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Crowd control: sex ratio affects sexually selected cuticular hydrocarbons in male *Drosophila serrata*

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Abstract

Although it is advantageous for males to express costly sexually selected signals when females are present, they may also benefit from suppressing these signals to avoid costly interactions with rival males. Cuticular chemical profiles frequently function as insect sexual signals; however, few studies have asked whether males alter these signals in response to their social environment. In *Drosophila serrata*, an Australian fly, there is sexual selection for a multivariate combination of male cuticular hydrocarbons (CHCs). Here, we show that the ratio of females to males that an adult male experiences has a strong effect on his CHC expression, with female-biased adult sex ratios eliciting greater expression of CHC profiles associated with higher male mating success. Classical models predict that male reproductive investment should be highest when there is a small but nonzero number of rivals, but we found that males expressed the most attractive combination of CHCs when there were no rivals. We found that male CHCs were highly sensitive to adult sex ratio, with males expressing higher values of CHC profiles associated with greater mating success as the ratio of females to males increased. Moreover, sex ratio has a stronger effect on male CHC expression than adult density. Finally, we explore whether sex ratio affects the variance among a group of males in their CHC expression, as might be expected if individuals respond differently to a given social environment, but find little effect. Our results reveal that subtle differences in social environment can induce plasticity in male chemical signal expression.

Introduction

Maintaining sexually selected traits can deplete male resources and may also elicit more harmful competitive interactions with rival males. One way for males to minimize these costs is to express sexually selected traits in response to social environment, reducing or eliminating their expression when costs are higher and/or potential benefits are lower. Behavioural plasticity in response to social environment, sometimes referred to as 'social plasticity' (Rodriguez *et al.*, 2013), is widespread (Bretman *et al.*, 2011). Sexual experience, density, sex ratio and the presence of competitors affect male visual, vibrational and acoustic signalling behaviour in different study systems (Bretman *et al.*, 2011). However, few studies examine the ability of males to alter chemical signals in a behaviourally plastic way.

Cuticular hydrocarbons (CHCs) are long-chain, mostly nonvolatile waxes produced by oenocyte cells and then deposited on the insect cuticle (Wigglesworth, 1970; Antony & Jallon, 1982). CHCs reduce water loss through the cuticle, increasing desiccation resistance (Gibbs *et al.*, 2003; Howard & Blomquist, 2005; Foley & Telonis-Scott, 2011). The CHC profile of an individual can be affected by extrinsic factors such as diet (Fedina *et al.*, 2012) and desiccation stress (Stinziano *et al.*, 2015), as well as intrinsic factors like age (Kuo *et al.*, 2012). Social environment has an effect on both male and female CHCs (Petfield *et al.*, 2005; Kent *et al.*, 2008; Farine *et al.*, 2012) such that both sexes exhibit social plasticity in CHC expression.

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In many insects, CHCs have been secondarily co-opted as a means of chemical communication. In *Drosophila serrata*, an Australian species of fruit fly, CHCs play a role in species recognition (Blows & Allan, 1998; Chenoweth & Blows, 2003, 2005), and within a given population, there is a specific multivariate combination of nine cuticular hydrocarbons associated with increased male mating success (Chenoweth & Blows, 2003, 2005). Sexually selected CHCs are costly to produce in *D. serrata* (Blows, 2002; Hine *et al.*, 2011), and their expression by males is condition-dependent (Delcourt & Rundle, 2011). Both male CHCs and female preferences for them have a genetic basis and respond to altered selection (Blows, 1998; Higgie *et al.*, 2000; Rundle *et al.*, 2005, 2009; Delcourt *et al.*, 2010; Hine *et al.*, 2011).

Gershman *et al.* (2014a) and Gershman & Rundle (2016) recently demonstrated that the presence and absence of both males and females affect male *D. serrata* CHC expression (Gershman *et al.*, 2014a; Gershman & Rundle, 2016). For the combination of CHCs targeted by sexual selection in a laboratory population (termed CHCβ after the sexual selection gradient β), males had higher values when there was greater opportunity to mate. In particular, a male housed with females had higher CHCβ values than a male housed without females, and a male housed alone with females had higher CHCβ values than a male housed with females and rival males (Gershman *et al.*, 2014a; Gershman & Rundle, 2016). These context-dependent changes in CHCs are likely to enhance mating success because they induce males to express higher values of the trait combination favoured by sexual selection, suggesting adaptive plasticity in sexual signal expression (Gershman *et al.*, 2014a; Gershman & Rundle, 2016).

Here, to characterize the effects of adult social environment on male CHC expression more finely, we move beyond simply the presence/absence of other males or females to manipulate adult sex ratio, allowing us to test whether social plasticity in CHCs is congruent with theoretical predictions of male competitive behaviour. Our study has three parts (Fig. 1). In Part 1, we quantify how expression of CHCβ by a male changes in response to the number of male rivals he encounters. Our predictions for how rival males affect male CHC expression are adapted from Parker & Ball's (2005) model of the effect of variable numbers of male rivals on sperm investment in ejaculate. Parker & Ball (2005) predict that male investment should peak in the presence of 1–2 competitors. As the numbers of rival males increase beyond this, the proportion of each males' sperm within the female's sperm storage organ decreases and the optimal male reproductive investment declines. Likewise, in the absence of any rival males, reproductive investment is predicted to be minimal, sufficient only to ensure successful fertilization. Optimal male reproductive investment is therefore predicted to follow a right-skewed distribution, with peak reproductive investment occurring in the presence of a small but nonzero number of rivals (Parker & Ball, 2005). Although this simple model uses sperm per ejaculate as a proxy for reproductive investment, it could be equally well applied to male–male competition via CHC expression. In this case, males have the capacity to rapidly up- or down-regulate their reproductive effort (investment in costly CHCs) in response to the presence of rival males. We first quantify how investment in a male precopulatory reproductive trait (CHCβ) scales with number of male rivals.

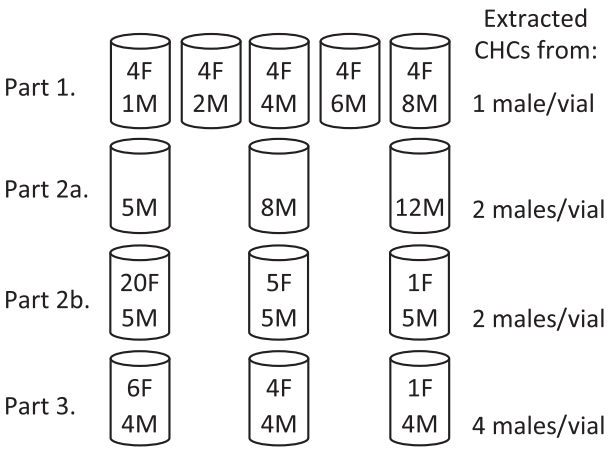

Fig. 1 Overview of sex ratio treatments used in parts 1–3. Each cylinder represents the number of females (F) and males (M) per vial, within each sex ratio treatment. CHCs were extracted from 1 to 4 males per vial.

Changing sex ratio must necessarily alter the number of individuals, either overall or of at least one of the sexes, making it challenging to separate the influence of sex ratio from that of density. In Part 2, we attempt to disentangle the effects of adult density and sex ratio on male CHC expression. There are multiple ways by which adult density could affect CHC expression, so we refrain from *a priori* predictions. For example, if male–male competition increases with absolute density and the harmful effects of this impair male condition, this could alter how males express costly CHCs. Competition for food (e.g. yeast) may also increase with density, and sexually antagonistic effects of adult diet have been shown for male condition (Chippindale *et al.*, 1993) and are known to alter male CHCβ (Rundle *et al.*, 2005; Gosden & Chenoweth, 2011).

Finally, in Part 3, we examine whether all males respond similarly to changes in sex ratio. If males compete with one another for mating opportunities, some males may consistently lose, whereas others may be repeat winners. Under such circumstances, losers may benefit from muting mate attraction cues to reduce aggression from other males. For example, after losing a fight, a male *Teleogryllus oceanicus* cricket will alter his

CHCs to avoid continued aggression from the rival male (Thomas & Simmons, 2011). The general phenomenon of 'sneaker' males altering sexually selected signals to avoid negative interactions with rival males is also common (Gross, 1996). To gain insight into variation among males in their response to sex ratio, we sample all males from each experimental replicate (vial) and then test for differences between sex ratio treatments in vial-mean, vial-maximum and the among-male within-vial variance in CHCβ values.

Materials and methods

For all experiments, we used laboratory-adapted, outbred stock populations of *D. serrata* that have been maintained at large population sizes (15–16 half-pint bottles) in nonoverlapping generations (see Rundle *et al.*, 2006; Chenoweth *et al.*, 2008 for a full description) for more than 10 years. Experimental animals and stocks were maintained at 25 °C and photoperiod (12L:12D), with the lights turning on at 07:00 and off at 19:00 daily.

Identifying sexually selected CHCs using binomial trials

To determine the effects of sex ratio on the expression of male CHCs, we focused our analyses on the combination of CHCs that are most strongly associated with increased mating success. Doing this reduces a high-dimensional data set to a single trait of biological interest and is an approach that has been frequently used in this species (e.g. McGuigan *et al.*, 2011; Sztepanacz & Rundle, 2012; Gershman *et al.*, 2014a,b; Hine *et al.*, 2014). To estimate the linear combination of CHCs most strongly associated with male mating success, we performed binomial choice mating trials and calculated the β vector of directional sexual selection gradients (i.e. partial regression coefficients) by standard polynomial regression of mating success against CHC values (Lande & Arnold, 1983). These trials are described in detail in Gershman *et al.* (2014b). In brief, in each trial, a virgin female was placed in a vial with two virgin males. The flies were continuously observed until a successful mating occurred, at which point the chosen and rejected males were anaesthetized and their CHCs were immediately extracted. Samples were analysed via gas chromatography, and individual CHC profiles were determined by integration of the area under nine peaks, as described below. CHC relative concentrations were calculated, transformed to log contrasts and standardized prior to estimating selection gradients.

CHCβ for individual males were calculated as CHC$\beta = \beta^{T}\mathbf{Z}$, where $\mathbf{Z}$ is the vector of the standardized log contrast-transformed CHCs that were measured for each male. The CHCβ score for an individual male represents his value for the linear combination of CHCs most strongly associated with male mating success. CHCβ has previously been interpreted as CHC-based attractiveness (Hine *et al.*, 2002, 2011; Gershman *et al.*, 2014a,b) under the assumption that female choice alone determines the outcome of the binomial trials used to estimate β. *Drosophila serrata* females can choose which males to associate with, and can also dislodge unwanted copulations (Hoikkala & Crossley, 2000; S.Gershman, personal observation). However, the role of male–male interactions in female *D. serrata* mate choice is unclear. *D. serrata* males do not display the extensive repertoire of physically aggressive behaviours that have been observed in *D. melanogaster* males (Chen *et al.*, 2002). *D. serrata* males will defend a food source and their success at doing so appears to affect their mating success (White & Rundle, 2014). However, CHCs do not appear to be associated with successful territory defence (White & Rundle, 2014), and the arenas for mating trials used in estimating β minimized the opportunity for this to occur (Gershman *et al.*, 2014b). Nevertheless, it is possible that subtle male–male interactions contribute to variation in male mating success and hence CHCβ values. Therefore, we refer to CHCβ as 'male sexually selected CHCs' and interpret this trait more broadly as the combination of CHCs associated with greater mating success.

CHC extraction and analysis

Samples were analysed by gas chromatography as described in Sztepanacz & Rundle (2012). CHC profiles of individual flies were determined by integrating the area under the nine peaks used in past studies of this species: (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-C_{26}; (Z,Z)-5,9-$C_{27:2}$; 2-Me-C_{28}; (Z,Z)-5,9-$C_{29:2}$; and 2-Me-C_{30} (Howard *et al.*, 2003). To correct for error associated with quantifying absolute abundances, 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. The resulting proportions are a form of compositional data (parts of a whole) for which standard statistical methods are not appropriate (Aitchison, 1986; Egozcue & Pawlowsky-Glahn, 2011). To address this, relative concentrations were transformed into eight log contrast values (Aitchison, 1986), using Z,Z-5,9-$C_{24:2}$ as the common divisor, and standardized (mean = 0, standard deviation = 1), following past studies on this species (e.g. Blows & Allan, 1998; Chenoweth & Blows, 2003, 2005; Gershman *et al.*, 2014a,b; Rundle *et al.*, 2008; Sztepanacz & Rundle, 2012). We used the Mahalanobis distance technique in the multivariate analysis procedure of JMP v. 9.02 (SAS Institute, Cary, NC; Sall *et al.*, 2005) to remove a small number of multivariate outliers, likely representing integration errors or contaminated samples (2% or fewer individuals were removed from each data set).

Part 1: The effect of sex ratio on male sexually selected CHCs

To characterize how male sexually selected CHCs (i.e. CHCβ) are affected by sex ratio, males were housed in one of five social environments. Virgin adults were collected within 24 h of eclosion using light CO_2 anaesthesia, separated by sex, and then housed in 95 mm × 25 mm vials with *ad libitum* food (standard medium with live yeast sprinkled on top) under one of five environments that varied only the number of males, yielding the following sex ratios (females:males): 4:1, 4:2, 4:4, 4:6 and 4:8. Four days after adult eclosion, CHCs were extracted from one male per vial, with the remaining individuals discarded. Extractions were performed from 09:00 to 12:00 and were blocked evenly across treatments. CHCs were collected from 28 to 30 males per social treatment. Three individuals were removed as outliers prior to analysis.

Part 2: The effect of adult density on male sexually selected CHCs

In Part 1, we altered sex ratio by changing the number of males, thereby confounding effects of sex ratio with adult male and total (i.e. male + female) adult density. In Part 2, we conducted two separate manipulative experiments to aid in the interpretation of these effects. The first directly tests for an effect of adult male density, whereas the second manipulates sex ratio by changing the number of females instead of the number of males, thereby reversing the confounding gradient in total density (i.e. in Part 1, the treatment with the highest female-to-male ratio had the highest total density, whereas here it had the lowest). Consistency of the latter results with those in Part 1 would provide strong evidence that sex ratio was the dominant factor affecting changes in male CHC expression.

For both experiments, virgin adults were collected within 24 h of eclosion using light CO_2 anaesthesia, separated by sex, and then housed in vials with *ad libitum* food under the appropriate social treatment environment. Four days after adult eclosion, CHCs were extracted from two males/vial, with the remaining individuals discarded. Using two males per vial reduced the total time per day that was required to extract CHCs, which is important: previous studies demonstrate substantial time of day effects on CHCβ, but no effect of vial identity (Rundle *et al.*, 2005; Gershman *et al.*, 2014a). All extractions were started at 09:00, the time of day that males express peak values of CHCβ (Gershman *et al.*, 2014a). CHCs were collected from 30 to 32 males per social treatment.

The first experiment involved only males and manipulated the numbers of adult males in a given vial. Although it is possible to alter density by varying the volume of the container instead, changing vial volume can alter humidity, which is known to affect CHCs. Therefore, to determine the effect of adult male density without altering the number of females or container volume, we held 5, 8 or 12 males per vial (using the same vials as in Part 1). The second experiment altered sex ratio by manipulating the number of females. To do this, at adult eclosion, males were housed in one of three social environments (females:males): 20:5, 5:5 and 1:5. Extractions were performed from 09:00 to 13:00. CHCs were collected from 30 males per social treatment. One sample was excluded from the analysis of the second experiment as an outlier (none were removed from the first).

Part 3: The effect of social environment on among-male variation in CHCs

In parts 1 and 2, we sampled only one or two males/vial, providing little insight into how different males may have responded to the same social environment. Here, we varied sex ratio and then extracted CHCs from all males in each replicate to explore variation among males in the effects of sex ratio.

Virgin adults were collected within 24 h of eclosion using light CO_2 anaesthesia, separated by sex, and then housed in 95 mm × 25 mm vials with *ad libitum* food (standard medium with live yeast sprinkled on top). As in Part 2, we manipulated the number of females while holding the number of males constant, yielding the following sex ratios (females:males): 6:4, 4:4, and 1:4. This was carried out to avoid effects of differing numbers of males. Four days after adult eclosion, CHCs were extracted from all males. Extractions were performed from 09:00 to 13:00. Forty-two replicate vials were performed for each social treatment. One male was excluded from the analysis due to GC error.

Variation in male CHCβs was analysed using a general linear mixed model that included the fixed effect of sex ratio (representing differences in mean CHCβ among social environments), the random effect of vial identity nested within treatment (representing among-vial variance) and the random effect residual variance representing the among-male variation within vials. The model was fit via restricted maximum likelihood using the mixed procedure in SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA). We used a likelihood ratio test to compare a model that included a single residual variance to one that allowed separate residual variances to be estimated for each level of the fixed effect treatment, using the 'group' option in the repeated statement (Littell *et al.*, 1996). This approach tests whether estimating separate within-vial residuals for each level of sex ratio significantly improves the fit of the model over a single residual. We allowed separate estimates of among-vial variance component for each level of sex ratio in both of these models, although results did not change qualitatively if a single among-vial variance was fit in both

models. Finally, we also tested the effect of sex ratio on the maximum CHCβ per vial (CHCβ_{max}) in a separate ANOVA.

Results

Part 1: The effect of sex ratio on male sexually selected CHCs

To determine the effect of sex ratio on male CHCs, males were housed in the following sex ratios (females:males): 4:1, 4:2, 4:4, 4:6 and 4:8. Sex ratio had an overall effect on male CHCβ (one-way ANOVA $F_{4,149} = 8.81$ $P < 0.0001$), with expression of this trait increasing as sex ratio became more female-biased (Fig. 2).

Part 2: The effect of adult density on male sexually selected CHCs

The effect of adult male density was tested by comparing CHCβ expression among groups of 5, 8 or 12 males (in the absence of females). Although there was some decrease in CHCβ expression with increasing male density (Fig. 3), the magnitude of the effect was small and it was not a statistically significant overall (one-way ANOVA $F_{2,93} = 2.58$, $P = 0.081$). Mean CHCβ also did not differ for any of the pairwise comparisons among treatment levels at $\alpha = 0.05$ (Tukey's HSD test of means).

When sex ratio was manipulated by altering the number of females instead of the number of males, thereby reversing the gradient in absolute density from that in Part 1, the effect of social treatments on male CHCβ was significant overall (ANOVA: $F_{2,176} = 38.3$, $P < 0.0001$) and the pattern of mean CHCβ values was consistent with the effect of sex ratio observed in Part 1: increasing CHCβ values as sex ratios become more female-biased (Fig. 4). Using Tukey's HSD test of means ($\alpha = 0.05$), males in the 1:5 (females:males) treatment had significantly lower values of CHCβ than males in the other two treatments, and the increased CHCβ value in males in the 20:5 treatment trended towards having higher CHCβs than males in the 5:5 treatment ($P = 0.057$).

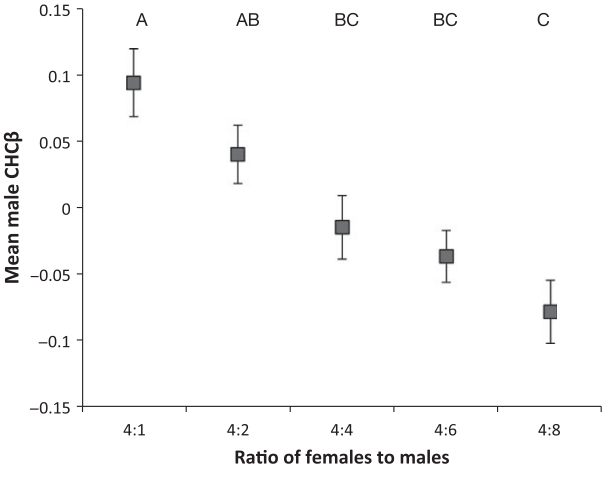

Fig. 2 Mean (± SE) CHCβs of males when held under one of four different ratios of females to males. Capital letters indicate statistically significant pairwise comparisons (Tukey's HSD $\alpha < 0.05$).

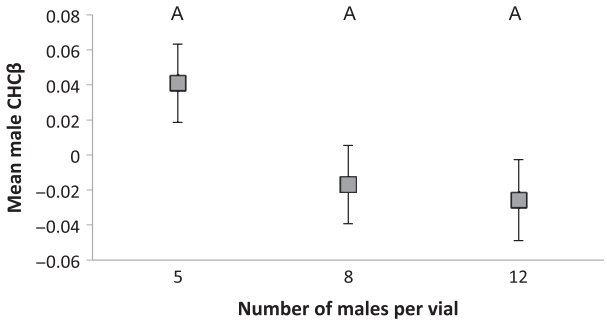

Fig. 3 Mean (± SE) CHCβs of males held at different adult densities. Capital letters indicate statistically significant pairwise comparisons (Tukey's HSD $\alpha < 0.05$).

Part 3: The effect of social environment on among-male variation in CHCs

To gain insight into the variation among males in their response to sex ratio, we sampled groups of four males that had experienced different social environments. Variation in CHCβ among males within a social group (vial) tended to increase as the sex ratio became more female-biased (residual variance for 6:4, 4:4 and 1:4 female:male sex ratios, respectively: 0.317, 0.324, 0.449), although a likelihood ratio test indicated that these differences were not statistically significant ($\chi^2_2 = 5.1$, $P = 0.078$; Fig. 5). As in previous experiments, mean CHCβ increased significantly as sex ratio became more female-biased ($F_{2,80.3} = 43.7$, $P < 0.0001$;

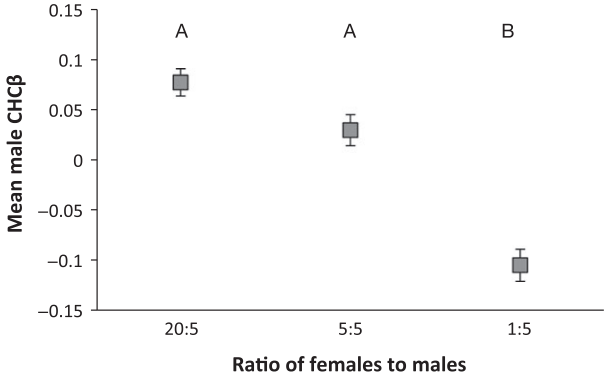

Fig. 4 Mean (± SE) CHCβs of males when held under one of four different ratios of females to males. Capital letters indicate statistically significant pairwise comparisons (Tukey's HSD $\alpha < 0.05$).

Figs 5 and 6). The maximum CHCβ value per vial (CHCβ_{max}) also increased significantly with increasing female bias in the sex ratio (ANOVA $F_{2,122} = 33.3$, $P < 0.0001$; Fig. 6).

Discussion

We demonstrate that *D. serrata* males are highly sensitive to their social environment and can respond to subtle differences in sex ratio by altering their sexually selected CHCs. In environments with a high ratio of females to males, males express a combination of sexually selected CHCs (CHCβ) associated with higher mating success; when there are more male competitors per female in an environment, male *D. serrata* express CHCs associated with lower CHCβ values. These results are consistent with Parker & Ball's (2005) prediction that when there are more than two rivals, a male's optimal strategy is to invest less in reproductive effort with each additional rival (Parker & Ball, 2005). Producing CHCs that improve mating success can be costly for males (Hine *et al.*, 2011), and the expression of male CHCs is condition-dependent (Delcourt & Rundle, 2011). Thus, male disinvestment in sexually selected CHCs when the possibility of mating is lower is consistent with an economic expression of a costly trait.

Parker & Ball (2005) predict that when a male has no rivals, he need only invest enough to fertilize all of a female's eggs to maximize his reproductive success (Parker & Ball, 2005). However, in our study, we found that a male housed alone with females had a higher CHCβ value than a male housed with both females and male rivals. Thus, male CHC expression is not entirely consistent with Parker and Ball's model. There are at least three possible explanations for why males have their highest CHCβ values when they lack rivals. First, although males were collected and placed in their respective social environment within 24 h of eclosion, they were raised in mixed-sex vials and spent up to 24 h in mixed-sex groups as adults prior to collection. It is possible that males perceive the presence of future rivals during the larval stage (Pontier & Schweisgurth, 2015) and/or after emergence but prior to sexual maturity. In the later case, although adult CHC profiles are not expressed until the cuticle has sclerotized (which takes more than 24 h in *D. serrata*), visual and/or other chemosensory cues could be used to detect the presence of other males.

A second possibility is that males may be unable to down-regulate CHC expression in the absence of rivals. For example, mating itself results in an increase in CHCβ expression in *D. serrata* (Gershman & Rundle,

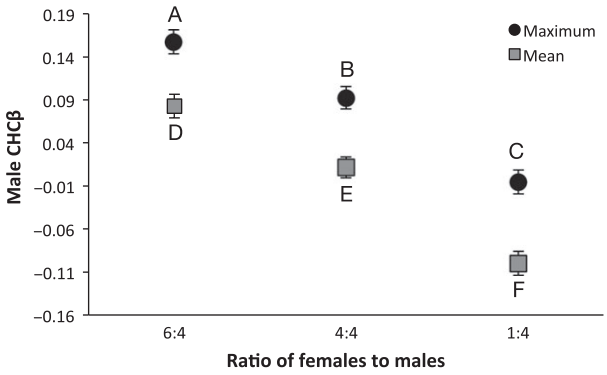

Fig. 6 Mean (± SE) and maximum CHCβ per vial for males held at varying female-to-male sex ratios. Capital letters indicate statistically significant pairwise comparisons (Tukey's HSD $\alpha < 0.05$; A-C for maximum CHCβ; D-F for mean CHCβ).

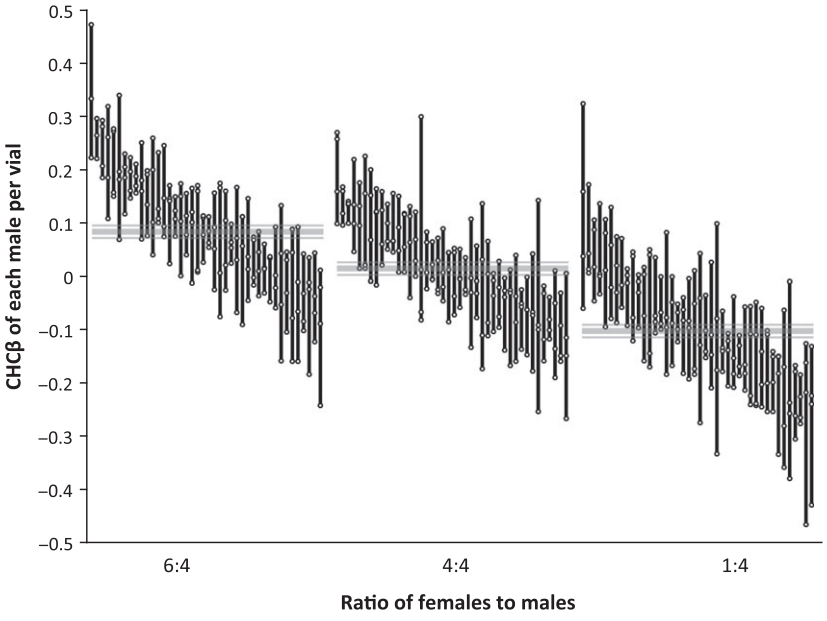

Fig. 5 CHCβs of each of four males per vial when held at varying female:male sex ratios. Each point represents a male CHCβ, and each vertical line connects the data points of males in the same vial. Horizontal grey lines represent mean (± SE) CHCβs per sex ratio treatment.

2016) and if this occurs as a physiological consequence of mating, the increased mating opportunities of males housed without rivals may necessarily result in them having higher CHCβ values. Linking CHCβ expression to past mating history could be adaptive if past mating success is a good predictor of optimal future reproductive investment. Finally, a third possibility is that females have strong preferences for CHCβ such that males must express a high value to successfully mate even in the absence of rivals. On its own, this does not explain why this minimum threshold declines (i.e. females become less 'choosy') as the number of rival males increases, although it is possible that increased harassment of females with more male-biased sex ratios males harms them, thereby lowering their resistance and allowing males to decrease investment in this costly trait. Parsing these three alternative hypotheses would require additional experimentation.

In parts 2 and 3, we examined factors that could mediate how sex ratio affects CHCs. In Part 2, we manipulated adult density and found no significant effect, although there was a trend of decreasing CHCβ expression with increasing number of males. Thus, our results demonstrate that if density has any effect on male CHCs, it is minor as compared to the effect of sex ratio. In Part 3, we asked whether interactions between males within a social group contribute to variance in male CHCβ expression. We found that the variance in CHCβ among males within a vial increased as the sex ratio became more female-biased, although the differences were not statistically significant. This suggests that males within a social group tend to respond similarly to differences in sex ratio. This finding is contrary to Thomas & Simmons (2011) in which losing a fight had a different effect on dominant and subordinate male CHC profiles. The effects of sex ratio on mean and maximum CHCβ values are also consistent with previous results (Fig. 6). Taken together, our results suggest that male–male interactions have little effect on CHCβ expression.

In previous studies, Gershman *et al.* (2014a) and Gershman & Rundle (2016) have shown that males increase their CHCβ values in the presence of females and decrease CHCβ in the presence of rival males (Gershman *et al.*, 2014a; Gershman & Rundle, 2016). Here, we demonstrate that even subtle differences in social environment can influence the expression of a sexually selected male chemical signal. In particular, male *D. serrata* are able to detect and respond to differences in sex ratio to become more attractive to females when males have a better chance of mating, and less attractive when the risk of competition is higher. Further, these results are repeatable and cannot be attributed to either within-vial interactions between males or adult density. Within the natural microhabitats where sexual selection occurs, there is likely variation in both the number of potential mates and the number of potential rivals. We have demonstrated that males may use this information to fine-tune their investment in a sexually selected signal. Researchers who use *Drosophila* should consider the effect of social environment on male cuticular hydrocarbons as they design experiments. Moreover, although this study and others have found variation among males in sexually selected CHCs, genetic variation in this social plasticity remains to be addressed. Finally, previous studies by Petfield *et al.* (2005) and Chenoweth *et al.* (2010) demonstrate that the genotype of individuals in a male's environment can affect his CHCs. How these indirect genetic effects contribute to the effect of sex ratio on male CHCs is an important topic for future work.

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References

Aitchison, J. 1986. *The Statistical Analysis of Compositional Data*. Chapman and Hall, London.

Antony, C. & Jallon, J.M. 1982. The chemical basis for sex recognition in *Drosophila melanogaster*. *J. Insect Physiol.* **28**: 873–880.

Blows, M.W. 1998. Evolution of a mate recognition system after hybridization between two *Drosophila* species. *Am. Nat.* **151**: 538–544.

Blows, M.W. 2002. Interaction between natural and sexual selection during the evolution of mate recognition. *Proc. Biol. Sci.* **269**: 1113–1118.

Blows, M.W. & Allan, R.A. 1998. Levels of mate recognition within and between two *Drosophila* species and their hybrids. *Am. Nat.* **152**: 826–837.

Bretman, A., Gage, M.J.G. & Chapman, T. 2011. Quick-change artists: male plastic behavioural responses to rivals. *TREE* **26**: 467–473.

Chen, S., Lee, A.Y., Bowens, N.M., Huber, R. & Kravitz, E.A. 2002. Fighting fruit flies: a model system for the study of aggression. *Proc. Natl. Acad. Sci. USA* **99**: 5664–5668.

Chenoweth, S.F. & Blows, M.W. 2003. Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. *Evolution* **57**: 2326–2334.

Chenoweth, S.F. & Blows, M.W. 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *Am. Nat.* **165**: 281–289.

Chenoweth, S.F., Rundle, H.D. & Blows, M.W. 2008. Genetic constraints and the evolution of display trait sexual dimorphism by natural and sexual selection. *Am. Nat.* **171**: 22–34.

Chenoweth, S.F., Rundle, H.D. & Blows, M.W. 2010. Experimental evidence for the evolution of indirect genetic effects: changes in the interaction effect coefficient psi (Ψ), due to sexual selection. *Evolution* **64**: 1849–1856.

Chippindale, A.K., Leroi, A.M., Kim, S.B. & Rose, M.R. 1993. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. *J. Evol. Biol.* **6**: 171–193.

Delcourt, M. & Rundle, H.D. 2011. Condition dependence of a multicomponent sexual display trait in *Drosophila serrata*. *Am. Nat.* **177**: 812–823.

Delcourt, M., Blows, M.W. & Rundle, H.D. 2010. Quantitative genetics of female mate preferences in an ancestral and a novel environment. *Evolution* **64**: 2758–2766.

Egozcue, J.J. & Pawlowsky-Glahn, V. 2011. Basic concepts and procedures. In: *Compositional Data Analysis: Theory and Applications* (V. Pawlowsky-Glahn & A. Buccianti A, eds), pp. 12–28. John Wiley & Sons, Chichester, UK.

Farine, J.-P., Ferveur, J.-F. & Everaerts, C. 2012. Volatile *Drosophila* cuticular pheromones are affected by social but not sexual experience. *PLoS ONE* **7**: e40396.

Fedina, T.Y., Kuo, T.-H., Dreisewerd, K., Dierick, H.A., Yew, J.Y. & Pletcher, S.D. 2012. Dietary effects on cuticular hydrocarbons and sexual attractiveness in *Drosophila*. *PLoS ONE* **12**: e49799.

Foley, B.R. & Telonis-Scott, M. 2011. Quantitative genetic analysis suggests causal association between cuticular hydrocarbon composition and desiccation survival in *Drosophila melanogaster*. *Heredity* **106**: 68–77.

Gershman, S.N. & Rundle, H.D. 2016. Level up: the expression of male sexually selected cuticular hydrocarbons is mediated by sexual experience. *Anim. Behav.* **112**: 169–177.

Gershman, S.N., Toumishey, E. & Rundle, H.D. 2014a. Time flies: time of day and social environment affect cuticular hydrocarbon sexual displays in *Drosophila serrata*. *Proc. Roy. Soc. B* **281**: 1792–1799.

Gershman, S.N., Delcourt, M. & Rundle, H.D. 2014b. Sexual selection on *Drosophila serrata* male pheromones does not vary with female age or mating status. *J. Evol. Biol.* **27**: 1279–1286.

Gibbs, A.G., Fukuzato, F. & Matzkin, L.M. 2003. Evolution of water conservation mechanisms in *Drosophila*. *J. Exper. Biol.* **206**: 1183–1192.

Gosden, T.P. & Chenoweth, S.F. 2011. On the evolution of heightened condition dependence of male sexual signals. *J. Evol. Biol.* **24**: 685–692.

Gross, M.R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *TREE* **11**: 92–98.

Higgie, M., Chenoweth, S. & Blows, M.W. 2000. Natural selection and the reinforcement of mate recognition. *Science* **290**: 519–521.

Hine, E., Lachish, S., Higgie, M. & Blows, M.W. 2002. Positive genetic correlation between female preference and offspring fitness. *Proc. R. Soc. B* **269**: 2215–2219.

Hine, E., McGuigan, K. & Blows, M.W. 2011. Natural selection stops the evolution of male attractiveness. *Proc. Natl. Acad. Sci. USA* **108**: 3659–3664.

Hine, E., McGuigan, K. & Blows, M.W. 2014. Evolutionary constraints in high-dimensional trait sets. *Am. Nat.* **184**: 119–131.

Hoikkala, A. & Crossley, S. 2000. Copulatory courtship in *Drosophila*: behavior and songs of *D. birchii* and *D. serrata*. *J. Insect Behav.* **13**: 71–86.

Howard, R.W. & Blomquist, G.J. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Ann. Rev. Ent.* **50**: 371–393.

Howard, R.W., Jackson, L.L., Banse, H. & Blows, M.W. 2003. Cuticular hydrocarbons of *Drosophila birchii* and *D. serrata*: identification and role in mate choice in *D. serrata*. *J. Chem. Ecol.* **29**: 961–976.

Kent, C., Azanchi, R., Smith, B., Formosa, A. & Levine, J.D. 2008. Social context influences chemical communication in *D. melanogaster* males. *Curr. Biol.* **18**: 1384–1389.

Kuo, T.-H., Yew, J.Y., Fedina, T.Y., Dreisewerd, K., Dierick, H.A. & Pletcher, S.D. 2012. Aging modulates cuticular hydrocarbons and sexual attractiveness in *Drosophila melanogaster*. *J. Exper. Biol.* **215**: 814–821.

Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D. & Schabenberger, O. 1996. *SAS for Mixed Models*. SAS Institute Inc, Cary, NC.

McGuigan, K., Petfield, D. & Blows, M.W. 2011. Reducing mutation load through sexual selection on males. *Evolution* **65**: 2816–2829.

Parker, G.A. & Ball, M.A. 2005. Sperm competition, mating rate and the evolution of testis and ejaculate sizes: a population model. *Biol. Lett.* **1**: 235–238.

Petfield, D., Chenoweth, S.F., Rundle, H.D. & Blows, M.W. 2005. Genetic variance in female condition predicts indirect genetic variance in male sexual display traits. *Proc. Nat. Acad. Sci. USA* **102**: 6045–6050.

Pontier, S.M. & Schweisgurth, F. 2015. A Wolbachia-sensitive communication between male and female pupae controls gamete compatibility in *Drosophila*. *Curr. Biol.* **25**: 2339–2348.

Rodriguez, R.L., Rebar, D. & Fowler-Finn, K.D. 2013. The evolution and evolutionary consequences of social plasticity in mate preferences. *Anim. Behav.* **85**: 1041–1047.

Rundle, H.D., Chenoweth, S.F., Doughty, P. & Blows, M.W. 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biol.* **3**: 1988–1995.

Rundle, H.D., Chenoweth, S.F. & Blows, M.W. 2006. The roles of natural and sexual selection during adaptation to a novel environment. *Evolution* **60**: 2218–2225.

Rundle, H.D., Chenoweth, S.F., & Blows, M.W. 2008. Comparing complex fitness surfaces: among-population variation in mutual sexual selection in Drosophila serrata. *Am. Nat.* **171**: 443e454. doi: 10.1086/528963.

Rundle, H.D., Chenoweth, S.F. & Blows, M.W. 2009. The diversification of mate preferences by natural and sexual selection. *J. Evol. Biol.* **22**: 1608–1615.

Sall, J., Creighton, L. & Lehman, A. 2005. *JMP Start Statistics: A Guide to Statistics and Data Analysis Using JMP and JMP IN Software*. Thomson Learning, Belmont, CA.

Stinziano, J.R., Sové, R.J., Rundle, H.D. & Sinclair, B.J. 2015. Rapid desiccation hardening changes the cuticular hydrocarbon profile of *Drosophila melanogaster*. *Compar. Biochem. Physiol. A: Molec. Integrat. Physiol.* **180**: 38–42.

Sztepanacz, J. & Rundle, H.D. 2012. Reduced genetic variance among high fitness individuals: inferring stabilizing selection on male sexual displays in *Drosophila serrata*. *Evolution* **66**: 3101–3110.

Thomas, M.L. & Simmons, L.W. 2011. Short-term phenotypic plasticity in long-chain cuticular hydrocarbons. *Proc. R. Soc. B* **278**: 3123–3128.

White, A.J. & Rundle, H.D. 2014. Territory defense as a condition-dependent component of male sexual fitness in *Drosophila serrata*. *Evolution* **69**: 407–418.

Wigglesworth, V.B. 1970. Structural lipids in the insect cuticle and the function of the oenocytes. *Tissue Cell* **2**: 155–179.

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