

## BIOLOGICAL SCIENCES

### **Sexual selection on wing interference patterns in *Drosophila melanogaster***

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## Abstract

Animals with color vision utilize 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 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 *Drosophila melanogaster* Genetic Reference Panel (DGRP), we compare attractiveness of different male WIPs against black and white visual backgrounds. We show that males with more vivid wings are more attractive for 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.

## **Significant Statement**

Recently, it was discovered that small insects like flies and wasps with seemingly transparent wings display vivid and stable coloration against black backgrounds due to so-called Wing Interference Patterns (WIPs). It was proposed that such wing coloration could function in sexual selection and species recognition, but direct evidence has been lacking to test this hypothesis. Here, we present the first experimental evidence that WIPs in males of *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 hence have evolved by sexual selection.

The visual world of animals comprises a complex combination of patterns, colors, contrast and motion (1), all of which may be utilized in mating strategies, signalling contexts and social behaviors (2, 3). Pronounced and remarkable color patterns in butterflies, for instance, has resulted in the evolution of extreme diversity of either more conspicuous color patterns used in mate choice (4), anti-predator defence (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 instance, passerine birds (*Phylloscopus* warblers) with bright wing and head patches live in darker habitats where these light signals increases 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 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 due to 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. S1A).

Unlike the angle-dependent iridescence effect of a flat thin film (11), microstructures in an insect's wing membrane act as diopters 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). Their possible role 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 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 WIPs among these lines, revealing a genetic basis of this recently described color trait. Using LH<sub>M</sub> (19) outbred (“wildtype”) 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, Fig. 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% 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<sub>M</sub> 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 analyse a broader range of male phenotypes, compared to the wild-type males, which is a genetic analogue to increasing male phenotypic variation using classical 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 non-invasive manipulation of female WIP perception (rather than manipulation of male WIP phenotype *per se*) in order 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 minutes, 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 male(s) and “0” to those which 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 dependent variable. However, no significant main effects of hue, saturation or brightness were found in both black and white backgrounds (TableS2). 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 are likely also affected by other factors than only WIPs, 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 lightning 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 hue, saturation and brightness 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 non-linear 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 to males with dull wings (Fig. 3A). This suggests that saturation may be subject to directional sexual selection by female choice in the LH<sub>M</sub> population. In contrast to this result for saturation, for hue we found evidence of a quadratic non-linear (monomodal) relationship (Fig. 3B), suggesting that males with intermediate trait values were most attractive. This suggests that wings with more magenta coloration were preferred compared to those with more blue or yellow coloration. Consistent with this preference, males from the LH<sub>M</sub> outbred line had more magenta coloration on their wings compared to the DGRP lines (Fig. 1). This suggests that stabilizing sexual selection in the LH<sub>M</sub> population might favour 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 to 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 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 standard errors 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 ( $S = 0.472$ ,  $SE = 0.200$ ;  $P = 0.024$ ). 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 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<sub>M</sub> population. However the fact that the stationary

point on the fitness surface of hue is within the 95% confidence interval for the population mean of the LH<sub>M</sub> 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<sub>M</sub> males (i.e. from the same population) revealed a strikingly similar pattern of selection (add ref: Li, Q. 2014. The Colors We Didn't See: The Heritability of Wing Interference Patterns (WIPs) and Their Roles in Female Choice in *Drosophila melanogaster*. Master's thesis, Lund University). 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. Comparing 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 during courtship, which was also the case during our experimental trials. Hence, females have 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 (25, 26). 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 behavioural data from LHM males suggests that this is unlikely to be an important factor. Out of three measures of male courtship behaviour (rate of wing display, rate of orientations towards 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 (add ref: Abbott,J.K., Li,Q. Svensson,E.I. & Kjaerandsen,J. 2014. Male courtship behaviour and female Wing Interference Pattern preference in *Drosophila*. [figshare. http://dx.doi.org/10.6084/m9.figshare.1109810](http://dx.doi.org/10.6084/m9.figshare.1109810)). 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* (27) and suggest that multiple aspects of the wing phenotypes are targets of sexual selection.

Although our experimental results do certainly not rule out an additional role of natural selection on WIPs, we also tentatively suggest that inter-sexual 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, due to the recent development of new analytical techniques (28).

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 (CHCs) in *D. serrata*; both are complex multivariate, sexually dimorphic traits known (or suspected) to be subject to both sexual and natural selection (add ref: <http://www.ncbi.nlm.nih.gov/pubmed/19549142>). As such, research on WIPs may offer similar scope for understanding sexual selection in *Drosophila* as CHC 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<sub>M</sub> outbred population was kindly donated by Edward H. Morrow. Flies of each line were cultured on a 14-day cycle in vials with cornmeal-molasses-yeast medium at 25°C with a 12:12 light/dark cycle and a

minimum of 50% relative humidity (19). Experiments were not initiated until after at least 2–3 standard culture cycles, in order to minimize the chance of maternal effects in the DGRP lines. We used males from the DGRP isogenic lines and females from the LH<sub>M</sub> outbred line. This allowed the repeated measurement of male fitness of a given genotype without confounding environmental effects. The choice of outbred females as the source of selection rather than a standardized line of inbred females was to ensure that the mate preferences we found would be representative of mate choice variation in natural populations.

**Mating assays.** Mating vials were produced by covering the back half of the vial (with medium) with a plastic sheet (matte black or white). Incident light could still enter the front half of the vial. The trials were performed under strong fluorescent desktop lamps at daylight. A virgin LH<sub>M</sub> female was added to each vial one day prior to the assay, in order to let females habituate to this new environment. During the trial, one isogenic DGRP male was introduced into the mating vial and time to mate was recorded. This procedure was repeated for each line. We performed 4–6 replicate mating trials for each line and each background, divided among four blocks. To exclude the effect among trial blocks, we normalized the variable “male attractiveness” ranging from 0 to 1: in each trial block, a value of “1” was assigned to the most successful male(s) and “0” to those which did not mate during the trial period. All of the mating assays were performed in the morning in order to ensure that the flies were at the same point in their daily cycle of activity during all assays.

**Quantifying wing color.** Digital photos of the wings were taken under identical illumination and magnification with a 5MP Nikon DS-L1 camera unit on a Nikon stereomicroscope (SMZ1500) fitted with an 80-LED ring light. The photos were imported to ImageJ 1.44o (National Institutes of Health, <http://imagej.nih.gov/ij/>). The area for measurement was selected to be the largest panel of the wing clearly demarcated by veins, and corresponds to the M-sector distal to cross-vein dM-Cu. The Red, Green and Blue (RGB) values were obtained from each pixel of the panel. The data was binarized by iteration method after noise filtering and the regions with low RGB values were eliminated. For each pixel, RGB values were then transformed into HSB values and the mean HSB values of the whole panel area were obtained for the analyses. Comparison of the observed frequency of original hue value (0–360) of individuals and the Newton color series reveals that the color patterns of wings range from a bluish (thicker) to a yellowish (thinner) wing membrane in a single color set, and that magenta is an intermediate trait (9) (Fig. S1A, S2). Taking into account the distribution of individuals of each isogenic line we calibrated the hue value at 60. The calibrated hue values used in this study range from 60 to 420 (Fig. S2).

**Statistical analyses.** All statistics were performed using R version 2.15.1 (R Development Core Team 2009). Differences of hue, saturation and brightness among lines were analyzed by ANOVA using the package “car.” Broad-sense heritability of color traits was calculated as the phenotypic variance attributable to line divided by the total phenotypic variance. Variance components were obtained from mixed models in the “lme4” package. Significance values for broad-sense heritability estimates were obtained using permutation

tests. Confidence intervals were obtained by calculating 1000 heritability estimates using simulated variance components derived from normal distributions with means and standard deviations as in table S1. The relationship between attractiveness in white background and black background was analyzed by Pearson correlation analysis and then the residual of attractiveness was calculated from a linear regression with attractiveness in white background as independent variable and that in black background as dependent variable. To quantify the effect of WIPs on residual of attractiveness, we performed a GAM (General Additive Model) analysis, using hue, saturation and brightness of WIP as independent variables and the residuals of attractiveness as dependent variable. We complemented this GAM with a more formal parametric selection analysis using a general linear model (GLM) with five terms: linear and quadratic effects of hue and saturation (the two significant terms in Table 1), their quadratic components and their interaction (crossproduct term), which reveal curvilinear selection terms (stabilizing, disruptive and correlational selection) (24) and two simple term analyses using hue and saturation were also performed. In the formal selection analysis, the difference between attractiveness in black background and that in white background in each line was divided by its mean across lines and these values were used as the dependent variable. Because the mean value of the difference across lines was negative, the dependent variable was multiplied by “-1” in the analysis so that larger values represent increased attractiveness in the black background. The quadratic coefficients and their standard errors were multiplied by two, before reporting the quadratic selection coefficients in the text (29). The full six-parameter model (including the intercept and the five other terms) is reported in table S3.

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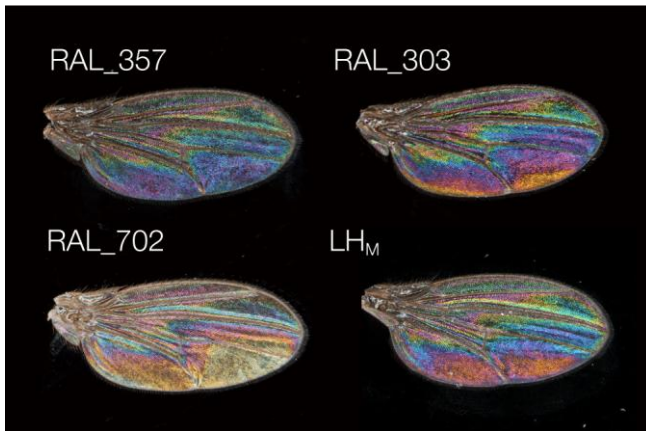
## References

1. Paulk A, Millard SS, van Swinderen B (2013) Vision in *Drosophila*: seeing the world through a model's eyes. *Annu Rev Entomol* 58:313–332.
2. Tori WP, Durães R, Ryder TB, Anciães M (2008) Advances in sexual selection theory: insights from tropical avifauna. *Ornitol Neotrop* 19:151–163.
3. Andersson MB (1994) *Sexual selection* (Princeton University Press).
4. Oliver JC, Robertson KA, Monteiro A (2009) Accommodating natural and sexual selection in butterfly wing pattern evolution. *Proc R Soc B* 276:2369–2375.
5. Janzen DH, Hallwachs W, Burns JM (2010) A tropical horde of counterfeit predator eyes. *Proc Natl Acad Sci USA* 107:11659–11665.
6. Skelhorn J, Rowland HM, Speed MP, Ruxton GD (2009) Masquerade: camouflage without crypsis. *Science* 327:51–51.
7. Marchetti K (1993) Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* 362:149–152.
8. Seehausen O, van Alphen JJ, Witte F (1997) Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277:1808–1811.
9. Shevtsova E, Hansson C, Janzen DH, Kjaerandsen J (2011) Stable structural color patterns displayed on transparent insect wings. *Proc Natl Acad Sci USA* 108:668–673.

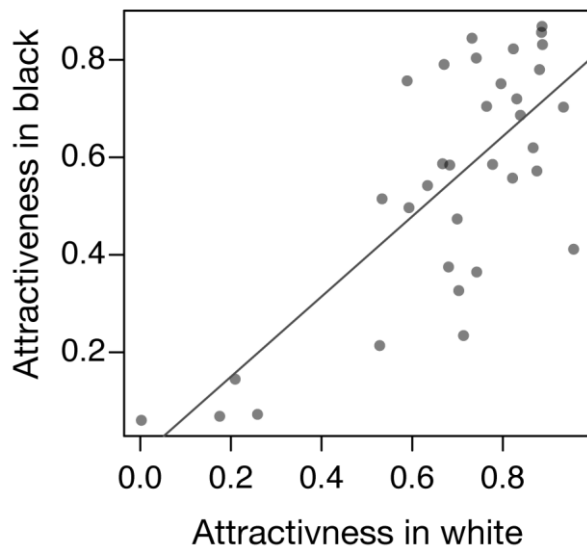


10. Denoël M, Doellen J (2010) Displaying in the dark: light-dependent alternative mating tactics in the Alpine newt. *Behav Ecol Sociobiol* 64:1171–1177.
11. Kinoshita S, Yoshioka S, Miyazaki J (2008) Physics of structural colors. *Rep Prog Phys* 71:076401.
12. Buffington ML, Sandler RJ (2011) The occurrence and phylogenetic implications of wing interference patterns in Cynipoidea (Insecta : Hymenoptera). *Invert Systematics* 25:586–597.
13. Shevtsova E, Hansson C (2011) Species recognition through wing interference patterns (WIPs) in *Achrysocharoides* Girault (Hymenoptera, Eulophidae) including two new species. *ZooKeys*:9–30.
14. Simon E (2013) Preliminary study of wing interference patterns (WIPs) in some species of soft scale (Hemiptera, Sternorrhyncha, Coccoidea, Coccidae). *ZooKeys*:269–281.
15. Devicari M, Lopes AR, Suesdek L (2011) Wing sexual dimorphism in *Aedes scapularis* (Diptera: Culicidae). *Biota Neotropica* 11:165–169.
16. Díaz-Fleischer F, Arredondo J (2011) Light conditions affect sexual performance in a lekking tephritid fruit fly. *J Exp Biol* 214:2595–2602.
17. Shevtsova E (2012) *Seeing the invisible: Evolution of wing interference patterns in Hymenoptera, and their application in taxonomy* (Ph.D. Thesis, Department of Biology, Lund University).
18. Mackay TFC et al. (2012) The *Drosophila melanogaster* Genetic Reference Panel. *Nature* 482:173–178.
19. Chippindale AK, Rice WR (2001) Y chromosome polymorphism is a strong determinant of male fitness in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 98:5677–5682.
20. Pitnick S (1991) Male size influences mate fecundity and remating interval in *Drosophila melanogaster*. *Anim Behav* 41:735–745.
21. Partridge L, Hoffmann A, Jones JS (1987) Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. *Anim Behav* 35:468–476.
22. Friberg U, Arnqvist G (2003) Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females. *J Evol Biol* 16:797–811.

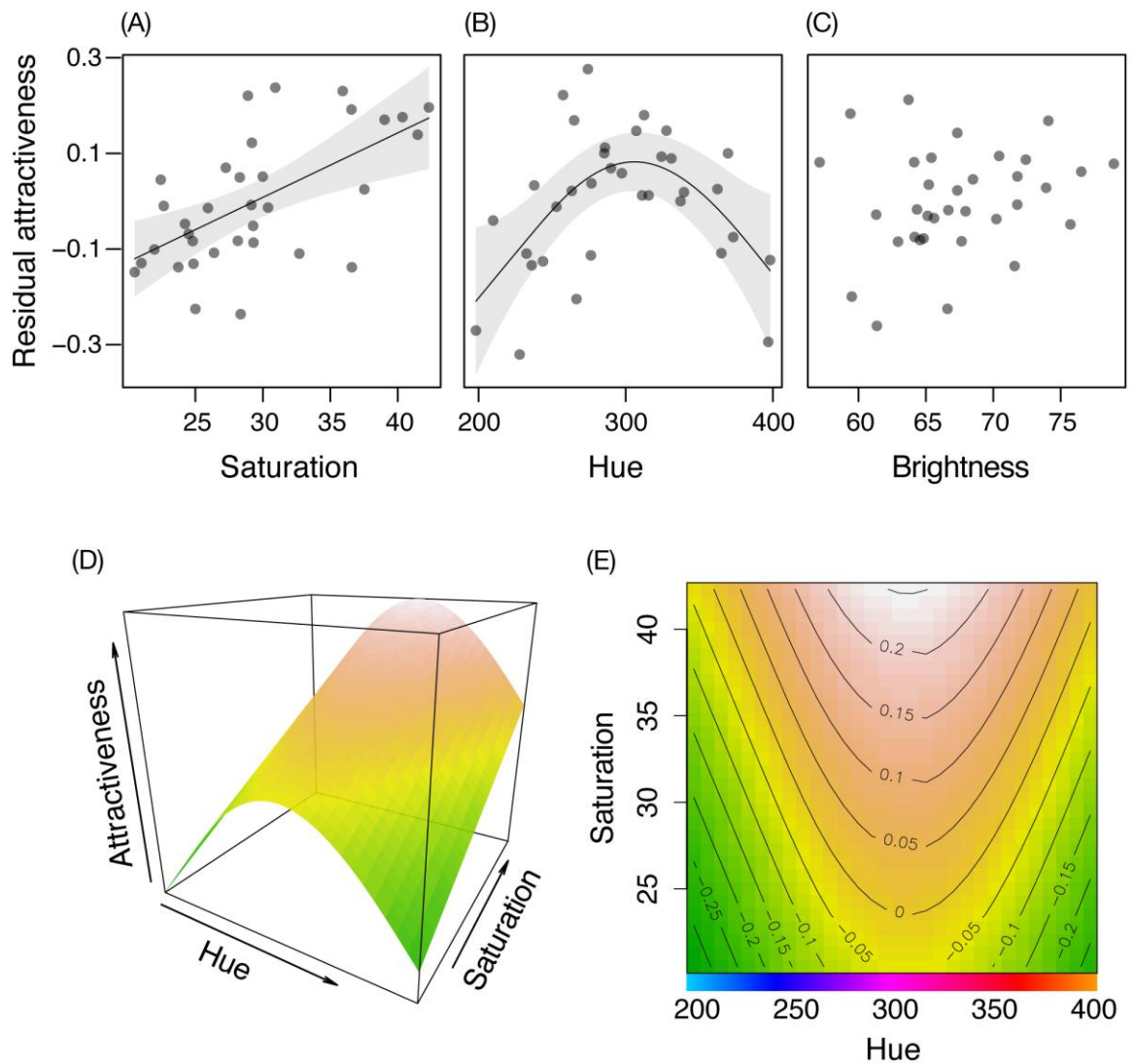
23. Rowe L, Houle D (1996) The Lek Paradox and the Capture of Genetic Variance by Condition Dependent Traits. *Proc R Soc B* 263:1415–1421.
24. Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
25. Ritchie M, Halsey E, Gleason J (1999) *Drosophila* song as a species-specific mating signal and the behavioural importance of Kyriacou & Hall cycles in *D. melanogaster* song. *Anim Behav* 58:649–657.
26. Trott AR, Donelson NC, Griffith LC, Ejima A (2012) Song choice is modulated by female movement in *drosophila* males. *PLoS ONE* 7:e46025.
27. Fabre CCG et al. (2012) Substrate-borne vibratory communication during courtship in *Drosophila melanogaster*. *Curr Biol* 22:2180–2185.
28. Chenoweth SF, Hunt J, Rundle HD (2012) in *The adaptive landscape in evolutionary biology*, eds Svensson E, Calsbeek R (Oxford University Press), pp 126–149.
29. Stinchcombe JR, Agrawal AF, Hohenlohe PA, Arnold SJ, Blows MW (2008) Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution* 62:2435–2440.



**Fig. 1.** Wing interference patterns (WIPs) in *Drosophila melanogaster*. Three representative WIPs found among the 34 DGRP lines and a WIP of LH<sub>M</sub> outbred line.



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

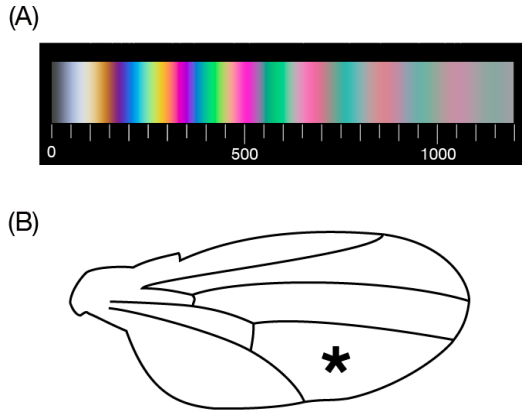


**Fig. 3.** Effects of saturation (A), hue (B) and brightness (C) on residual attractiveness estimated by GAM analyses. Significant relationships were found for hue and saturation, but not for brightness (see Table 1). Three- and two-dimensional fitness surface (D, E). Fitness (residual attractiveness) monotonically increased with saturation, though it peaked at moderate degree of hue.

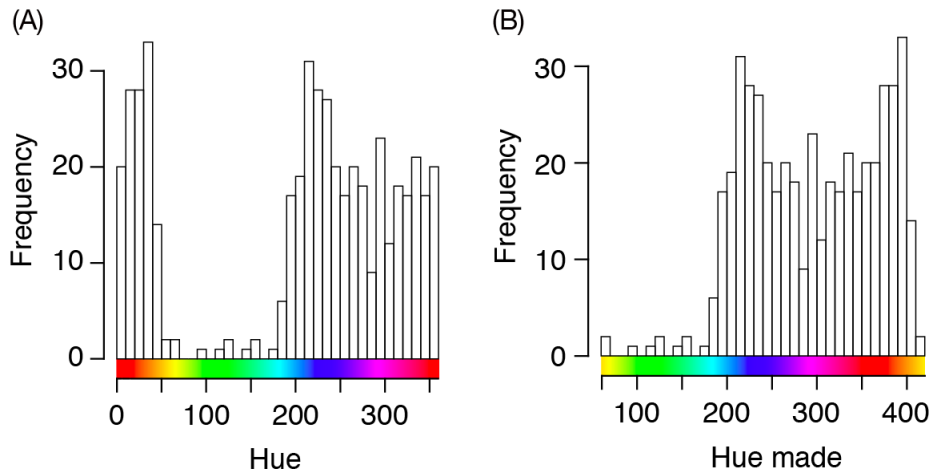
**Table 1. Estimated non-parametric components of GAM model, with the corresponding effective degrees of freedom (edf), *F*-statistic and *P* value.**

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

## Supporting Information



**Fig. S1.** (A) Computer generated Newton series scale of two-beam interference colors calibrated for the refractive index of chitin (1.57). Scale shows approximate thickness (nm) of a wing membrane. (B) Wing area (asterisk) used for analyses in this study.



**Fig. S2.** Histogram of hue value of individuals examined. Histogram using original value (A) and histogram using calibrated hue value (B).

**Table S1. Variance components used in the calculation of broad-sense heritabilities**

Trait	Effect	Variance component	Standard deviation
Hue	Line	4091	63.96
	Residual	6678	81.72
Saturation	Line	43.68	6.609
	Residual	59.91	7.740
Brightness	Line	25.57	5.057
	Residual	38.80	6.229

**Table S2. Estimated non-parametric components of GAM model in black and white background, with the corresponding effective degrees of freedom (edf), *F*-statistic and *P* value.**

Background	Smooth effect of variable	edf	<i>F</i> -statistics	<i>P</i> value
Black	Hue	1.892	0.928	0.415
	Saturation	3.301	1.576	0.211
	Brightness	1.000	0.022	0.884
White	Hue	1.000	0.018	0.894
	Saturation	3.247	0.899	0.478
	Brightness	1.000	0.049	0.826

**Table S3. Estimated parametric components of six-parameter model, with the corresponding coefficient, standard error and *P* value.** Adjusted  $r^2$ : 0.507, *F*-statistic: 7.79 on 5 and 28 degree of freedom, *P*-value: 0.0001

	Coefficient	Standard error	<i>P</i> value
Intercept	-0.455	0.256	0.086
Hue	0.358	0.171	0.045
Saturation	0.590	0.184	0.003
Hue × Saturation	-0.190	0.205	0.362
Squared Hue	-0.638	0.169	< 0.001
Squared Saturation	0.153	0.193	0.434