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Morphometrical evolution in a *Drosophila* clade: the *Drosophila obscura* group

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Abstract

Five morphometrical traits (wing and thorax length, ovariole number, and thoracic and female abdomen pigmentation) were investigated in laboratory stocks of 20 species belonging to the *Drosophila obscura* group (subgenus *Sophophora*). These species originated from four biogeographical regions and represent all five of the presently recognized, taxonomic subgroups. Size-related traits (wing and thorax length) were highly variable across species, and interspecific variation explained more than 90% of total variability. In both traditional and phylogenetic analyses, wing size was positively correlated with latitude of origin. These interspecific correlations were however notably weaker than those for intraspecific correlations. Wing/thorax ratio, which may be related to flight capacity, showed little variation. Ovariole number was highly variable (range 27–53) both within and between species, and was positively correlated with the wing/thorax ratio, suggesting that species with relatively large ovaries have relatively low wing loading. Although many species are completely dark, 11 had some regions of light coloration. A light thorax with a median darkening was observed in six species. A variable pigmentation of abdominal tergites, in females only, was found in nine species, belonging to three subgroups only. With respect to both molecular phylogeny and morphometrical evolution, the *D. obscura* subgroup is probably now the best investigated clade in *Drosophila*.

Key words: *Drosophila obscura* group – species comparison – body size – ovariole number – body pigmentation – phylogenetic patterns

Introduction

Understanding adaptive capacities of natural populations and species is a central problem of evolutionary biology, and the comparative method has long been a powerful tool for exploring such capacities. One example of the use of comparative evidence of adaptation comes from the common observation that species facing similar selective pressures often have similar phenotypes (i.e. convergence; Endler 1986). Interpretations of such comparative patterns face, however, various challenges (Leroi et al. 1994). For example, two species may appear similar for two opposite reasons: either they have independently evolved similar traits, or they inherited those traits from a common ancestor. Resolving this ambiguity is often only possible with specific analytical techniques (Harvey and Pagel 1991), provided the true phylogeny is known.

With more than 3000 described species (many of which can be reared in the laboratory), drosophilid flies appear as an irreplaceable model for investigating phenotypic adaptations. Most studies to date have concentrated on intraspecific comparisons, especially those investigating clines with latitude or climate. In this respect, *Drosophila subobscura* has been a most investigated species, with latitudinal clines documented in Europe (Prevosti 1955; Misra and Reeve 1964) and more recently on the American continent (Pegueroles et al. 1995; Huey et al. 2000). Latitudinal clines are documented in several other widespread species. For example, in *D. melanogaster* they concern a diversity of traits, such as morphology, physiology, behaviour, chromosome inversions or allozyme frequencies (David and Capy 1988).

From a biogeographical point of view, species can be classified, as a first approximation, either as tropical (cold sensitive) or temperate (cold tolerant). A few are able to proliferate under tropical and temperate environments, being widespread and often cosmopolitan (David and Tsacas 1981;

Parsons and Stanley 1981; Parsons 1983; Powell 1997; Gibert et al. 2001).

Despite the wealth of comparative studies at the intraspecific level in flies (above), remarkably few studies have been conducted at the species level. Most of these investigations have focused on tolerance to environmental stresses, including starvation, desiccation and temperature (Parsons 1983; Van Herrewege and David 1997; Gibert et al. 2001); and surprisingly few have examined morphological traits, except as regards phylogenetic reconstruction. Consequently, it is not known whether latitudinal increases in body size, which are usually seen within species (David and Capy 1988), also occur between species.

An interspecific, comparative analysis of several morphometrical traits in the *obscura* group of *Drosophila* (*Sophophora* subgenus) has been undertaken. This clade has several attributes that are attractive from a comparative perspective: (1) It is manageable in size (about 35 species are described) and a majority can be grown in the laboratory. (2) A large amount of information already exists on diverse aspects of the biology of this group and is reviewed in Lakovaara and Saura (1982) and Krimbas (1993). (3) Its phylogeny has been repeatedly investigated with molecular techniques (Lakovaara and Saura 1982; Cariou et al. 1988; Barrio et al. 1992; Ruttkay et al. 1992; Beckenbach et al. 1993; Gleason et al. 1997; Watabe et al. 1997; O'Grady 1999) and, although a definitive consensus is not yet achieved, the phylogeny is relatively well known. (4) Morphometrical studies have mostly considered diagnostic taxonomic features with the objective to work out a morphological phylogeny (Buzzati-Traverso and Scossiroli 1955). (5) As a group the species have a broad geographical distribution (mostly in the Holarctic region, but some have colonized the Afrotropical and the Neotropical regions). So, adaptations to climate may be expected.

Material and methods

We investigated morphometrical traits for 20 different species (Table 1), which represent about 57% of the extant species in the group. (Note that we consider *D. bogotana*, which is a South American subspecies of *D. pseudoobscura*, as a species). All flies came from laboratory strains that had generally been in culture for several years. As most species are cold tolerant and heat sensitive, we used a rearing temperature of 20–21°C for all investigations. All species were kept as mass cultures on a 16:8 LD cycle.

To obtain flies for measurement, we selected groups of 10 males and 10 females of each species as parents. These were allowed to oviposit in culture vials (on a high nutrient, killed yeast food) for only 24 h, thereby preventing overcrowding (Karan et al. 1999). Oviposition was repeated over successive days, and several culture vials were thus obtained for each species.

After emergence, adults were transferred to fresh vials kept at 21°C and measured a few days later. For each species, we measured 25 individuals of each sex taken haphazardly from several culture vials. Total wing length was measured on a lateral view of the left side, from the thoracic articulation to the tip. Thorax length was measured also on a lateral view, from the neck to the tip of the scutellum. Measurements were carried out with an ocular micrometer in a binocular microscope, and values were transformed to mm × 100. For some analyses, we used principal component scores for these two size measurements. Ovariole number was determined by dissecting females aged 4–8 days.

All species in the *D. obscura* group are dark, but not all are completely dark. In particular, how far black pigment extends onto the abdominal tergites of females (but not males) can differ and is used in taxonomy (Bächli and Burla 1985). We quantified for females the extent of the non-pigmented area, following methods used in *D. melanogaster* (David et al. 1990; Gibert et al. 2000). Phenotypic classes were visually assigned [ranging from 0 (no black pigment) to 10 (completely dark tergite)], although no *obscura*-group flies had completely un-pigmented tergites (class 0). For each species, we measured pigment extension on segments 2–7 in 25 females. In *D. melanogaster*, pigmentation variation on the thorax (mesonotum) can also be investigated by monitoring the intensity of a pigmented area with a trident shape (David et al. 1985). For the trident, four phenotypic classes can be established, ranging from 0 (no visible trident) to 3 (dark pigmented trident). We applied this approach to the *D. obscura* group.

Table 1. List of the 20 species investigated with indication of their geographical and latitudinal origin

Species	Origin	Latitude (°N)
<i>Drosophila affinis</i>	Athens, GA, USA.	33.1
<i>D. ambigua</i>	Moscow, Russia	55.8
<i>D. athabasca</i>	Seattle, WA, USA	47.4
<i>D. azteca</i>	Chilpancingo, Mexico	17.5
<i>D. bifasciata</i>	Kuusamo, Finland	66.0
<i>D. bogotana</i>	Bogota, Colombia	4.34
<i>D. guanche</i>	Canary Islands, Spain	29.3
<i>D. helvetica</i>	Gif sur Yvette, France	48.9
<i>D. imaii</i>	Sapporo, Japan	43.0
<i>D. madeirensis</i>	Madeira, Portugal	32.7
<i>D. microlabis</i>	Mt. Elgon, Kenya	1.00
<i>D. miranda</i>	Mather, CA, USA	37.9
<i>D. narragansett</i>	Bastrop State Park, TX, USA	30.1
<i>D. obscura</i>	Utrecht, Netherlands	52.0
<i>D. persimilis</i>	Mt San Jacinto, CA, USA	33.8
<i>D. pseudoobscura</i>	Flagstaff, AZ, USA	35.2
<i>D. subobscura</i>	Montgenève, France	48.4
<i>D. subsilvestris</i>	Gif sur Yvette, France	48.9
<i>D. tolteca</i>	Coroico, Bolivia	16.5
<i>D. tristis</i>	Gif-sur-Yvette, France	48.9

Results

Size-related traits in both sexes

Average wing and thorax lengths, as well as average wing/thorax ratios, are presented in Table 2. Size variation is marked across species, as is evidenced by the interspecific coefficient of variations (CVs), which average 7.96 ± 0.04 for the four cases. For wing length, *D. miranda* is the biggest species and *D. affinis* is the smallest. For thorax length, *D. miranda* and *D. tristis* are big, whereas *D. affinis* is the smallest.

For each trait and species, phenotypic variability among individuals is indexed by the associated CVs (Table 2). Intraspecific CVs were analysed with a three-way ANOVA (not shown), which showed significant main effects for sex, trait and species (all $p < 0.01$). Average CVs were slightly less for females (1.99) than for males (2.28) and also slightly less for the wing (2.02) than for the thorax (2.24). In these two cases, traits with lesser average values are more variable.

In another investigation of wing parts (Imasheva et al. 2000), shorter traits exhibited higher CVs, because of a higher relative magnitude of measurement errors. The same interpretation may be given for explaining that, on average, males are apparently more variable than females and that the thorax is apparently more variable than the wing length. If this interpretation also applies to interspecific differences, average body size should be negatively correlated with average CV. For the *obscura* group, this correlation was negative ($r = -0.33$) but non-significant ($p > 0.1$): thus measurement errors do not explain interspecific variations. However, because laboratory stocks were studied here, the low CVs of some species might reflect a prior loss of genetic and phenotypic variation from inbreeding. Alternatively, high CVs might reflect the unsuitability of the laboratory food for some species, which are notoriously hard to breed.

Wing and thorax length are positively correlated not only within species (Table 2), but also between species (Fig. 1; $r = 0.92$ and 0.93 , females and males, respectively). The distributions for the sexes overlap broadly (Fig. 1), such that males of some big species are much bigger than females of some small species. At the intraspecific level, wing–thorax correlations (Table 2) are low relative to interspecific correlations (above), and average only 0.70 ± 0.03 and 0.71 ± 0.02 in females and males, respectively. An average of 0.70 is also found in *D. melanogaster* (Karan et al. 1999) and this stability might reflect some strong internal constraints in the development of wing and thorax of *Drosophila*.

The wing/thorax ratio (Table 1) is an interesting parameter because it provides some information on wing loading, wing beat frequency and presumably flight capacity (Pétavy et al. 1997). Average values among species are identical (2.51) in males and females. Also the interspecific CVs (2.93 and 3.09) are much less variable than the traits themselves. At the intraspecific level, variability between individuals is also less than that found for wing or thorax length (Table 2). This general observation is due to the positive correlation existing between wing and thorax (David et al. 1994).

In all investigated species, males are consistently smaller than females (*t*-tests, not shown); but size variations between sexes are highly correlated ($r = 0.93$ and 0.89 for wing and thorax length, respectively). For each trait, the female/male ratio is an estimate of sexual dimorphism (David et al. 1994).

Table 2. Mean values of size-related traits in females and males of 20 species belonging to the *Drosophila obscura* group. *m*: mean (mm × 100); CV: coefficient of variation. *n* = 25 for each sex and each species

Species	Wing length				Thorax length				Wing–thorax correlation		Wing/thorax ratio			
	Female		Male		Female		Male		Female	Male	Female		Male	
	<i>m</i>	CV	<i>m</i>	CV	<i>m</i>	CV	<i>m</i>	CV			<i>m</i>	CV	<i>m</i>	CV
<i>D. affinis</i>	259.68	2.13	226.56	2.22	105.84	2.11	92.72	2.71	0.75	0.80	2.45	1.48	2.44	1.64
<i>D. ambigua</i>	322.40	1.90	280.80	0.71	127.12	2.08	111.28	1.79	0.69	0.64	2.54	1.58	2.52	1.56
<i>D. athabasca</i>	302.56	1.82	266.40	2.21	115.84	2.05	99.04	2.54	0.72	0.57	2.61	1.47	2.69	2.28
<i>D. azteca</i>	293.44	1.88	251.04	2.36	113.92	1.64	100.96	2.15	0.62	0.80	2.58	1.42	2.49	1.43
<i>D. bifasciata</i>	328.16	1.62	291.04	1.79	128.32	2.05	115.04	2.30	0.80	0.73	2.56	1.22	2.53	1.59
<i>D. bogotana</i>	304.96	1.33	267.36	2.98	115.28	1.93	105.52	1.91	0.63	0.64	2.65	1.50	2.53	2.29
<i>D. guanache</i>	286.24	2.36	277.12	2.65	117.76	3.00	114.56	2.87	0.84	0.80	2.43	1.67	2.42	1.76
<i>D. helvetica</i>	276.80	2.00	233.76	1.98	113.36	2.14	95.44	2.22	0.78	0.71	2.44	1.37	2.45	1.61
<i>D. imaii</i>	273.76	2.39	238.88	2.88	114.32	1.72	101.36	3.59	0.47	0.72	2.39	2.18	2.36	2.43
<i>D. madeirensis</i>	317.92	1.50	283.36	1.56	130.64	1.86	113.76	2.40	0.26	0.43	2.43	2.05	2.49	2.32
<i>D. microlabis</i>	271.52	2.49	242.24	2.03	108.88	2.38	98.64	2.17	0.79	0.65	2.49	1.60	2.46	1.75
<i>D. miranda</i>	343.36	1.70	308.64	1.82	130.00	1.88	115.28	1.80	0.84	0.70	2.64	1.04	2.68	1.40
<i>D. narragansett</i>	263.84	1.93	226.72	2.70	105.04	2.14	87.84	2.70	0.74	0.85	2.51	1.47	2.58	1.42
<i>D. obscura</i>	304.32	1.82	277.44	2.56	121.20	2.13	110.32	2.95	0.81	0.82	2.51	1.24	2.52	1.73
<i>D. persimilis</i>	289.12	1.76	262.88	1.35	115.60	1.58	103.04	1.69	0.55	0.73	2.50	1.60	2.55	1.15
<i>D. pseudoobscura</i>	296.96	1.71	267.68	1.61	116.24	2.00	104.40	1.83	0.71	0.62	2.56	1.42	2.55	1.50
<i>D. subobscura</i>	290.76	1.54	261.92	1.98	118.48	1.56	104.32	2.40	0.67	0.72	2.45	1.27	2.51	1.66
<i>D. subsilvestris</i>	304.00	2.40	262.72	1.85	122.96	2.11	106.00	2.61	0.81	0.67	2.47	1.42	2.48	1.94
<i>D. tolteca</i>	274.08	1.74	224.64	2.60	108.40	1.76	89.44	3.20	0.67	0.80	2.53	1.42	2.51	1.92
<i>D. tristis</i>	319.52	2.38	285.12	2.67	130.64	2.99	115.04	2.71	0.76	0.83	2.45	1.96	2.48	1.62
Mean	296.23	1.92	261.82	2.13	117.98	2.06	104.2	2.43	0.70	0.71	2.51	1.52	2.51	1.75
CV	7.61		9.04		6.89		8.3				2.93		3.09	

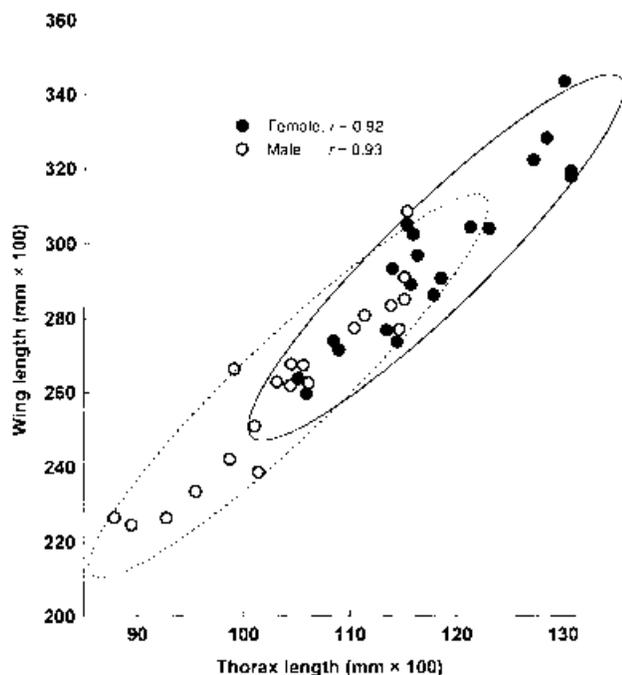


Fig. 1. Scatterplot of wing and thorax length variation observed in 20 species of the *D. obscura* group. Ellipses of 85% probability are drawn to help visualize the distributions

Average values are 1.13 ± 0.01 and identical for wing and thorax, and these values are slightly lower than those in *D. melanogaster* (David et al. 1994).

Ovariole number

Mean values for the sum of both ovaries are given in Table 3. Interspecific correlations of ovariole number with wing or thorax length were low ($r = 0.40$ and 0.22 , respectively) and non-significant. However, ovariole number was positively correlated with the wing/thorax ratio ($r = 0.54$, $p = 0.018$), although a phylogenetic correlation based on O'Grady's (1999) phylogeny was not significant ($r = 0.335$, $p > 0.10$).

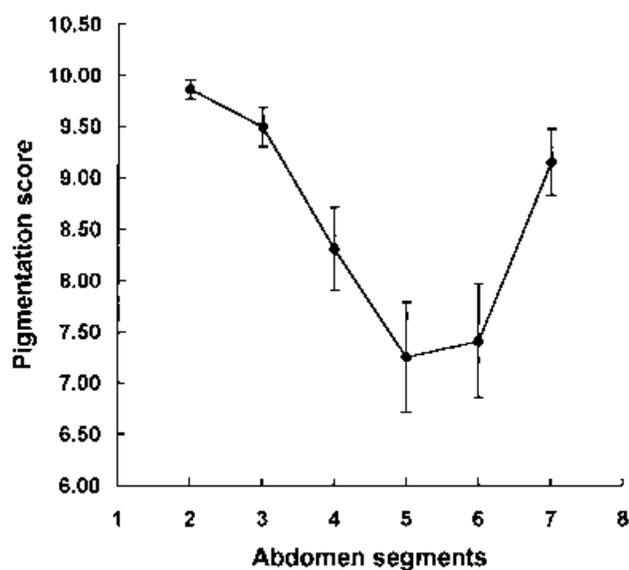
At the intraspecific level, ovariole number is much more variable than size related traits, with an average CV of 9.17 ± 0.46 , as has been found in *D. melanogaster* and *D. simulans* (Capy et al. 1994). Between species, ovariole number is also highly variable (average CV = 17.5). The lowest number (27.0 in *D. narragansett*) is only half that (53.4) found in *D. azteca*. A statistical comparison with the CVs of size traits can be carried out by considering that the square of a CV is the variance of a variable having a mean of 1, so that the variances of two different traits can be compared with a variance ratio, *F*. Comparisons of the variability of ovariole number and that of wing or thorax length gives *F* ratios of 5.3 and 6.5 (both $p < 0.01$).

Body pigmentation

Species included in the *D. obscura* group are dark in colour, but not all are uniformly black. As explained in Methods, variation in the extent of the median darkening ('trident') on the thorax could be investigated in both sexes in six of the species (Table 3). The average intensity of the median dark spot ranged from 0.68 (*D. guanache* females) to 2.40 (*D. subsilvestris* males).

Table 3. Mean values of body pigmentation traits and of ovariole number in 20 species of the *D. obscura* group. F: female; M: male; *m*: mean; CV: coefficient of variation; SD: standard deviation. *n* = 25 for each species

Species	Thoracic trident		Abdomen segments, female					Ovariole number		
	F	M	2	3	4	5	6	7	<i>m</i>	CV
<i>D. affinis</i>	1.44	1.60	9.64	8.16	6.80	5.12	5.60	8.04	32.12	7.21
<i>D. ambigua</i>	3.00	3.00	9.92	9.24	8.04	8.00	9.08	10.00	44.56	8.42
<i>D. athabasca</i>	3.00	3.00	10.00	10.00	9.76	8.88	8.64	10.00	34.24	9.32
<i>D. azteca</i>	3.00	3.00	10.00	10.00	10.00	10.00	10.00	10.00	53.44	11.10
<i>D. bifasciata</i>	3.00	3.00	10.00	10.00	9.48	8.16	7.76	8.56	41.08	11.02
<i>D. bogotana</i>	3.00	3.00	10.00	10.00	10.00	10.00	10.00	10.00	40.40	7.07
<i>D. guancho</i>	0.68	1.28	10.00	10.00	10.00	10.00	10.00	10.00	29.96	8.43
<i>D. helvetica</i>	3.00	3.00	10.00	10.00	10.00	10.00	10.00	10.00	33.32	7.83
<i>D. imaii</i>	1.64	1.73	10.00	9.44	7.76	6.00	5.96	8.96	35.00	12.01
<i>D. madeirensis</i>	1.28	1.84	10.00	10.00	10.00	10.00	10.00	10.00	30.00	7.20
<i>D. microlabis</i>	2.28	2.16	10.00	9.64	8.68	7.16	7.16	9.36	32.24	8.88
<i>D. miranda</i>	3.00	3.00	10.00	10.00	10.00	10.00	10.00	10.00	41.20	8.95
<i>D. narragansett</i>	3.00	3.00	10.00	10.00	10.00	10.00	10.00	10.00	27.04	11.32
<i>D. obscura</i>	3.00	3.00	10.00	9.36	7.40	6.52	7.48	10.00	35.92	8.87
<i>D. persimilis</i>	3.00	3.00	10.00	10.00	10.00	10.00	10.00	10.00	38.52	8.45
<i>D. pseudoobscura</i>	3.00	3.00	10.00	10.00	10.00	10.00	10.00	10.00	47.04	7.09
<i>D. subobscura</i>	3.00	3.00	10.00	10.00	10.00	10.00	10.00	10.00	32.80	7.72
<i>D. subsilvestris</i>	2.28	2.40	9.20	9.64	6.92	5.52	5.00	7.40	40.12	8.61
<i>D. tolteca</i>	1.12	1.21	10.00	10.00	10.00	10.00	10.00	10.00	36.92	8.49
<i>D. tristis</i>	3.00	3.00	10.00	9.96	9.92	9.92	10.00	10.00	43.88	15.35
Mean	2.50	2.57	9.94	9.79	9.29	8.89	8.96	9.64	37.49	9.17
SD	0.79	0.69	0.19	0.45	1.16	1.72	1.68	0.77	6.56	2.06

Fig. 2. Variation of female pigmentation score according to abdomen segment in the nine variable species of the *D. obscura* group. Vertical lines are standard errors

With respect to abdomen pigmentation, males of all species have uniformly black tergites (i.e. all scores = 10). Females of some species were, however, not uniformly black. As shown in Table 3, 11 species had completely black tergites (for segments 2–7), whereas nine others did not. One of these latter species (*D. tristis*) was very dark, with only small white areas restricted to segments 4, 5 and 6. Other species were much lighter and some had scores around 5, indicating that half the tergite area was unpigmented.

A general pattern of progressive lightening of more posterior segments becomes evident after averaging the pigment scores by segment for the nine variable species (Fig. 2). Segment 2 is very dark on average and more posterior segments become progressively lighter, except for the last segment (7).

Relationship with taxonomy

A recurrent issue in comparative studies concerns the relationship between morphology and phylogeny. In the *D. obscura* group, numerous molecular investigations have provided important insights on interspecific relationships (reviewed in Powell 1997; O'Grady 1999, Renard 2000), although a definitive evolutionary tree is not yet available. Nevertheless, five monophyletic clades or subgroups are currently recognized (O'Grady 1999). The distribution of the 20 investigated species among these subgroups is presented in Fig. 3. [Note: O'Grady (1999) did not study *D. helvetica*, but we have assigned it here to the *D. affinis* subgroup, based on chromosomal structures (Lakovaara and Saura 1982) and subsequent molecular data (Renard 2000)].

Using this phylogenetic framework, it was analysed whether species included in a given subgroup are more similar than species in different subgroups. This analysis was performed by ANOVA, species being nested within subgroups (Table 4). (Note: While a nested ANOVA is technically invalid statistically for phylogenetic data, it is nonetheless heuristically useful in indicating the relative variation associated at various taxonomic levels.) For all size-related traits and ovariole number, the mean square between subgroups was always superior to the mean square within (*F* greater than unity), but the pattern was significant in three cases only: wing length in males and thorax length in both sexes. Thus traits have a slight tendency to run in subgroups, but whether this reflects a phylogenetic constraint

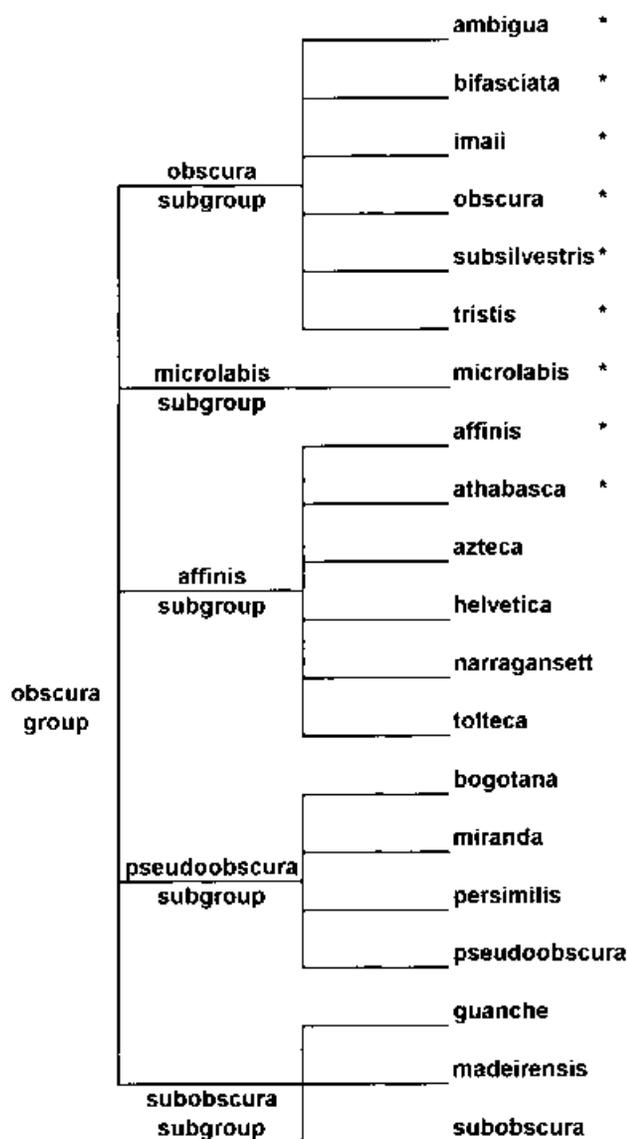


Fig. 3. Phylogenetic – taxonomic dendrogram showing the position of the 20 investigated species (after O’Grady 1999). Species marked “*” exhibit a variable abdomen pigmentation in females

or merely having recent common ancestors is not clear. It is also interesting to note that, for wing and thorax length, the subgroups explain half (51.6%) the total variability, whereas the individual level explains only 7.1% of the variability. For the wing/thorax ratio and ovariolo number, subgroup variation explains much less (27.3%), but that of individuals explains more (23.7%).

We also investigated variation in abdomen pigmentation. As shown in Fig. 3, pigmentation of females is either variable or completely black in four of five subgroups; and only the *affinis* subgroup contains both types of females. Thus pigmentation variability might be constrained by phylogeny. We checked further this hypothesis by submitting data of Table 3 to ANOVA (not shown). For two segments, nos 5 and 6, variation between segments approached significance ($p = 0.06$). Adding the values of these segments did not improve the discrimination. Therefore, female variation in coloration may be influenced by phylogeny, but more extensive investigations are needed.

Relationship with geographical origin and climate

The species investigated here originated from a very broad range of latitudes (1° to 66° ; Table 1). Because many intraspecific studies document marked clines of traits with latitude, we investigated whether interspecific clines with latitude occur in the *obscura* group flies (Table 5). We initially checked traditional (non-phylogenetic) correlations. Wing and thorax length were positively correlated with latitude (on average 0.44) but only thorax length in females was significant. Correlations of wing/thorax ratio, ovariolo number and body pigmentation with latitude were all near zero.

As wing and thorax size are strongly correlated (Table 2), we re-analysed the correlation with latitude following a principal component analysis. Both traits loaded positively on first axis of principal component analysis (PC1) (0.94, 0.34, respectively) and PC1 accounted for 99.1% of the variance. In a general linear model with latitude and sex, PC1 was weak but significantly correlated with latitude ($p = 0.026$). Next we conducted a phylogenetic correlation (a standardized independent-contrasts) of the correlation between size (PC1 score) and latitude (Felsenstein 1985; Garland et al. 1999), based on the phylogeny in O’Grady (1999). As males and females differ in size, we ran separate phylogenetic correlations for each sex and later combined probabilities by using Fisher’s combined probability test. Size was positively correlated with latitude for both sexes, although the individual correlations were insignificant in one-tailed tests [$p = 0.051$ (males); $p = 0.166$ (females)]. However, the combined correlation was significant ($p = 0.049$), confirming a weak relationship with latitude.

Discussion and conclusions

Flies of the *obscura* group are very broadly distributed. The 20 species investigated here are native to four of the world’s biogeographical regions: Palearctic, Nearctic, Neotropical and Afrotropical. Further, they occupy a broad range of latitudes, ranging from 1° (*D. microlabis*) to 66° (*D. bifasciata*). One might expect, therefore, to observe major morphological differentiations within this group and also clear correlations of traits with climate. We find that size traits do indeed show marked interspecific variability, confirming that this group is favourable for comparative investigations. However, interpreting the causes behind that differentiation is difficult for two reasons. First, the ecological niches of *obscura* group flies are often poorly known, if at all. For example, no information is available on the larval breeding sites of *D. helvetica*, although this species is very common around Paris in summer. Secondly, the exact phylogeny is not completely resolved, although the subdivision of the group into five monophyletic subgroups (O’Grady 1999) appears solid.

The interspecific differentiation of body size traits (wing and thorax length) is illustrated by two observations. First, the interspecific CV is about 8%. Secondly, the amount of variability explained by interspecific differences always accounts for more than 90% of the total variance. The wing/thorax ratio is a useful index, which might be related to flight capacity: a higher ratio implies a lesser wing loading and may be considered as an adaptation to flying in a colder climate (Stalker and Carson 1980; Pétavy et al. 1997). The ratio is far less variable than the traits themselves, with an intraspecific CV of only 1.63. This is a statistical consequence of the fact that wing and thorax length are positively correlated. Such a

Table 4. Results of a one-way ANOVA showing the relative importance of species and subgroups in the total phenotypic variability; species are nested in subgroups. df: degree of freedom; *F*: variance ratio

Source	df	Female			Male				
		Mean square	<i>F</i>	Variance explained (%)	Mean square	<i>F</i>	Variance explained (%)		
Wing length									
Subgroup	4	25455.21	2.69	ns	39.28	36086.67	4.44	*	51.30
Species	15	9451.38	290.85	***	54.70	8126.67	257.85	***	43.32
Individuals	480	32.50			6.02	31.52			5.38
Thorax length									
Subgroup	4	4464.73	4.98	**	52.12	6147.29	8.44	***	63.56
Species	15	896.31	145.33	***	39.24	728.39	110.33	***	28.24
Individuals	480	6.17			8.64	6.60			8.20
W/T ratio									
Subgroup	4	0.2665	2.87	ns	33.64	0.2129	1.55	ns	21.99
Species	15	0.0927	62.44	***	43.87	0.1374	68.66	***	53.21
Individuals	480	0.0015			22.49	0.0020			24.80
Ovariole no.									
Subgroup	4	1762.36	1.98	ns	26.31				
Species	15	891.47	67.14	***	49.91				
Individuals	480	13.28			23.78				

Significance level: ns, non significant. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

Table 5. Correlation between latitude of origin and various morphometrical traits. *p*-Values are based on one-tailed tests, and are not corrected for multiple comparisons

Trait	Female		Male	
	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
Wing length	0.42	0.10	0.40	0.07
Thorax length	0.55*	0.02	0.39	0.10
Wing/thorax ratio	-0.20	0.39	0.19	0.43
Ovariole number	0.06	0.61		

* *p* < 0.05.

strong correlation may be considered as a developmental constraint, which both reduces the variability at the intra and interspecific level. However, the proportion of the total variability explained by interspecific differences remains fairly high, above 75%. Interestingly, several species with a high ratio originate from high altitudes (e.g. *D. bogotana* and *D. miranda*), where a high ratio (thus low wing loading) might be an adaptation to flying in a cold environment or in an atmosphere with reduced air density.

Positive clines of body size with latitude are well known in several species of *Drosophila* (Parsons 1983; Capy et al. 1993; James et al. 1995; Karan et al. 1998, 2000; Huey et al. 2000). These intraspecific correlations are generally strong and the coefficients may exceed 0.90. The general interpretation is that a colder climate favours a bigger size, although the functional interpretation is far from clear (Atkinson 1994).

Between-species correlations of size and latitude are positive in the *D. obscura* group, but the correlations are notably weak (Table 5, on average, $r = 0.44 \pm 0.05$, $n = 4$) relative to intraspecific correlations. Indeed, the only significant (non-phylogenetic) correlation is that for thorax length of females. Among the phylogenetic correlations, that for size (PC1) and latitude is significant, although weakly so (combined $p = 0.049$).

The much stronger correlations for the within-species (above) than for between-species comparisons is surprising, given that size can be a very fast evolving trait in this group. For example, wing length in *D. subobscura* evolved a latitudinal cline in fewer than 20 years (Huey et al. 2000). Consequently, the relative weak correlations for the between-species comparisons suggest that size is likely to be subject to diverse selective pressures (e.g. resource utilization) that are not acting on within-species trends.

Ovariole number was also very variable, both at the intra and interspecific levels, and differences among species account for more than 75% of the total variability. Nevertheless, the magnitude of interspecific differentiation is much less than among Hawaiian species (see Kambyzellis and Heed 1971). Ovariole number is independent of body size traits ($r = 0.40$ and 0.22) and seemingly unrelated to phylogenetic subgroupings.

Interestingly, ovariole number was positively and significantly correlated with the wing/thorax ratio ($r = 0.54$). Thus species with many ovarioles have wings that are long relative to their thorax. A functional explanation can be suggested. If ovary mass is proportional to ovariole number (David 1979; Karan et al. 2000), then a higher wing/thorax ratio might be an adaptation to reducing wing loading in females with big ovaries. However, given that the phylogenetic correlation was weaker ($r = 0.335$) and non-significant, an adaptive interpretation is premature.

Although many *obscura*-group flies are uniformly dark, several species have a fairly light, not black colour, permitting a quantification of the median darker region of the thorax. This so-called 'trident' pattern was observed in six species belonging to four subgroups and is clearly independent of phylogeny. More interesting was the pigmentation of the female abdominal tergites. Nine species exhibited a variable pattern; and, as in *D. melanogaster* (David et al. 1990), the posterior margin of each tergite was always dark, while the anterior part could be almost white. The extension of the light area follows a quite regular

pattern over successive segments (Fig. 2) with a maximum in segments 5 and 6. There is no obvious explanation for females to have light pigmentation only on the anterior part of some abdomen segments. Also this pattern seems to be related to phylogeny, as only one subgroup (*D. affinis* subgroup) harbours females of the two types. In these species, perhaps variable pigmentation is a visual cue for mate recognition.

In the future, it may be expected that the phylogeny of the species in the *D. obscura* group will be completely resolved and that the morphometrical analyses should then be extended to encompass additional species in this group. Physiological data, related to the cold adaptation of some species, are also available (Moreteau et al. 1997; Gibert and Huey 2001; Gibert et al. 2001) and worth of exploration. Extant data already suggest that, among all taxonomic clades of similar size, the *D. obscura* group is the best investigated. It will be interesting to undertake similar investigations in other *Drosophila* clades, thereby providing insights into the magnitude and evolutionary speed of interspecific variability of various morphometrical traits.

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Zusammenfassung

Morphometrische Evolution in einer Klade der Gattung Drosophila: Die D. obscura-Gruppe

Fünf morphometrische Merkmale (Flügel- und Thoraxlänge, Zahl der Ovariolen, Thoraxpigmentierung und Pigmentierung des weiblichen Abdomens) wurden in Laborstämmen von 20 Arten der *Drosophila obscura*-Gruppe (Subgenus *Sophophora*) untersucht. Die Arten dieser Studie stammen aus vier verschiedenen biogeographischen Regionen und repräsentieren alle fünf gegenwärtig unterschiedenen taxonomischen Untergruppen. Die größenbezogenen Merkmale (Flügel- und Thoraxlänge) erwiesen sich als hoch variable über die Arten hinweg und die zwischenartliche Variation erklärt mehr als 90% der Gesamtvariabilität. Sowohl in den traditionellen als auch in den phyletischen Analysen zeigte sich die Flügelgröße mit der geographischen Breite des Herkunftsortes positiv korreliert. Die zwischenartlichen Korrelationen waren jedoch deutlich schwächer als die innerartlichen. Die Flügel-Thorax-Proportion, die mit der Flugfähigkeit zusammenhängen mag, zeigte wenig Variation. Die Zahl der Ovariolen war sowohl innerhalb als auch zwischen den Arten sehr variabel (Bereich 27 – 53) und positiv korreliert mit den Flügel-Thorax-Proportionen, was vermuten läßt, daß dadurch Arten mit relativ großen Ovarien eine relativ geringere Flügelbelastung haben. Obgleich viele Arten vollkommen schwarz sind, zeigte sich bei 11 Arten in einzelnen Regionen doch eine hellere Zeichnung. Ein heller Thorax mit einer medianen Verdunkelung wurde bei 6 Arten gefunden. Eine variable Pigmentierung der abdominalen Tergite, die nur bei den Weibchen auftritt, wurde bei 9 Arten von drei Untergruppen beobachtet. In Bezug auf molekulare Phylogenie und morphologische Evolution erscheint die *D. obscura*-Gruppe nun als die derzeit vermutlich best untersuchte Klade von *Drosophila*.

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