

Wing Shape in the *Drosophila simulans* Species Complex

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INTRODUCTION

The *Drosophila* wing is seen as an ideal organ with which to examine developmental processes, and increasingly as an important quantitative genetic model system. Weber (1990) has demonstrated that substantial selectable genetic variation exists for wing shape in *D. melanogaster*, and QTL (Quantitative Trait Locus) mapping studies have shown that the trait is under the control of many small-effect genes (Weber *et al.* 1999, 2001), which can have different effects on separable components of wing shape (Zimmerman *et al.* 2000).

Two recent studies have measured geographic variation in wing shape in natural populations: Gilchrist *et al.* (2000) measured shape changes along three body size clines on different continents in *D. melanogaster*, and Hoffmann and Shirriffs (2002) examined a single Australian cline in *D. serrata*. Both studies found that wing size and shape vary independently, and that shape changed within each cline, but that the change was different across clines. However, while shape variation was significant, the range of values was very small, possibly indicating stabilizing selection (Gilchrist *et al.* 2000). It is unclear what relevance these subtle differences in shape have in natural populations, as very little is known about the functional implications of variation in wing morphology (Grodzinsky 1999).

Thus far wing shape has only been investigated within *Drosophila* species, and the degree to which different *Drosophilid* species differ is unknown. Here I assess interspecies variation in wing shape using the three species of the *D. simulans* complex – *D. simulans*, *D. mauritiana*, and *D. sechellia*.

MATERIALS AND METHODS

Isofemale lines: *D. simulans* – simiso1, simiso24 (obtained from A. G. Clark), S130, S131, S132, S134, S145 (European *Drosophila* Stock Center, Umea); *D. mauritiana* – 14021-0241.1, 14021-0241.2, 14021-0241.4, 14021-0241.5 (Tucson Stock Center), maur535 (A. G. Clark); *D. sechellia* – JDsec (J. David), 14021-0248.8, 14021-0248.15, 14021-0248.19 (Tucson Stock Center), S-9, S-32 (J. Roote).

Fly culture: Each of the 18 laboratory-adapted lines was maintained in vial culture on standard cornmeal/yeast/sugar medium, at 25°C on a 12:12 h light:dark cycle. To generate test flies a large population were allowed to lay, and the eggs collected into

vials at a density of 30 eggs per vial. Parents of the test flies were cultured in the same way, such that the density was controlled for two generations.

Wing size/shape data: Ten males and 10 females were collected from each line (aside from the *D. sechellia* line S-32, for which only 5 males and 5 males could be collected due to low egg hatching success). Single wings were removed from each fly and mounted on microscope slides in DPX (Merck, Ltd).

Images of wings were taken with a digital camera connected to a light microscope, and the PC software *SigmaScan Pro v.4* (Jandel Corporation) was used to map six wing vein landmarks about the perimeter of the wing. Wing size was defined as the area of the polygon formed by joining the landmark coordinates with line segments.

To investigate shape variation, landmarks from all test wings were subjected to a full Procrustes superimposition, implemented using the public domain software *tpsSuper* (version 1.07, F. J. Rohlf, <http://life.bio.sunysb.edu/morph>). This process uses iterative reflection, rotation and scaling of the original shape data to produce the best least square fit of all the landmarks of all the specimens, to minimize deviation from a consensus shape (described fully in Rohlf and Slice 1990). In order to reduce the dimensionality of the wing shape data, the resulting size-independent Procrustes coordinates were rotated to their principal components, computed using the covariance matrix, using the free statistical programming language R (<http://www.r-project.org>).

RESULTS AND DISCUSSION

PCA (Principal Components Analysis) yields a number of independent PC (Principal Components) that account for different proportions of the total variation in the data. Figure 1 plots PC1 against PC2, which account for 64.2% and 11.8%, respectively of the total shape variation. The remaining components each account for less than 9% of the variation, and as such are less likely to be biologically meaningful.

Figure 1 clearly shows that within each species the sexes are separable along the PC1 axis, with females consistently having higher values of PC1 than males. Plotting the mean of each of the six Procrustes *xy*-coordinates for each species and sex shows that males have very slightly broader wings than females (not shown), confirming previous results in *D. melanogaster* (Gilchrist *et al.* 2000).

The PC1 axis (Figure 1) also shows that the *D. sechellia* lines cluster separately from all other lines (aside from the *D. mauritiana* line maur535 at the top of the figure). The shape difference between *D. simulans* and *D. sechellia* male wings is shown in Figure 2, highlighting the broader wings of *D. sechellia* (the same is also true for female wing shape). *D. sechellia* is endemic to the Seychelles archipelago, and has reduced molecular variation compared to its sibling species (Cariou *et al.* 1990; Hey and Kliman 1993). Therefore, it is possible the species originated by a founder event, with genetic

drift largely responsible for the wing shape difference. However, without knowing any of the ecological factors that might influence *Drosophila* wing shape in nature, one cannot rule out the action of some form of selection.

The PC2 axis (Figure 1) seems to represent within-species variation. However, insufficient variation exists along this axis to assess what aspect of shape this component may represent.

Comparison of the pattern of variation across species in Figure 1 and Table 1 confirms previously published observations that wing size and shape are uncoupled. For wing size, *D. sechellia* and *D. mauritiana* are similar, while *D. simulans* has larger wings. To examine size/shape independence more formally I tested area vs. PC1 and area vs. PC2 correlations within each species and sex. Only four of the 12 tests were significant ($p < 0.05$), falling to zero after Bonferroni correction ($p > 0.05/12$).

It is unfortunate that the ecological consequences, if any, of variation in wing shape are unknown. However, any trait that varies both between populations and species is clearly of interest from an evolutionary perspective. One of the attractive features of morphological variation in the *D. simulans* complex is its amenability to genetic analysis. The species can be intercrossed to yield fertile hybrids, enabling standard QTL mapping techniques to be employed, for instance backcross designs (Macdonald and Goldstein 1999; Zeng 2000), and RIL (Recombinant Inbred Line) designs (Civetta *et al.* 2002). Studies of this nature would allow one to test whether morphological variation between species shows similar genetic architecture to variation within species.

REFERENCES

- Cariou ML, Solignac M, Monnerot M, David JR. Low allozyme and mtDNA variability in the island endemic species *Drosophila sechellia* (*D. melanogaster* complex). *Experientia* 1990; 46(1):101-4.
- Civetta A, Waldrip-Dail HM, Clark AG. An introgression approach to mapping differences in mating success and sperm competitive ability in *Drosophila simulans* and *D. sechellia*. *Genet. Res.* 2002; 79(1):65-74.
- Gilchrist AS, Azevedo RB, Partridge L, O'Higgins P. Adaptation and constraint in the evolution of *Drosophila melanogaster* wing shape. *Evol. Dev.* 2000; 2(2):114-24.
- Grodzinsky DL. *Form and Function of Insect Wings*, Johns Hopkins University Press, 1999.
- Hey J, Kliman RM. Population genetics and phylogenetics of DNA sequence variation at multiple loci within the *Drosophila melanogaster* species complex. *Mol. Biol. Evol.* 1993; 10(4):804-22.

Hoffmann AA, Shirriffs J. Geographic variation for wing shape in *Drosophila serrata*. *Evolution* 2002; 56(5):1068-73.

Macdonald SJ, Goldstein DB. A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia*. *Genetics* 1999; 153(4):1683-99.

Rohlf FJ, Slice D. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* 1990; 39(1):40-59.

Weber KE. Selection on wing allometry in *Drosophila melanogaster*. *Genetics* 1990; 126(4):975-89.

Weber K, Eisman R, Morey L, Patty A, Sparks J, Tausek M, Zeng ZB. An analysis of polygenes affecting wing shape on chromosome 3 in *Drosophila melanogaster*. *Genetics* 1999; 153(2):773-86.

Weber K, Eisman R, Higgins S, Morey L, Patty A, Tausek M, Zeng ZB. An analysis of polygenes affecting wing shape on chromosome 2 in *Drosophila melanogaster*. *Genetics* 2001; 159(3):1045-57.

Zeng ZB, Liu J, Stam LF, Kao CH, Mercer JM, Laurie CC. Genetic architecture of a morphological shape difference between two *Drosophila* species. *Genetics* 2000; 154(1):299-310.

Zimmerman E, Palsson A, Gibson G. Quantitative trait loci affecting components of wing shape in *Drosophila melanogaster*. *Genetics* 2000; 155(2):671-83.

Figure 1. Wing shape PCA

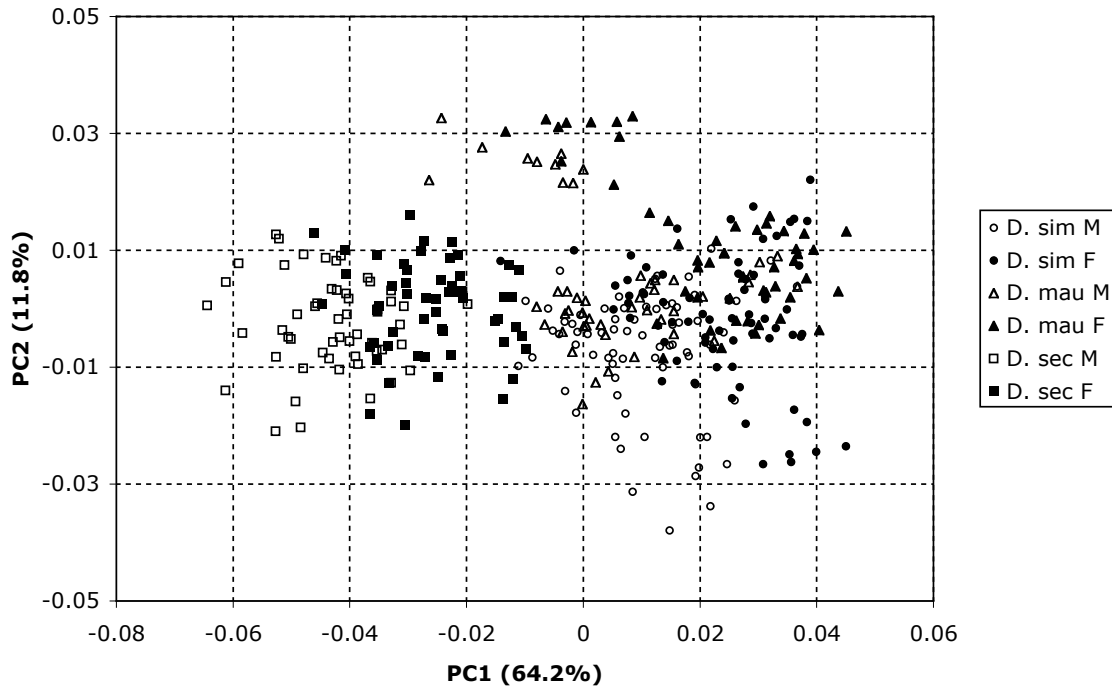


Figure 1. Plot of principal component 1 (PC1) against principal component 2 (PC2) to show variation in wing shape due to sex and species. Values in parentheses indicate the percentage of the total variation explained by each component. In the legend, “M” refers to males, and “F” refers to females.

Figure 2. Shape difference between *D. simulans* and *D. sechellia*

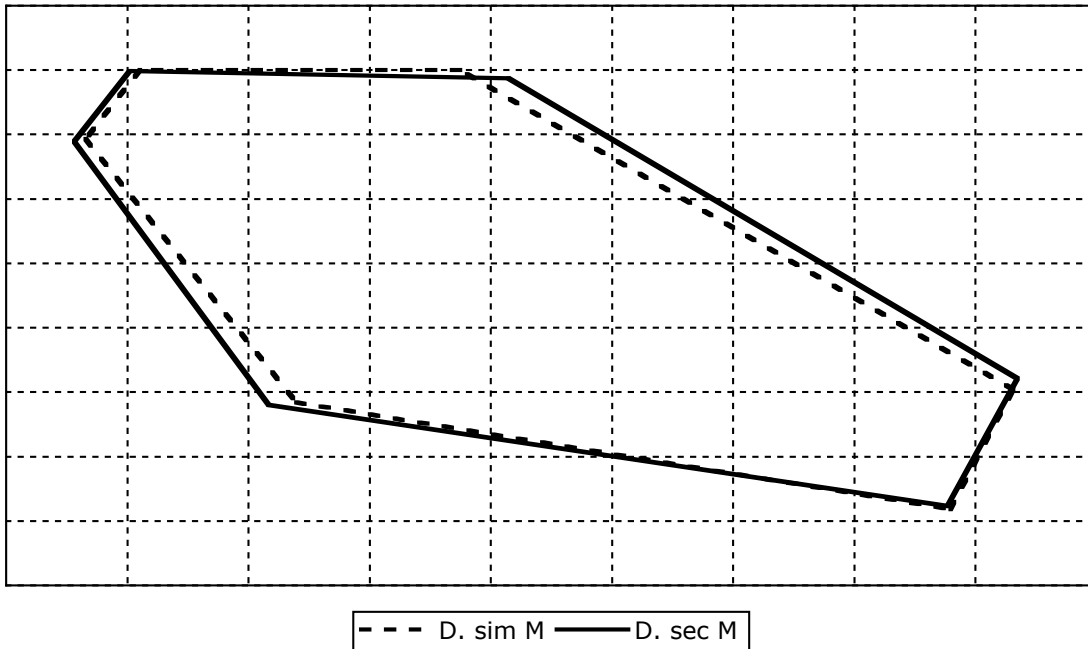


Figure 2. Plot of the six Procrustes coordinates averaged across wings within sex and species, to show the broader wing shape of *D. sechellia* males compared to *D. simulans* males.

Table 1. Mean wing area by species and sex

species	sex	N^1	wing area (mm ²)			
			mean	SD ²	max.	min.
<i>D. simulans</i>	male	70	0.788	0.057	0.884	0.641
	female	70	0.971	0.075	1.099	0.819
<i>D. mauritiana</i>	male	50	0.667	0.024	0.719	0.618
	female	50	0.843	0.034	0.900	0.730
<i>D. sechellia</i>	male	55	0.682	0.036	0.752	0.577
	female	55	0.816	0.049	0.896	0.651

¹ Number of individual wings measured.

² standard deviation.