

SHORT COMMUNICATION

Sexual selection on *Drosophila serrata* male pheromones does not vary with female age or mating statusS. GERSHMAN*†¹, M. DELCOURT*² & H. D. RUNDLE*

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Abstract

Mate preferences are costly and are thought to evolve due to the direct and/or indirect benefits they provide. Such costs and benefits may vary in response to intrinsic and extrinsic factors with important evolutionary consequences. Limited attention has been given to quantifying such variation and understanding its causes, most notably with respect to the direction and strength of preferences for multivariate sexual displays. In *Drosophila serrata*, female preferences target a pheromone blend of long-chain cuticular hydrocarbons (CHCs). We used a factorial design to test whether female age and mating status generated variation in the strength and direction of sexual selection on male CHCs. Replicate choice mating trials were conducted using young and old females (4 or 10 days post-emergence) that were either virgin or previously mated. The outcome of such trials is known to capture variation in female mate preferences, although male–male interactions may also contribute. Directional sexual selection on male CHCs was highly significant within each treatment, but there was little evidence of any variation among treatments. The absence of treatment effects implies that the multivariate combination of male CHCs preferred by females was constant with respect to female age and mating status. To the extent that male–male interactions may also contribute, our results similarly imply that these did not vary among treatments groups. With respect to *D. serrata* mate preferences, our results suggest that either plasticity with respect to age and mating status is not beneficial to females, or preference expression is somehow constrained.

Introduction

Female preference for specific male signals or displays can generate differential reproductive success among males and drive the exaggeration of these male traits over evolutionary time (Darwin, 1871). Determining the factors that influence female choice is therefore integral to our understanding of evolution by sexual

selection. Female mate preferences are often quantified by assaying the mating success of a range of male phenotypes across a particular group of females. If these females are randomly sampled from a larger population, this approach provides an estimate of the average female preference in the population, equivalent to the sexual selection gradient resulting from precopulatory female mate choice on these male traits (Wagner, 1998; Kingsolver *et al.*, 2001; Chenoweth & Blows, 2006).

In quantifying mate preferences, however, individuals are often not randomly sampled from a population of interest but instead represent a particular subsample, often consisting of young (but sexually receptive) females that are generally either virgin (Wagner *et al.*, 1995; Ritchie, 1996; Basolo, 1998; Hine *et al.*, 2002; Chenoweth & Blows, 2003; Delcourt & Rundle, 2011) or have mated once previously (Thomas & Simmons,

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2010). Whereas such females are possibly chosen to represent those that are at the peak of their reproductive potential, and hence likely also to be the choosiest, extrapolating the preference of these females to the population level relies on the ancillary assumption that this subset of females is representative of those in the population as a whole. However, we know that females can vary in their mate preferences (Houde, 1994; Ritchie *et al.*, 2005; Delcourt *et al.*, 2010), and examining the causes and consequences of such behavioural variation will improve our understanding of female choice and the resulting sexual selection it generates.

Variation in preference can arise in response to both intrinsic and extrinsic factors. For example, mate preferences are thought to be costly (Pomiankowski, 1987; Reynolds & Gross, 1990; Byers *et al.*, 2005; Rundle *et al.*, 2009), and if their costs and benefits depend on an individual's quality, their expression may evolve to be condition dependent (Cotton *et al.*, 2006). Various factors could cause individual condition itself to vary within and among individuals. In addition, extrinsic factors such as predation risk (Hedrick & Dill, 1993; Endler & Houde, 1995), search effort (Milinski & Bakker, 1992), mate availability (Uetz & Norton, 2007) and the risk of contracting sexually transmitted diseases/parasites (Sheldon, 1993; Knell & Webberley, 2004) could affect the costs and/or benefits of preference expression. Intrinsic factors such as female age and mating status may also be important. In terms of female mating status, virgin females (or any females that are sperm depleted) may relax their preferences to increase the likelihood of mating. Conversely, females that have a surplus of stored sperm may become choosier, or select males that provide different genetic or material benefits (Gabor & Halliday, 1997; Kodric-Brown & Nicoletto, 2001; Lynch *et al.*, 2005; Uetz & Norton, 2007; Mautz & Sakaluk, 2008; Judge *et al.*, 2010; Bergman *et al.*, 2011). With respect to the effect of female age on mate choice, younger females may also be choosier than older ones if the consequence of their choice has a greater effect on their lifetime fitness, or if older females are more sensitive to the costs of expressing preference (Gray, 1999; Kodric-Brown & Nicoletto, 2001; Moore & Moore, 2001; Uetz & Norton, 2007; Mautz & Sakaluk, 2008; Bergman *et al.*, 2011).

In *Drosophila serrata*, a native Australian fruit fly, female mate preferences target a specific combination of nonvolatile long-chain hydrocarbons (CHCs) found on the epicuticle of males (Chenoweth & Blows, 2003, 2005; Rundle *et al.*, 2008; Delcourt *et al.*, 2010). These preferences and their evolutionary consequences have been extensively investigated via a series of quantitative genetic studies, evolution experiments and behavioural assays (Blows *et al.*, 2004; Hine *et al.*, 2004, 2011; Rundle *et al.*, 2005, 2009; Delcourt *et al.*, 2010, 2012; Rundle & Chenoweth, 2011). Although among-individual variation in multivariate mate preferences exists and

has been shown to have a genetic basis (Delcourt *et al.*, 2010), little attention has been directed towards understanding this within-population variation beyond a single test for condition-dependent preference expression (Delcourt *et al.*, 2010). In addition, past studies have generally employed virgin females of a similar, young age when estimating mate preferences. Whether the results of such assays accurately reflect population-level mate preferences, and hence sexual selection gradients, is an open question.

Here, we test whether female preferences for male CHCs are affected by female age and mating status (i.e. whether females are virgins or have previously mated). Although a number of previous studies have examined the effects of one or both of these factors on aspects of mate choice in other species (Gabor & Halliday, 1997; Gray, 1999; Lynch *et al.*, 2005; Uetz & Norton, 2007; Mautz & Sakaluk, 2008; Judge, 2010; Bergman *et al.*, 2011), the separate and combined effects of female age and mating status on multivariate preference functions have not been previously examined. To do this, we use a 2 × 2 factorial design in which sexual selection on male CHCs is estimated via mating trials using either young or old females (4 or 10 days post-eclosion) that are either virgin or have previously mated once.

In choice trials, female mate preferences and male–male competition may both contribute to variation in male mating success. In *D. serrata*, however, there is clear experimental evidence of female mate choice and little indication of male–male competition during trials of this design. With respect to female choice, a previous quantitative genetic study detected significance variance at the sire level from trials in which single daughters were placed with five random stock males (Delcourt *et al.*, 2010). Such genetic variance in male mating success could only result from female mate preferences given the design of the study. With respect to male–male competition, unlike in *D. melanogaster*, *D. serrata* males rarely make any physical contact with one another, or otherwise interact in any detectable way, during trials (S. Gershman and H.D. Rundle, pers. obs.). *D. serrata* females also have the capacity to dislodge males who attempt to force copulation, allowing females an additional means of exerting choice. Finally, whereas territorial behaviour has been shown in other *Drosophila* species in which males will aggressively defend a limited food resource consisting of a single spot of live yeast (Hoffmann, 1987), presumably to gain access to females coming to feed, the design of our trials minimizes the opportunity for this by introducing a novel group of four males (i.e. that have not previously interacted) into a vial containing abundant live yeast immediately prior to starting a mating trial. Mating generally occurs quickly under these conditions, often within minutes (see Methods for details). Nevertheless, we cannot rule out some contribution of male–male competition during our trials, and we therefore interpret our results accordingly.

Materials and methods

Mate choice assays

Sexual selection on male CHCs was assayed via replicate mate choice trials using flies from a previously described laboratory-adapted stock population of *D. serrata* (Rundle *et al.*, 2006; Chenoweth *et al.*, 2008). In each trial, a single female was placed in a vial together with four haphazardly selected virgin males. Four males were presented to each female because, given that male CHC attractiveness cannot be determined prior to the mate choice assays (CHC extraction is fatal to the fly), additional males provide females with a greater range of phenotypes to choose among, better permitting them to express any preference. Vials were continuously observed until the female began mating with one of the four males, at which point the chosen male (mating success scored as '1'), along with one randomly selected rejected male (mating success scored as '0'), was removed for CHC extraction using CO₂ anaesthesia. The remaining flies were discarded.

Females used in the mating trials were from one of four treatment combinations in a two-way factorial design that independently manipulated their age (4 vs. 10 days post-emergence) and mating status (virgin vs. previously mated). *D. serrata* females are sexually mature by 48 h after emergence, and previous studies have varied in the post-emergence age of females used in mating trials, ranging from 3–8 days (see Hine *et al.*, 2002; Chenoweth & Blows, 2003; Blows *et al.*, 2004; Rundle *et al.*, 2005, 2009; Delcourt & Rundle, 2010; Sztepanacz & Rundle, 2012). Female age was manipulated by collecting the 700 virgin females at emergence either four or ten days prior to the mating trials. Females were collected using light CO₂ anaesthesia and were housed in groups of 8 in standard 95 mm × 25 mm vials containing 10 mL food medium with abundant live yeast added on top. Mating status was manipulated by either holding females as virgins (300 females) until the mating trials or by allowing each female to mate with a single, 4-day-old virgin male (400 females) one day prior to the mating trials. The day before mating trials, all females were transferred into individual vials, and the subsequent mating trials were conducted within these same vials. We collected male CHCs from approximately 260 choice trials per treatment. The few females that failed to mate within 2 h (< 5%) were discarded.

Males used in the mating trials were collected as virgins at emergence using light CO₂ anaesthesia and then housed in groups of five. Males were 4 days post-eclosion at the time of the trials. Rival males in each mating trial were aspirated (without CO₂) from different holding vials, thus were unfamiliar to each other. For each trial, the four competing males were rapidly drawn into the aspirator and simultaneously introduced

into the vial containing an individual female. During the mating trials, male–female pairs that copulated for more than approximately 2 min were considered to have mated successfully (Hoikkala *et al.*, 2000). All flies were housed at 28 °C, with a 12:12 light cycle. All choice trials were conducted from 10 a.m. to 12 noon (during the light phase of the daily cycle) at 28 °C. Trials were performed in two blocks separated by 28 days. Each block included equal numbers of trials from all four treatment combinations.

CHC extraction and analysis

Cuticular hydrocarbons were extracted from single flies and analysed using a dual-channel Agilent Technologies (Wilmington, DE, USA) 6890N fast gas chromatograph as described in Sztepanacz & Rundle (2012). Individual CHC profiles were determined by integration of the area under nine peaks, corresponding to those used in past studies of this species, and identified in order of their retention times as: (Z,Z)-5,9-C_{24:2}; (Z,Z)-5,9-C_{25:2}; (Z)-9-C_{25:1}; (Z)-9-C_{26:1}; 2-Me-C₂₆; (Z,Z)-5,9-C_{27:2}; 2-Me-C₂₈; (Z,Z)-5,9-C_{29:2}; and 2-Me-C₃₀ (Howard *et al.*, 2003). After integration, relative abundances were calculated separately for each individual by dividing the area integrated for each of their CHCs by the total area for all nine CHCs. Expressing abundances as proportions corrects for technical error associated with quantifying absolute abundances. To break the unit-sum constraint inherent in such compositional data, proportions were transformed into eight logcontrast values (Blows & Allan, 1998) following Atchison (1986), using Z,Z-5,9-C_{24:2} as the common divisor. We used the Mahalanobis distance technique, described in Sall *et al.* (2005) and implemented in the multivariate package JMP v. 9.02 (SAS Institute, Cary, NC, USA), to identify and remove several multivariate outliers. 2085 remaining males were included in the analyses below.

Statistical analyses

Female choice implies some dependence among observations on different males, and in multiple choice mating trials in particular, the number of females (i.e. trials) represents the number of independent observations. Our statistical analyses nevertheless treated the two males from a single trial as independent because a previous randomization procedure has shown that such a large number of trials are sufficient to capture all the variation among females, thereby preventing any detectable increase in type I error (Rundle *et al.*, 2005). For additional details, Rundle *et al.* (2005) provide a detailed discussion of the issue of pseudoreplication in such mating trials.

Standardized linear sexual selection gradients on the eight logcontrast CHCs were estimated using first-order multiple regression (Lande & Arnold, 1983):

$$w = \beta_0 + B + A + M + AM + \sum_{i=1}^8 \beta_i Z_i + \varepsilon, \quad (1)$$

in which w is the relative mating success score of individual males, Z_i is the eight logcontrast CHC values for a given male (standardized to mean = 0, standard deviation = 1), and ε is unexplained error. Fixed effects include the intercept (β_0), experimental block (B), age (A) indicating young vs. old females, mating status (M) indicating virgin vs. previously mated females, and the interaction of age and mating status (AM). This regression yielded a column vector of linear selection gradients (β) characterizing directional sexual selection overall on each of the eight logcontrast CHCs. If variation in mating success arose solely from female mate choice, such gradients would estimate the average linear preference function for these male traits across all experimental females (Wagner, 1998; Chenoweth & Blows, 2006). Although the selection gradients were estimated using standard least squares, significance was determined using logistic multiple regression because mating success is binomially distributed (Fairbairn & Preziosi, 1996; Rundle *et al.*, 2009; Rundle & Chenoweth, 2011). This was carried out using a generalized linear model with a logistic link function, fit via maximum likelihood, as implemented in JMP v. 9.02 (SAS Institute). Significance of sexual selection overall was tested using a likelihood ratio test comparing the above model to one lacking the eight $\beta_i z_i$ terms.

Nonlinear sexual selection was tested using a second-order polynomial regression that added to eqn (1) the 36 pairwise interactions between the eight logcontrast CHCs (i.e. $\gamma_{ij} z_i z_j$) that comprise the full γ matrix (Lande & Arnold, 1983; Brodie *et al.*, 1995). A generalized model was again fit via maximum likelihood, and a likelihood ratio test (LRT) was used to compare this model to one lacking the 36 interaction terms, thereby providing a test of the overall significance of nonlinear selection.

Variation in sexual selection was compared among treatments using a sequential model building approach for response surface design (Draper & John, 1988; Rundle & Chenoweth, 2011; Chenoweth *et al.*, 2012). The approach involves the addition of interaction terms between the eight logcontrast CHCs and the fixed treatment effects (i.e. age and mating status), thereby allowing directional sexual selection on these traits to vary between these fixed-effect levels. A series of nested LRTs were then used to compare the fit of a particular model to one lacking some of these interaction terms. We chose to focus on variation in directional (i.e. linear) sexual selection because it appears to be the predominate form of sexual selection in both the current experiment (see Results) and in a previous analysis of a set of independent mating trials (Delcourt *et al.*, 2010). In addition, even with our substantial sample sizes, testing for variation in nonlinear selection would result

in an over-parameterized model as it would require not only the inclusion of the 36 second-order trait interactions representing quadratic and correlational selection, but also the separate interaction of each of these with age, mating status and age \times mating status. Parameter reduction cannot be achieved in this context via a canonical rotation of the γ matrix (Blows & Brooks, 2003) because the resulting trait combinations would be different in every treatment combination, preventing comparisons among them.

The full model testing for variation in sexual selection, including the three-way interaction between age (A), mating status (M) and the logcontrast male traits (Z_i), is given by:

$$w = \beta_0 + B + A + M + AM + \sum_{i=1}^8 \beta_i Z_i + \sum_{i=1}^8 \alpha_i Z_i A + \sum_{i=1}^8 \mu_i Z_i M + \sum_{i=1}^8 \alpha_i \mu_i Z_i A M + \varepsilon, \quad (2)$$

with terms as defined in eqn (1). Variation in sexual selection arising from the interaction of the fixed effects of female age and mating status ($\alpha_i \mu_i Z_i A M$) was tested by comparing the fit of the above model to one lacking this three-way interaction. The three-way interaction was nonsignificant (see Results) and was therefore excluded from further models. The main effects of age and mating status were then tested by comparing the fit of models that included and excluded the respective interaction of each with the eight male traits. As expected given the lack of any interaction, results differed little depending on the inclusion or exclusion in both models of the trait interactions with the other main effect (i.e. including or excluding the trait \times age interactions in both models when testing the mating status \times trait interactions), and results excluding the other interactions are therefore presented.

Variation in sexual selection was also tested with respect to a univariate trait of particular biological interest: the combination of CHCs most strongly associated with male mating success overall (termed CHC β). This trait was calculated by applying to each male's logcontrast CHC phenotype the vector of linear sexual selection gradients (i.e., β) as calculated from the global analyses of all treatment combined. Variation among treatments in sexual selection on CHC β was tested using the same sequential model building approach as above.

Results and discussion

Linear sexual selection on male logcontrast CHCs was significant overall (LRT: $\chi^2 = 117.9$, d.f. = 8, $P < 0.0001$; Table 1), explaining 5.1% of the variance in male mating success (r^2_{adj}). Nonlinear selection was also significant overall (LRT: $\chi^2 = 71.7$, d.f. = 36,

Table 1 Vectors of standardized directional sexual selection gradients (β) and adjusted r^2 for eight logcontrast CHCs in males. Gradients were estimated from a standard first-order multiple regression using data from all females (global) and from each of the four combinations of the factorial treatments of female age (young vs. old) and mating status (virgin vs. previously mated).

Logcontrast CHC	β (global)	β (young, virgin)	β (young, mated)	β (old, virgin)	β (old, mated)
(Z,Z)-5,9-C _{25:2}	-0.036	-0.036	-0.102	0.028	-0.032
(Z)-9-C _{25:1}	-0.031	-0.074	-0.014	-0.111	0.040
(Z)-9-C _{26:1}	0.016	0.047	0.162*	-0.022	-0.097
2-Me-C ₂₆	-0.146**	-0.136	-0.235*	-0.089	-0.116
(Z,Z)-5,9-C _{27:2}	-0.003	0.013	-0.123	-0.019	0.080
2-Me-C ₂₈	0.502**	0.486*	0.633**	0.415*	0.450*
(Z,Z)-5,9-C _{29:2}	0.068	0.021	0.164*	0.106	-0.019
2-Me-C ₃₀	-0.557**	-0.467**	-0.696**	-0.536**	-0.495**
r^2 adjusted	0.050	0.018	0.078	0.054	0.048

* $P < 0.05$, ** $P < 0.001$.

CHCs, cuticular hydrocarbons.

$P = 0.0004$). Its inclusion, however, explained only an additional 1.1% of the variance in male mating success, indicating that sexual selection on these traits is predominantly directional, consistent with previous studies of this species (Chenoweth & Blows, 2005; Delcourt *et al.*, 2010; Szepeanacz & Rundle, 2012).

There was little evidence of any difference in sexual selection on male logcontrast CHCs arising from an interaction between female age and mating status. Although the individual trait \times age \times mating status interaction approached significance for one of the eight logcontrast CHCs ((Z,Z)-5,9-C_{29:2}; Table 2), this was insufficient to generate significant variation overall (LRT: $\chi^2 = 8.03$, d.f. = 8, $P = 0.430$). Evidence of an age \times mating status interaction was also lacking in the univariate analysis of CHC β , the linear combination of logcontrast CHCs most strongly associated with male mating success (LRT: $\chi^2 = 2.00$, d.f. = 1, $P = 0.157$).

There was likewise little evidence that sexual selection varied in response to the main effects of female age or mating status. Although the effect on mating success of one logcontrast CHCs differed significantly with age ((Z)-9-C_{26:1}; Table 2), neither of the overall

tests for variation with age (LRT: $\chi^2 = 11.23$, d.f. = 8, $P = 0.188$) or mating status (LRT: $\chi^2 = 2.54$, d.f. = 8, $P = 0.960$) was significant. Likewise, there was no evidence for variation in sexual selection for the univariate trait CHC β with either age (LRT: $\chi^2 = 0.05$, d.f. = 1, $P = 0.832$) or mating status (LRT: $\chi^2 = 0.76$, d.f. = 1, $P = 0.382$). Consequently, the linear sexual selection vectors on male logcontrast CHCs had very similar orientation in multivariate phenotypic space among the four treatment combinations (Table S1).

In *D. serrata*, female mate preferences are known to contribute to variation in male mating success during mating trials (Delcourt *et al.*, 2010), and the lack of any significant treatment effects therefore implies that average female mate preferences in this species do not vary in response to these treatments. To the extent that any male–male interactions also contribute, our results further imply that their effect on mating success also does not vary with the age and mating status of the female involved. This apparent constancy of female mate preferences in *D. serrata* contrasts with results from other species in which effects of age and mating status have been found (Gabor & Halliday, 1997; Gray, 1999; Lynch *et al.*, 2005; Uetz & Norton, 2007; Mautz & Sakaluk, 2008; Judge, 2010; Bergman *et al.*, 2011). However, many of these studies focus on other aspects of mate choice (e.g. choosiness, latency to mate) and therefore do not address preferences themselves. No previous study has examined the effect of female age or mating experience using a multivariate measure of female preference, despite the fact that females often assess multiple traits when choosing mates (Candolin, 2003; Chenoweth & Blows, 2006).

The apparent constancy in female mate preferences appears to extend across multiple studies in *D. serrata*, with a correlation between the current vector of directional sexual selection (β_{global} ; Table 1) and two past estimates (both using young, virgin females) ranging from 84.9% (Hine *et al.*, 2011) to 91.9% (Blows *et al.*, 2004). This is remarkable given almost a decade between these studies, conducted in different laboratories, and using different stock populations. In addition,

Table 2 Logistic regression coefficients quantifying the effect of female age (young vs. old), mating status (virgin vs. previously mated) and their interaction, on sexual selection on male logcontrast CHCs. Significance values are given in parentheses.

Logcontrast CHC	Age \times mating status	Age	Mating status
(Z,Z)-5,9-C _{25:2}	-0.042 (0.863)	-3.071 (0.256)	0.782 (0.771)
(Z)-9-C _{25:1}	-0.157 (0.434)	-0.204 (0.784)	-0.532 (0.477)
(Z)-9-C _{26:1}	0.444 (0.095)	3.712 (0.005)*	-0.153 (0.907)
2-Me-C ₂₆	-0.249 (0.455)	-1.488 (0.308)	1.229 (0.399)
(Z,Z)-5,9-C _{27:2}	-0.525 (0.095)	-1.167 (0.195)	0.021 (0.981)
2-Me-C ₂₈	0.465 (0.459)	1.544 (0.603)	-1.546 (0.604)
(Z,Z)-5,9-C _{29:2}	0.594 (0.052)	0.643 (0.449)	0.024 (0.978)
2-Me-C ₃₀	-0.694 (0.142)	0.007 (0.999)	1.220 (0.490)

* $P \leq 0.05$.

CHCs, cuticular hydrocarbons.

little effect on female mate preferences themselves was found within the context of a recent diet manipulation (Delcourt *et al.*, 2010), suggesting that female preference expression is also not condition dependent in *D. serrata*.

Statistical power is of concern whenever a treatment effect is nonsignificant. However, a lack of power is an unlikely explanation for the failure to detect significant variation in the current case. Sample sizes were sufficient to detect significant sexual selection overall within each treatment separately. Replication within treatments level was also approximately twice that in a past study that found significant differences in sexual selection both between the sexes and among geographical populations of *D. serrata* (Rundle *et al.*, 2008; Rundle & Chenoweth, 2011). Moreover, even in a univariate analysis of $\text{CHC}\beta$, treatment effects remained nonsignificant.

Although we detected no treatment effects with respect to age or mating status, it is of course possible that female mate preferences, and hence sexual selection, could change in even older females or in females that have mated several times. We used virgin 4-day-old females as this is an age and mating status commonly used in previous studies of this species (females take approximately 48 h to become sexually mature after emergence). Adults in the stock population of *D. serrata* that we used have been evolving for a few hundred (nonoverlapping) generations in an environment in which adults are unlikely to live longer than 3–4 days. Ten-day-old females are therefore quite old relative to the stock's recent evolutionary history. Female *D. serrata* do appear to remate at a fairly high rate, generating extensive polyandry in both laboratory and field populations (Frentiu & Chenoweth, 2008). We contrasted virgins with once-mated females to focus on the initial effect of reproductive assurance (i.e. having sperm or not). Whether preferences vary with subsequent matings therefore remains an outstanding question. Females may also target other traits, in addition to CHCs, when choosing mates, and it is possible that preference for such traits could vary with respect to female age and mating status.

Aside from an insufficient manipulation of the treatment factors, there are two potential explanations for the absence of an effect of female age and mating status on mate preferences in this species. First, such plasticity in preferences may provide little or no fitness advantage to *D. serrata* females such that selection does not favour it. Whereas there is no evidence of any direct benefit to females from their choice of mates in this species, there is some evidence suggesting possible indirect benefits (Hine *et al.*, 2002; but see Rundle *et al.*, 2006; Delcourt *et al.*, 2009). Such good genes benefits, if present, may select for specific and constant female mate preferences that target the combination of male CHCs that best indicate male condition (Delcourt & Rundle, 2011). Alternatively, female mate preferences

may be unaffected by age and mating status because, despite such plasticity being beneficial, preferences are constrained in some way, preventing changes in their expression within individuals. That females (and males) of other species of *Drosophila* can learn from past courtship experience and alter subsequent mate choice decisions (Dukas, 2005; Dukas *et al.*, 2012) suggests that preferences can be flexible within individuals, although this form of plasticity has not been tested in *D. serrata*.

Although the majority of studies that have examined the effect of female age on female preference found that virgins were choosier than mated females and young females were choosier than older females, our study suggests that the opposite may be occurring in *Drosophila serrata*. In our experiment, sexual selection was weakest in young, virgin females, with CHCs only explaining 1.8% of the variance in male mating success (compared to 4.8–7.8% in the other treatments). This result is consistent with the general phenomenon that mating causes female insects to become less sexually receptive to males (Leopold *et al.*, 1971; Koudele *et al.*, 1987; Mair & Blackwell, 1998; Ortigosa & Rowe, 2003; Fleischman & Sakaluk, 2004; Judge *et al.*, 2010), suggesting that virgin females become choosier after mating. This result is also consistent with the observation that, on average, virgin *D. serrata* females mate more rapidly than mated females (S. Gershman, pers. obs). Whereas our difference in sexual selection between young virgin females and the other three treatments was insufficient to produce a significant treatment effect, a rigorous statistical test for variation in preference strength alone would require replicate populations within each treatment (Rundle *et al.*, 2009).

In conclusion, *D. serrata* has been a model system for the study of mate preferences, including the evolutionary genetics of the sexual selection they generate. The apparent constancy of female mate preferences, as revealed by highly concordant sexual selection estimates, suggests that evolutionary inferences involving sexual selection in this species, such as the depletion of standing genetic variance in the preferred combination of male CHCs (Blows *et al.*, 2004; Hine *et al.*, 2004) and the genetic basis of the limits to CHC exaggeration (Hine *et al.*, 2011; Delcourt *et al.*, 2012; Sztepanacz & Rundle, 2012), are robust to variation in mate preferences, at least with respect to our manipulation of female age and mating status.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Similarity in female mate preferences for the suite of eight logcontrast CHCs in males as characterized by the vector correlation of the linear sexual selection gradients (Table 1) between pairs of treatments (lower left), along with the corresponding angle (in degrees) between these vectors in multivariate phenotypic space (upper right).

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