



## Evolutionary experiments on mate recognition in the *Drosophila serrata* species complex

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### Abstract

It is becoming increasingly apparent that at least some aspects of the evolution of mate recognition may be amenable to manipulation in evolutionary experiments. Quantitative genetic analyses that focus on the genetic consequences of evolutionary processes that result in mate recognition evolution may eventually provide an understanding of the genetic basis of the process of speciation. We review a series of experiments that have attempted to determine the genetic basis of the response to natural and sexual selection on mate recognition in the *Drosophila serrata* species complex. The genetic basis of mate recognition has been investigated at three levels: (1) between the species of *D. serrata* and *D. birchii* using interspecific hybrids, (2) between populations of *D. serrata* that are sympatric and allopatric with respect to *D. birchii*, and (3) within populations of *D. serrata*. These experiments suggest that it may be possible to use evolutionary experiments to observe important events such as the reinforcement of mate recognition, or the generation of the genetic associations that are central to many sexual selection models.

### Introduction

Much of what we understand about the genetic basis of speciation comes from the identification of chromosomal regions that code for differences between divergent populations or species (Coyne & Orr, 1998; Orr, 2001). With the advent of genomic techniques, these analyses will become more precise, leading to a better understanding of the number of genes that may be involved in speciation, and in some cases, to the characterization of candidate loci. However, the genetic analysis of speciation will require more than the mapping and characterization of genes underlying sexual isolation using these new techniques. If the process of speciation is to be dissected, rather than simply the pattern it leaves behind, the genetic basis of sexual isolation will need to be placed in the context of models of how mate recognition evolves.

Sexual selection theory provides a quantitative genetic framework within which the genetic basis of

mate recognition can be related to how sexual isolation may evolve (Lande, 1981; Iwasa & Pomiankowski, 1995; Kirkpatrick & Barton, 1997; Houle & Kondrashov, 2002). Although natural selection may often play an important role in the initiation of divergence in mate recognition between speciating populations (Turelli et al., 2001), sexual selection is likely to contribute to the generation of further divergence in many instances (Schluter, 2001). Determining the genetic basis of the response to sexual selection within populations, and how this process may result in the divergence of mate recognition between populations, is therefore of general importance in understanding the process of speciation.

The evolution of genetic associations as a consequence of linkage disequilibrium are central to the operation of many models of sexual selection (Lande, 1981; Kirkpatrick & Barton, 1997). The evolution of genetic correlation between female preference, male attractiveness and other fitness components, are

critical in determining how males and females may coevolve. Since these genetic associations evolve as sexual selection proceeds, evolutionary experiments are well-suited to the study of the quantitative genetic basis of mate recognition.

We review a series of evolutionary experiments which are part of a continuing investigation into the quantitative genetic basis of mate recognition at three levels of divergence within the *Drosophila serrata* species complex; between species, between populations within species, and within populations. We highlight how experiments that disrupt populations from equilibrium, or directly manipulate evolutionary processes such as natural and sexual selection, may be able to illustrate how the genetic basis of mate recognition evolves. At the between-species level, hybridization between species was used to generate a series of non-equilibrium populations to investigate the evolution of the genetic basis of mate recognition under unmanipulated conditions. Natural and sexual selection were then directly manipulated in hybrid populations to determine the influence of these evolutionary processes on mate recognition evolution. At the level of divergence between populations within species, we manipulated natural selection on mate recognition in populations of *D. serrata* to determine the quantitative genetic basis of the reinforcement process. Finally, a quantitative genetic analysis of the sexual selection process was conducted to determine how males and females may coevolve within populations of *D. serrata*.

### Genetics of mate recognition between species

*D. serrata* and *D. birchii* are sibling species that are endemic to Australia, and surrounding islands, and are part of the melanogaster species group (Bock, 1976). They represent one of the few cases in the genus *Drosophila* where hybrids between two species are viable and fertile, and crosses are obtainable in both directions (Bock, 1984), albeit at a very low frequency (Ayala, 1965; Blows, 1998). As a consequence of the historical nature of speciation events, it has been difficult to determine if the genetic basis of sexual isolation between species is a direct consequence of the process of speciation (Coyne, 1992; Wu et al., 1995). Strong prezygotic isolation, but weak postzygotic isolation (Haldane's rule as yet to evolve), suggests that this species complex may be a suitable system for determining the genetic basis of sexual isolation as it may

represent that which evolved during speciation, rather than changes accumulating after the event.

### *Genetic correlation between mate recognition and mechanistic traits*

Even with animals that are renowned for their ease of experimentation such as *Drosophila*, it has not been straightforward to determine the relative contribution of various traits to mate recognition in specific cases. Perhaps of more importance than identifying traits involved in mate recognition, is the determination of which traits contribute most of the genetic variation in mate recognition. In other words, a trait may be involved in a critical step in the process of mate recognition, but without genetic variation, it is unable to be involved in the subsequent evolution of mate recognition. Therefore, determining the genetic correlation between a behavioural measure of mate recognition with mechanistic traits allows their potential importance in the evolution of mate recognition to be determined.

*Drosophila* use a number of different mechanisms to recognise and choose mates, including tactile, visual, acoustic, and chemical cues (Spieth & Ringo, 1983). In members of the melanogaster species group, cuticular hydrocarbons (CHCs) have been shown to contribute to mate choice within species (Jallon, 1984) and sexual isolation between species (Cobb & Jallon, 1990; Coyne et al., 1994; Buckley et al., 1997; Coyne & Charlesworth, 1997). To determine the importance of CHCs in mate recognition in the *D. serrata* complex, a genetic analysis after hybridization between sympatric populations of *D. serrata* and *D. birchii* was conducted (Blows & Allan, 1998). Hybrid male and female mate recognition were highly genetically correlated with the multivariate CHC profile across 30 iso-female lines ( $r_g = 0.90$  and  $0.78$ , respectively), indicating that CHCs explained most of the genetic variation between the two species in male mate recognition, and to a lesser extent in female mate recognition. Unfortunately, such analyses of mate recognition must necessarily be oversimplifications of how male and female mating decisions are made. For instance, although hybrid female mating decisions were genetically associated with CHCs, this experiment did not investigate aspects of signal reception by females. Despite this drawback, a high genetic correlation between a behavioural measure of mate recognition and mechanistic traits suggests that when mate recognition is placed under selection, it will be

those traits that respond to selection. This genetic analysis successfully predicted that both male and female CHCs would respond to selection on mate recognition in the evolutionary experiments we describe below (Higgie et al., 2000; Blows, 2002).

The same multivariate genetic approach has been used in guppies, where multiple colour traits were highly genetically correlated with a measure of male attractiveness (Brooks, 2000). Such genetic analyses provide evidence that a single mechanism (pheromones in flies or colour in guppies) may be largely responsible for the genetic variation in male attractiveness, although both traits have complex multivariate phenotypes. One of the major practical problems in the study of the genetics of speciation has been that behavioural measures are notoriously labile (Wu et al., 1995), and often extremely logistically demanding. Such analyses may therefore allow readily quantifiable mechanistic traits to be substituted for mate recognition *per se* in genetic and evolutionary analyses to some extent, where the large number of treatments and/or geographic populations may often effectively prohibit behavioural assays.

#### *Genetic correlation between male and female components of mate recognition*

The genetic correlation between male and female components of mate recognition is the central genetic relationship in many models of sexual selection, and consequently, speciation (Lande, 1981; Iwasa & Pomiankowski, 1995; Kirkpatrick & Barton, 1995, 1997). Despite the importance of the preference–trait genetic correlation, there are very few direct estimates of this critical parameter. Genetic studies of sexual selection using either breeding designs (Bakker, 1993; Gray & Cade, 1999, 2000) or selection experiments (Houde, 1994; Wilkinson & Reillo, 1994; Brooks & Coullidge, 1999), invariably conclude linkage disequilibrium underlies the correlations and correlated responses. Although the results of a number of these experiments are consistent with the presence of linkage disequilibrium between trait and preference (Pomiankowski & Sheridan 1994; but see Breden et al., 1994 for alternative explanations), only Gray and Cade (1999) have employed an experimental design that attempted to exclude pleiotropy as a cause of the genetic correlation. The (full-sib) genetic correlation between male pulse rate and female preference for rate in the field cricket *Gryllus integer* decreased after one generation of random mating from 0.51 to  $-0.20$ .

Unfortunately, the longitudinal and unreplicated nature of this test makes it difficult to identify the treatment of random mating as the cause of the decline in genetic correlation. Ideally, future experiments will need to incorporate replication of the treatments of sexual selection and random mating to test for the presence of linkage disequilibrium between female preference and male traits.

Not surprisingly then, given the difficulties associated with estimating genetic correlations with precision, and distinguishing between pleiotropy and linkage disequilibrium as causes of genetic correlation, it has yet to be established unequivocally that such a genetic correlation as a consequence of linkage disequilibrium was generated by sexual selection. Blows (1998, 1999) used an alternative approach of hybridizing *D. serrata* and *D. birchii* to disrupt the mate recognition system to determine the level of pleiotropic genetic correlation between male and female components, and how the genetic correlation subsequently evolved. Five generations after hybridization the genetic correlation between male and female components was 0.39, indicating that pleiotropy may make a significant contribution to the genetic association between male and female components. The genetic correlation rapidly evolved, first increasing to a value of 1.02 after 37 generations, then decreasing again to  $-0.04$  after a further 19 generations. Although the increase in genetic correlation was consistent with the predictions of sexual selection theory, these experiments have a number of limitations, some of which are shared by studies estimating genetic correlations in undisturbed populations: (1) Without manipulating sexual selection, the changes in genetic correlation cannot be directly attributed to sexual selection. (2) Linkage disequilibrium or pleiotropy cannot be distinguished between as contributing factors to the increase and/or decrease in the genetic correlation. (3) Female preference and male traits were not directly measured as in most other studies. However, this evolutionary experiment does demonstrate that male and female components of mate recognition may rapidly coevolve at the genetic level, consistent with the requirements of sexual selection models.

#### *Manipulation of the evolutionary forces acting on mate recognition*

It is probably clear from the previous discussion that what has been missing from most studies of how

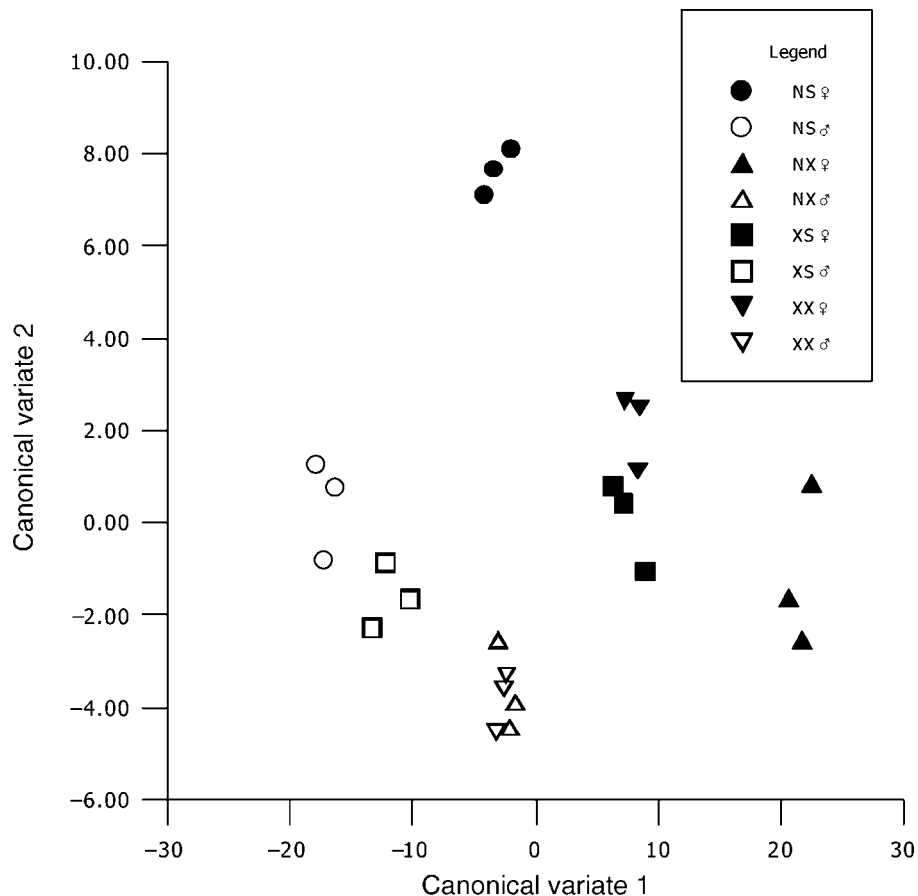


Figure 1. The response of CHCs in hybrid populations after 20 generations of natural and sexual selection. Natural and sexual selection were manipulated in a 2-way factorial design, with three replicate populations per treatment; XX: no natural or sexual selection, NX: natural but not sexual selection, XS: sexual but not natural selection, NS: natural and sexual selection. Population means for each sex based on 20 individuals are displayed. The response to the various combinations of natural and sexual selection are interpreted in the text by using the XX populations as a reference point.

sexual selection results in the evolution of mate recognition, is the direct manipulation of the evolutionary forces involved. Although there have been many attempts to determine if divergent natural selection results in the evolution of mate recognition, and subsequently sexual isolation (Rice & Hostert, 1993), there is little experimental evidence for how sexual selection, and particularly the interaction between natural and sexual selection, affect the evolution of mate recognition. Since mate recognition in unmanipulated hybrid populations evolves rapidly (Wallace et al., 1983; Carson et al., 1994; Blows, 1998, 1999), they provide a system in which levels of sexual and natural selection may be manipulated by simply denying the opportunity for these forces to operate. This experimental design has the advantage that the sexual

selection process is determined by the animals themselves, rather than by the experimenter as in artificial selection experiments.

In a subsequent experiment (Blows, 2002), natural and sexual selection were directly manipulated over 20 generations in hybrid populations between *D. serrata* and *D. birchii* (Figure 1). Natural selection, sexual selection, but particularly their interaction, had large and highly specific effects on the evolution of CHCs over this period. Total hydrocarbon content of both sexes was reduced in the presence of natural selection suggesting they are expensive to produce. In addition, CHC composition of females changed (Figure 1), suggesting that some CHCs were more expensive to produce than others. Male CHCs evolved in response to sexual selection

as a consequence of female choice. Most importantly, the interaction between sexual and natural selection resulted in male CHCs becoming even more exaggerated than under sexual selection in isolation, and female CHCs evolved in the direction opposite to that when in the presence of natural selection in isolation.

These evolutionary responses were consistent with females gaining indirect genetic benefits from their choice of males with particular CHC profiles. Females were less able to benefit from their choice of males in XS populations as natural selection on females had been greatly reduced in these populations. When females were allowed to gain all indirect benefits from their choice of males (the interaction between natural and sexual selection in NS populations), male sexually-selected CHCs became more exaggerated than under sexual selection in isolation (XS populations).

### Genetics of mate recognition under natural selection within species

Species that may have experienced reinforcement of their mate recognition systems are of great interest to the study of speciation as there is the potential to catch part of the process of speciation in action. Reinforcement may occur in the final stages of speciation, after there has been initial divergence between two incipient species, through genetic drift or adaptation to local conditions (Schluter, 2001). The occurrence of reproductive character displacement in a diverse range of taxa (Howard, 1993; Noor, 1995; Rundle & Schluter, 1998) suggests that reinforcement may be an important component of speciation (Dobzhansky, 1951).

*D. serrata* has both allopatric and sympatric populations with respect to *D. birchii*. When the CHCs of sympatric and allopatric *D. serrata* were examined, the pattern of reproductive character displacement was found, consistent with the process of reinforcement (Higgie et al., 2000). However, the presence of reproductive character displacement does not demonstrate that natural selection on mate recognition produced this pattern, as the pattern is confounded with geographic distribution (Butlin, 1995), and other processes may also produce such patterns (Howard, 1993; Rundle & Schluter, 1998).

A natural selection experiment in which both field allopatric and sympatric populations of *D. serrata*

were submitted to nine generations of experimental sympatry with *D. birchii* was carried out to test if natural selection on mate recognition had resulted in the field pattern of reproductive character displacement (Higgie et al., 2000). After nine generations, the CHCs of sympatric populations remained unchanged with respect to their paired control populations which did not contain *D. birchii*. In contrast, allopatric populations of *D. serrata* displayed significant directional changes in their CHCs after experimental sympatry, and had evolved in the direction of the field sympatric populations. The process of reinforcement under laboratory conditions had therefore reproduced the pattern of reproductive character displacement seen in field populations of *D. serrata*.

The process of reinforcement in this case appears to have operated in the absence of any significant gene flow between the two species, as successful hybridisations between *D. serrata* and *D. birchii* are rare. Reinforcement without gene flow has been called both the process of 'facilitated reproductive character displacement' (Templeton, personal communication, cited in Howard, 1993) and 'reproductive character displacement' (Butlin, 1987, 1989). Alternatively, Howard (1993) has argued that reinforcement is the process leading to the evolution of the pattern of reproductive character displacement, irrespective of whether gene flow still occurs or not. Our experiment suggests that reinforcing natural selection may play an important role in the evolution of mate recognition in the *D. serrata* complex. It remains to be determined whether reinforcement has played a significant role in the speciation event between *D. serrata* and *D. birchii*.

### Dynamics of genetic variance during reinforcement

Noor (1995) highlighted that a genetic analysis of the displaced reproductive character in an example of reinforcement, would determine the genetic basis of traits that are currently under natural selection for increased reproductive isolation. The response of CHCs to experimental sympatry was measured in a half-sib experimental design (Higgie et al., 2000), which allowed a genetic analysis of the process of reinforcement using two approaches (Blows & Higgie, in press). First, we used a comparative approach to determine the similarity in the genetic basis of CHCs between field sympatric and allopatric populations. Second, we used an experimental approach by determining if the genetic basis of CHCs in field allopatric

populations had been changed by the application of experimental sympatry in the selection experiment.

Field sympatric populations have higher levels of genetic variance than field allopatric populations for both male and female CHCs (Table 1). Field allopatric populations which had evolved in response to experimental sympatry (Higgie et al., 2000), displayed large increases in genetic variances over nine generations, similar in magnitude to the difference between field allopatric and field sympatric populations. Field sympatric populations exposed to experimental sympatry displayed no consistent increase in genetic variance, suggesting that the response to selection seen in field allopatric populations exposed to experimental sympatry was associated with the increase in genetic variance.

Such large increases in genetic variance after selection have rarely been observed (Barton & Turelli, 1987), if at all (Keightley & Hill, 1989), and are consistent with a selection response based on rare alleles (Barton & Turelli, 1987). Large increases in genetic variance are more likely to occur if the traits responding to directional selection were previously under strong stabilizing selection (Keightley & Hill, 1989; Burger & Lande, 1994). The genetic basis of the selection response to sympatry in field and experimental populations was therefore consistent with the presence of stabilizing selection on mate recognition in allopatric populations. Mate recognition has been anticipated to be under stabilizing selection to maintain coordination between males and females, particularly in the presence of closely related species (Butlin et al., 1985; Paterson, 1985), however there is surprisingly little experimental evidence for this.

Table 1. Genetic variances of CHCs in field and experimental populations of *D. serrata*

Trait	Field sympatric control	Field allopatric control	Field allopatric experimental sympatry
CV1♀	41.982	32.945	41.695
CV2♀	22.864	8.235	16.570
CV3♀	13.292	1.497	8.533
CV4♀	12.039	4.066	9.680
CV1♂	38.442	23.490	36.926
CV2♂	15.148	4.615	5.141
CV3♂	6.720	7.634	5.003
CV4♂	9.161	3.412	5.628

This experiment appears to have been able to reproduce not only the pattern of reproductive character displacement in means, but the associated changes in genetic variance as a consequence of the response to selection under experimental sympatry. It is always a concern in laboratory-based evolutionary experiments that the experimental application of selection may not accurately reflect how selection operates under field conditions (Harshman & Hoffmann, 2000). Traits or genes that are not the target of selection in the field may then respond to selection under experimental conditions. Our results suggest that the selection response to experimental sympatry in the laboratory was likely to be based on the same genetic variation as that in the field. This has important implications for future experiments designed to characterize which loci may be involved in the selection response during reinforcement. QTL mapping after the application of experimental sympatry to generate divergence in reproductive characters, in addition to mapping the field pattern of reproductive character displacement, may provide manipulative evidence for which loci may be under selection during reinforcement. Such an experimental design could potentially overcome the major limitation of QTL analyses which may map genetic changes that have accrued after the evolutionary event of interest, rather than those loci actually involved in the process.

#### *G* eigenstructure and the evolution of reproductive character displacement

The replicated evolution of reproductive character displacement in field and laboratory populations of *D. serrata* suggested that mate recognition may have been constrained to evolve in a particular direction. Genetic constraints have received a great deal of attention in relation to life-history and morphological evolution (Arnold, 1992), but the influence of genetic constraints on the evolution of mate recognition has had little consideration. As we have pointed out above, even in cases where genetic variation in a single mechanistic trait explains most of the genetic variation in mate recognition (Blows & Allan, 1998; Brooks, 2000), these traits have complex multivariate phenotypes. The genetic basis of multiple-trait phenotypes is best described by the **G** matrix, which will influence the speed and direction of the response to selection on that set of traits (Lande, 1979). The eigenstructure of **G**, representing the genetic covariance structure between traits, indicates in which direction genetic

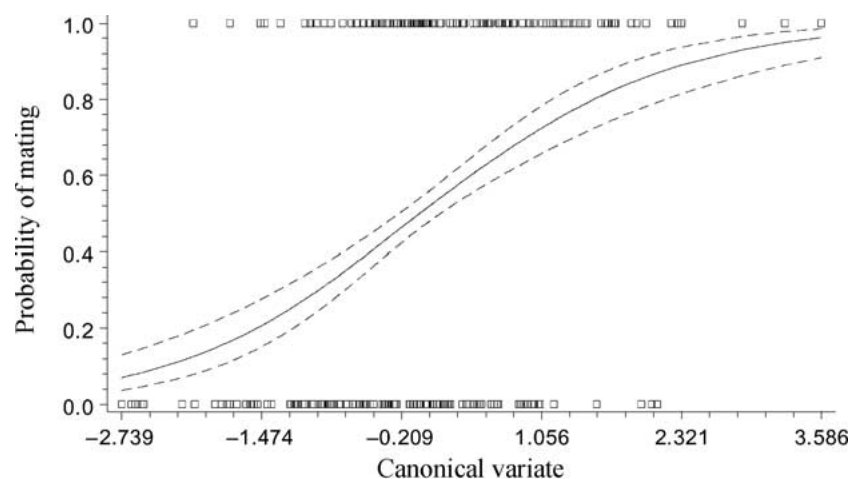


Figure 2. Fitness surface of sexual selection on cuticular hydrocarbons. The probability of mating is displayed as a function of the value of the canonical variate distinguishing between chosen and rejected males. The fitness function (solid line) was estimated using the non-parametric cubic spline method of Schluter (1988). Dashed curves are  $\pm 1$  SE of the predicted probability of mating. Squares indicate the values of the canonical variate for each male. From Hine et al. (in press).

variation exists in the multivariate space described by the set of traits. In particular, the first principal component of  $\mathbf{G}$ ,  $g_{\max}$ , has been used to determine if populations have been constrained to diverge in the direction of greatest genetic variance (Schluter, 1996; Arnold & Phillips, 1999).

To determine the influence that genetic constraints may have had on the repeatable evolution of reproductive character displacement,  $\mathbf{G}$  eigenstructure was compared to the direction that field and experimental populations had evolved in (Blows & Higgie, in press).  $\mathbf{G}$  eigenstructure was compared to the eigenstructure of the divergence variance–covariance matrix ( $\mathbf{D}$ ), which represented the phenotypic variation around population means. The first principal component of  $\mathbf{D}$  is equivalent to  $\mathbf{z}$ , the major axis of variation among populations employed by Schluter (1996).  $\mathbf{G}$  eigenstructure in allopatric field populations was closely associated with the direction that CHC traits had evolved in under field sympatric conditions. In contrast, there was less similarity between  $\mathbf{G}$  eigenstructure before selection in allopatric control populations and the direction of evolution after nine generations of experimental sympatry. However, the predictive power of  $\mathbf{G}$  in experimental populations was restored by using an average of the elements of  $\mathbf{G}$  (from before and after selection), taking into account any changes in  $\mathbf{G}$  during the response to selection. Genetic constraints therefore seem to be associated with

the direction of mate recognition evolution over short term (experimental populations) and long term (field populations) evolutionary time scales.

#### Genetics of mate recognition under sexual selection within populations

In the previous two sections we have dealt with mate recognition in the context of two species; how do hybrids between the two species evolve, and has mate recognition been reinforced to avoid recognising the other species as a potential partner? We now consider how mate choice occurs within a single species, *D. serrata*. In particular, we address the question; does *D. serrata* use (CHCs) within populations during mate choice, and if so, why?

##### *Mate choice in D. serrata*

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). When a female is given a choice between two males, chosen and rejected males differ significantly in CHC composition (Howard et al., in preparation.). There are at least four hydrocarbons, three of which are methyl-branched alkanes, that seem to be associated with female choice

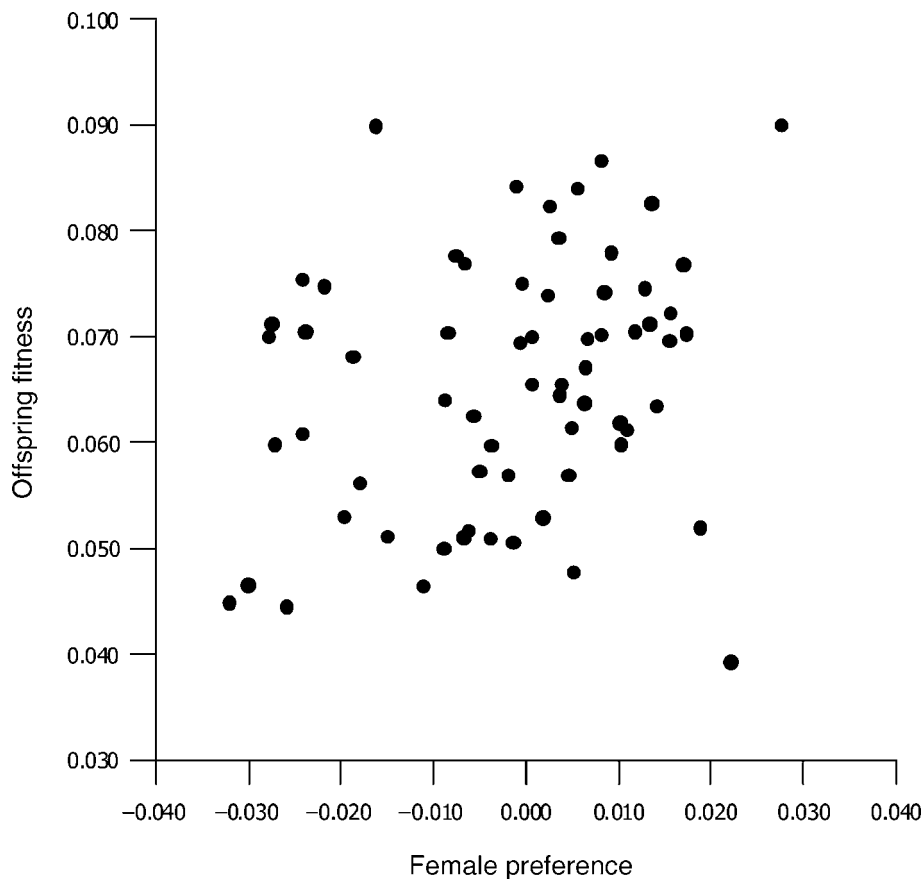


Figure 3. Genetic correlation between the ability of female *D. serrata* to chose males that confer high offspring fitness, with offspring fitness itself. Offspring fitness was measured as the weight (g) of all offspring produced by each individual female. Female preference was calculated as the difference in offspring weight conferred by chosen and rejected males. Each point represents a sire mean from the half-sib experiment. The product-moment correlation across sire means is 0.260 ( $P = 0.035$ ), which supplies a conservative test of the significance of the genetic correlation from zero (Lynch & Walsh, 1998).

in *D. serrata*. Chosen males have greater relative concentrations of 2-Me-C<sub>28</sub>, 2-Me-C<sub>30</sub>, (Z,Z)-5,9-C<sub>29:2</sub>, and to a lesser extent, 2-Me-C<sub>26</sub>. From a canonical discriminant analysis, the canonical variate that best distinguished between chosen and rejected males was used to estimate the sexual selection fitness function for male CHCs (Figure 2). The standardised linear sexual selection gradient of 0.756 estimated by linear regression suggested that strong sexual selection acts on male CHCs. (Hine et al., in press).

It is more difficult to determine why females favour these CHCs. From Figure 1, it can be seen that natural selection, in the absence of sexual selection, favoured CHCs in hybrid females (NX populations) that were in the opposite direction to those preferred by females on males (XS populations), suggesting that females may prefer costly CHCs. Methyl-branched hydrocarbons have been suspected to have impor-

tant biological functions as they are likely to be very expensive to produce (Nelson, 1993). The greater exaggeration of male CHCs in the presence of natural and sexual selection (NS populations), over sexual selection in isolation (XS populations), in turn suggested that females may have gained genetic benefits from their choice of males. If this is so, a genetic correlation will evolve between female preference and genes contributing to fitness (Iwasa et al., 1991; Kirkpatrick & Barton, 1997). Although there are a number of examples of positive genetic covariance between male sexually-selected traits and offspring fitness (Norris, 1993; Petrie, 1994; Hasselquist et al., 1996), a genetic association between female preference genes and fitness genes has not been estimated in any system.

While female preference is usually considered in relation to male sexually-selected traits, perhaps in this context a more direct relationship to

consider empirically is the preference females have for a male's contribution to fitness. A half-sib experimental design (Hine et al., in press) was used to determine the genetic correlation between female preference for males conferring high offspring fitness and offspring fitness conferred by males. Daughters of half-sib families were allowed to choose between males, and then random females were mated to those males to measure the effect of choice (chosen or rejected males) on offspring fitness. Female preference for male genetic quality was then calculated as the difference between the offspring fitness of chosen and rejected males. Sons of half-sib families were mated to random females to assess male contribution to offspring fitness. The positive genetic correlation between female preference for male contribution to offspring fitness and the offspring fitness conferred by males (Figure 3), suggested that good genes sexual selection may have occurred in this population.

Interestingly, females do not appear to benefit from their choice of male CHCs as the mean productivity (biomass of offspring that survive to eclosion) of females inseminated by chosen or rejected males is the same. Choice does however significantly reduce the variance in mean productivity by over 16%. This experiment indicated two aspects about sexual selection in *D. serrata*. First, females may be able to classify males with respect to their potential contribution to the fitness of their offspring based on male CHCs. Second, no change in mean productivity, but a reduction in the variance of productivity suggests that the choice of male CHCs by females may currently be under stabilizing natural selection, even though male CHCs are under strong directional sexual selection (Figure 2). Females seem to be able to avoid males that confer very low offspring fitness. Conversely, males that confer very high offspring fitness may also be missing from the chosen group presumably since the cost of being attractive trades off with offspring fitness at equilibrium (Kirkpatrick, 1987).

## Discussion

The study of mate recognition has often been conducted within two levels of biological organisation; sexual isolation between groups (populations or species), and mate choice within populations. With respect to genetic analyses, the division in approach is wide. Studies of the genetics of sexual isolation have been usually concerned with determining the number

of loci underlying differences in trait levels. In contrast, genetic studies of mate choice within species more often use the approaches of quantitative genetics since the critical parameters in models of sexual selection are couched in those terms. This difference in methodology has perhaps contributed to an artificial divide between these two levels of mate recognition.

Mate recognition in the *D. serrata* complex highlights some similarities that may link mate recognition within populations to recognition at the species level (Spieth & Ringo, 1983; Ryan & Rand, 1993; Endler & Houde, 1995). There is strong sexual selection on male CHCs in *D. serrata* populations, which is applied through female choice. When sexual selection was manipulated in hybrid populations, male CHCs rapidly evolved in a directional manner, indicating that female choice caused the rapid evolution of this male sexually-selected trait. Furthermore, natural selection appeared to operate on female choice of male CHCs, suggesting that females may be using male CHCs as indicators of male genetic quality. At the level of species, the CHCs of *D. serrata* evolve rapidly as a consequence of natural selection on mate recognition when in the presence of *D. birchii*, indicating they are an important mechanism of species recognition. A single mechanism of mate choice is therefore closely associated with the evolution of mate recognition from sexual selection within populations to sexual isolation between species. However, even in this system, where we have manipulative evidence for the importance of the same mechanism of mate recognition involved in mate choice within populations and between species, we are left with virtually no clues as to whether the processes of sexual and natural selection actually contributed to the historical speciation event between the two species.

### *Genetic analysis of mate recognition in populations at equilibrium*

At equilibrium, most mate recognition systems which experience sexual selection are likely to be under stabilizing natural selection (Kirkpatrick, 1987). Two lines of evidence suggested that stabilizing selection operates on mate recognition in undisturbed populations of *D. serrata*. First, female preference for male CHCs may be under stabilising natural selection within populations of *D. serrata*; female choice reduces the variance in offspring biomass, but does not increase the mean. While many studies of sexual selection search for positive phenotypic associations

between choice and offspring fitness (Andersson, 1994), such a relationship is only to be expected when the population is not at equilibrium. Although increases in individual fitness components as a consequence of female choice (Jennions et al., 2001) may suggest that a population is not in equilibrium, other (unmeasured) fitness components may be negatively correlated with the one under study (Partridge, 1980).

Second, the large increases in genetic variance from allopatric to sympatric populations, both in the field and under experimental conditions, suggests that CHCs may be under stabilizing selection at least in allopatric populations. It has long been held that species recognition characters will experience strong stabilizing selection in the presence of closely-related species, presumably at least after any reproductive character displacement has evolved. Our data suggest that stabilizing natural selection may be the predominant force currently acting on mate recognition at least in allopatric populations of *D. serrata*. Although the presumably equilibrium genetic variance in sympatric populations is higher than allopatric populations, this should not be interpreted as evidence for weaker stabilizing selection on mate recognition in sympatric populations. Direct measures of the form and strength of selection by estimating fitness surfaces will be required to determine the relative strength of stabilizing selection in sympatric and allopatric populations.

#### *Evolution of mate recognition under non-equilibrium conditions*

The intraspecific analysis of sexual selection in *D. serrata* suggests that females may use CHCs as an indicator of males that carry alleles for high fitness. Unfortunately, estimates of genetic correlations do not provide direct evidence for processes that may have generated them, nor do they, for example, indicate whether the level of genetic correlation is sufficient to allow indirect selection on female preferences in the case of female preference and fitness genes. Evolutionary experiments provide a way of determining the evolutionary consequences of these genetic associations.

The response of the interspecific hybrid populations to the interaction between natural and sexual selection suggested the operation of good genes sexual selection over 20 generations (Blows, 2002). Female preference for male CHCs resulted in the further exaggeration of the male sexually-selected trait in the presence of natural selection, suggesting indirect

selection on female preferences for male CHCs may have occurred. The strength of indirect selection on female preferences will in part depend on the genetic correlation between male and female components of mate recognition (Kirkpatrick & Barton, 1997). This genetic correlation was very high 37 generations after hybridization (Blows, 1999), indicating that favourable conditions were present for the operation of indirect selection in hybrid populations. It remains to be seen if the evolution of the genetic correlation between female preference and offspring fitness can be observed in populations of *D. serrata* in an experiment that can directly attribute its generation to sexual selection.

#### **Conclusion**

Mate recognition in the *D. serrata* complex evolves rapidly under laboratory conditions at both the phenotypic and genetic levels. Critical steps in the evolution of mate recognition may therefore occur over a timescale amenable to manipulation in evolutionary experiments (Barton, 2000). Surprisingly, mate recognition seems to respond to natural and sexual selection in a remarkably repeatable and deterministic fashion, leaving little role for genetic drift in the divergence of mate recognition systems, at least over the evolutionary periods explored in these experiments. Quantitative genetic analyses that focus on the genetic consequences of evolutionary processes that result in mate recognition evolution, may eventually provide an understanding of the genetic basis of the process of speciation.

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#### **References**

- Andersson, M., 1994. *Sexual Selection*. Princeton University Press, New Jersey.
- Arnold, S.J., 1992. Constraints on phenotypic evolution. *Am. Nat.* 140: S85–S107.
- Arnold, S.J. & P.C. Phillips, 1999. Hierarchical comparison of genetic variance–covariance matrices. II. Coastal-inland divergence in the garter snake, *Thamnophis elegans*. *Evolution* 53: 1516–1527.

- Ayala, F.J., 1965. Sibling species of the *Drosophila serrata* group. *Evolution* 19: 538–545.
- Bakker, T.C.M., 1993. Positive genetic correlation between female preference and preferred male ornament in sticklebacks. *Nature* 363: 255–257.
- Barton, N., 2000. The rapid origin of reproductive isolation. *Science* 290: 462–463.
- Barton, N.H. & M. Turelli, 1987. Adaptive landscapes, genetic distance and the evolution of quantitative characters. *Genet. Res.* 49: 157–173.
- Blows, M.W., 1998. Evolution of a mate recognition system after hybridization between two *Drosophila* species. *Am. Nat.* 151: 538–544.
- Blows, M.W., 1999. Evolution of the genetic covariance between male and female components of mate recognition: an experimental test. *Proc. R. Soc. Lond. B* 266: 2169–2174.
- Blows, M.W., 2002. Interaction between natural and sexual selection during the evolution of mate recognition. *Proc. R. Soc. Lond. B* 269: 1113–1118.
- Blows, M.W. & R.A. Allan, 1998. Levels of mate recognition within and between two *Drosophila* species and their hybrids. *Am. Nat.* 152: 826–837.
- Blows, M.W. & M. Higgie. Genetic constraints on the evolution of evolution of mate recognition under natural selection. *Am. Nat.* (in press).
- Bock, I.R., 1976. *Drosophilidae* of Australia. I. *Drosophila* (Insecta: Diptera). *Aust. J. Zool., Supp. Ser. No.* 40.
- Bock, I.R., 1984. Interspecific hybridization in the genus *Drosophila*. *Evol. Biol.* 17: 41–70.
- Breden, F., H.C. Gerhardt & R.K. Butlin, 1994. Female choice and genetic correlations. *Trends Ecol. Evol.* 9: 343.
- Brooks, R., 2000. Negative genetic correlation between male sexual attractiveness and survival. *Nature* 406: 67–70.
- Brooks, R. & V. Couldridge, 1999. Multiple sexual ornaments co-evolve with multiple mating preferences. *Am. Nat.* 154: 37–45.
- Burger, R. & R. Lande, 1994. On the distribution of the mean and variance of a quantitative trait under mutation–selection–drift balance. *Genetics* 138: 901–912.
- Butlin, R.K., G.M. Hewitt & S.F. Webb, 1985. Sexual selection for intermediate optimum in *Chorthippus brunneus* (Orthoptera: Acrididae). *Anim. Behav.* 33: 1281–1292.
- Butlin, R., 1987. Speciation by reinforcement. *Trends Ecol. Evol.* 2: 8–13.
- Butlin, R., 1989. Reinforcement of premating isolation, pp. 158–179 in *Speciation and Its Consequences*, edited by D. Otte & J.A. Endler. Sinauer Associates, Sunderland.
- Butlin, R., 1995. Reinforcement: an idea evolving. *Trends Ecol. Evol.* 10: 432–434.
- Buckley, S.H., T. Tregenza & R.K. Butlin, 1997. Speciation and signal trait genetics. *Trends Ecol. Evol.* 12: 299–301.
- Carson, H.L., F.C. Val & A.R. Templeton, 1994. Change in male secondary sexual characters in artificial interspecific hybrid populations. *Proc. Natl. Acad. Sci. USA* 91: 6315–6318.
- Cobb, M. & J.M. Jallon, 1990. Pheromones, mate recognition and courtship stimulation in the *Drosophila melanogaster* species sub-group. *Anim. Behav.* 39: 1058–1067.
- Coyne, J.A., 1992. Genetics of speciation. *Nature* 355: 511–515.
- Coyne, J.A., A.P. Crittenden & K. Mah, 1994. Genetics of a pheromonal difference contributing to sexual isolation in *Drosophila*. *Science* 265: 1461–1464.
- Coyne, J.A. & B. Charlesworth, 1997. Genetics of a pheromone difference affecting sexual isolation between *Drosophila mauritiana* and *D. sechellia*. *Genetics* 145: 1015–1030.
- Coyne, J.A. & H.A. Orr, 1998. The evolutionary genetics of speciation. *Phil. Trans. R. Soc. Lond. B* 353: 287–305.
- Dobzhansky, T., 1951. *Genetics and the Origin of Species*. Columbia University Press, New York, 3rd edn.
- Endler, J.A. & A.E. Houde, 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* 49: 456–468.
- Gray, D.A. & W.H. Cade, 1999. Quantitative genetics of sexual selection in the field cricket *Gryllus integer*. *Evolution* 53: 848–854.
- Gray, D.A. & W.H. Cade, 2000. Sexual selection and speciation in field crickets. *Proc. Natl. Acad. Sci. USA* 97: 14449–14454.
- Harshman, L.G. & A.A. Hoffmann, 2000. Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends Ecol. Evol.* 15: 32–36.
- Hasselquist, D., S. Bensch & T. von Schantz, 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature* 381: 229–232.
- Higgie, M., S. Chenoweth & M.W. Blows, 2000. Natural selection and the reinforcement of mate recognition. *Science* 290: 519–521.
- Hine, E., S. Lachish, M. Higgie & M.W. Blows. Positive genetic correlation between female preference and offspring fitness. *Proc. R. Soc. Lond. B* (in press).
- Hoikkala, A. & S. Crossley, 2000. Copulatory courtship in *Drosophila*: behaviour and songs in *D. birchii* and *D. serrata*. *J. Insect Behav.* 13: 71–87.
- Houde, A.E., 1994. Artificial selection on male colour patterns shifts mating preferences of female guppies. *Proc. R. Soc. Lond. B* 256: 125–130.
- Houle, D. & A.S. Kondrashov, 2002. Coevolution of costly mate choice and condition-dependent display of good genes. *Proc. R. Soc. Lond. B* 269:97–104.
- Howard, D.J., 1993. Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis, pp. 46–69 in *Hybrid Zones and the Evolutionary Process*, edited by R.G. Harrison. Oxford University Press, New York.
- Howard, R.W., L.L. Jackson, H. Banse & M.W. Blows. Cuticular hydrocarbons of *Drosophila birchii* and *D. serrata*: identification and role in mate choice in *D. serrata*. (in preparation).
- Iwasa, Y., A. Pomiankowski & S. Nee, 1991. The evolution of costly mate preferences. II. The “Handicap” principle. *Evolution* 45: 1431–1442.
- Iwasa, Y. & A. Pomiankowski, 1995. Continual change in mate preferences. *Nature* 377: 420–422.
- Jallon, J.M., 1984. A few chemical words exchanged by *Drosophila* during courtship and mating. *Behav. Genet.* 14: 441–478.
- Jennions, M.D., A.P. Moller & M. Petrie, 2001. Sexually-selected traits and adult survival: a meta-analysis. *Quart. Rev. Biol.* 76: 3–36.
- Keightley, P.D. & W.G. Hill, 1989. Quantitative genetic variability maintained by mutation-stabilizing selection: sampling variation and response to subsequent directional selection. *Genet. Res.* 54: 45–57.
- Kirkpatrick, M., 1987. Sexual selection by female choice in polygynous animals. *Ann. Rev. Ecol. Syst.* 18: 43–70.
- Kirkpatrick, M. & N.H. Barton, 1995. Deja vu all over again. *Nature* 377: 388–389.
- Kirkpatrick, M. & N.H. Barton, 1997. The strength of indirect selection on female mating preferences. *Proc. Natl. Acad. Sci. USA* 94: 1282–1286.

- Lande, R., 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution* 33: 402–416.
- Lande, R., 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. USA* 78: 3721–3725.
- Lynch, M. & B. Walsh, 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Massachusetts.
- Nelson, D.R., 1993. Methyl-branched lipids in insects, pp. 271–315 in *Insect Lipids: Chemistry, Biochemistry and Biology*, edited by D.W. Stanley-Samuelson & D.R. Nelson. University of Nebraska Press, Lincoln.
- Noor, M.A., 1995. Speciation driven by natural selection in *Drosophila*. *Nature* 375: 674–675.
- Norris, K., 1993. Heritable variation in a plumage indicator of viability in male great tits *Parus major*. *Nature* 362: 537–539.
- Orr, H.A., 2001. The genetics of species differences. *Trends Ecol. Evol.* 16: 343–350.
- Partridge, L., 1980. Mate choice increases a component of offspring fitness in fruit flies. *Nature* 283: 290–291.
- Paterson, H.E.H., 1985. The recognition concept of species, pp. 21–29 in *Species and Speciation*, edited by E. Vrba. Transvaal Museum Monograph 4, Pretoria, South Africa.
- Pomiankowski, A. & L. Sheridan, 1994. Linked sexiness and choosiness. *Trends Ecol. Evol.* 9: 242–244.
- Petrie, M., 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature* 371: 598–599.
- Rice, W.R. & E.E. Hostert, 1993. Laboratory experiments on speciation: what have we learned in forty years. *Evolution* 47: 1637–1653.
- Rundle, H.D. & D. Schluter, 1998. Reinforcement of stickleback mate preferences: sympatry breeds contempt. *Evolution* 52: 200–208.
- Ryan, M.J. & A.S. Rand, 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* 47: 647–657.
- Schluter, D., 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* 42: 849–861.
- Schluter, D., 1996. Adaptive radiation along genetic lines of least resistance. *Evolution* 50: 1766–1774.
- Schluter, D., 2001. Ecology and the origin of species. *Trends Ecol. Evol.* 16: 372–380.
- Spieth, H.T. & J.M. Ringo, 1983. Mating behaviour and sexual isolation in *Drosophila*, pp. 223–284 in *The Genetics and Biology of Drosophila*, Vol. 3C, edited by M. Ashburner, H.L. Carson & J.N. Thompson. Academic Press, London.
- Turelli, M., N.H. Barton & J.A. Coyne, 2001. Theory and speciation. *Trends Ecol. Evol.* 16: 330–343.
- Wallace, B., M.W. Timm & M.P.P. Strambi, 1983. The establishment of novel mate recognition systems in introgressive hybrid *Drosophila* populations. *Evol. Biol.* 16: 467–488.
- Wilkinson, G.S. & P.R. Reillo, 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. *Proc. R. Soc. Lond. B* 255: 1–6.
- Wu, C.-I., H. Hollocher, D.J. Begun, C.F. Aquadro, Y. Xu & M.-L. Wu, 1995. Sexual isolation in *Drosophila melanogaster*: a possible case of incipient speciation. *Proc. Natl. Acad. Sci. USA* 92: 2519–2523.