

# The diversification of mate preferences by natural and sexual selection

H. D. RUNDLE,\*,† S. F. CHENOWETH† & M. W. BLOWS†

\*Department of Biology and Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, Ontario, Canada

†School of Integrative Biology, University of Queensland, St Lucia, Queensland, Australia

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## Abstract

The evolution of sexual display traits or preferences for them in response to divergent natural selection will alter sexual selection within populations, yet the role of sexual selection in ecological speciation has received little empirical attention. We evolved 12 populations of *Drosophila serrata* in a two-way factorial design to investigate the roles of natural and sexual selection in the evolution of female mate preferences for male cuticular hydrocarbons (CHCs). Mate preferences weakened in populations evolving under natural selection alone, implying a cost in the absence of their expression. Comparison of the vectors of linear sexual selection revealed that the populations diverged in the combination of male CHCs that females found most attractive, although this was not significant using a mixed modelling approach. Changes in preference direction tended to evolve when natural and sexual selection were unconstrained, suggesting that both processes may be the key to initial stages of ecological speciation. Determining the generality of this result will require data from various species across a range of novel environments.

## Introduction

Ecological speciation occurs when divergent natural selection results in the evolution of different mating preferences, and ultimately reproductive isolation, among populations (Schluter, 2000; Rundle & Nosil, 2005). When populations experience divergent environmental conditions, different favourable alleles affecting male sexually selected traits, or the female preferences for them, may respond to natural selection in the different environments (Turelli *et al.*, 2001). Parallel mating preferences (Rundle *et al.*, 2005), and the reproductive isolation that results from their divergence (Kilias *et al.*, 1980; Dodd, 1989; Rundle *et al.*, 2000; Nosil *et al.*, 2002), have been observed to evolve in correlation with environment, indicating that divergent natural selection may be a common mechanism of speciation.

Although divergent natural selection may initiate the divergence of male display traits and female preferences for them, sexual selection is also likely to play a key role in ecological speciation (Schluter, 2000). This is because

mate preferences are maintained within populations as a consequence of the direct benefits gained by females (e.g. resources, parental care, fewer parasites, the avoidance of male-imposed costs; Chapman *et al.*, 2003), and/or by the indirect benefits that may accrue to females as a consequence of the genes that the males carry (e.g. good genes mate choice; Kirkpatrick & Barton, 1997; Houle & Kondrashov, 2002; Cameron *et al.*, 2003; Kokko *et al.*, 2003). Consequently, evolutionary changes in male display traits or female preferences in response to divergent natural selection will necessarily affect how sexual selection operates within a population (Lande, 1981). Although comparative studies have implicated sexual selection in speciation in nature (Coyne & Orr, 2004), the extent to which divergence in male traits and female preferences during adaptation to a novel environment is dependent on the operation of sexual selection has not been addressed empirically. Here, we experimentally investigate the roles of both natural and sexual selection in the evolution of female mate preferences during adaptation to a novel environment.

Empirical investigation of the mechanisms involved in the evolutionary diversification of mate preferences may be critical not only for understanding their role in the origin of new species, but may also be key to reconciling conflicting evidence concerning their effect

Correspondence: Howard D. Rundle, Department of Biology and Centre for Advanced Research in Environmental Genomics, 30 Marie-Curie, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada.  
Tel.: +1 613 562 5800 (ext. 2835); fax: +1 613 562 5486;  
e-mail: hrundle@uottawa.ca

on adaptation. According to recent good genes models, preferences for mates with high breeding values for fitness can have beneficial effects on population mean nonsexual fitness, increasing both the rate and extent of adaptation (Lorch *et al.*, 2003). Despite some support for good genes mate choice (Jennions *et al.*, 2001; Bonduriansky & Rowe, 2005; Head *et al.*, 2005; Reid *et al.*, 2005; Dolgin *et al.*, 2006; Fricke & Arnqvist, 2007), two recent evolution experiments that manipulated the opportunity for sexual selection failed to find any benefit in promoting adaptation to a novel environment (Holland, 2002; Rundle *et al.*, 2006). While this may reflect the general absence of good genes indirect benefits in any environment, another potential explanation involves genotype  $\times$  environment interactions that may initially disrupt a good genes process in a novel environment (Rundle *et al.*, 2006). In the presence of such interactions, mate preferences may need to evolve in response to nonadaptive plastic changes in the novel environment, or to find the new combination of male display traits indicative of high genetic quality. Evaluating such possibilities will require an understanding of preference evolution in novel environments.

Using the fruit fly *Drosophila serrata*, we manipulated the opportunities for both natural and sexual selection within the context of experimental evolution to provide direct insight into the evolution of mate preferences during adaptation to a novel environment. *Drosophila serrata* is an ideal candidate for such an experiment because mate preferences have been well characterized in a number of evolutionary and quantitative genetic experiments. Both sexes use a suite of contact pheromones, composed of a series of nonvolatile cuticular hydrocarbons (CHCs), in mate choice within populations (Hine *et al.*, 2002, 2004; Chenoweth & Blows, 2003, 2005; Blows *et al.*, 2004; Petfield *et al.*, 2005; Skroblin & Blows, 2006) and species recognition (Blows & Allan, 1998; Higgie *et al.*, 2000; Higgie & Blows, 2007). CHCs and preferences for them have been shown to evolve in replicate laboratory populations during adaptation to a novel corn-based food environment (Rundle *et al.*, 2005). We now determine the importance of sexual selection in mate preference evolution during adaptation to this same novel environment.

## Materials and methods

### Experimental populations

The derivation and maintenance of the experimental populations is described in detail elsewhere (Rundle *et al.*, 2006; Chenoweth *et al.*, 2008). Briefly, from a laboratory stock population of *D. serrata* adapted to a standard yeast-based larval food, we derived 12 replicate populations and independently propagated them in a novel corn-based larval food (Rundle *et al.*, 2005) environment. Generations were discrete and involved trans-

ferring 55 adults of each sex. These populations were assigned in a two-way factorial design to one of four treatments that independently manipulated the opportunities for natural and sexual selection, yielding three populations that experienced both natural and sexual selection (NS), three that experienced reduced natural selection with sexual selection present (XS), three that experienced natural selection with reduced sexual selection (NX) and three that experienced reduced natural and reduced sexual selection (XX). The latter treatment serves as an internal control because there is little opportunity for evolutionary change in it: evolution by natural and sexual selection are prevented and the duration of the experiment minimizes the input of novel mutations.

Natural selection was permitted by allowing females to contribute to the next generation in proportion to the number of adult progeny she produced, whereas it was greatly reduced by equalizing the contribution to the next generation of all females in the population. The success of this treatment in manipulating natural selection was confirmed by a significant effect on female productivity when tested on these same populations after 13–16 generations of experimental evolution (Rundle *et al.*, 2006). Sexual selection was independently manipulated by either mixing virgin adults together every generation for 3–6 days for mating, thus providing extensive opportunities for both intrasexual competition and mutual mate choice (sexual selection present treatment), or by enforcing random monogamy, thereby equalizing the sexual fitness of all individuals (sexual selection reduced). The success of this treatment was confirmed by a significant treatment effect on the evolution of CHCs during this experiment (Chenoweth *et al.*, 2008).

### Mate preference assays

After 13 generations of experimental evolution, an average of 213 (range 209–221) replicate binomial female choice mating trials were conducted in each of the 12 populations (a total of 2557 choice trials) to estimate female mate preferences for male CHCs. In every trial, a single virgin female from one of the experimental populations was given a choice between two virgin males from the stock population. The use of stock males ensured that any differences among populations in female preferences can be attributed directly to changes in the preferences themselves by presenting females with the same range of male phenotypes among which to choose. Stock males were raised for a generation in the corn environment prior to their use to account for potential environmental effect on male CHCs. Because preferences were assayed under the same experimental conditions in all populations (i.e. a 'common garden'), including the corn environment in which all of the flies were raised, among-population differences can be interpreted as genetically based.

Mating trials were conducted over 5 days in glass vials containing 10 mL corn media using males and females that were 4–6 and 8 days post-emergence respectively. Trials were blocked by day such that approximately one-fifth of all replicate trials for each population were performed on each day. During the trials, vials were observed and once intromission occurred between the female and one of the two males, all flies were anaesthetized using CO<sub>2</sub> and either the male that was chosen by the female (50% of the time, randomly determined) or the male that was rejected by the female (the remaining 50% of the time) was removed for subsequent CHC extraction following established methods (Blows & Allan, 1998). Using only a single male from each trial ensured the independence of all individuals in subsequent statistical analyses.

Samples were analysed on an HP6890N gas chromatograph (Agilent Technologies, Wilmington, Delaware, USA) employing flame ionization detection and using a previously described method (Rundle *et al.*, 2005). Individual CHC profiles were subsequently determined by integration of the area under nine peaks. These are the same peaks as used in past studies (Blows *et al.*, 2004; Chenoweth & Blows, 2005; Petfield *et al.*, 2005; Rundle *et al.*, 2005), identified in order of their retention times as: (Z,Z)-5,9-C<sub>24:2</sub>, (Z,Z)-5,9-C<sub>25:2</sub>, (Z)-9-C<sub>25:1</sub>, (Z)-9-C<sub>26:1</sub>, 2-Me-C<sub>26</sub>, (Z,Z)-5,9-C<sub>27:2</sub>, 2-Me-C<sub>28</sub>, (Z,Z)-5,9-C<sub>29:2</sub> and 2-Me-C<sub>30</sub> (Howard *et al.*, 2003). Peak areas were transformed into proportions by dividing the area of each peak by the total area of all nine peaks for each individual. Proportions are used in place of total amounts because they control for variation in both the extraction process and injection quantities into the gas chromatograph, and are therefore less prone to experimental error than the use of internal standards (Blows & Allan, 1998; Savarit & Ferveur, 2002). Proportions were then transformed to logcontrasts to remove the unit-sum constraint that exists in such compositional data (Atchison, 1986; Blows *et al.*, 2004), resulting in eight logcontrast variables:

$$\text{logcontrast CHC}_n = \log_{10} \frac{\text{proportion (CHC}_n\text{)}}{\text{proportion}[(Z) - 9 - C_{26:1}]}$$

(Z)-9-C<sub>26:1</sub> was used as the divisor when calculating logcontrasts to permit comparison with other results from this experiment (Chenoweth *et al.*, 2008). These logcontrast CHCs were individually standardized ( $\sim N(0,1)$ ) prior to conducting the analyses below.

### Statistical analyses

Female mate preferences for the eight male logcontrast CHCs were estimated separately in each population by employing population-level linear sexual selection gradients (Wagner, 1998; Chenoweth & Blows, 2005). Selection gradients were calculated using the standard linear regression model (Lande & Arnold, 1983):

$$w = \alpha + \sum_{i=1}^8 \beta_i z_i, \quad (1)$$

where  $w$  is the relative mating success (0 = rejected, 1 = chosen) and  $z_i$  are the eight logcontrast CHC values for a given individual. These regressions yielded, for each population, a column vector of linear selection gradients ( $\beta$ ) characterizing directional sexual selection on each of the eight logcontrast CHCs. The overall importance of CHCs in explaining variation in male mating success was given by the adjusted coefficient of determination ( $R^2_{\text{adj}}$ ) in each population. Significance of the model fit was determined using logistic multiple regression rather than standard least squares because mating success is binomially distributed (Fairbairn & Preziosi, 1996; Rundle *et al.*, 2008). Differences among treatments in the importance of male CHCs in explaining variation in mating success was tested using a two-way analysis of variance (ANOVA) on the 12 replicate  $R^2_{\text{adj}}$  values (natural log transformed). When the independent variables (e.g. CHCs) are uncorrelated, this is equivalent to testing for differences in the magnitudes of the vectors of linear selection gradients ( $\beta$ ) in each population (i.e. the strength of sexual selection) because  $R^2 = \Sigma \beta_i^2$  (Bring, 1996). Identical results were therefore obtained when the analysis was performed on  $\|\beta\|$  calculated from a multiple regression (eqn 1) using the principal components of the logcontrast CHCs in place of the original traits.

Variation in the combination of male CHCs that females found attractive would be manifested as among-population differences in the multivariate direction of sexual selection on male CHCs. The appropriate statistical approach for quantifying and comparing such variation is unclear. One approach is to extend the linear regression model of eqn (1) by including population and/or treatment as fixed effects, resulting in an analysis of covariance (ANCOVA) that tests whether selection varies among populations or treatments. A partial  $F$ -test could then be used to compare the fit of models that include and exclude these interaction terms. A drawback of this approach, however, is that as the number of traits increases, substantial degrees of freedom can be consumed in estimating all of the resulting interactions (Rundle *et al.*, 2008). Here, we employ two alternative, recently described techniques. The first directly compares the vectors of linear selection gradients ( $\beta$ ) by assembling a similarity matrix describing variation in multivariate sexual selection among all pair wise combinations of populations (Rundle *et al.*, 2008) (Table S1). Elements of this matrix were the vector correlations of linear sexual selection between every pair of populations, calculated as the dot-product of their standardized  $\beta$  vectors from eqn 1. Structure in this similarity matrix was visualized using multidimensional scaling (Borg & Groenen, 1997) and tested using a matrix randomization approach (Mantel tests) that compared the similarity matrix to model

matrices specifying treatment-level structure corresponding to the main effects of natural and sexual selection (Manly, 1985; Rundle *et al.*, 2008).

Second, we employed a random regression (also known as random coefficient) mixed modelling approach to the analysis of function-valued traits (Meyer & Kirkpatrick, 2005; McGuigan *et al.*, 2008). Sexual and natural selection had to be collapsed into a single treatment factor with four levels (XX, NX, XS and NS) in this analysis because of computing limitations. The multivariate random coefficient model was:

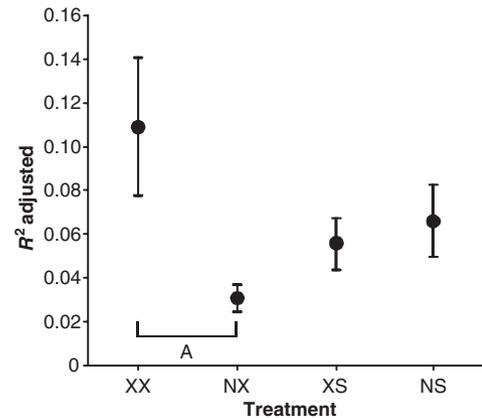
$$y_{ijk} = \alpha + \mathbf{X}_{jk}\beta + \delta_{jk}^{(p)} + \delta_k^{(t)} + \varepsilon_{ijk} \quad (2)$$

in which  $y_{ijk}$  is a column vector containing the binomial mating success scores of the  $i$  males modelled as a function of the fixed effect intercept ( $\alpha$ ) and population-wide regression coefficients ( $\beta$ ) for the set of eight continuous CHCs ( $\mathbf{X}$ ).  $\delta_k^{(t)}$  and  $\delta_{jk}^{(p)}$  represent the random effect variances among these regression coefficients at the level of treatment and population nested within treatment. These variances are assumed to have distributions  $\sim N(\mathbf{0}, \Sigma_t)$  and  $\sim N(\mathbf{0}, \Sigma_p)$  respectively. We used factor analytic modelling to estimate the first eigenfunction of preference from the covariance matrix of random regression coefficient at the levels of both treatment and population nested within treatment (Kirkpatrick & Meyer, 2004; Meyer & Kirkpatrick, 2005; Hine & Blows, 2006).

## Results

Female mate preferences were a source of significant sexual selection on CHCs in males in all 12 populations ( $P < 0.005$  in all cases; Table S2). To address the evolution of these mate preferences, two aspects of among-population divergence can be distinguished. First, populations may vary in extent to which females utilize CHCs when choosing among males (i.e. the strength of their mate preference), and hence the importance of CHCs in determining male attractiveness. When estimating preferences using sexual selection gradients, such differences would be manifested as among-population variation in the overall explanatory power of male CHCs (i.e. the adjusted coefficient of determination;  $R^2_{\text{adj}}$ ). Second, populations may vary in the specific combination of display traits that females prefer, or in other words, the blend of CHCs that confer high male attractiveness. Such variation would be manifested as among-population differences in the multivariate direction of sexual selection on male CHCs. We address both of these aspects in turn.

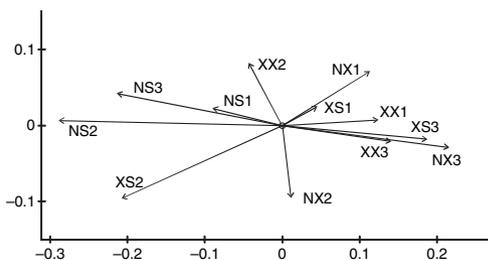
The strength of female mate preferences for male CHCs diverged significantly among treatments, as revealed by a significant interaction between natural and sexual selection (two-way ANOVA on  $R^2_{\text{adj}}$  values:  $F_{1,8} = 7.68$ ,  $P = 0.024$ ) (results do not change qualitatively if non-adjusted  $R^2$  values are used instead). The treatment in which both forms of selection were reduced (XX)



**Fig. 1** Proportion of variance in male mating success explained by their cuticular hydrocarbons (CHCs). Values are means  $\pm$  1 SE of the three replicate populations that evolved under each treatment combination. Labels indicate the natural selection treatment (N, present; X, reduced) followed by the sexual selection treatment (S, present; X, reduced). Comparison 'A' indicates treatments that are significantly different in *post hoc* pairwise comparisons.

provides an internal control because evolution by natural and sexual selection were both prevented and little decay of preferences as a neutral trait is expected via novel mutation in only 13 generations. Preferences in this treatment therefore reflect those present upon colonization of the novel environment. Relative to this treatment, preferences for CHCs tended to weaken in the other treatments, although this decrease was only significant in the populations experiencing natural selection alone (NX treatment; Fig. 1).

Variation was also present in the combination of male CHCs that females found attractive, manifested as differences among populations in the multivariate direction of sexual selection (Fig. 2). The main component of this variation tended to contrast the three populations in which both natural and sexual selection were unconstrained (NS), along with a population from the sexual selection alone treatment (XS), with the remaining populations. Although this generated a positive correlation with a model matrix specifying a main effect of sexual selection that approached significance ( $r = 0.192$ ,  $P = 0.072$ ) and a nonsignificant correlation with a model matrix specifying a main effect of natural selection ( $r = -0.022$ ,  $P = 0.44$ ), it suggests that evolutionary changes in direction of female preferences depended on the joint presence of both forms of selection. It is not possible to construct a model matrix that provides an overall test for any interaction between the natural and sexual selection treatments. Nevertheless, a strong and significant positive correlation was detected with a model matrix that contrasted the NS treatment with the others ( $r = 0.443$ ,  $P = 0.009$ ), suggesting that both forms of selection were required for evolutionary change in the combination of male CHCs that females found most attractive.



**Fig. 2** Multidimensional scaling plot depicting the structure in the similarity matrix of linear sexual selection on male CHCs among the 12 replicate populations in two dimensions. Labels as in Fig. 1, with the addition of population replicate number.

In contrast to the above results involving the pair-wise comparison of the vectors of linear selection gradients, random regression was unable to detect significant variation in female mate preferences among treatments. Using factor analytic modelling, the first eigenfunction of preference at the treatment level was not statistically supported (log likelihood ratio test:  $\chi^2 = 4.48$ , d.f. = 8,  $P = 0.81$ ). This approach was also unable to detect significant variation in female mate preferences among populations, with the first eigenfunction of preference at the population level also not statistically supported (log likelihood ratio test:  $\chi^2 = 4.54$ , d.f. = 8,  $P = 0.81$ ).

Selection arises from variation in the contribution of individuals to subsequent generations, so experimental manipulations of natural and sexual selection necessarily confound variation in effective population size ( $N_e$ ) with the selection treatments. A key question in such experiments is therefore whether there is any evidence that variation in  $N_e$  could explain any treatment effects (Rice & Holland, 2005). The  $N_e$  hypothesis predicts that the response to selection and  $N_e$  will be positively correlated, and that within treatments, among-population variance will increase as  $N_e$  decreases. Neither of these predictions are supported in our data. First, for changes in preference direction, the response to selection was greatest in the treatment in which  $N_e$  should be lowest (i.e. populations in which both natural and sexual selection were unconstrained; NS treatment). Second, among-population variance in both the strength and direction of mate preferences did not increase with decreasing  $N_e$ . Finally, neither of these  $N_e$  predictions were upheld for the evolution of three other traits in these same populations during this experiment (female productivity and male and female CHCs; Rundle *et al.*, 2006; Chenoweth *et al.*, 2008), strongly implying that variation in effective population size was not responsible for any treatment effects.

## Discussion

The evolutionary divergence of mate preferences (Rundle *et al.*, 2005) and the premating isolation they generate (Kilias *et al.*, 1980; Dodd, 1989; Rundle *et al.*, 2000; Nosil *et al.*, 2002) have been shown to evolve in correlation with

environment, providing experimental confirmation of the ecological model of speciation. The contribution of sexual selection to this process has received little attention, however. Here, we used an experimental evolution approach in which the opportunities for both natural and sexual selection were independently manipulated for 13 generations in a factorial design to evaluate their effects on the divergence of female mate preferences for male sexual display traits in replicate populations of *D. serrata*. The optimal statistical procedure for the analysis of such data is unclear and we therefore employed two recently described approaches: the direct comparison of vectors of sexual selection and a random regression mixed model. Results differed, with the former detecting significant treatment effects in terms of changes in preference direction while the latter failing to do so (nor even to detect any significant preference differences among populations). Below we summarize the changes in preference that were detected, consider their potential biological implications, and discuss possible explanations for why results differed using these two analytical approaches.

Female mate preferences decreased significantly in strength in the NX as compared to the XX treatment, with variation in CHCs explaining less than one-third of the variance in male mating success in the former as compared to the latter treatments (Fig. 1). Although costs of female preferences are generally assumed in most models of sexual selection, they have rarely been demonstrated (Byers *et al.*, 2005), especially within the context of a manipulative experiment. In our case, this decrease in preference strength in the absence of sexual selection during adaptation (i.e. the NX treatment) implies a cost to female preference alleles in the novel environment that is not associated with preference expression itself (because preference expression was prevented by enforcing lifelong monogamy in this treatment). One way that female preference could be selected against in this fashion is if preference alleles are in nonrandom association with other loci that are under selection in the novel environment. Female preference has been shown to be genetically correlated with male fitness in *D. serrata* (Hine *et al.*, 2002), suggesting that selection on male fitness in the novel environment could generate indirect selection on female preferences.

Female preferences also appeared to evolve with respect to the combination of male CHCs that females found attractive, although as noted earlier, results contrasted between the two analytical techniques we employed. Using a random regression approach, we failed to detect significant variation at the treatment or population level, suggesting not only that preferences failed to respond to selection, but that there was no among-population genetic variation for them. Random regression provides a seemingly elegant mixed linear modelling approach to the analysis of function-valued traits (Meyer & Kirkpatrick, 2005) and has been successfully employed recently within the context of a

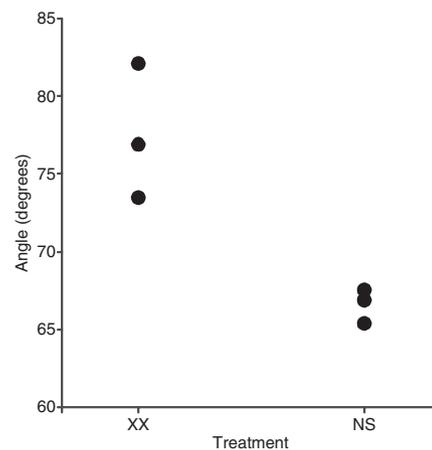
quantitative genetic breeding design to demonstrate a significant genetic basis of female mate preferences for male CHCs in the closely related *Drosophila bunnanda* (McGuigan *et al.*, 2008). As an approach to the analysis of evolution experiments, however, it may not be ideal for two reasons. First, restricted maximum likelihood estimation of the covariance matrices of random regression coefficients is memory intensive and, in our case, we had to collapse our two-way factorial design into a single treatment with four levels to run the model using SAS V. 9.1 (SAS Institute Inc., Cary, NC, USA). Second, the random effect variances among the regression coefficients at the level of treatment involve a limited number of subjects (12 replicate populations from four treatments) in our case; in comparison, it required 500 females from 122 sires to detect a significant sire-level treatment effect demonstrating genetic variance in female preference for male CHCs in *D. bunnanda* (McGuigan *et al.*, 2008).

Among population differences in female preferences were evident in the direct comparison of the  $\beta$  vectors of linear sexual selection (Fig. 2), with the populations in which both natural and sexual selection were unconstrained (NS) tending to cluster. Differences in preference strength cannot contribute to this observed structure ( $\beta$  vectors were standardized to unit length), indicating a shift in mate preference direction that evolved primarily in the presence of both natural and sexual selection. Perhaps surprisingly, natural selection in the absence of sexual selection had no consistent effect on the evolution of mate preferences, suggesting the possibility that natural selection alone may be unlikely to cause preference evolution during the early stages of ecological speciation. Although this may seem to contradict a wealth of past speciation experiments in which assortative mating has been observed to evolve as a by-product of divergent natural selection (Rice & Hostert, 1993; Coyne & Orr, 2004), as far as we are aware, sexual selection was unconstrained in all of these experiments, making its contribution unknown. Our results suggest that the operation of sexual selection may be critical for mate preferences evolution after this particular change in environment, although the generality of this conclusion awaits further study in other species and across a range of novel environments.

Determining why sexual selection appeared to interact with natural selection during adaptation will require a detailed understanding of how female preferences are maintained in the ancestral environment and how this was affected by the novel environment. This has yet to be conclusively established in any species. In our case, measurements of female productivity demonstrated that the presence of sexual selection did not promote adaptation to the novel environment during this experiment (Rundle *et al.*, 2006), despite previous evidence suggesting the possible operation of good genes mate choice in *D. serrata* (Hine *et al.*, 2002, 2004). Our estimate of female mate preferences in the treatment in which both natural and sexual selection were reduced (XX) is consistent with

this result and provides a possible explanation: preferences for male CHCs in these populations were oriented 77.5 degrees on average from the vector of divergence representing the response of male CHCs to natural selection alone during this experiment (Fig. 3; see Supporting Information Appendix S1 for further details). This suggests that upon colonization of this novel environment, females were initially preferring a combination of CHCs in males that was almost orthogonal to that favoured by natural selection in this environment.

If changes in preference direction evolved in response to good genes indirect benefits, then this should have brought the vector of sexual selection generated by these preferences closer in line with the vector of natural selection on these same traits (CHCs). Consistent with this, changes in the direction of female preferences that evolved when selection was unconstrained (NS treatment) decreased the angle between them and the divergence vector representing the response of male CHCs to natural selection (Fig. 3). This decrease from that observed in the XX treatment is significant (Watson–Williams *F*-test for angular data:  $F_{1,4} = 17.7$ ,  $P = 0.014$ ), indicating that the changes in preferences occurring in the presence of natural and sexual selection (NS treatment) appear to have been adaptive. This suggests that the failure of sexual selection to promote adaptation in this experiment (Rundle *et al.*, 2006) may have represented a temporary disruption of a good genes process upon colonization of the novel environment; given sufficient time, mate preferences may have evolved to bring them in line with the combination of male CHCs favoured by natural selection. Such a scenario requires direct investigation because it contrasts with good genes models (Lorch *et al.*, 2003) in which such



**Fig. 3** Variation among populations in the orientation of female mate preferences with respect to natural selection on male CHCs, as given by the angle between these vectors. The vector of natural selection on male CHCs was estimated as the divergence vector between the XX and NX treatments in male CHCs measured after 12 generations of experimental evolution (see Supporting Information for further details). Treatment labels as in Fig. 1.

genotype  $\times$  environment interactions are assumed absent. The rapid evolution of assortative mating that has been seen to evolve in some laboratory speciation experiments (Kilias *et al.*, 1980; Dodd, 1989; Coyne & Orr, 2004), however, implies that mate preferences may often diverge during adaptation to a novel environment (Rundle *et al.*, 2005); such divergence will necessarily affect a good genes process and therefore requires further study.

## Conclusions

Thirteen generations of experimental evolution in a novel environment caused changes in the strength and direction of female mate preferences for male CHCs that were detectable by direct comparison of selection vectors but not via a random regression mixed modelling approach. This suggests that such treatment effects may be small, although random regression may be poorly suited to analysing the outcome of evolution experiments. More work is needed with respect to the optimal statistical approach for investigating among-population divergence in function-valued traits like mate preferences.

The changes in preference that did occur included a weakening in populations experiencing natural selection alone (NX treatment) as compared to the XX (control) treatment. This decrease in preference strength suggests natural selection against preferences in absence of their expression because little decay of a neutral trait is expected over such a small number of generations. Experimental evolution provides a useful approach to detecting such costs that are otherwise difficult to measure directly. Changes in the combination of CHCs that females preferred appeared to arise primarily in the treatment in which both natural and sexual selection were unimpeded, suggesting that sexual selection may be an important component of ecological speciation although additional data are needed from multiple species in a variety of environments. Although much attention has been given to the role of divergent natural selection in the evolution of premating isolation, a detailed understanding of the contribution of sexual selection is lacking (Rundle *et al.*, 2005). In future studies, it will be important to directly link preference divergence with the buildup of premating isolation. Finally, changes in the combination of CHCs that females preferred appeared to bring them closer in line with the combination of traits favoured by natural selection in this environment. A comprehensive understanding of the contribution of sexual selection to adaptation will therefore require knowledge of how display traits and preferences change upon colonization of novel environments, as well as their subsequent evolution in these environments.

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## Supporting information

Additional supporting information may be found in the online version of this article.

**Appendix S1** Estimating natural selection on male CHCs.

**Table S1** Between-population comparisons in the similarity of linear sexual selection on eight logcontrast cuticular hydrocarbons (CHCs) in males.

**Table S2** Strength of linear sexual selection on male CHCs in each of the 12 experimental populations, as generated by female mate preferences.

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