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Phenotypic Plasticity Across 50 MY of Evolution: Drosophila Wing Size and Temperature

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Abstract

We studied the response in wing size to rearing at different temperatures of nine strains of Drosophila representing six species. The species varied in their natural habitats from tropical to temperate and one cosmopolitan. The evolutionary divergence of the species spans 50 million years. While some quantitative differences were found, all species responded to temperature very similarly: females increased an average of ~11% and males ~14% when reared at 19°C compared to 25°C. The phenotypic plasticity in wing size in response to temperature appears to be a fixed trait in Drosophila across long evolutionary time and diverse ecological settings. This likely reflects the close relationship between wing area (and thus wing loading) and insect body mass that is a crucial factor for flight regardless of ecology and is, thus, maintained across long evolutionary time.

Keywords

Drosophila; phenotypic plasticity; temperature; wing size

1. Introduction

It is well-established that insects grown at cooler temperatures are larger than when grown at higher temperature (Reviewed in Atkinson, 1998; Schlichting and Pigliucci, 1998). Drosophila, being the quintessential laboratory model insect, has been particularly well-studied. However, the vast majority of analyses of the effect of temperature on body size has been done on a single species, *Drosophila melanogaster*. Given the great ecological diversity of the genus *Drosophila* (Powell, 1997) and the well known phylogenetic relationships over ~50 million years (Powell and DeSalle, 1995; Remsen and O'Grady, 2002), we undertook a study of the effect of temperature on body size (as reflected in wing length) of species from distinct ecologies, especially with regard to tropical and temperate environments, with the expectations that species adapted to temperate and tropical climates may have different phenotypic plasticity responses with respect to temperature.

2. Materials and Methods

We analyzed the response to temperature of six species, nine strains, of *Drosophila* (Table 1). These include two primarily temperate in distribution (*pseudoobscura* and *hydei*), three tropical (*willistoni*, *tropicalis*, and *insularis*), and one cosmopolitan (*melanogaster* although it

originated in the Old World tropics). Adult females, without anesthetizing, were aspirated into 150 ml bottles with 30 ml of standard Drosophila medium (cornmeal/corn sugar/yeast/agar) and allowed to oviposit overnight at room temperature (~22° C). One to five females were in each bottle depending on species. Adults were removed after 12 hours and the bottles placed in a 19° C or 25° C incubator until adults eclosed. Replicate bottles produced between five and 20 adults, indicating minimal, if any, crowding.

Wing length was used as an estimate of body size. The distance from the r-m cross vein to the tip of the wing was measured. We used a Zeiss dissecting microscope with a Zeiss AxioCam HRc live camera attachment fed into a computer with the AxioVision software. Imaged wings were measured in pixels and converted to absolute lengths (in μm). Fifteen to 56 eclosed adults of each sex for each temperature for each strain were measured. The resulting coefficients of variation (standard deviation/mean; Sokal and Rolf, 1981) ranged from 1% to 5.1%, i.e., flies of the same species, sex, and treatment were remarkably uniform in size. This low variation indicates that the flies were affected only minimally by density or other environmental variables other than temperature.

3. Results

Statistical tests

Pair-wise T-tests as well as ANOVA across replicates all indicated highly significant differences ($P<0.01$ in all cases, $P<0.001$ in 90% of cases): (a) Between sexes of a species reared at the same temperature (females > males) and (b) Between the same sex reared at different temperatures (19° C > 25° C). Therefore in the tables we do not indicate statistical parameters, but only make conclusions based on differences with high statistical confidence.

Table 1 summarizes the results. As expected females were always larger than males and flies reared at 19° C were larger than flies reared at 25° C. The species varied in size, in order, *D. hydei* > *D. pseudoobscura* > *D. melanogaster* ≈ *willistoni* group species (*willistoni*, *tropicalis*, *insularis*). However, there were some interesting differences among strains of the same species. The strain of *D. melanogaster* obtained from the National Drosophila Species Resource Center (a *white eye* mutant strain that was used for complete genome sequencing and has been under laboratory culture since the 1930s) was larger than a wild type strain collected in St. Lucia in 2006. Evidently, the long term laboratory culturing has led to larger size despite being homozygous for a recessive mutation. The *D. willistoni* strain from the Species Stock Center was derived by four generations of single pair inbreeding from the Guadeloupe strain. The non-inbred progenitor strain is slightly larger than the inbred derivative strain.

To compare species response to temperature as well as sexual dimorphism, we present in Table 2 the proportionate differences in sexes and temperatures. The percentages shown are the difference over the smaller number, e.g., (female size - male size)/(male size), (size at 19 - size at 25)/(size at 25). For all species/strains studied, females are between 3.3% and 17.2% larger than males. On average, the sexual dimorphism was greater at 25° C (12.2%) compared to 19° C (9.4%), although not consistent across species/strains. Similarly, males tended to respond more to temperature change (13.7%) than females (11.0%) although not consistently across species/strains. Of all the strains tested, the one *D. insularis* strain from St. Lucia stands out. The sexual dimorphism at 19° C is the smallest seen (3.3%) and the male response to temperature (20.4%) was the largest.

4. Discussion

It is difficult to detect any clear-cut difference between species that are tropical in origin and those temperate in origin with regard to wing length and rearing temperature. All species have

sexual dimorphism of the same sort and all attain larger size when reared at lower temperature. This is somewhat surprising given the long evolutionary history of these species; the divergence between *D. hydei* in the subgenus *Drosophila* and the rest of the species in the subgenus *Sophophora*, occurred 40–50 mya; the *D. willistoni* group split from other Sophophorans 20–25 mya, and *D. pseudoobscura* 15–20 mya (Powell and DeSalle, 1995). While there are some quantitative differences in the level of response, the phenotypic response to temperature in the genus Drosophila appears to be a fixed trait.

The fact that tropical and temperate species of Drosophila respond to temperature in regard to size is consistent with previous studies of maintaining these species at the different temperatures for several years. Populations maintained at lower temperature become genetically larger than replicates maintained at higher temperature. This is true for the cosmopolitan species we studied here, *D. melanogaster* (Partridge et al., 1994), the temperate species *D. pseudoobscura* (Anderson, 1973), and the tropical species *D. willistoni* (Powell, 1974). Differences in wing size may be due to differences in either cell size or cell number (reviewed in Nijhout, 2003). Generally in Drosophila, the size differences in wings of flies reared at different temperatures is due to change in cell size (Robertson, 1959; Azevedo et al., 2002). Where it has been studied (*D. melanogaster*) these evolved changes under laboratory conditions reflect the phenotypic plasticity in that differences are due to change in cell size (Nijhout, 2003).

The rigidity of this phenotypic response to temperature likely reflects the close linkage between body mass and wing area that, in turn, determines wing loading (Dudley 2000). Flying insects that attain greater body mass at lower temperature need to simultaneously develop larger wings in order to maintain flight. At least in the Drosophila studied here, this linkage seems to dominate over adaptation to ecological differences such as temperature regimes in tropical versus temperate climates and, subsequently, is also evolutionarily stable.

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Table 1

Strains used and results. The numbers in the Strain Origin column are stock numbers for the National Drosophila Species Stock left, U. C. San Diego. Other strains are wildtype maintained in the author's laboratory. The numbers in the measurement columns are distance from the r-m cross vein to the wing tip in μm ± S.D.

Species	Strain Origin	Females 25C	Males 25C	Females 19C	Males 19C
melanogaster	14021-0231.36	1513 ± 30	1291 ± 30	1710 ± 34	1516 ± 63
melanogaster	St. Lucia	1278 ± 60	1101 ± 31	1435 ± 45	1268 ± 45
Pseudoobscura	14011-0121.94	1628 ± 26	1481 ± 32	1838 ± 37	1667 ± 38
willistoni	14030-0811.24	1353 ± 26	1205 ± 30	1458 ± 21	1337 ± 23
willistoni	Guadeloupe	1380 ± 19	1242 ± 25	1519 ± 30	1364 ± 28
willistoni	Ecuador	1399 ± 23	1224 ± 24	1464 ± 75	1369 ± 38
insularis	St. Lucia	1286 ± 24	1171 ± 25	1456 ± 30	1410 ± 21
tropicalis	14030-0801.00	1389 ± 18	1232 ± 67	1515 ± 18	1341 ± 29
hydei	Yale KBT	1975 ± 19	1843 ± 44	2250 ± 37	2149 ± 48

Table 2

Percent differences between sexes and percent increase from 25°C to 19°C. In both it is the difference over the smaller valued, i.e., (female – male)/male and (25 - 19)/25.

Species/strain	Δ sexes 25	Δ sexes 19	Female temp response	Male temp response
mel-SC	17.2%	12.8%	13.0%	17.5%
mel-St. Lucia	16.1%	13.2%	12.3%	15.2%
pseudoobscura	9.9%	10.2%	12.9%	12.5%
willi- SC	12.2%	9.1%	7.8%	10.9%
willi-Guad	11.1%	11.4%	10.1%	9.8%
willi- Ec	14.3%	7.0%	7.0%	11.8%
ins-St. Lucia	9.8%	3.3%	13.2%	20.4%
trop-SC	12.7%	12.9%	9.1%	8.8%
hydei-Yale	7.2%	4.7%	13.9%	16.6%
Mean	12.3%	9.4%	11.0%	13.7%