

The Depletion of Genetic Variance by Sexual Selection

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Summary

Sexually selected traits display substantial genetic variance [1, 2], in conflict with the expectation that sexual selection will deplete it [3–5]. Condition dependence is thought to resolve this paradox [5–7], but experimental tests that relate the direction of sexual selection to the availability of genetic variance are lacking. Here, we show that condition-dependent expression is not sufficient to maintain genetic variance available to sexual selection in multiple male sexually selected traits. We employed an experimental design that simultaneously determined the quantitative genetic basis of nine male cuticular hydrocarbons (CHCs) of *Drosophila bunnanda*, the extent of condition dependence of these traits, and the strength and direction of sexual selection acting upon them. The CHCs of *D. bunnanda* are condition dependent, with 18% of the genetic variance in male body size explained by genetic variance in CHCs. Despite the presence of genetic variance in individual male traits, 98% of the genetic variance in CHCs was found to be orientated more than 88° away from the direction of sexual selection and therefore unavailable to selection. A lack of genetic variance in male traits in the direction of sexual selection may represent a general feature of sexually selected systems, even in the presence of condition-dependent trait expression.

Results and Discussion

Sexually selected traits that reflect male fitness are under both sexual selection and natural selection, and consequently genetic variance in these traits is expected to be depleted. In species in which females gain no direct benefits from exercising choice, the long-standing controversy surrounding the persistence of female choice despite expected low genetic variance in male traits is known as the lek paradox [1–3]. In contrast to this expectation, male sexually selected traits often display high levels of genetic variance [4, 5], a phenomenon that has been attributed to the evolution of condition-dependent male trait expression [3, 6, 7].

There are two key genetic requirements for the evolution of condition-dependent trait expression to maintain genetic variance in traits under sexual selection. First, male sexually selected traits must be genetically

correlated with condition. Little direct evidence is available for condition-dependent expression of male traits [8], and in particular, evidence for this critical genetic association between condition and male traits has been shown on very few occasions [5, 9].

Second, genetic variance in male sexually selected traits, and consequently male fitness, must be available to sexual selection. Within a single species, females often have preferences for more than one male trait [10], and even single male traits often have multiple components [11]. Although there is evidence for substantial genetic variance in individual male sexually selected traits [4, 5, 7], such metrics have been shown to be inadequate for assessing levels of genetic variance when multiple traits are under selection [12, 13]. This is because the level of genetic variance in the direction of selection can vary greatly from the levels of genetic variance in individual traits as a consequence of the genetic covariance structure among traits [14, 15]. Therefore, the general conclusion that genetic variance in sexually selected traits is maintained by the evolution of condition-dependent expression may be premature [16], because no studies have determined the level of genetic variance in combinations of condition-dependent traits under sexual selection.

Extensive investigations of mate choice in the *Drosophila serrata* species complex have demonstrated that cuticular hydrocarbons (CHCs) play a key role in the mating systems of species in this group. CHCs are involved in species recognition among closely related members of this group [17], the CHCs of male *D. serrata* are under sexual selection within populations [18–20], and these traits have been shown to respond to manipulations of the processes of both sexual selection within populations [21] and natural selection on mate recognition [17]. In particular, in two previous experiments we have demonstrated that even though substantial genetic variance was present in individual CHCs of *D. serrata*, there was little genetic variance in the direction of sexual selection under either laboratory [18] or field [19] conditions. However, strong evidence for the condition dependence of CHC expression was not available in either experiment.

Drosophila bunnanda is a newly described member of the *Drosophila serrata* species complex that is native to the rainforests of northeastern Australia [22]. Initial investigation of the CHCs of *D. bunnanda* found that they were highly sexually dimorphic, suggesting they too may be targets of sexual selection. Here, we report the results of an experiment that simultaneously determined the quantitative genetic basis of a suite of male sexually selected traits and the direction of sexual selection acting upon them, enabling a test of whether condition-dependent trait expression maintains genetic variance that is available to sexual selection in *Drosophila bunnanda*. First, we show that male CHCs of *D. bunnanda* are under sexual selection. Second, by using a large quantitative genetic experiment to determine

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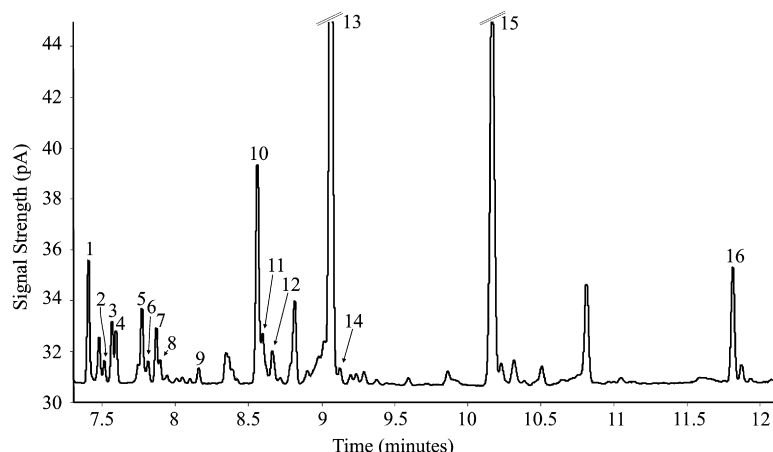


Figure 1. Typical Gas Chromatograph of a *D. bunnanda* Male

The 16 CHCs used in this study were identified by mass spectrophotometry as: 1. 2-Me-C₂₄, 2. C_{25:1} (A), 3. C_{25:1} (B), 4. C₂₅, 5. C₂₅H₄₆ (A), 6. C₂₅H₄₆ (B), 7. C₂₅H₄₈ (A), 8. C₂₅H₄₈ (B), 9. C₂₆, 10. 2-Me-C₂₆, 11. 7,11-C_{27:2}, 12. C_{27:1}, 13. C₂₇H₅₀ (A), 14. C₂₇H₅₀ (B), 15. 2-Me-C₂₈, 16. 2-Me-C₃₀. Peak 4 was used as the divisor to generate logcontrasts, and the remaining 15 peaks, transformed to logcontrasts, were used in initial analyses.

the extent of genetic correlation between CHCs and a metric of male body size, we show that male CHCs are condition dependent. Finally, we determine the level of genetic variance in male CHCs and show that the vast majority of genetic variance in these sexually selected traits is effectively unavailable to selection.

Sexual Selection on Male CHCs

To determine which of the 15 male CHCs of *D. bunnanda* (Figure 1) may be under directional sexual selection, 976 male offspring from a quantitative genetic half-sib breeding design were competed against males from the stock population in a standard mate-choice experiment [18, 19]. Multiple regression was used to estimate standardized directional selection gradients (β) [23] in conjunction with model selection via Mallow's *Cp* statistic [24]. A model involving nine CHCs ($F_{[9, 906]} = 12.37$, $p < 0.001$), explaining 10.9% of the phenotypic variance in mating success, was found to best satisfy the Mallow's *Cp* selection criteria. We therefore conducted all subsequent analyses on this subset of nine CHCs.

Significant directional sexual selection gradients were found on six male CHCs in the favored nine-trait model (Table 1). Of those individual traits under significant sexual selection, two were 2-methyl-alkanes, a class of

CHCs that have been shown to be important targets of sexual selection in *D. serrata* [18, 19]. Directional sexual selection was relatively strong, with five CHCs experiencing selection stronger than the median magnitude of directional sexual selection gradients (0.18) found across all taxa by a recent comprehensive review [25].

Genetic Correlation between Body Size and Male CHCs

Male size is commonly used as a surrogate for condition in insects [9, 26], because condition itself is notoriously difficult to empirically quantify [5, 26]. Although male body size clearly does not represent all potential resources that were available to individuals as required by the theoretical definition of condition [6], it nevertheless represents a substantial competing resource sink when lipid allocation is made during the juvenile stages in insects [27]. Wing size has been used as a measure of body size in *Drosophila* [28], and here we use wing centroid size [29] as the metric of body size. Wing centroid size was not under sexual selection ($F_{[1, 802]} = 0.65$, $r^2 = 0.001$, $p = 0.421$).

Bivariate genetic correlations between body size and each CHC would have given an indication of the genetic association between size and CHCs on a trait-by-trait basis, and therefore the condition dependence of each CHC [8]. Here we needed to know how much of the genetic variance in body size was explained by genetic variance in not one, but nine, CHCs. Therefore, to quantify the extent of genetic association between wing centroid size and CHCs, best linear unbiased predictors [30] (BLUPs) of the 125 sire breeding values were generated by univariate models for all traits. The BLUPs were then used in a multiple regression, with wing size as the response variable and the nine CHCs as independent variables (Figure 2). The genetic variance in the nine CHCs accounted for 18.6% of the genetic variance in wing centroid size ($F_{[9, 115]} = 2.93$, $r^2 = 0.186$, $p = 0.004$) (Figure 2). This relationship is not simply due to allometric scaling [8] because variation in total CHC content across individuals, and therefore size-related increases in CHC content, was removed prior to genetic analysis by dividing by total CHC content (see Experimental Procedures). Consequently, the sexually selected CHCs of *D. bunnanda* may represent a substantial resource

Table 1. Measures of Heritability, Sexual Selection, and the Predicted Response to Sexual Selection in Nine Cuticular Hydrocarbons of *D. bunnanda*

Trait	h^2	β	Δz
2-Me-C ₂₄	0.396 ^a	0.135 ^b	-0.0271
C _{25:1} (A)	0.276 ^c	-0.876 ^a	-0.0270
C _{25:1} (B)	0.332 ^a	-0.163	-0.0359
C ₂₅ H ₄₈ (B)	0.184 ^b	0.265 ^a	-0.0289
7,11-C _{27:2}	0.396 ^a	0.827 ^a	0.0038
C _{27:1}	0.172 ^b	0.156	0.0028
C ₂₇ H ₅₀ (A)	0.228 ^b	-0.478 ^a	-0.0376
2-Me-C ₂₈	0.128 ^d	0.294 ^c	0.0095
2-Me-C ₃₀	0.168 ^b	-0.115	0.0181

Abbreviations: h^2 , heritability; β , vector of sexual selection gradients; Δz , vector of predicted response to selection.

^a $p < 0.001$.

^b $p < 0.05$.

^c $p < 0.01$.

^d $p = 0.090$.

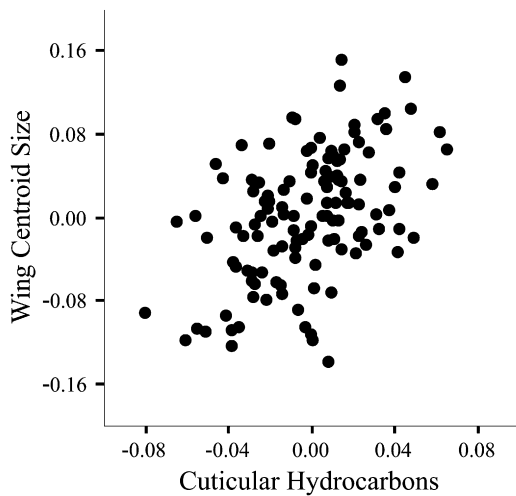


Figure 2. Genetic Association between Wing Centroid Size and Nine Cuticular Hydrocarbons of *D. bunnanda*

Each point represents the best linear unbiased predictor (BLUP) of the breeding value of each of 125 sires. Values for the independent variable are the predicted values from a multiple regression associating the BLUPs of the nine CHCs with wing centroid size. The equation for this genetic regression was $y = 0.032*t_1 + 0.022*t_2 - 0.234*t_3 + 0.163*t_7 + 0.324*t_{11} - 0.606*t_{12} + 0.051*t_{13} - 0.179*t_{15} + 0.328*t_{16}$, where trait numbers are defined in the Figure 1 legend. From this equation, it can be seen that some CHCs have a negative genetic association with size when genetic variance in other traits is accounted for, while others have a positive genetic association with size. The correlation between the predicted values of the CHCs and body size is 0.432 ($p = 0.004$).

investment that will affect resource allocation to male body size. This competition for resources between male size and CHCs suggests that the first key requirement of the condition-dependent hypothesis is met in *D. bunnanda*: the condition-dependent expression of male sexually selected traits.

Genetic Variance in Male CHCs

As has been commonly found in many species, the sexually selected CHCs of *D. bunnanda* displayed significant levels of genetic variance. Univariate quantitative genetic analyses revealed significant genetic variance in all CHCs with the exception of 2-Me-C₂₈, which displayed a marginally nonsignificant heritability of 12.8% (Table 1). However, the multivariate availability of genetic variance can differ greatly from that indicated by univariate levels when traits are genetically correlated [12, 13]. Despite the presence of significant univariate

genetic variance in all but one CHC, application of the multivariate breeders equation, $\Delta z = G\beta$, where **G** is the additive genetic variance-covariance matrix (Table 2), indicated that all CHCs would respond only very slowly to selection. The largest predicted response of an individual CHC was only 3.8% of a phenotypic standard deviation for C₂₇H₅₀ (A), as indicated by its coefficient in the vector of standardized predicted responses (Table 1). Such a small response to selection suggested that the genetic covariance structure among the CHCs was limiting the availability of genetic variance in the direction of selection.

To directly investigate the multivariate availability of genetic variance, we first established what part of the 9-dimensional genetic space had statistical support. Factor-analytic modelling of the number of dimensions of **G** [31] found strong statistical support for four genetic dimensions (log-likelihood ratio test; $\chi_6 = 18.08$, $p = 0.006$) and marginal support for a fifth dimension ($\chi_5 = 9.79$, $p = 0.081$). We therefore had statistical evidence for the presence of at least 95% (4 dimensions), but up to 98% (5 dimensions) of the estimated genetic variance. In other words, the phenotypes of the nine CHCs we measured were adequately described by five independent genetic traits. This analysis demonstrated that many of the CHCs share a genetic basis to some extent, consistent with known shared biosynthetic pathways for lipid production in insects [32].

Matrix projection [18] indicated that 98% of the genetic variance, described by the first five eigenvectors of **G**, was effectively unavailable to sexual selection. An angle of 88.2° between β and closest vector of genetic variance in this 5-dimensional subspace was found, indicating that the closest available genetic variance was almost at right angles to the direction of selection. This result was more extreme (89.1°) if only four eigenvectors accounting for 95% of the genetic variance were included in the subspace. To place this result in a univariate context, the genetic variance of the composite trait represented by β can be calculated by $\beta^T G \beta$ [33] and is only 0.021, six times lower than the lowest genetic variance of an individual CHC. Therefore, genetic variance in the CHCs of *D. bunnanda* was not maintained in the direction of sexual selection, and the second key requirement of the condition-dependent hypothesis is not met.

The restriction of genetic analyses of male traits to univariate descriptions of genetic variance, when multiple traits are likely to be under sexual selection, has resulted in the generally held view that genetic variance in sexually selected traits is maintained through the

Table 2. Genetic Variance-Covariance Matrix of Nine Cuticular Hydrocarbons of *D. bunnanda*

	2-Me-C ₂₄	C _{25:1} (A)	C _{25:1} (B)	C ₂₅ H ₄₈ (B)	7,11-C _{27:2}	C _{27:1}	C ₂₇ H ₅₀ (A)	2-Me-C ₂₈	2-Me-C ₃₀
2-Me-C ₂₄	0.345								
C _{25:1} (A)	0.057	0.276							
C _{25:1} (B)	0.175	0.262	0.325						
C ₂₅ H ₄₈ (B)	0.076	0.067	0.070	0.185					
7,11-C _{27:2}	0.032	0.302	0.279	0.008	0.397				
C _{27:1}	-0.084	0.178	0.131	0.026	0.230	0.172			
C ₂₇ H ₅₀ (A)	0.134	0.122	0.157	0.068	0.160	0.075	0.229		
2-Me-C ₂₈	0.112	0.085	0.129	-0.010	0.132	0.051	0.107	0.135	
2-Me-C ₃₀	-0.018	0.099	0.084	-0.055	0.163	0.101	0.063	0.119	0.174

evolution of condition-dependent expression. Here we have demonstrated that such univariate analyses can be misleading. By using multivariate quantitative genetics, we have shown that the lek paradox is a statistically verifiable phenomenon in condition-dependent male traits in *D. bunnanda*. Taken together with previous experiments demonstrating a virtual absence of genetic variance in the direction of sexual selection in *D. serrata* [18, 19], lack of genetic variance in male display traits in the direction of sexual selection may represent a general feature of sexually selected systems.

How then can the observation that single condition-dependent male sexually selected traits display substantial genetic variance [4, 5] be reconciled with the fact that the combinations of multiple traits that are preferred by females display virtually no genetic variance? One reason why condition dependence may be unable to maintain genetic variance in multiple male CHCs is that multitrait phenotypes may pay a cost in terms of adaptation [34, 35]. The frequency of favorable mutations in n -dimensional trait space is expected to be low, because the probability of a random mutation being able to satisfy all the constraints of selection will be inversely proportional to n . As shown in this study on *D. bunnanda* and previously in *D. serrata* [18, 19], multiple male traits share a genetic basis, indicating that a structure of up to 8 dimensions is under sexual selection. Therefore, to be favorable, and thus supply genetic variance in the direction of sexual selection, a mutation needs to affect CHC expression in a way consistent with the signs of the selection gradients in Table 1. Consequently, although the evolution of condition dependence may be able to maintain the substantial genetic variance commonly observed in single traits [36], it may not be as effective in maintaining genetic variance in the combination of multiple male traits under sexual selection.

Conclusions

We have shown that condition-dependent trait expression is insufficient to maintain genetic variation in multiple male sexually selected traits. Consequently, females may be unable to gain indirect genetic benefits from standing genetic variation by choosing among males. Continuing to exercise choice in the absence of any remaining genetic benefit is problematic because females are likely to incur costs associated with choice [37]. How female choice is maintained in populations where direct phenotypic benefits to females are absent therefore remains a key unresolved problem in evolutionary biology [16].

Experimental Procedures

Quantitative Genetic Experiment

A standard half-sib breeding design was employed by the use of 125 sires each mated to four dams. Two virgin 6- to 8-day-old males from each half-sib family (976 individuals in total) were each competed in a mate-choice experiment against a virgin male raised under the same conditions and of the same age that came from the same mass-bred population as the flies that initiated the half-sib breeding design. To allow identification, males from the breeding design had a small piece of wing clipped on their left wing, and males from the stock were clipped on the right. A single virgin 6- to 8-day-old female from the stock population was placed with the two males. After intromission by one of the males was achieved, the male from the half-sib family was recorded as either being chosen or rejected,

and this male was immediately removed and prepared for gas chromatography. The male from the stock population was not considered further in this study.

Gas Chromatography

Quantification of CHCs of the males from the breeding design was conducted by chromatography. CHCs were extracted from single flies by placing each fly in 100 μ l of hexane for 3 min, followed by vortexing for 1 min and subsequently removing the fly. Samples were run on an Agilent 6890N gas chromatograph with a HP-5 column of 50 m in length, 320 μ m in diameter, and 0.17 μ m film thickness. An Agilent 7683 autosampler was used to inject 1 μ l of sample into a 200°C pulsed-pressure splitless inlet. The oven temperature program had an initial temperature of 57°C that was held for 0.9 min, then ramped at 100°C per min to 230°C, 100°C per min to 270°C, 15°C per min to 300°C, and 100°C per min to 315°C for a total run time of 15.28 min. The flame ionization detector was at 320°C. An Agilent 5975B mass selective detector attached to an Agilent 6890 gas chromatograph was used to obtain mass spectra of compound peaks at 70 eV. Compounds were identified to carbon chain length, and number and position of double bonds where possible, via characteristic EI-MS fragmentation patterns [38]. After integration to calculate peak areas, all areas were divided by the total CHC content of the sample, and proportions were transformed to logcontrasts with C_{25} as the divisor (peak 4 from Figure 1), so that multivariate analyses could be performed [18–20]. We have refrained from analyzing total CHC content of individuals as a trait in its own right because we use it here as a way of controlling for experimental error in the extraction of each CHC sample.

Measurement of Wing Centroid Size

Right wings were collected from each male after CHC sampling. Wing images were captured from a Leica MZ6 microscope with a Panasonic digital video camera and the software Video Trace. Wing centroid size [29] was estimated as the square root of the sum of squared distances of each of the nine landmarks to their centroid. The tps package (F.J. Rohlf, <http://life.bio.sunysb.edu/morph/>) was used to record nine landmarks [39] and to then to rotate and align individual wings and estimate centroid size.

Statistical Analysis

All analyses were conducted on standardized traits with mean of zero and standard deviation of one. Multiple regression was first used to determine which of the 15 CHCs were under sexual selection by the Mallow's C_p model selection criteria [24]. A plot of Mallow's C_p and the number of parameters (p) in the model was inspected, and the model that fell closest to the line $p = C_p$ and had the lowest value of C_p was selected. The vector of standardized directional selection gradients β was then calculated by multiple regression [23].

Genetic analyses were conducted by the MIXED procedure in SAS. Restricted maximum likelihood was used to estimate variance components according to the standard half-sib nested model. Univariate analyses were first conducted to estimate heritabilities. Significance of heritabilities was determined by holding the estimate for each trait to zero and rerunning the model, and then comparing the $-2 \log$ likelihood of the two model fits with a chi-square test with one degree of freedom, and halving the resulting probability level as variance components are constrained to be greater than zero [40]. Best linear unbiased predictors (BLUP) of the breeding values of each sire were saved from these analyses and used to estimate the multivariate genetic association between wing centroid size and the nine CHCs [41]. Finally, a multivariate model was used to estimate the 9-dimensional G matrix.

Factor-analytic modelling of G [31] was conducted by the sequential dropping of a dimension from a factor-analytic model at the sire level implemented in the MIXED procedure. A series of nested log likelihood ratio tests determined when the dropping of a dimension resulted in a significantly worse fit, and therefore statistical support for the presence of the dimension that had been dropped. Matrix projection [18] was used to determine the orientation of the multivariate distribution of genetic variance in relation to β . First, G was diagonalized and the first five eigenvectors, which accounted for 98% of the genetic variance, formed the columns of a square matrix A . The projection of genetic variance (p) that is closest to β was then

found with the formula $p = A(A^T A)^{-1} A^T \beta$. The angle between p and β was then calculated.

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