. Data are indicated by squares. The fitness function (solid curve) ± 1 s.e. (dashed curves) displayed was estimated using a non-parametric cubic spline (Schluter 1988).

Genetic correlations were estimated as sire-mean Pearson's product-moment correlations. Because the traits we correlated were measured on different individuals, sire-mean correlations provided a straightforward way to make conservative tests for significance of genetic correlations, as the product-moment correlations will be biased towards zero as a consequence of the inclusion of residual variance in the between-sire variances (Lynch & Walsh 1998, p. 664). The genetic correlations are therefore likely to be larger than indicated by our analyses.

3. RESULTS

(a) Female choice experiment

Drosophila serrata females presented with two males in mate-choice tests exhibited a strong preference for male CHCs (MANOVA; Wilks's lambda = 4.983, $F_{8,238} = 93\ 327.75$, $p < 0.001$). Paired *t*-tests were used to indicate differences between chosen and rejected males in the relative abundance of individual CHCs in the mate-choice experiment. Of the CHCs on the cuticle of *D. serrata*, all three 2-methylalkanes were found in higher relative abundances on chosen males, although 2-Me-C₂₆ was not significantly elevated on chosen individuals (2-Me-C₂₆: $t_{122} = 1.667$, $p = 0.098$; 2-Me-C₂₈: $t_{122} = 3.312$, $p = 0.001$; 2-Me-C₃₀: $t_{122} = 1.999$, $p = 0.048$). One additional hydrocarbon ((Z,Z)-5,9-C_{29:2}: $t_{122} = 2.315$, $p = 0.022$), also displayed elevated levels on chosen individuals.

The canonical discriminant analysis distinguishing between chosen and rejected males generated a univariate description of CHCs that females preferred (Endler 1986, p.180), which was used to estimate the sexual selection fitness function for male CHC profile (figure 1). Linear regression estimated the unstandardized linear sexual selection gradient (β) as 0.815. Parametric significance testing of β was not appropriate as the errors had a binomial distribution, so a randomization approach was used to evaluate this estimate. Fitness (0 or 1) was randomly reassigned to male trait values and β recalculated 1000 times. The estimate of β was significant at $p < 0.001$.

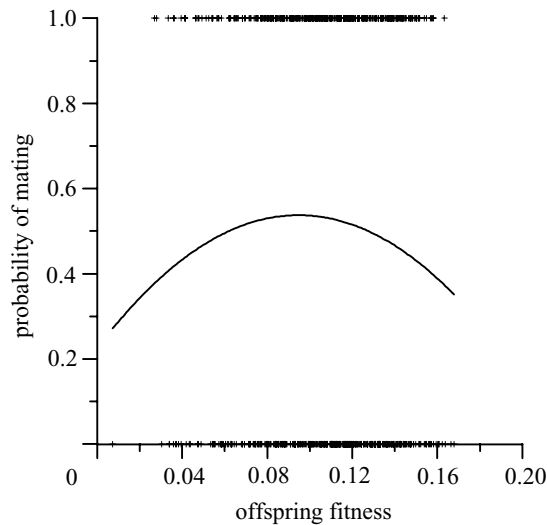


Figure 2. Natural selection fitness function of female choice. The probability of a male mating is displayed as a function of the offspring fitness conferred by that male. Data are indicated by crosses. The fitness function displayed was estimated using quadratic regression ($y = 0.225 + 6.600x - 34.797x^2$).

(b) Genetic experiment

A daughter's choice of male affected the offspring fitness of random females. Although levels of offspring fitness conferred by rejected (mean \pm variance, $0.0684 \text{ g} \pm 8.82 \times 10^{-4}$) and chosen ($0.0671 \text{ g} \pm 7.49 \times 10^{-4}$) males were not significantly different (paired t -test: $t_{353} = -0.730$, $p = 0.466$), the variance in offspring fitness of chosen males displayed a marginally non-significant reduction in comparison with rejected males (F -test; $F_{371,376} = 1.1776$, $p = 0.057$). A reduction in variance, with no change in the mean, indicated that female choice was under stabilizing natural selection.

To determine the form of natural selection operating on female choice, we estimated the fitness function between female choice and offspring fitness. The fitness function was first estimated using a cubic spline, which indicated that the shape of the function was quadratic. We therefore proceeded to estimate the fitness function using quadratic regression (Brodie *et al.* 1995), and calculated the unstandardized directional ($\beta = -0.417$) and stabilizing ($\gamma = -34.797$) selection gradients in separate regressions (figure 2). Significance of β ($p = 0.228$) and γ ($p = 0.015$) was determined by the randomized reassignment of fitnesses where β and γ were estimated 1000 times in separate regressions. Stabilizing natural selection on female choice was confirmed by the presence of a significant stabilizing selection gradient. Males with the highest probability of mating conferred intermediate levels of offspring fitness.

The sons and daughters of half-sibling families were used to estimate the genetic correlations between female preference, male attractiveness and offspring fitness. The negative genetic correlation between the attractiveness of sons and offspring fitness conferred by sons (figure 3a) indicated that genes which conferred male attractiveness had adverse effects on offspring fitness. The positive genetic correlation between daughters' preference for male genetic quality and offspring fitness conferred by sons (figure 3b) indicated that families in which daughters pre-

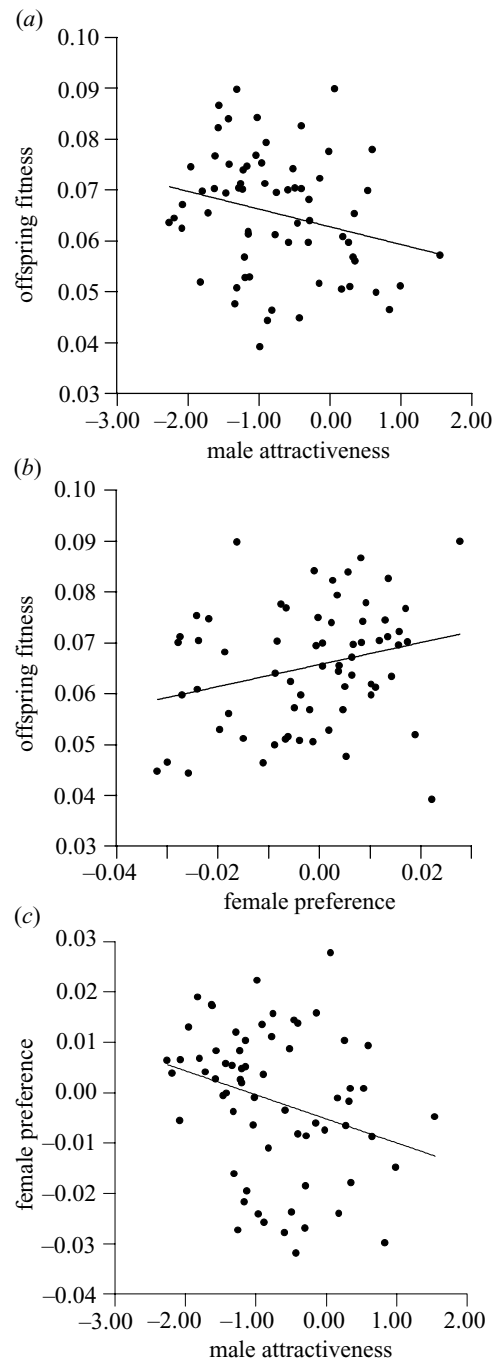


Figure 3. Genetic relationships between female preference, male attractiveness and offspring fitness. (a) Negative genetic correlation between the relative abundance of the attractive combination of CHCs on the cuticle of sons and offspring fitness conferred by sons ($r = -0.242$, $p = 0.051$). (b) Positive genetic correlation between a daughter's preference for male contribution to offspring fitness and offspring fitness conferred by sons ($r = 0.260$, $p = 0.035$). (c) Negative genetic correlation between the relative abundance of the attractive combination of CHCs on the cuticle of sons and daughters' preference for male genetic quality ($r = -0.281$, $p = 0.022$).

ferred males that conferred high offspring fitness had sons that conferred high offspring fitness. Although female preference genes may have become associated with genes that increase offspring fitness (figure 3b) they have at the same time become associated with genes that reduce the attractiveness of male offspring (figure 3c). This is a conse-

quence of the negative genetic correlation between male attractiveness and offspring fitness (figure 3a).

4. DISCUSSION

Female *D. serrata* display a strong preference for an extreme male CHC blend. The standardized linear sexual selection gradient of 0.756 represents one of the larger linear selection gradients detected thus far (Kingsolver *et al.* 2001). Such strong linear sexual selection indicates that male CHCs could evolve in a directional manner. However, depictions of the form of selection are simply descriptive, and an experimental manipulation is required to demonstrate how selection operates on the traits under consideration (Brodie *et al.* 1995). Sexual selection has been manipulated in populations of hybrids between *D. serrata* and *D. birchii*, demonstrating that male CHCs rapidly respond to the presence of sexual selection in a directional manner (Blows 2002).

A female's choice of male affected the fitness of offspring, although chosen males did not increase the fitness of offspring in this population, the more common finding in sexual selection studies (Møller & Alatalo 1999). Instead, males with the highest probability of mating conferred intermediate levels of offspring fitness, indicating that female choice is under stabilizing natural selection. The standardized stabilizing selection gradient of -0.028 indicated that stabilizing selection on female choice was relatively weak. This is consistent with most published estimates of stabilizing selection in other systems when based on similarly large sample sizes (Kingsolver *et al.* 2001). Most male sexually selected traits and female preferences in undisturbed populations are likely to conform to an overall pattern of stabilizing selection (Kirkpatrick 1987), as the directional evolutionary phase of male-trait exaggeration by female choice is likely to be extremely rapid (Fisher 1930; Lande 1981). Stabilizing natural selection on female choice may therefore be representative of a population currently at equilibrium, at which the cost to males of becoming attractive may have evolved to balance the benefits of attractiveness (Fisher 1930; Kirkpatrick 1987).

The genetic relationships between female preferences, male sexually selected traits and fitness will depend on which evolutionary phase a population is in (Heisler 1994). At equilibrium, the trade-off between the costs and benefits of attractiveness to males may result in a negative genetic correlation between the male trait and fitness (Heisler 1994), and has been found in other species (Etges 1996; Wedell & Tregenza 1999; Brooks 2000). The benefit to *D. serrata* males in being attractive is considerable, given the strong preference that females display for attractive male CHCs. The negative genetic correlation found between male attractiveness and offspring fitness in *D. serrata* in turn indicates that there is a cost to males in becoming attractive. Females prefer increased relative concentrations of methylalkanes on the cuticle of males (R. W. Howard, L. L. Jackson, H. Bouse and M. W. Blows, unpublished data). Methylalkanes are likely to be very expensive to produce (Nelson 1993), indicating a possible physiological basis for the trade-off between attractiveness and fitness that could result in the evolution of antagonistic pleiotropy. A balance between natural

selection (offspring fitness) and sexual selection (male attractiveness) may therefore have been reached in this population.

Under the assumption that pleiotropy between female preference genes and genes contributing to productivity is negligible (Lande 1981; Bakker & Pomiankowski 1995), female choice for male genetic quality appears to have generated linkage disequilibrium between preference genes and offspring fitness genes, as predicted by models of good genes' sexual selection (Iwasa *et al.* 1991; Kirkpatrick & Barton 1997). The level of genetic correlation between female preference and offspring fitness detected was small, indicating that the strength of indirect selection on female preference generated by the positive association between preference and fitness genes may be relatively weak (Kirkpatrick & Barton 1997). Nevertheless, male CHCs were observed to evolve in a manner consistent with females gaining genetic benefits from their choice of males in an evolutionary experiment (Blows 2002). Future experiments, which enable the generation of the genetic correlation between female preference and offspring fitness to be directly observed in experiments that manipulate sexual selection, will be critical in demonstrating the importance of genetic benefits for the evolution of female preference.

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