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PHENOTYPIC PLASTICITY IN COLONIZING POPULATIONS OF *Drosophila subobscura*

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ABSTRACT

The phenotypic plasticity of some quantitative traits of two colonizing populations of *Drosophila subobscura* (Davis and Eureka, California) was studied. Temperature effects and the effect of rearing in the laboratory were studied. Laboratory rearing during four generations at 18ºC significantly increased the wing and tibial length. This increase was similar to that obtained when the flies were reared at 13ºC during two generations.The low temperature environment can be considered more stressful for females than for males, as shown by the increase of phenotypic variance. The two populations analyzed had great phenotypic plasticity in spite of the genetic bottleneck during the colonization event. Our study shows that keeping flies for a relatively short time in the laboratory significantly changes some quantitative traits, emphasizing the need to analyze flies immediately after collecting them in order to obtain reliable estimates for the analysis of these traits in natural populations.

INTRODUCTION

In general, flies from cooler regions tend to be larger than flies from warmer regions. Both Bergmann's and Allen's rules express the relationship between body surface and environmental temperature, but these rules may or may not concern heat conservation in poikilotherms (Ray, 1960). Size seems to be genetically controlled. Populations maintained in the laboratory at different temperatures diverge genetically with respect to body size (Anderson, 1966, 1973; Powell, 1974; Cavicci *et al*., 1989). Furthermore, *Drosophila* flies from humid tropical and temperate zones grown at different temperatures show a similar trend in body size and weight phenotypic differentiation (Ray, 1960). Powell (1974) suggested that some populations show genetic variation for body size for reasons other than temperature adaptations and it is only in the artificial, laboratory environment that temperature is the selective force on this variance. On the other hand, phenotypic variance has been shown to increase in stressful environments (Burla and Taylor, 1982; Barker and Krebs, 1995), suggesting that this phenomenon could contribute to the increase observed in genetic variation in marginal environments, with more rapid evolution during periods of special stress.

Phenotypic plasticity is exhibited as environmentally mediated change in the phenotype (Via *et al*., 1995). Although the study of phenotypic responses has a long history recently there has been a new interest in this phenomenon at both theoretical and experimental levels (review in Via, 1994). Laboratory conditions constitute a completely new environment for the flies, in which genetic variation for body size could be expressed in a different way from the wild. *Drosophila subobscura* is a Palearctic species distributed all over Europe (Krimbas, 1993). This species was detected for the first time in Puerto Montt (Chile) in February 1978 (Brncic *et al*., 1981) and subsequently it has spread very quickly all over the country. It was also detected in North America in summer 1982 (Beckenbach and Prevosti, 1986) and has become established all over the Western Pacific coast, from British Columbia to Ojai (California) (Prevosti *et al*., 1989). We examined two colonizing populations to ascertain whether the genetic bottleneck that took place during the founder event (Prevosti *et al*., 1983; Ayala *et al*., 1989; Mestres *et al*.; 1990, Balanyà *et al*., 1994) had any influence on the phenotypic plasticity of this species.

MATERIAL AND METHODS

Two American populations of *D. subobscura*, Davis and Eureka (California) were analyzed. The population of Davis (38º 32'N, 121º 46'W) is located in the Californian Central Valley (altitude 18 m) and has extreme weather conditions (the range of temperatures is between -6.1ºC and 43.9ºC, annual average 16.2ºC, annual rain 345 mm). Eureka (40º 48'N, 124º 10'W) is a coastal locality (altitude 18 m) in Northern California (the range of temperatures is between -2.8ºC and 27.8ºC, annual average 12.2ºC; annual rain 803 mm). Six quantitative traits were studied in males: wing length was measured along longitudinal vein IV, divided into two segments, L_1 (from the base of the fourth longitudinal vein to the posterior cross vein) and L_2 (from the posterior cross vein to the extreme of the media, according to Robertson and Reeve (1952) and Prevosti (1955)); wing width (W) from the extreme of the V vein to the costal border, running perpendicular to the third vein (Figure 1); tibial length (TL), and two meristic traits: number of teeth of the proximal (PC) and distal (DC) sex combs (on the right leg). In females only the continuous variables L_1, L_2, W and TL were analyzed.

The experimental procedure for each population

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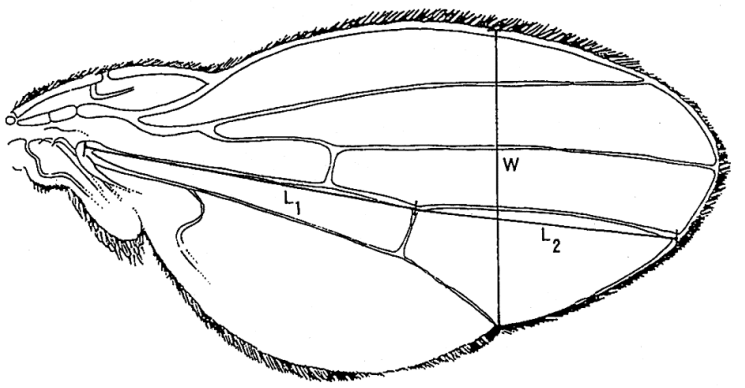

Figure 1 - Continuous variables measured in the wing of *D. subobscura* (L_1, L_2, and W). See text for details.

was as follows: 10 wild females were placed in individual rearing vials at 18ºC. All the F_1 offspring from each vial were put into plastic chambers measuring 13 x 9 x 6 cm (one plastic chamber for each vial) to obtain eggs. One day later, 100 eggs were collected from each of the 10 chambers and placed in individual vials (100 eggs per vial) in order to prevent larval competition and kept at 18ºC. The same procedure was repeated with another independent sample of 10 wild females but the vials were keat at 13ºC. For measuring the quantitative traits 10 F_2 males and 10 F_2 females were selected at random from each vial. Thus, 100 F_2 males and 100 F_2 females were analyzed for each temperature (18º and 13ºC). All these adult flies were preserved in glycerine-alcohol (1:2) until the measurement of the quantitative traits. The remaining F_2 offspring kept at 18ºC were maintained for two generations at the same temperature (100 eggs were chosen at random from each chamber in each generation to prevent larval competition), and the same procedure for the measurement of quantitative traits was carried out in the F_4 generation. The measurements were made under a compound microscope at 50X magnification with an ocular micrometer. Wing length and width and tibial length were recorded to the nearest unit of the micrometer scale, which corresponded to 0.029 mm.

The data were statistically analyzed by ANOVAS for each variable and by three one-way multivariate analyses of variance (MANOVA), considering as factors the temperature (13º and 18ºC), the population (Davis and Eureka) and the number of generations in the laboratory at 18ºC (F_2 and F_4), respectively. In all cases sexes were analyzed separately due to the significant sexual differences in the variables analyzed. A canonical analysis to study the differences among the six characterized groups (Eureka 18ºC F_2, Davis 18ºC F_2, Eureka 13ºC F_2, Davis 13ºC F_2, Eureka 18ºC F_4 and Davis 18ºC F_4) was also performed.

RESULTS

The mean values for the continuous variables L_1, L_2, W and TL (Tables I and II) were very similar for the

Table I - Mean value (M) and standard error (SE) of the continuous variables L1, L2, W and TL, for the *Drosophila subobscura* females of each group - Eureka 18º F_2, Davis 18º F_2, Eureka 13º F_2, Davis 13º F_2, Eureka 18º F_4 and Davis 18º F_4 (100 individuals measured in each group).

		18º F_2		13º F_2		18º F_4	
		Eureka	Davis	Eureka	Davis	Eureka	Davis
L_1	M	41.3	41.5	43.4	43.4	43.9	43.1
	SE	0.14	0.18	0.23	0.24	0.18	0.18
L_2	M	35.3	35.4	37.4	37.1	37.3	36.7
	SE	0.13	0.14	0.16	0.18	0.11	0.11
W	M	33.6	33.6	35.5	35.5	35.7	35.1
	SE	0.11	0.11	0.14	0.17	0.11	0.10
TL	M	16.8	16.6	17.4	17.2	17.7	17.3
	SE	0.06	0.07	0.08	0.08	0.06	0.06

L1 and L2: Segments from the base of the fourth longitudinal vein to the posterior cross vein and from the posterior cross vein to the extreme of the media, respectively; W = wing width from the extreme of the V vein to the costal border, running perpendicular to the third vein; TL = tibial length.

Table II - Mean value (M) and standard error (SE) of the continuous variables L1, L2, W and TL and the meristic variables DC and PC, for the *Drosophila subobscura* males of each characterized group - Eureka 18º F_2, Davis 18º F_2, Eureka 13º F_2, Davis 13º F_2, Eureka 18º F_4 and Davis 18º F_4 (100 individuals measured in each group).

		18º F_2		13º F_2		18º F_4	
		Eureka	Davis	Eureka	Davis	Eureka	Davis
L_1	M	37.1	37.1	39.2	38.9	38.9	38.5
	SE	0.13	0.17	0.16	0.21	0.20	0.16
L_2	M	32.7	32.8	34.4	33.8	33.8	33.8
	SE	0.10	0.12	0.12	0.14	0.12	0.11
W	M	30.8	30.9	32.4	32.1	32.3	32.1
	SE	0.10	0.11	0.11	0.16	0.12	0.09
TL	M	17.3	17.4	18.0	17.7	17.9	18.0
	SE	0.08	0.07	0.08	0.08	0.08	0.07
DC	M	9.5	9.2	9.8	9.8	9.8	9.8
	SE	0.12	0.11	0.12	0.11	0.12	0.11
PC	M	10.4	10.6	11.4	11.4	10.9	11.3
	SE	0.11	0.13	0.12	0.13	0.11	0.11

DC and PC = Distal and proximal sex combs. For other abbreviations see legend to Table I.

Table III - Results of F-test for the comparisons of variances between 18ºC (F_2 and F_4) and 13ºC (F_2) for *Drosophila subobscura* females in both populations (Davis and Eureka).

		L_1		L_2		W		TL	
		F	P	F	P	F	P	F	P
Eureka	18º F_2 - 13ºF$_2$	2.746	0.0001	1.433	0.0376	1.871	0.0010	1.979	0.0004
	18º F_4 - 13ºF$_2$	1.661	0.0061	1.955	0.0005	1.803	0.0018	1.631	0.0079
Davis	18º F_2 - 13ºF$_2$	1.848	0.0012	1.694	0.0047	2.495	0.0001	1.490	0.0243
	18º F_4 - 13ºF$_2$	1.912	0.0007	2.589	0.0001	2.974	0.0001	2.120	0.0001

For abbreviations see Table I.

13ºC F_2 and 18ºC F_4 groups, in both sexes. Furthermore, the 13ºC environment could be classified as more stressful for the females, as shown by the higher values of the standard deviation in this sex. The differences between the standard deviation at 13º and 18ºC were significant at the 0.05 level for the females in all cases (Table III).

A comparison was made between F_2 and F_4 females from Davis reared at 18ºC to determine the effect of rearing in the laboratory (Table IV). For the multivariate case, there was homogeneity of the variance-covariance matrices (P = 0.174) and the difference between the mean vectors was significant (F = 34.78 with 4 and 195 d.f.). The results were similar for the males from Davis and for both sexes in Eureka.

A comparison between F_2 males from Eureka at 13º and 18ºC was made to determine the effect of temperature (Table V). Although in this case the Box-M test of homogeneity of the variance-covariance matrices is significant (P = 0.011), the use of the MANOVA procedure is still justified because most of the signs of the correlation coefficients coincide. A decrease of 5ºC provoked a clear increase in the variances (Table III), which could reflect a loss of homeostasis. Furthermore, the mean vectors were also significantly different. This result is in agreement with those obtained by other authors (Ray, 1960; Anderson, 1966, 1973; Sokoloff, 1966; Powell, 1974). The corresponding results for females and for both sexes in Davis were equivalent. The effect of the population (Davis versus Eureka) was determined for F_4 males at 18ºC (Table VI). The variance-covariance matrices were homogeneous (P = 0.139) and the difference between the mean vectors was significant, but when considering the analysis for each variable separately, some of the groups did not differ significantly (P = 0.158 for variable L_1; P = 0.850 for L_2; P = 0.399 for W and P = 0.194 for TL).

A canonical analysis was made to determine the similarities between groups (Table VII, Figure 2). Although there was no homogeneity among the variance-covariance matrices, the elements of these matrices have, in general, the same sign, which justifies the application of the method (Cuadras, 1991). The first two canonical axes explain 89.5 and 97.91% of the total variance in males and females, respectively, which is more than sufficient for the bi-di-

Table IV - Effect of rearing in the laboratory on the quantitative variables L_1, L_2, W and TL with the statistical values (F), degrees of freedom (d.f.) and significance levels. Comparison of Davis 18ºC F_2 and Davis 18ºC F_4 populations (200 *Drosophila subobscura* females measured).

Variable	F	d.f.	Significance of F
L_1	40.082	1,198	0.000
L_2	54.024	1,198	0.000
W	113.075	1,198	0.000
TL	67.438	1,198	0.000
Multivariate case	34.780	4,195	0.000

For abbreviations see Table I.

Table V - Effect of temperature on the quantitative variables L_1, L_2, W and TL and meristic variables PC and DC with the statistical values (F), degrees of freedom (d.f.) and significance levels. Comparison of Eureka 18ºC F_2 and Eureka 13ºC F_2 populations (200 *Drosophila subobscura* males measured).

Variable	F	d.f.	Significance of F
L_1	101.653	1,198	0.000
L_2	114.672	1,198	0.000
W	111.928	1,198	0.000
TL	36.394	1,198	0.000
PC	40.064	1,198	0.000
DC	2.837	1,198	0.094
Multivariate case	38.290	6,193	0.000

For abbreviations see Tables I and II.

Table VI - Effect of population on the quantitative variables L_1, L_2, W and TL and the meristic variables PC and DC based on the statistical values (F) with degrees of freedom (d.f.). Comparison of Davis 18ºC F_4 and Eureka 18ºC F_4 populations (200 *Drosophila subobscura* males measured).

Variable	F	d.f.	Significance of F
L_1	2.0119	1,198	0.158
L_2	0.036	1,198	0.850
W	0.713	1,198	0.399
TL	1.698	1,198	0.194
PC	6.887	1,198	0.009
DC	0.015	1,198	0.092
Multivariate case	3.435	6,1193	0.003

For abbreviations see Tables I and II.

Table VII - Results of the canonical analysis of the characterized groups (see legend to Figure 2).

	MALES		FEMALES	
Function	Eigenvalue	Cum. Pct.	Eigenvalue	Cum. Pct.
1	0.424	80.40	0.556	86.83
2	0.048	89.50	0.071	97.91
3	0.038	96.76	0.011	99.68
4	0.012	99.03	0.002	100.0
5	0.0051	100.0		

Number of significant dimensions (after Wilk's) for males:

Function	Lambda	Chi-square	d.f.	Significance
0	0.634	269.882	30	0.0
1	0.904	60.186	20	0.0
2	0.947	32.387	12	0.0012
3	0.983	10.088	6	0.1210
4	0.995	3.029	2	0.2199

Number of significant dimensions (after Wilk's) for females:

Function	Lambda	Chi-square	d.f.	Significance
0	0.592	311.100	20	0.0
1	0.921	48.613	12	0.0
2	0.987	7.895	6	0.2459
3	0.998	1.223	2	0.5425

Coordinates of the mean groups:

Group	Index	Function 1	Function 2	Function 1	Function 2
Eureka 18º F_2	1	-0.92001	-0.14032	-1.01323	0.23210
Davis 18º F_2	2	-0.84654	0.08096	-1.02951	-0.17601
Eureka 13º F_2	3	0.77856	-0.00881	0.55582	-0.14499
Davis 13º F_2	4	0.44738	-0.28424	0.41042	-0.42213
Eureka 18º F_4	5	0.31569	-0.06450	0.81868	0.31388
Davis 18º F_4	6	0.22502	0.41691	0.25782	0.19715

Cum. Pct., Cumulative percentage.

mensional representation of the characterized groups. The 18ºC F_2 group from both populations clearly separates from the other two groups (18ºC F_4 and 13ºC F_2), both in males and females.

DISCUSSION

In general, the response of body size to temperature is considered to be adaptive and due to natural selection. The developmental system responding to the growth environment could be a phenomenon of adaptive plasticity (Schmalhausen, 1949; Bradshaw, 1965; Gomulkiewicz and Kirkpatrick, 1992). The increase in body size and cell size resulting from development at low temperature has also been considered a case of adaptive phenotypic plasticity (Partridge *et al*., 1994). David *et al.* (1994) also detected a response of wing and thorax lengths to temperature but found significant variations between lines and significant line-temperature interactions, demonstrating different norms of reaction among the various lines. Altogether, in the present study the main conclusions that can be drawn from the MANOVA results and the canonical analyses of the characterized groups are the following: temperature, although important, is not the only factor that explains the phenotypic differentiation of *Drosophila* flies kept in the laboratory. The size of the flies reared in the laboratory at 18ºC for four generations was equivalent to the size of the flies reared at 13ºC for only two generations. The response was much clearer for continuous variables. Meristic variables (number of teeth of the sex combs) did not differentiate appreciably. The multivariate mean tests were always significant, whether we consider laboratory rearing, temperature or the population effects. This is expected due to the sensitivity of the multivariate techniques and to the sample size. On the other hand, the univariate tests show similarity between the Davis and Eureka populations. As pointed out above, the colonization of the American continent by *D. subobscura* is a recent phenomenon. The colonizing populations are very much alike genetically (Prevosti *et al*., 1988 and 1989; Ayala *et al*., 1989; Mestres *et al*., 1990, 1992, 1995; Balanyà *et al*., 1994). This genetic similarity could explain the resemblance between Eureka and Davis populations in terms of quantitative traits: the multifactorial genotype controlling these traits would not have differentiated significantly since the colonization took place, in spite of the environmental differences between these two localities (Pascual *et al.*, 1993). The two populations analyzed still show great phenotypic plasticity in spite of the genetic bottleneck during the founder event (Prevosti *et al.*, 1989). This result is in agreement with the empirical evidence obtained from *Drosophila* and housefly populations, supported by several theoretical models (Bryant *et al*. 1986; Goodnight, 1987; Lewin, 1987; Carson, 1990) indicating that genetic variance available to selection may actually increase following a population bottleneck.

Finally, analysis of the correlation of quantitative traits with environmental factors has been widely used to detect natural selection in the wild (Ford, 1975; Endler, 1986), and some data are available on the existence of latitudinal clines for quantitative traits in Palearctic populations of *D. subobscura* (Prevosti, 1955, Misra and Reeve, 1964; Pfriem, 1983; Pegueroles *et al*., 1995). Nevertheless, studies of this kind rely heavily on all samples being reared under the same laboratory conditions prior to measurement. As it is clearly shown in our analysis of the effect of rearing in the laboratory, all samples should be measured immediately after being collected, or at least be kept in the laboratory for the same period of time, to get reliable estimates of the existence of natural selection acting on quantitative traits in natural populations.

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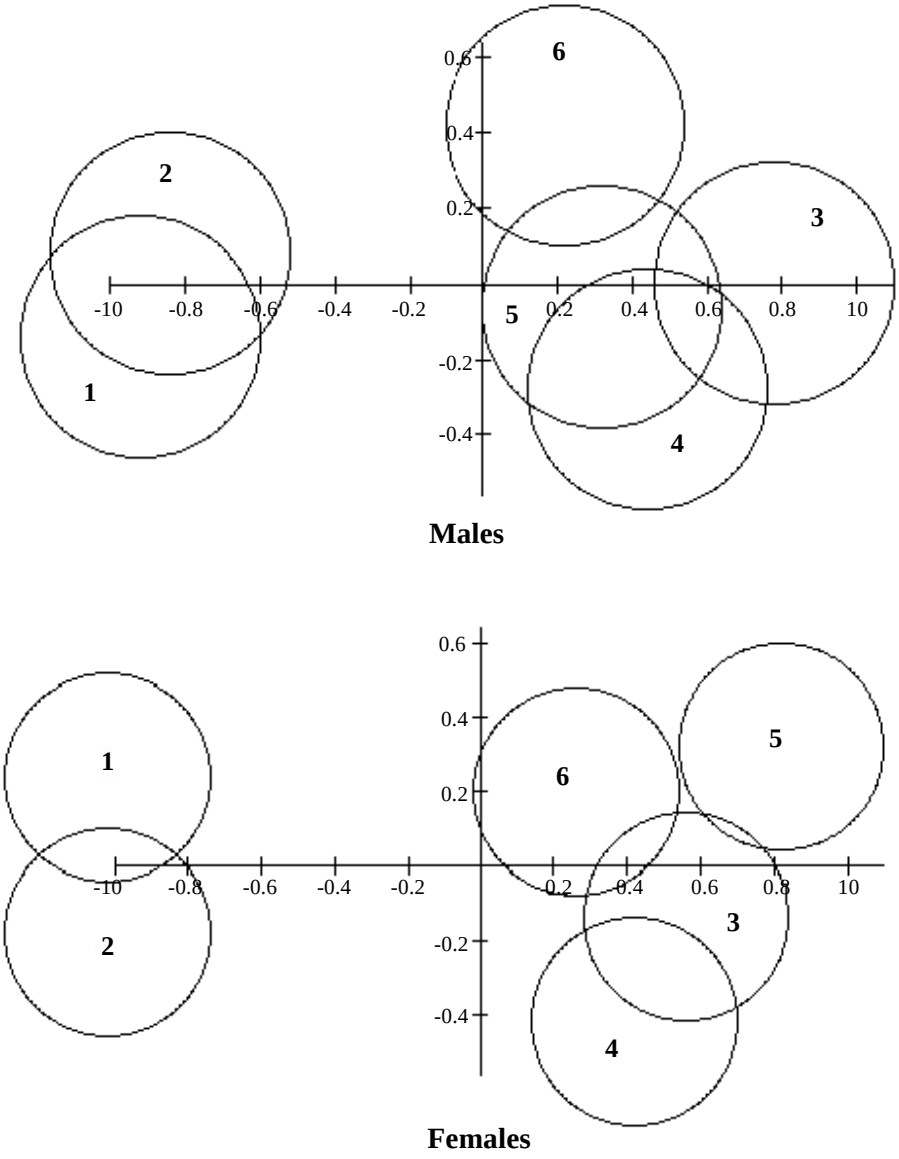

Figure 2 - Canonical representation of the characterized groups (1. Eureka 18ºC F_2, 2. Davis 18ºC F_2, 3. Eureka 13ºC F_2, 4. Davis 13ºC F_2, 5. Eureka 18ºC F_4, and 6. Davis 18ºC F_4) at the 90% confidence level (radii = 0.33 for males and 0.28 for females). For both populations (Eureka and Davis) the group 18ºC F_2 clearly separates from the other two groups (18ºC F_4 and 13ºC F_2).

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RESUMO

A plasticidade fenotípica de alguns caracteres quantitativos foi estudada em duas populações colonizadoras de *Drosophila subobscura* (Davis e Eureka, Califórnia). Analisaram-se tanto o efeito da temperatura como o da criação em laboratório. A criação em laboratório durante quatro gerações a 18 ºC aumentou significativamente o comprimento da asa e da tíbia. Este incremento foi semelhante ao obtido quando as moscas foram cultivadas a 13 ºC durante duas gerações. O ambiente de temperatura baixa pode ser considerado mais estressante para as fêmeas, pois elas apresentaram um aumento na variância fenotípica. As duas populações analisadas apresentaram uma grande plasticidade fenotípica, apesar do "gargalo" genético produzido durante o processo colonizador. Nossos estudos mostram que a manutenção das moscas no laboratório por um período de tempo relativamente curto é capaz de mudar significativamente alguns caracteres quantitativos, sendo fundamental analisar as moscas imediatamente após capturá-las, para se obterem estimativas confiáveis na análise de tais caracteres nas populações naturais.

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