Variation in wing length in Eurasian natural populations of *Drosophila melanogaster*

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A study of 16 natural populations of *Drosophila melanogaster* from Eastern Europe, the Caucasus and Central Asia has revealed a cline in wing length associated with geographical position of the populations. Wing length was shown to be positively correlated with temperature. The coefficient of variation in wing length was significantly different in town and orchard populations. The existence of a cline in wing length in the northern part of the species range and in the region where migration must be substantial suggests strong selection pressure acting in natural populations of *D. melanogaster*.

Keywords: clines, *Drosophila melanogaster*, genetic variation, natural populations, wing.

Introduction

Among natural populations of *Drosophila melanogaster* considerable genetic differentiation in quantitative characters has been demonstrated (Lemeunier *et al.*, 1986; David & Capy, 1988; Singh, 1989). Some morphological and physiological characters exhibit latitudinal clines in this species. One of them is a well-documented cline for numerous characters, including wing length, which extends from France to tropical Africa (David & Bocquet, 1975a, b; Allemand & David, 1976; David *et al.*, 1977; Cohet & David, 1980; Cohet *et al.*, 1980). Another cline for wing length and abdominal bristle number has been reported in populations of the east coast of the U.S.A. (Coyne & Beecham, 1987).

In this paper we present evidence for clinal variation in wing length in wild populations of *D. melanogaster* that are located in the Palearctic zoogeographic region, i.e. in the northern part of the species range. The existence of three parallel clines in wing length is a strong argument for an adaptive significance of variation in this character.

Materials and methods

The flies were collected in 16 localities of Eastern Europe, the Caucasus region and Central Asia (Fig. 1). The list of collection sites, their latitudes, longitudes,

temperatures and dates of collection are given in Table 1. Since the size of the wing in natural populations of *Drosophila* is subject to seasonal changes (see e.g. Tantawy & Mallah, 1961), we have restricted the sampling period to the two hottest summer months (from mid-July to early September) when *D. melanogaster* is most abundant in the surveyed region. Collections were made in the large commercial apricot or apple orchards situated in rural areas ('orchard' samples), or on fruit stalls in city markets and in garbage bins outside wineries or fruit-processing factories ('town' samples). About 30 isofemale lines were started for each of the localities with females taken from the sample at random. After oviposition the females of the parental generation were removed from vials and their left wings were measured.

From the progeny of each isofemale line five pairs of flies were put into a separate vial, left for three days to lay eggs and discarded. To make the population density for the analysed flies uniform for all cultures, 60 eggs from each vial were removed and placed in a vial with fresh medium. After emergence of adults they were collected, five females chosen at random, their left wings removed, mounted on a slide and measured with an ocular micrometer. This gave a total sample size of about 150 wings per locality. The length of the wing was measured as the distance from the outer margin of the anterior crossvein along the third longitudinal vein to the wing tip and given in micrometer units (1 unit = 0.014 mm).

All cultures were kept on standard *Drosophila* medium at 25°C, the physiologically optimal tempera-

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Fig. 1 Collection sites. For explanation, see Table 1.

Table 1 Populations of *Drosophila melanogaster* used in this study

Locality	Country	Latitude (N)	Longitude (E)	$T_{\max}^{\dagger}$	$T_{\text{year}}^{\ddagger}$	Habitat	Time of sampling
Tartu (Tar)	Estonia	58°23'	26°44'	17.4°	5.0°	Town	Aug. 1989
Rybnoe (Ryb)	Russia	54°43'	39°30'	18.4°	3.8°	Orchard	Jul. 1989
Pyany Rog (Pya)	Russia	52°54'	33°35'	19.0°	5.3°	Orchard	Aug. 1990
Gomel (Gom)	Byelorussia	52°26'	30°59'	18.5°	6.1°	Town	Sep. 1991
Khrystinovka (Khr)	Ukraine	48°49'	29°58'	19.2°	7.3°	Orchard	Aug. 1991
Kishinev (Kis)	Moldavia	47°01'	28°50'	21.7°	9.5°	Orchard	Aug. 1990
Zabuzan (Zab)	Russia	46°32'	48°18'	25.5°	9.4°	Orchard	Sep. 1991
Astrakhan (Ast)	Russia	46°22'	48°03'	25.3°	9.4°	Town	Sep. 1990
Vilino (Vil)	Ukraine	44°52'	33°36'	23.0°	11.3°	Orchard	Jul. 1989
Alma-Ata (Alm)	Kazakhstan	43°16'	77°57'	23.4°	8.7°	Town	Jul. 1991
Akzhar (Akz)	Kazakhstan	43°07'	71°38'	23.6°	9.1°	Orchard	Jul. 1992
Jambul (Jam)	Kazakhstan	42°54'	71°23'	23.6°	9.1°	Town	Aug. 1991
Gergebil (Ger)	Russia	42°31'	47°04'	18.3°	7.2°	Orchard	Jul. 1992
Makhinjauri (Mak)	Georgia	41°40'	41°42'	22.0°	14.5°	Orchard	Jul. 1990
Cheptura (Che)	Tajikistan	38°32'	68°21'	27.9°	14.2°	Town	Sep. 1991
Bezmein (Bez)	Turkmenistan	38°03'	58°11'	30.3°	16.4°	Town	Aug. 1992

† Average temperature of the hottest calendar month.

‡ Average year temperature.

ture for this species (David *et al.*, 1983). Although in the present study there was not much danger of confusing *D. melanogaster* with its sibling species *D. simulans*, since the latter is rarely found in the region covered by the survey (Imasheva, A. G. *et al.*, unpublished data), all F_1 cultures were checked by examining males; no *D. simulans* individuals were found.

Temperature data for weather stations adjacent to collection sites were obtained from the Committee for Meteorology of the Russian Federation (Rosgidromet). The distance from collection sites to weather stations was in all cases less than 50 km (20 km on average). Two temperature variables were used: average year temperature (T_{year}) and average temperature of the hottest calendar month (T_{max} ; July for all localities except Makhinjauri for which it was August); for both parameters, 10-year averages (1980–1990) were used. Temperature values are listed in Table 1.

Statistical analysis was done using SYSTAT (version 4.1).

Results

In Table 2 average wing lengths and coefficients of variation are given for wild-caught (P) and laboratory-reared F_2 flies from the 16 studied populations (to eliminate the dependence of variance on the mean value of wing length we have used coefficients of variation rather than variance as the measure of within-population variation). The results of the nested

ANOVA for laboratory-reared flies are presented in Table 3.

Variation among populations is highly significant, showing that populations are geographically differentiated according to wing length. The isofemale lines also contribute significantly to the variation. This is to be expected since genetic determination of wing length in *Drosophila* is a firmly established fact, and heritability of this character estimated in laboratory conditions is high, varying from 0.2 to 0.6 (see e.g. Robertson & Reeve, 1952; Reeve & Robertson, 1953; Latter & Robertson, 1962).

The data in Table 2 clearly show that the average wing length in the populations under study decreases from north to south and from west to east, thus forming a cline. This is confirmed by regression analysis, which demonstrated a significant relationship between wing length and latitude and longitude of populations (Fig. 2a, b). Regression slopes were significantly different from zero both in wild-caught parents (latitude: $b = 0.782$, $P < 0.01$, $r^2 = 0.498$; longitude: $b = -0.294$, $P < 0.001$, $r^2 = 0.618$) and in laboratory-reared offspring (latitude: $b = 0.188$, $P < 0.001$, $r^2 = 0.595$; longitude: $b = -0.062$, $P < 0.001$, $r^2 = 0.574$). As can be seen from Fig. 2, the regression slope is steeper in the wild-caught flies compared with the laboratory-reared ones, which must be due to the fact that in nature flies live in a variety of habitats while in laboratory cultures environmental conditions are uniform.

No significant association with altitude was found for either wild-caught or laboratory-reared flies (the

Table 2 Mean wing length ($\bar{x}$) and coefficient of variation (CV) in 16 Eurasian populations of *Drosophila melanogaster*

Population	Habitat	Wild-caught flies (P)				Laboratory-reared flies (F_2)			
		<i>n</i>	$\bar{x}$	S.E.	CV (%)	<i>n</i>	$\bar{x}$	S.E.	CV (%)
Tartu	Town	55	116.82	0.70	4.44	150	114.03	0.30	3.26
Rybnoc	Orchard	53	115.53	0.95	5.98	150	113.50	0.33	3.53
Pyany Rog	Orchard	50	119.74	0.61	3.60	140	112.86	0.23	2.43
Gomel	Town	60	116.85	0.95	6.32	150	113.15	0.28	3.02
Khristinovka	Orchard	60	116.63	0.99	6.57	150	114.55	0.23	2.46
Kishinev	Orchard	45	114.64	1.07	6.24	150	112.68	0.22	2.37
Zabuzan	Orchard	55	102.93	1.03	7.44	150	111.45	0.24	2.67
Astrakhan	Town	33	113.64	0.55	2.77	150	112.02	0.28	3.07
Vilino	Orchard	53	110.81	1.30	8.57	150	111.20	0.28	3.11
Alma-Ata	Town	60	105.07	1.32	9.75	150	111.49	0.25	2.80
Akzhar	Orchard	60	106.73	0.82	5.94	150	110.29	0.22	2.45
Jambul	Town	55	98.24	1.41	10.65	135	111.31	0.30	3.12
Gergebil	Orchard	29	106.38	0.99	5.00	145	112.86	0.23	2.47
Makhinjauri	Orchard	27	114.00	1.68	7.67	135	112.66	0.20	2.09
Cheptura	Town	60	108.45	1.20	8.57	150	109.85	0.41	4.56
Bezmein	Town	50	101.78	0.87	6.02	150	109.94	0.29	3.25

results of regression analysis are not presented), probably because none of the populations under study was located higher than 800 m above sea level, so altitudinal climatic differences were probably not sufficient to cause population differentiation.

The regression analysis on temperature (Fig. 2c, d) has shown strong association between the wing length

of laboratory-reared populations and the hottest calendar month temperature, $T_{\max}$ ($b = -0.330$, $P < 0.001$, $r^2 = 0.769$), as well as average year temperature, T_{year} ($b = -0.281$, $P < 0.01$, $r^2 = 0.502$). In wild-caught populations, this relationship was statistically significant for $T_{\max}$ ($b = -1.112$, $P < 0.01$, $r^2 = 0.422$) and non-significant for T_{year} ($b = -0.857$, $P > 0.05$, $r^2 = 0.225$).

In order to determine the relative magnitudes of the contributions of the variables under study (latitude, longitude and the two temperature parameters) to the wing length variation, we performed multiple regression analysis, the results of which are presented in Table 4. Only the regression on $T_{\max}$ in laboratory-reared flies was significant. The association between the variables and wing length was tighter in laboratory-reared flies where it accounted for 84 per cent of between-population variation.

Table 3 Nested ANOVA of wing length for Eurasian populations of *Drosophila melanogaster*

Source of variation	d.f.	SS	MS	F
Among localities	15	4445.83	296.39	9.99***
Among lines	455	13505.33	29.68	4.47***
Error	1884	12498.80	6.63	

*** $P < 0.001$.

Fig. 2 The relationship of wing length in wild-caught (open circles) and laboratory-reared (solid circles) populations of *Drosophila melanogaster* to latitude (a), longitude (b), temperature of the hottest calendar month (c) and average year temperature (d). Regression lines: dotted, wild-caught; solid, laboratory-reared.

Table 4 Linear multiple regression of wing length on geographical and temperature variables

Variables	Wild-caught flies (P)		Laboratory-reared flies (F ₂)	
	<i>b</i>	<i>R</i> ²	<i>b</i>	<i>R</i> ²
Latitude	0.638		0.019	
Longitude	-0.127	0.700**	-0.026	0.843***
<i>T</i> _{max} †	-0.663		-0.232*	
<i>T</i> _{year} ‡	0.851		-0.001	

b, partial regression coefficient; *R*², coefficient of determination.

P* < 0.05, *P* < 0.01, ****P* < 0.001.

†Average temperature of the hottest calendar month.

‡Average year temperature.

A comparison of populations occupying different habitats showed that average wing length was not influenced by habitat either in wild-caught or in laboratory-reared flies. The same was shown for variation in wild-caught flies. In laboratory-reared flies, however, coefficients of variation were dependent on the habitat, being on average higher in populations sampled in fruit-processing plants and in markets, and lower in those sampled in fruit orchards. The difference between them was significant as shown by the Kruskal-Wallis test (Table 5).

Discussion

Geographical clines similar to the one we have revealed theoretically could result from two causes (Endler, 1977). One is the pressure of natural selection associated with some climatic variable, which in turn depends on the geographical position of the population. The alternative explanation is the interaction of genetic drift and migration. At present there is little doubt that latitudinal clines in wing length – and in body size in general – in *Drosophila* are caused by selection. The cline demonstrated in the present study (as has been) is the third reported in *D. melanogaster*. Similiar clines more or less well documented have been demonstrated for several other *Drosophila* species: *D. robusta* (Stalker & Carson, 1947), *D. subobscura* (Prevosti, 1955; Misra & Reeve, 1964; Pfriem, 1983), *D. virilis* (David & Kitagawa, 1982) and *D. simulans* (Hyytiä *et al.*, 1985).

In all of them there was positive correlation of wing size with latitude: the wings in the northern populations were larger. It is extremely unlikely that such a number of parallel clines can result from chance events.

Table 5 Kruskal-Wallis test for differences in mean wing length ($\bar{x}$) and coefficient of variation (CV) of wild-caught (P) and laboratory-reared (F₂) flies from different habitats

Habitat	Sample size	Average rank of $\bar{x}$		Average rank of CV	
		P	F ₂	P	F ₂
Orchards	9	9.33	9.44	7.94	6.00
Towns	7	7.43	7.29	9.21	11.71
Test statistic, <i>H</i>		0.63	0.81	0.28	5.67*

**P* < 0.05.

The selective factor that forms these clines is almost certainly temperature. Whenever it is colder flies' wings are larger; this is evident in seasonal variation (Tantawy & Mallah, 1961) and in association with altitude (Stalker & Carson, 1948; Louis *et al.*, 1982). This view is also confirmed by laboratory selection experiments where flies kept at a colder temperature for many generations eventually acquire bigger wings (Anderson, 1966, 1973; Powell, 1974).

In our experiment, wing length showed significant correlation with both temperature parameters used in the analysis, but the association with the temperature of the hottest month, *T*_{max}, was much stronger than that with the average year temperature, *T*_{year}. In view of the interpretation involving selection, these results seem quite reasonable. *T*_{max} is an indicator of the temperature of the warm season when selection must operate most strongly, since it is at this time that *D. melanogaster* populations are most abundant and live outdoors. On the other hand, the *T*_{year} parameter includes temperatures of colder months when in northern regions *D. melanogaster* overwinters indoors and is not subjected to factors of the external environment.

Though temperature seems to be generally accepted as a selective agent in this case, the actual mechanism of selection is not yet understood. Since wing size is positively correlated with body size (Reeve & Robertson, 1953), it is presumed that selection works directly on body size. Bergmann's rule (body size increases with latitude) is usually invoked though it does not really clarify the issue, since in poikilothermic animals like *Drosophila* it is not clear how it works. A review of possible mechanisms of selection for size by temperature is given by Coyne and Beecham (1987). One hypothetical explanation discussed by them is selection connected with developmental time of flies.

Since at higher temperatures flies develop faster and are smaller, selection favouring rapid development

could lead to smaller fly size. This hypothesis is confirmed by the results of Cohet *et al.* (1980) who demonstrated faster developmental rate for African populations of *D. melanogaster* compared with European ones. However, our preliminary experiments have not shown any differences between time of development of Central Asian and European populations (Bubli & Imasheva, 1994). There is also a possibility that selection might act on wing length in a way connected with efficiency of flight (Stalker, 1980) or with thermoregulation via the wing surface (Kingsolver & Koehl, 1985).

The two previously reported clines for *D. melanogaster* (David *et al.*, 1977; Coyne & Beecham, 1987) concerned populations inhabiting much more southern areas with climatic conditions ranging from mild to hot. Our survey includes localities from as far north as Estonia, which is probably the extreme northern edge of the range of the species (David & Capy, 1988). The fact that a stable cline exists so far north and in such drastic temperature conditions supports the view that populations of *D. melanogaster* living there are not a result of frequent (each season) recolonization from warmer regions, but are continuously living and reproducing there.

A noteworthy fact is the existence of a cline over a rather small geographical distance in a cosmopolitan domestic species like *D. melanogaster*. Even supposing that by active migration *D. melanogaster* does not cover hundreds of miles (though there is evidence to the contrary: see Coyne *et al.*, 1982; Coyne & Milstead, 1987; Coyne *et al.*, 1987), the passive transportation (with food products, etc.) must be substantial, especially in the region in question, where rotten fruits and vegetables are often transported by train in mass quantities. The existence of a cline in such conditions proves the existence of strong selection pressure.

The regressions of wing length on geographical and temperature parameters in wild-caught flies were stronger than those in laboratory ones, i.e. the clines in the wild-caught flies were much steeper. This is to be expected because of the effect of local temperature on individual development. Flies from northern parts of the range have an additional source of variation since they develop in a cold habitat which causes larger body size (and *vice versa* for flies from southern parts of the range). Note, though, that in *D. melanogaster* populations of the east coast of North America, the opposite trend was found: the latitudinal cline in wing length was shallower in wild-caught flies compared with their laboratory progeny (Coyne & Beecham, 1987).

As expected, phenotypic variation of wing length was much higher in wild-caught than in laboratory-reared flies. The ratio of the variances of wild-caught to

laboratory-reared flies was about 12, which is close to a previous estimate of 15 for a French natural population of *D. melanogaster* (David, 1979). The low estimate of this ratio reported by Coyne & Beecham (1987) for populations of the east coast of North America (between 1.5 and 3, depending on the temperatures at which laboratory flies were raised) seems puzzling as it is not obvious what differences could exist between the ecology of the sampling sites in their case and ours.

One further point to consider is the difference in variation in populations occupying different habitats. Although mean wing length did not differ in this case, the coefficient of variation was higher in populations sampled in the town areas (wineries, fruit-processing factories and markets) compared with large gardens situated in the rural areas. This might be due to a greater genetic heterogeneity of the town samples, which could consist of a mixture of flies transported there from different neighbouring localities, or to the fact that the town habitats are less uniform.

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