Heredity **61** (1988) 263–268

Received 29 February 1988

Thoracic trident pigmentation in natural populations of *Drosophila simulans*: a comparison with *D. melanogaster*

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By contrast with *Drosophila melanogaster*, wild living *D. simulans* adults generally do not exhibit a thoracic dark pigmentation with a trident shape. This trident however appears when development occurs at low temperature. A study of 26 natural populations from various parts of the world revealed a significant latitudinal cline, expressed only at 17°C. The slope of this cline and the amplitude of the variations were, however, much lower than in *D. melanogaster*. Laboratory selection on equatorial and temperate populations of *D. simulans* led to the production of lines with a very dark trident expressed at all temperatures. Crosses between selected and unselected lines showed a main or partial influence of the X chromosome, a maternal effect and a reversal of dominance according to growth temperature. Thus, *D. simulans* exhibits a large amount of genetic interpopulational variability which is not fully expressed among its geographic populations. For thoracic pigmentation, a clear contrast exists between the homogeneity of *D. simulans* natural populations and the great geographic variation found in *D. melanogaster*, an observation comparable to that made for other genetical traits in the two sibling species.

INTRODUCTION

Geographic differentiation in gene frequency may occur either as a consequence of local adaptations, or of stochastic events such as founder effects and genetic drift. Choosing between these interpretations must usually be based on indirect arguments. For a cosmopolitan species like *Drosophila melanogaster* the existence of parallel clines over different continents is a powerful argument in favour of the adaptive hypothesis (Endler, 1986). Moreover, when two closely related species, like *D. melanogaster* and *D. simulans*, which have similar geographic distributions and population dynamics (David and Tsacas, 1981), exhibit parallel genetical trends, the argument for adaptation becomes still stronger (Tantawy and Mallah, 1961; Parsons, 1983; Anderson and Oakeshott, 1984; Hyytia *et al.*, 1985; Lemeunier *et al.*, 1986).

Drosophila melanogaster is a very geographically differentiated species; less is known of its sibling (Lemeunier *et al.*, 1986), although a few studies have suggested that the two may exhibit quite different patterns of genetic variability (Ashburner and Lemeunier, 1976; Dowsett and Young, 1982; Hyytia *et al.*, 1985; Singh *et al.*, 1987).

Thoracic trident pigmentation has long been known (Morgan and Bridges, 1919; Dubinin *et al.*, 1934 cited in Merrel, 1981) as a highly polymorphic trait in natural populations of *D. melanogaster*. David *et al.* (1985) studied the phenotypic variability of that species, especially its response to environmental temperature and geographic variability. A very steep cline towards increased pigmentation was observed between 30 and 50° of latitude on different continents. Here we show that, in spite of several analogies, the interpopulational variability of *D. simulans* is much lower than in *D. melanogaster*.

MATERIALS AND METHODS

Natural populations

Wild collected females were isolated to initiate isofemale lines. The F1 progeny (in almost all cases of more than ten lines) was then mixed to produce a mass culture which was analysed either immediately after collection or after a few generations in the laboratory.

Pigmentation score

Flies were grown at low larval density on a killed yeast medium under controlled temperature. Two days after emergence, adults were examined with a binocular microscope and partitioned into four phenotypic classes, ranging from 0 (no visible trident) to 3 (dark trident). This qualitative procedure was used with *D. melanogaster* and produces very consistent results with different trained observers (David *et al.*, 1985). For each population, temperature and set, 100 individuals were observed and the average score considered as a single measure.

Selection procedure

Thoracic pigmentation is far less pronounced in *D. simulans* than in *D. melanogaster*, and is generally not visible in flies grown at 25°C. Selection was initiated by growing the flies at a low temperature (14°C) which enforces dark pigmentation. About a quarter of the most pigmented individuals (never less than 20 individuals) were taken to produce the next generation. After a few generations, pigmentation increased and a higher selection temperature could be used, first 17°C and then room temperature (between 19° and 25°C). After about 30 generations, pigmentation did not increase further but selection was continued to stabilize the phenotype.

RESULTS

Geographical variation in natural populations

Drosophila simulans pigmentation scores for the available populations, at 17 and 25°C are given in table 1. Within each population and temperature, male and female scores are highly correlated ($r = 0{\cdot}96$ and $0{\cdot}92$ at 17 and 25°C respectively). Females are on average slightly darker than males. At 25°C, all populations are very light and phenotypically similar, and their limited variations are not correlated with latitude of origin ($r = 0{\cdot}05$). At 17°C, on

Table 1 Origin and pigmentation scores of *D. simulans* populations. Average difference between sexes is significant at 17°C ($d = 0{\cdot}068 \pm 0{\cdot}017$) but not at 25°C ($d = 0{\cdot}026 \pm 0{\cdot}005$). At each temperature, values of both sexes are highly correlated (17°C: $r = 0{\cdot}94$; 25°C: $r = 0{\cdot}82$)

			17°C			25°C		
Countries	Population	Latitude	Female	Male	*d*	Female	Male	*d*
France	Draveil	48·6°N	0·91	0·75	+0·16	0·07	0·01	+0·06
France	Villeurbanne	45·7°N	0·94	0·90	+0·04	0·04	0·03	+0.01
France	Montpellier	43·4°N	0·63	0·45	+0·18	0·00	0·00	0·00
France	Moulis	42·6°N	0·64	0·53	+0·11	0·03	0·03	0·00
France	Porquerolles	43·0°N	1·29	1·18	+0·11	0·08	0·01	+0·07
France	Perpignan	42·4°N	1·00	0·95	+0·05	0·03	0·00	+0·03
Greece	Athens	37·6°N	0·63	0·65	−0·02	0·02	0·04	−0.02
Sicilly	Erice	38·0°N	0·62	0·35	+0·27	0·02	0·00	+0·02
California	Carmel	36·3°N	0·91	0·86	+0·05	0·00	0·00	0·00
Tunisia	Nasrallah	35·0°N	0.74	0·64	+0·10	0·07	0·04	+0·03
California	Pomona	34·0°N	0·91	1·03	−0·12	0·16	0·09	+0.07
Iraq	Baghdad	33·2°N	0·71	0·71	0·00	0·08	0·03	+0·05
Arizona	Tempe	33·2°N	0·91	0·84	+0·07	0·04	0·00	+0·04
Canary Is.	Puerto Cruz	32·5°N	0·89	0·82	+0.07	0·05	0·00	+0·05
Arizona	Tucson	32·1°N	1·03	1·15	−0·12	0·14	0·07	+0·07
Egypt	Alexandria	31·1°N	1·02	1·03	−0·02	0·09	0·05	+0·04
Texas	Austin	30·2°N	0·80	0·78	+0·02	0·09	0·04	+0·05
Hawaii	Hilo	19·4°N	0·96	0·87	+0·09	0·01	0·00	+0·01
Botswana	Victoria Falls	17·6°S	0·51	0·44	+0·07	0·02	0·00	+0·02
Polynesia	Moorea	17·3°S	1·12	0·84	+0·28	0·06	0·01	+0·05
Guiana	Cayenne	4·6°N	0·27	0·14	+0·13	0·00	0·00	0·00
Congo	Brazzaville	4·0°S	0·32	0·23	+0·09	0·03	0·00	+0·03
Seychelles	Mahé	4.6°S	0·27	0·15	+0·12	0·01	0·00	+0·01
Tahiti	Papeete	17·4°S	0·58	0·58	0·00	0·02	0·02	0·00
S. Africa	Johannesburg	26·0°S	0·60	0·40	+0·20	0·00	0·00	0·00
S. Africa	Cape Town	33·0°S	0·80	0·82	−0·02	0·00	0·01	−0.01
Mean	(*n*=26)		0·752	0·678	+0·068	+0·044	0·018	+0·026

the other hand, a significant increase of trident pigmentation is seen ($r = 0{\cdot}68$; $P \leqq 0{\cdot}05$) as shown in fig. 1.

Directional selection and crosses between dark and light strains

Pigmentation intensity depends on growth temperature and a significant trident is observed only in flies reared at a low temperature, 14°C for tropical populations and 17°C for temperate populations (figs. 1 and 2). Do, in spite of their low level of phenotypic pigmentation, natural populations contain sufficient genetic variability to produce phenotypically dark flies? Two populations were selected, one from the tropics (Seychelles) and the other from a temperate country (Villeurbanne, France). In 30 generations, very dark flies were obtained from each, with a pigmentation score higher than 2·5 at 25°C (fig. 2 and table 2), thus demonstrating a large amount of hidden variability in each natural population.

Crosses were made between the selected lines and their original populations. The Seychellian flies were investigated over a broad range of growth temperature (fig. 2). Males and females of the selected line were very dark and their score is close to the possible maximum value of 3. Some decrease in pigmentation is however noticed with increasing temperature. Non-selected flies were very light, and a significant darkening was found only at 14°C. F1 results differed greatly according to sex. In females which are genetically identical, a regular decrease of pigmentation is observed with increasing temperature, but the offspring of dark females are always darker than the offspring from the reciprocal cross, showing a maternal effect. A

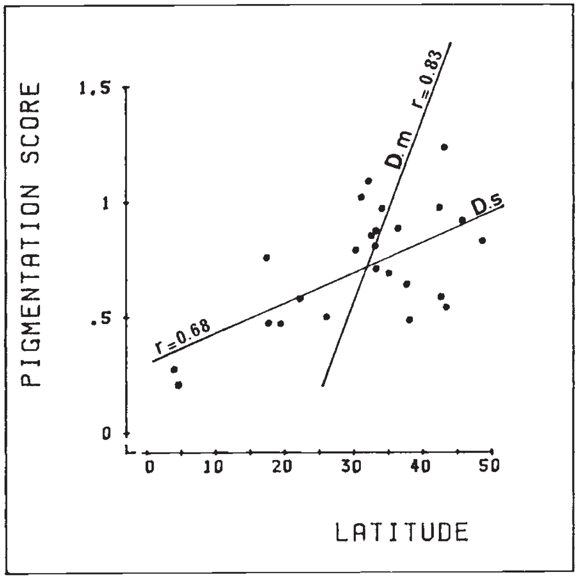

Figure 1 Relationship between pigmentation score of thoracic trident and latitude of origin in *D. simulans*. Flies were grown at 17°C (both sexes averaged). Points give the values for *D. simulans* (D.s.) populations ($r = 0{\cdot}68$; $b = 0{\cdot}010 \pm 0{\cdot}003$). For comparison the regression obtained in *D. melanogaster* (D.m.) between 30° and 50° of latitude is shown ($r = 0{\cdot}83$; $b = 0{\cdot}090 \pm 0{\cdot}014$).

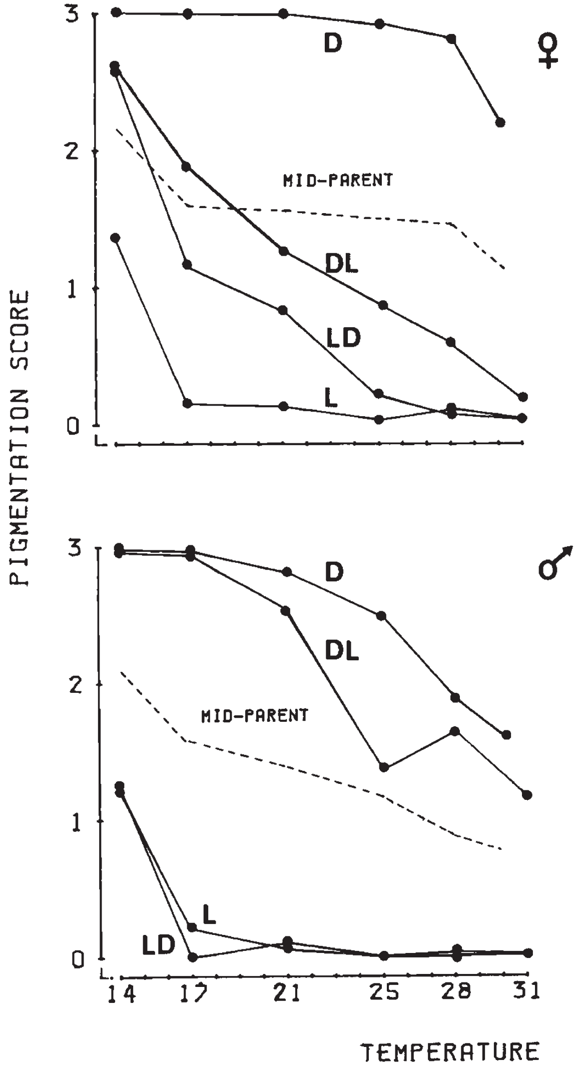

Figure 2 Relationship between growth temperature and pigmentation score in the Seychelles population of *D. simulans*. D: dark, selected line; L: light, unselected, natural strain; DL and LD: F1 individuals from respectively dark and light mothers. Confidence intervals are not shown but very small. The greatest standard error of all average values is 0·07.

Table 2 Results from crosses between a selected dark line from Villeurbanne (France) and an unselected population of the same locality

	17°C		25°C	
	Females	Males	Females	Males
Selected parent (D)	2·99	2·99	2·64	2·58
Unselected parent (L)	0·63	0·65	0·02	0·04
Mid-parent	1·81	1·82	1·33	1·31
F1♀D×♂L	1·56	2·03	1·04	1·29
F1♀L×♂D	1·32	1·27	0·90	0·70
Difference between Fl's	0·24	0·76	0·14	0·59

modification of dominance with growth temperature is also noticed: at low temperatures, dark is dominant over light, while the reverse is true at high temperatures. F1 males, on the other hand, are almost phenotypically identical to their female parent, suggesting besides a possible maternal effect, an X-linked inheritance of pigmentation.

The French selected population was studied only at 17 and 25°C and the results are given in table 2. We again find a difference between the reciprocal F1, which is more pronounced in males than in females. In females, the difference may be accounted for by a maternal effect. In males, a greater divergence presumably arises from an interaction between an X chromosome influence and a maternal effect. However, F1 males are not identical to those of their maternal strain suggesting that, in this European population, selection involved not only X-linked, but also autosomal genes.

Comparison of D. simulans with D. melanogaster

In *D. melanogaster* (David *et al.*, 1985), the geographic variability of natural populations had to be split into two parts: between the equator and 20° of north and south latitude, no significant correlation was observed; between 30° and 50° of latitude, a very steep cline was observed, at both 17° and 25°C of growth temperature. The slope of the regression line in *D. melanogaster* was 0·090 ± 0·014 and 0·055 ± 0·011 at 17° and 25°C respectively.

Overall geographic variability is much lower in *D. simulans* than in *D. melanogaster.* For example, at 17°C, the highest pigmentation score was 1·29 in the former species and 2·34 in the latter. The difference is still greater at 25°C (0·16 against 1·32: fig. 3).

DISCUSSION AND CONCLUSION

Several of these observations on the thoracic pigmentation of *D. simulans* parallel those made on *D. melanogaster* (David *et al.*, 1985). Thus, in crosses between dark and light lines, there is a maternal effect in the F1 and a modification of dominance according to growth temperature, dark being dominant at a low temperature and recessive at high temperature. There is also a significant increase of pigmentation with latitude of origin. Such an analogy is a strong argument for assuming some adaptive significance of the cline (*e.g.*, Anderson and Oakeshott, 1984). Several physiological responses may account for the pigmentation cline (see David *et al.*, 1985) among which

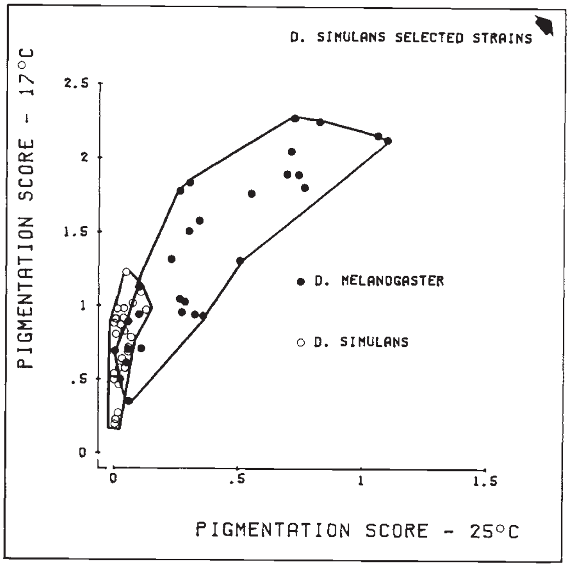

Figure 3 Relationship between pigmentation score at 17 and 25°C in the two sibling species. Note that the scores for *D. simulans* selected strains (see fig. 3 and table 2) are located outside the frame of the picture.

some relationship with heat-desiccation tolerance is most likely. In the case of *D. simulans*, the fact that the cline is only expressed at a low temperature suggests that the adaptive phenotype concerns darker flies in countries where low temperatures are encountered. A possible explanation would be that during the cold season in temperate countries, a darker cuticule increases light absorption and improves the thermal balance of the fly (Jacobs, 1982). Also, an adaptive interpretation helps to understand the dominance reversal correlated to growth temperature.

However, besides these similarities, large differences exist between the two species. In *D. melanogaster*, the temperature response curve for pigmentation intensity is U-shaped with a minimum at about 25°C, while, in *D. simulans*, there is no indication of any increase at high temperature. This difference may be due to different genetic bases for thoracic pigmentation. There is evidence that in *D. melanogaster*, several genes, carried by chromosomes 1, 2 and 3 are involved but the major effect is autosomal (see David *et al.*, 1985). In *D. simulans*, on the other hand, we have found a strong X-linked effect which could be due to a gene homologous to the *pentagon* gene (*ptg*, 1-23·2) of *D. melanogaster.*

The most striking divergence between the two species concerns the amount of variation between geographic populations, as shown in figs. 1 and 2. A similar observation, i.e. a limited geographic variability in *D. simulans*, was already observed for a diversity of genetical traits, including chromosomal polymorphism (Ashburner and Lemeunier, 1976), allozymes (Hyytia *et al.*, 1985; Watada *et al.*, 1986a; Singh *et al.*, 1987), morphometrics (Hyytia *et al.*, 1985; Watada *et al.*, 1986b) and alcohol tolerance (David and Bocquet, 1975). However, an opposite result, *i.e.*, greater variability in *D. simulans*, was found for the inter-pulse interval of courtship songs (Kawanishi and Watanabe, 1980).

Intrapopulational variability is less clear since many traits remain to be studied. For several cases such as chromosomal polymorphism (Ashburner and Lemeunier, 1976), mitochondrial DNA polymorphism (Baba-Aïssa and Solignac, 1983 and personal communication), bidimensional electrophoretic polymorphism (Onishi *et al.*, 1983), and the number of repeated genomic sequences (Dowsett and Young, 1982), the genetic diversity is much lower in *D. simulans*. For quantitative, morphological or physiological traits, the results are usually not clear and further work is needed. In the present study, thoracic pigmentation, considered as a quantitative trait, responded quickly to directional selection, thus demonstrating the presence of a large amount of genetic diversity within natural populations.

Comparative ecological genetic studies suggest that, in spite of their similar colonizing ability and ecological success, the two sibling species exhibit different genetic structures. *D. simulans* may have colonized the world more recently than *D. melanogaster*, after a founder effect (Baba Aïssa and Solignac, 1983; Singh *et al.*, 1987) and has thus had less time to adapt itself to local conditions. However, other interpretations may also be suggested, including a narrower and more uniform ecological niche in *D. simulans* than in *D. melanogaster*. There may also be genomic constraints opposing the diversification of the *D. simulans* populations. Further investigations involving more numerous genetical traits are obviously needed to understand the genetic strategies of the two sibling species.

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