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Genetic basis of some morphological differences between temperate and equatorial populations of *Drosophila melanogaster*

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Abstract. The genetic basis of three morphological traits (ovariole number, sternopleural bristle number and wing length) of *Drosophila melanogaster* has been investigated in natural populations that show great differences in these traits, i.e. Bordeaux (France) and Loua (Congo). F1 and F2 crosses, and chromosome substitutions between these two populations, were analysed. Maternal and/or X chromosome effects were found for sternopleural bristle number and wing length. For all traits, significant effects from each of the three chromosomes were found, but in general only one or two chromosomes had a major effect. Moreover, in all cases significant interactions between chromosomes were observed, suggesting the existence of epistatic effects. Our results are discussed and compared to those obtained from the analysis of selected laboratory strains.

Key words. Morphometry; geographic races; chromosome transfers; *Drosophila melanogaster*; natural populations.

Numerous quantitative genetic analyses have been made on *Drosophila melanogaster*, which is often considered as a model organism for such investigations. In most cases, heritability was estimated from strains kept under laboratory conditions for a long time. Divergent directional selections generally showed a large amount of intrapopulational variability[1,2]. In some cases, chromosome localization of major genes was investigated, such as for bristle number on chromosome 3[3,4].

On the other hand, studies of natural populations have demonstrated the existence of significant geographical quantitative variations in several *Drosophila* species. In *D. melanogaster* and *D. simulans*, latitudinal clines are observed for various morphometrical traits[5-7]. Although the precise mechanisms leading to these clines are not known, they are probably maintained by natural selection. Moreover, the genetic basis of the differences observed between natural populations remains almost unexplored. An exception is the analysis of ovariole number divergence between French and Japanese populations[8].

Therefore, the aim of this work was to investigate the genetic basis of the differences between temperate and tropical populations for 3 morphological traits. Two experimental procedures were used: first, interstrain crosses comparing parents, F1 and F2; second, chromosome transfers allowing an estimate of the contribution of each major chromosome.

Material and methods

In the present study, we investigated the genetic basis of 3 traits for which latitudinal clinal variations are known to exist between tropical Africa and Europe[6]. The 3 traits are ovariole number, sternopleural bristle number and wing length. Of these, ovariole number and wing length are presumably related to fitness and submitted to selective pressures in natural conditions, while the sternopleural bristle number is probably a more neutral trait.

The two natural populations that have been compared were taken at the two ends of the cline i.e. an Afrotropical population collected at Loua near Brazzaville (Congo, latitude 4°), and a temperate French population collected near Bordeaux (France, latitude 45°). Compared to the African population, the French one is characterized by higher mean values for the 3 traits investigated[6].

For these populations, mean values measured on the first laboratory generation are available[6]. They were then kept for one year in *Drosophila* bottles, at 20 ± 2 °C on cornmeal-sugar medium seeded with live yeast. At each generation, a large number (more than 100) of adults was transferred as parents in order to prevent any demographic bottleneck and rapid genetic drift. During each experiment (crosses and chromosome transfers) these populations were again measured. They did not differ significantly from the initial values obtained in the first laboratory generation, showing that genetic drift was negligible. To demonstrate the genetic basis of the parental differences and to investigate possible maternal effects, crosses were done between the parental populations.

A second set of experiments investigated the role of the 3 major chromosomes and their interactions. Chromosome substitutions were performed using balancer strains with dominant markers on the 2d and 3d chromosomes (CyO and Plum for chromosome 2 and TM 3

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Table 1. Means values ± SE of ovariole number, sternopleural bristle number and wing length of parental strains and their F1 and F2.

Strains (n)	Ovariole number (M ± SE)	Sternopleural bristle (M ± SE)	Wing length (M ± SE)
Bordeaux (30)	50.77 ± 0.78	19.03 ± 0.31	198.10 ± 0.96
Loua (30)	38.27 ± 0.47	15.57 ± 0.23	183.53 ± 0.64
Mean parent	44.37 ± 0.45	17.30 ± 0.19	190.00 ± 0.58
F1A (30)	43.10 ± 0.40	16.73 ± 0.44	188.83 ± 0.62
F1B (30)	43.30 ± 0.38	18.03 ± 0.18	193.23 ± 0.90
F2A (30)	43.33 ± 0.63	16.97 ± 0.51	192.17 ± 0.93
F2B (30)	43.93 ± 0.63	17.87 ± 0.48	193.10 ± 1.05

A = female of Loua × male of Bordeaux, B = reciprocal cross. Thirty flies were measured in each case. Wing length is expressed in mm × 100.

Table 2. Analysis of variance (ANOVA) of the effects of the 3 major chromosomes in substituted lines.

Sources	Ovariole number			Sternopleural bristle			Wing length		
	d.f.	MS	F	d.f.	MS	F	d.f.	MS	F
Residual	231	10.32		232	3.01		232	20.80	
Chr 1	1	66.15	6.41*	1	120.42	39.97**	1	774.00	37.22**
Chr 2	1	534.02	51.75**	1	25.35	8.42*	1	2633.44	126.63***
Chr 3	1	1325.40	128.45***	1	173.40	57.56**	1	5050.84	242.87***
Chr1 × Chr2	1	6.67	0.65 ns	1	0.07	0.02 ns	1	53.20	2.56 ns
Chr1 × Chr3	1	30.82	2.99 ns	1	0.42	0.14 ns	1	690.20	33.19**
Chr2 × Chr3	1	252.15	24.44*	1	20.42	6.78**	1	40.84	1.96 ns
Chr1 × Chr2 × Chr3	1	9.6	0.93 ns	1	29.40	9.76*	1	4.00	0.19 ns

d.f. = degree of freedom, MS = mean square, F = F-test value.
Significance: * < 5%, ** < 1%, *** < 1‰ and ns = non significant.

and Sb for chromosome 3)[9]. No marker was used for the X (chromosome 1); this chromosome was manipulated by balancing it with the Y chromosome of males. The general procedure is similar to that published by Allemand and David[10]. At the end of the crosses (6 generations), 8 different genotypes were obtained, associating the 3 chromosomes in all possible homozygous combinations.

All experimental files were grown at 25 °C, with low larval density (about 30 individuals per vial), on a killed yeast, high nutrient food. Ovariole number was measured on females while the two other traits were measured on males, as in ref. 6.

Results and discussion

Ovariole number. A highly significant difference ($p < 0.001$) characterized the two parental populations (table 1). The two F1s were almost identical, showing an absence of maternal effect. The average number (43.2) was a little less than the mid-parent value, but this possible dominance effect was not significant. The means of the two F2s were not different from the F1s, but there was an indication of an increase in variance (11.91 versus 4.51, $p < 1\%$) as expected. The analysis of the substituted lines showed that the two reconstructed parental genotypes were similar to those of the initial strains, while all the other genotypes were intermediate (fig.). An analysis of variance (table 2) indicated major effects of the second and third chromosomes, while the effect on the X chromosome, although significant, was relatively small. The interaction between chromosomes 2 and 3 was also significant. The proportion of parental difference which was explained by the direct effects of the three chromosomes corresponds to 70% (table 3). The remaining 30% was presumably due to epistatic interactions. In all cases, the chromosomes from Bordeaux increase the mean value of the trait.

These results, showing a distribution of the genes over the 3 major chromosomes with a predominant effect of chromosomes 2 and 3, are in agreement with previous data obtained by Thomas-Orillard[8,11], who compared French and Japanese populations. In a recent study, comparing two sibling species of *D. melanogaster* (*D. simulans* and *D. sechellia*)[12], significant effects of chromosomes 2 and 3, and no effect of the X, were found. This may suggest that ovariole determinism involves homologous genes among related species.

In her studies Thomas-Orillard[8,11] found a significant maternal effect which was later shown to be due to a picornavirus[13]. Although picornaviruses seem to be frequent in natural populations of *Drosophila*[14], we failed to detect any such effect. This may be due either to the presence of the same virus in both parental strains or to a complete absence.

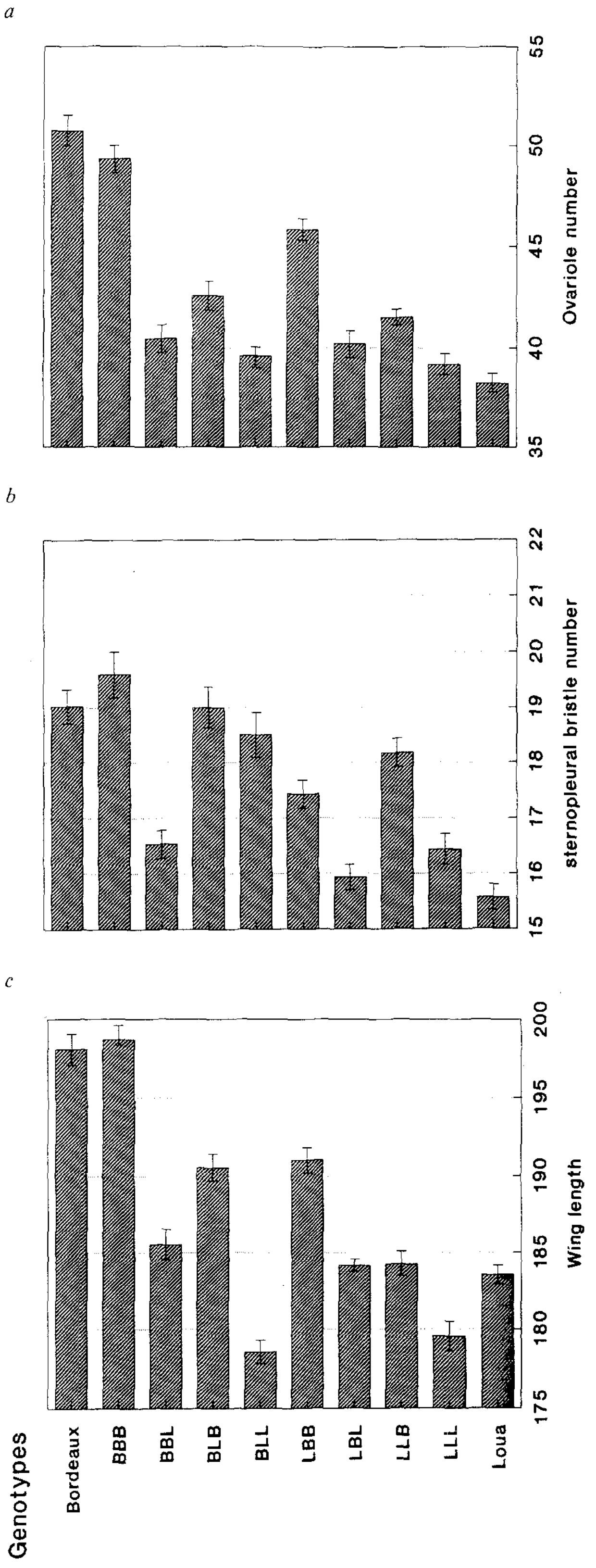

Figure. Variation in ovariole number, sternopleural bristle number and wing length in substituted chromosome lines. Bordeaux and Loua: parental populations. In the substituted genotype, B and L designate the Bordeaux and Loua chromosomes. In each genotype, the letter order corresponds to chromosomes 1, 2 and 3. Therefore, LLL corresponds to the reconstituted Loua parental genotype. *a* = ovariole number. *b* = sternopleural bristle number. *c* = wing length.

Sternopleural bristle number. The difference between the parental populations (3.46 bristles) is highly significant ($p < 1‰$, table 1). The reciprocal F1 are statistically different, but their mean value (17.38) is close to the mid-parent (17.30). Since only males were measured, this suggests an effect of the X chromosome. This interpretation is confirmed by the chromosome substitution data (table 2). A difference still occurs between the F2, as could be expected from an X chromosome effect, but its amplitude (0.9 bristle) is not significant. An increase in the variance is also found (8.15 versus 2.03).

The analysis of the substituted lines shows an intermediate position of the lines containing chromosomes of different origins, while the two lines corresponding to the parental genotypes do not differ statistically from the initial parental strains (fig.). Significant effects of all chromosomes and of interactions 1.2 and 1.2.3 are demonstrated by ANOVA (table 2). The analysis of the mean effect of each chromosome (table 3) also shows that major effects due to chromosomes 1 and 3 explain about 90% of the total difference between the two parents. Chromosomes 1 and 3 from Bordeaux increase the average value of the trait. Surprisingly, a reverse influence is observed for chromosome 2, i.e. the chromosome originating from Loua increases the mean value of the trait. From the analysis of selected laboratory strains, several authors have mentioned the existence of major genes on the third chromosome[3,4,15–17]. These observations are in agreement with our results in which the largest effect is due to the third chromosome. However, although less important, the effects of the other chromosomes remain significant, as well as several interactions. These data from natural populations distribute the effects quite evenly over the three major chromosomes, while in selected lines the effect of chromosome 3 was about 88% of the total difference[15]. This suggests that intrapopulation polymorphism is more pronounced in chromosome 3.

Wing length. For this trait, the two F1 are significantly different and they are also different from the mid-parent value. Again, the existence of a maternal and/or X chromosome effect is likely, since the F1 from Bordeaux mothers presents a higher value. In F2, the reciprocal values are not significantly different, suggesting that the difference observed in F1 was mainly due to a maternal effect, rather than an effect of the X chromosome. Both values are significantly higher than the mid-parent. A significant increase of the trait value was observed between F1A and F2A. This is difficult to explain and deserves further investigations. The analysis of the substituted lines (table 2) shows significant effects of each of the three chromosomes, with higher effects of chromosomes 2 and 3 compared to that of chromosome 1 (table 2). This result is confirmed by the analysis of the mean effects of each chromosome, which all act in the same direction (table 3). Surprisingly, the total additive contribution of the 3 chromosomes to the parental

Table 3. Mean effects of each chromosome contribution to the differences between parents.

Traits	Chromosomes	Bordeaux	Loua	Difference	Percent
Ovariole number	I	42.74	41.69	1.05	8.40
	II	43.70	40.72	2.98	23.84
	III	44.56	39.87	4.69	37.60
Sternopleural bristle	I	18.41	16.99	1.42	41.04
	II	17.37	19.25	−0.65	−18.93
	III	18.53	16.35	1.70	49.13
Wing length	I	188.35	184.76	3.59	24.64
	II	189.87	183.25	6.62	45.44
	III	191.14	181.97	9.17	69.94

Differences are also expressed in percentage of the total difference of the parent. A total percentage different from 100% is due to epistatic interactions.

difference is more than 100% (table 3). This may be explained by the existence of epistatic interactions between the different chromosomes.

From the analysis of selected laboratory strains, several authors[18,19] showed that all chromosomes were involved in the determination of wing length, with a major effect of the third chromosome and significant interactions between chromosomes. On the other hand, the estimate of the average heritability between isofemale lines from a large number of natural populations suggested that epistatic interactions were important[7]. These previous results are in agreement with the present observations. Compared with the other traits here studied, wing length is probably under the control of a general regulation during development[18,20–22]. Several phenotypic and genetic correlations have been reported between wing length and traits related to size and weight. Therefore, it is likely that the number of genes acting on this trait, including genes with pleiotropic effects, is higher than for the first two traits.

Compared to the results of directional selection, morphological differences between Afrotropical and European populations are relatively small. For ovariole number, the natural difference is 12.5 ovarioles while, by directional selection starting from a single population, it was possible to obtain an increase of more than 60 ovarioles[23]. For sternopleural bristles, we have a difference of 3.46, while, after directional divergent selections, differences of more than 30 bristles could be obtained[24]. A similar, although less striking, conclusion may be obtained from wing length studies.

Any natural population contains a large amount of hidden genetic variability. At a given place, an average phenotype is maintained over years, presumably as a consequence of stabilizing selection. Differences between natural populations imply that the stabilized phenotypes are not exactly the same. The fact that 'bigger' phenotypes are maintained in temperate populations is usually considered as a temperature adaptation[5] and some experimental confirmation was found in *D. melanogaster*[25], although one does not know why it is better to be bigger in colder places.

For each quantitative trait here investigated, a significant effect of each of the 3 major chromosomes was demonstrated. The geographic difference thus appears as a quantitative trait and is not due to the major effect of a single gene. The increase of variance, which was observed in the F2 generation, suggests that the genes are not exactly the same in the two parental populations, or that their frequencies are different.

For ovariole number and wing length, all chromosome effects are in the same direction, i.e. all the Bordeaux chromosomes contribute to an increase of the mean phenotypic values. For ovariole number, the chromosome interaction appears also to increase the parent difference, while for wing length, the interaction seems to decrease the length. It could be suggested that such an interaction, if it exists within a population, helps to stabilize the average phenotype. Finally, for sternopleural bristle number, we obtain a very surprising conclusion, i.e. the second chromosome from Bordeaux has genes which contribute to decrease the mean phenotype. This is difficult to explain if we assume that the average bristle number is the direct target of natural selection and again suggests that this number is a more neutral trait. If such is the case, the effect of chromosome 2 from Bordeaux could be a by-product of some other adaptive trait carried by this chromosome.

In conclusion, the genetic analysis of the phenotypic variability observed between natural populations of different geographic races does not provide exactly the same results as the comparison of strains obtained by directional selection under laboratory conditions. For instance, the effects of chromosome interactions, which probably correspond to epistatic effects, are greater in the differences between natural populations than between selected laboratory strains. This is probably due to different strategies of selection, i.e. selective pressures on a single trait in laboratory conditions versus selective constraints on several traits simultaneously under natural conditions.

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