Sexual selection on wing interference patterns in *Drosophila melanogaster*

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Animals with color vision use color information in intra- and interspecific communication, which in turn may drive the evolution of conspicuous colored body traits via natural and sexual selection. A recent study found that the transparent wings of small flies and wasps in lower-reflectance light environments display vivid and stable structural color patterns, called “wing interference patterns” (WIPs). Such WIPs were hypothesized to function in sexual selection among small insects with wing displays, but this has not been experimentally verified. Here, to our knowledge we present the first experimental evidence that WIPs in males of *Drosophila melanogaster* are targets of mate choice from females, and that two different color traits—saturation and hue—experience directional and stabilizing sexual selection, respectively. Using isogenic lines from the *D. melanogaster* Genetic Reference Panel, we compare attractiveness of different male WIPs against black and white visual backgrounds. We show that males with more vivid wings are more attractive to females than are males with dull wings. Wings with a large magenta area (i.e., intermediate trait values) were also preferred over those with a large blue or yellow area. These experimental results add a visual element to the *Drosophila* mating array, integrating sexual selection with elements of genetics and evo-devo, potentially applicable to a wide array of small insects with hyaline wings. Our results further underscore that the mode of sexual selection on such visual signals can differ profoundly between different color components, in this case hue and saturation.

sexual selection | wing interference patterns | mate choice | color | signaling

The visual world of animals comprises a complex combination of patterns, colors, contrast, and motion (1), all of which may be used in mating strategies, signaling contexts, and social behaviors (2, 3). Pronounced and remarkable color patterns in butterflies, for example, has resulted in the evolution of extreme diversity of either more conspicuous color patterns used in mate choice (4), antipredator defense (5), or more cryptic patterns, such as in camouflage (5, 6). The physical environment, with its variable light conditions, can also strongly affect the visibility and appearance of animal coloration. For example, passerine birds (*Phylloscopus* warblers) with bright wing and head patches live in darker habitats, where these light signals increase the conspicuousness of the signaler, providing an advantage in intraspecific communication and sexual selection (7). Conversely, impaired visibility in some aquatic environments following eutrophication has led to a collapse in color-associated species diversity in cichlid species in Lake Victoria, Eastern Africa, revealing the strong link between the visual environment and the evolution of color signals (8).

The recent discovery that stable wing interference patterns (WIPs) are present in the majority of small insects with thin and transparent wings (9) provides an excellent opportunity to study mating behavior and sexual selection under different visual environments, such as under different light regimes (10). The extremely thin wing membranes of small insects reflect vivid color patterns because of thin film interference (11). In a bright

environment, the wings might therefore be expected to appear transparent when the relatively weak WIPs are overpowered by the background reflectance. In contrast, in a dark and light-absorbing environment with incoming external light (sunshine), conspicuous WIPs would be expected to be displayed on the wing membranes. Such WIPs vary greatly among species, moderately within species, but also to some extent between sexes within a species (9). The observed Newton color series is similar to that appearing on a soap bubble and is directly proportional to the thickness of the wing membrane at any given point (9) (Fig. S14). Unlike the angle-dependent iridescence effect of a flat thin film (11), microstructures in an insect's wing membrane act as dioptrics, ensuring the WIPs appear essentially noniridescent (9).

The biological significance of WIPs as originally proposed by Shevtsova et al. (9) has so far mainly received attention for their potential as diagnostic species identification traits in taxonomic and systematic studies (12–14). The possible role of WIPs as signaling traits at the intraspecific level where they might be targets of sexual selection (15–17) has only been hypothesized and has not been experimentally investigated. Here we use males from 34 *Drosophila melanogaster* Genetic Reference Panel (DGRP) isogenic lines of *Drosophila melanogaster* Meigen (18) to investigate the role of WIPs in mate choice by females, using experiments where we manipulate background color to control the light environment. We demonstrate significant variation in

Significance

Recently, it was discovered that small insects like flies and wasps, with seemingly transparent wings, display vivid coloration against dark backgrounds because of so-called “wing interference patterns” (WIPs). It was proposed that such wing coloration could function in sexual selection and species recognition, but direct evidence for this hypothesis has been lacking. Here, to our knowledge we present the first experimental evidence that WIPs in male *Drosophila melanogaster* are targets of mate choice from females. Comparison of attractiveness of different male WIPs between black and white backgrounds revealed that two different color traits, saturation and hue, experience directional and stabilizing sexual selection, respectively. Our results suggest that vivid coloration in WIPs is a target of mate choice and might have evolved by sexual selection.

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WIPs among these lines, revealing a genetic basis of this recently described color trait. Using LH_M (19) outbred (“wild-type”) females in mate choice trials, we confirm the recent hypothesis (9) that WIPs do indeed have an important function in intersexual selection by female choice.

Results and Discussion

We quantified wing color patterns of the largest wing panel surrounded by wing veins (Fig. S1B), estimating the hue, saturation, and brightness (HSB in the color space). Based on the observed frequency of wing color patterns and the Newton color series with repeating sets of a sequential color pattern, the color patterns of wings range from a bluish (thicker) to a yellowish (thinner) wing membrane in a single color set, with magenta as the intermediate trait value (Fig. 1 and Figs. S1A and S2).

There was significant variation among the 34 DGRP lines in their WIP-patterns (hue: $F_{33,441} = 16.964$, $P < 0.001$; saturation: $F_{33,441} = 6.016$, $P < 0.001$; and brightness: $F_{33,441} = 6.498$, $P < 0.001$). The significant variation among the different isogenic DGRP lines reveals that all these three WIP components are partly heritable and can evolve by natural and sexual selection, presumably because different alleles have been fixed in these different isogenic lines. The broad-sense male heritabilities for the three different traits were 0.3799 for hue [$P < 0.001$, 95% confidence interval (CI): 0.3703, 0.3898], 0.4217 for saturation ($P < 0.001$, 95% CI: 0.3210, 0.5174), and 0.2682 for brightness ($P < 0.001$, 95% CI: 0.2778, 0.5200). See Table S1 for variance component estimates.

To investigate female mate choice and preferences for WIPs of the different male phenotypes in the DGRP lines, we performed several replicated blocks of mating trials, where an outbred virgin LH_M female was paired with a male from one of the 34 isogenic lines, and exposed to one of two different background treatments (black or white). By using males from the DGRP lines rather than outbred lines, we were able to analyze a broader range of male phenotypes compared with the wild-type males, which is a genetic analog to increasing male phenotypic variation using classic and more traditional experimental phenotypic manipulation of secondary sexual traits (3). This experimental procedure also allowed us to easily obtain replicated measurements from the same genotype. We elected to use this noninvasive manipulation of female WIP perception (rather than manipulation of male WIP phenotype per se) to ensure that male phenotype would not be changed in unanticipated ways, keeping our results relevant to natural conditions. Female mating behavior and mating responses were observed over a period of 75 min, and male attractiveness to females was calculated by transforming the continuous time-to-mating variable to a general

(and also continuous) attractiveness score, as described further below. To compare the mating success of different male phenotypes from the different lines, we standardized the variable “male attractiveness” within each trial block and assigned a value of “1” to the most successful males and “0” to those that did not mate during the trial period.

First, we examined the effect of WIPs on male attractiveness. We performed a generalized additive model (GAM) analysis, using color hue, color saturation, and color brightness of the WIP as independent variables and the attractiveness in the black or white background as a dependent variable. However, no significant main effects of HSB were found in both black and white backgrounds (Table S2). Instead, the mean attractiveness of the different male lines as observed against a black background was positively and significantly correlated with the mean attractiveness of males from the same line against the white background ($r = 0.751$; $P < 0.001$) (Fig. 2). These results suggest that the attractiveness of male phenotypes from the different lines is likely to also be affected by factors other than WIPs alone, consistent with previous studies (20–22). Alternatively, but certainly not mutually exclusively, females could, at least to some extent, detect variation in male WIP-phenotypes even in the environment with white background. A third possibility is that male WIP-phenotype is correlated with overall male attractiveness or condition, as suggested in some models of sexual selection based on indirect fitness benefits and so-called “good genes” (23). Nevertheless, to control for the potentially confounding effects of male attractiveness variation among lines that was independent of lighting environment, we calibrated the attractiveness in the black background against the attractiveness in the white background by analyzing residual attractiveness from a regression of attractiveness in white vs. black backgrounds (Fig. 2).

To quantify the effect of WIPs on male attractiveness, we performed a GAM analysis, using HSB of the WIP as independent variables and the residual attractiveness as dependent variable. A main-effects only model revealed that the effects of saturation and hue on residual attractiveness were significant, but that of brightness was not significant (Table 1). Note that in this main-effect model using the GAM-approach, the main effects can contain both linear and nonlinear effects, and hence these terms do not necessarily imply that sexual selection is only directional (see further below).

The analysis further revealed that for saturation, attractiveness monotonically increased as WIP-saturation increased, revealing that males with more vivid wings were more attractive for females compared with males with dull wings (Fig. 3A). This finding suggests that saturation may be subject to directional sexual selection by female choice in the LH_M population. In contrast to this result for saturation, for hue we found evidence of a quadratic nonlinear (monomodal) relationship (Fig. 3B), suggesting that males with intermediate trait values were most attractive. This finding suggests that wings with more magenta coloration were preferred compared with those with more blue or yellow coloration. Consistent with this preference, males from the LH_M outbred line had more magenta coloration on their wings compared with the DGRP lines (Fig. 1). This finding suggests that stabilizing sexual selection in the LH_M population might favor males with magenta coloration, and males with blue or yellow coloration suffer from lower attractiveness. This conclusion was visually supported by inspection of the univariate cubic splines, which revealed directional selection on saturation, stabilizing selection on hue, but no significant relationship between brightness and attractiveness (Fig. 3A–C). Incorporating both saturation and hue in a joint fitness surface of both these traits confirmed this (Fig. 3D and E).

We performed a more formal selection gradient analysis using a general linear model that involved both the main effects of hue and saturation and their squared components (24), and

Fig. 1. Wing interference patterns in *D. melanogaster*. Three representative WIPs found among the 34 DGRP lines and a WIP of LH_M outbred line.

calculated the stabilizing selection gradient for the bell-shaped fitness function on hue [$\gamma_{\text{hue}} = -1.276 \pm 0.338$ (SE); $t_{1,33} = -3.772$; $P < 0.001$]. In contrast, there was no evidence of significant quadratic selection on saturation [$\gamma_{\text{saturation}} = 0.306 \pm 0.386$ (SE); $t_{1,33} = 0.794$; $P = 0.434$]. Note that the quadratic coefficients and their SEs were multiplied by two (see original values in Table S3). We also performed the univariate selection analyses on the two traits. Directional selection was suggested on both saturation ($S = 0.502$, SE = 0.198; $P = 0.017$) and hue ($S = 0.472$, SE = 0.200; $P = 0.025$). However, because hue and saturation are significantly correlated with each other (Pearson's $r = 0.405$; $P < 0.017$), the other effect cannot be excluded in these single-term analyses. Therefore, significant directional selection on hue in this univariate analysis is likely to partly reflect the quadratic selection on the same trait that we documented in the multivariate selection analysis.

Our results suggest that these two different components of WIPs may experience directional selection and stabilizing selection, respectively, in an outbred laboratory population. The mismatch in population of origin between the males and females used in our experiments means that we cannot conclude unambiguously that WIP traits are subject to exactly this form of selection in the LH_M population. However, the fact that the stationary point on the fitness surface of hue is within the 95% CI for the population mean of the LH_M outbred males ($n = 11$, mean = 307.4, 95% CI = 272.4–342.5) supports the interpretation of stabilizing selection on this trait. In addition, in a set of continuing experiments within our research group, an analysis of female mate preference for LH_M males (i.e., from the same population) revealed a strikingly similar pattern of selection (25). Furthermore, as the analyses were performed at a line mean level, our results have essentially revealed a genetic correlation between WIP traits and mating success, which is necessary for any genetic evolution of WIPs through sexual selection.

Hue and saturation of WIPs are both related to wing thickness because thin film interference produces specific color patterns generated by the two layers of transparent chitin of the wings. Compared to the Newton color series that shows repeating sets of a sequential color pattern, yellow areas are thinner and blue areas are thicker than magenta areas on a color set. Thus, these WIP color differences among males should also reveal difference in wing thickness among these DGRP lines. An interesting possibility is that females might use WIPs as an indicator of the genetic and phenotypic quality of foraging ability or courtship performance of their partners, if flight performance is critically affected by the thickness of the wing. It should also be emphasized that *D. melanogaster* males display their wings to females

Fig. 2. Relationship between mean attractiveness in white background and that in the black background ($r = 0.751$; $P < 0.001$).

Table 1. Estimated nonparametric components of GAM model, with the corresponding effective degrees of freedom (edf), F statistic, and P value

Smooth effect of variable	edf	F statistics	P value
Hue	2.522	5.828	0.003
Saturation	1.000	12.491	0.001
Brightness	1.000	0.678	0.417

during courtship, which was also the case during our experimental trials. Hence, a female has ample opportunities to judge male quality through WIPs during the courtship phase before she decides to mate (or not). These wing displays are associated with the production of courtship song, and have never been considered visual signals (26, 27). However, the fact that we found significant WIP effects in the black background that were absent in the white background strongly suggests that the wing display behavior also functions as a visual signal. An alternative explanation could be that male courtship behavior differs according to light level, but behavioral data from LH_M males suggests that this is unlikely to be an important factor. Of the three measures of male courtship behavior (rate of wing display, rate of orientations toward the female, and rate of mating attempts), only one (wing display) differed significantly between backgrounds using a paired t test. However, the relationship between attractiveness and rate of wing display was consistent between backgrounds (i.e., no interaction effect in a mixed-model analysis), suggesting that selection on rate of wing display was independent of background (28). Although it may appear surprising that the visual role the wings play during courtship display has been previously overlooked, our results complement the recent discovery of vibratory courtship signals in *Drosophila* (29) and suggest that multiple aspects of the wing phenotypes are targets of sexual selection.

Although our experimental results certainly do not rule out an additional role of natural selection on WIPs, we also tentatively suggest that intersexual selection can drive the evolution of wing saturation via a good genes process. More generally, stabilizing sexual selection has not been documented in many past studies, although it is increasingly becoming detected because of the recent development of new analytical techniques (30).

The present study demonstrates that WIPs act as a visual signal during mate choice in the model organism *D. melanogaster*. This previously unknown trait calls for further experimental studies of sexual selection on WIPs in other groups of small insects. Once the genetic basis of WIP traits is better understood, then genetic manipulations will be a fruitful area for manipulative experiments of the role of WIPs in sexual selection. In addition, WIPs are likely to be affected by light environment of their habitats and correlated with other traits (e.g., flight ability), suggesting that WIPs could potentially be an underestimated factor in the evolution of color patterns in small flies, and possibly also other small insects, such as wasps (9). The study of WIPs thus potentially shares many characteristics with cuticular hydrocarbons in *Drosophila serrata*; both are complex multivariate, sexually dimorphic traits known (or suspected) to be subject to both sexual and natural selection (31). As such, research on WIPs may offer similar scope for understanding sexual selection in *Drosophila* as cuticular hydrocarbons research.

Materials and Methods

Fly Culture. Altogether, we used 34 DGRP isogenic lines obtained from the Bloomington Stock Center, (RAL_208, RAL_310, RAL_303, RAL_304, RAL_307, RAL_313, RAL_315, RAL_324, RAL_335, RAL_357, RAL_358, RAL_360, RAL_362, RAL_365, RAL_375, RAL_379, RAL_380, RAL_391, RAL_399, RAL_437, RAL_517, RAL_555, RAL_639, RAL_705, RAL_712, RAL_730, RAL_732, RAL_765, RAL_774, RAL_786, RAL_799, RAL_820, RAL_852, RAL_859). The LH_M outbred population was kindly donated by Edward H. Morrow, University of Sussex, Falmer, UK. Flies of each line were cultured

(32). The full six-parameter model (including the intercept and the five other terms) is reported in [Table S3](#).

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