Heritability of Two Morphological Characters Within and Among Natural Populations of *Drosophila melanogaster*

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ABSTRACT

Heritabilities of wing length and abdominal bristle number, as well as genetic correlations between these characters, were determined within and among populations of *Drosophila melanogaster* in nature. Substantial "natural" heritabilities were found when wild-caught flies from one population were compared to their laboratory-reared offspring. Natural heritabilities of bristle number approximated those derived from laboratory-raised parents and offspring, but wing length heritability was significantly lower in nature than in the laboratory. Among-population heritabilities, estimated by regressing population means of wild-caught flies against those of their laboratory-reared descendants, were close to 0.5. The genetic differentiation of populations was clinal with latitude, and was accompanied by significant geographic differences in the norms of reaction to temperature. These clines are similar to those reported on other continents and in other Drosophila species, and are almost certainly caused by natural selection. Genetic regressions between the characters reveal that the cline in bristle number may be a correlated response to geographic selection on wing length, but not vice versa. Our results indicate that there is a sizable genetic component to phenotypic variation within and among populations of *D. melanogaster* in nature.

MORPHOLOGICAL differences among natural populations are frequently attributed to natural selection, but the role of nongenetic modification by the environment has often been neglected. Controlled breeding or transplant experiments sometimes given surprising results: population differences observed in nature may disappear, dwindle, or even be reversed when animals are reared under identical conditions (LEVINS 1969; BERVEN, GILL and SMITH-GILL 1983; JAMES 1983). The interpretation of population differences expressed in a common environment depends on the pattern of geographic variation in nature. Samples of Drosophila, for example, often show latitudinal clines in body size when reared in the laboratory (PREVOSTI 1955; TANTAWY and MALLAH 1961; DAVID and BOCQUET 1975). This suggests the action of natural selection, but the target of selection cannot be body size itself unless similar geographic differences are observed in free-living flies.

Genetic analysis of variation among individuals *within* a population in nature may, on the other hand, show the susceptibility of the character to evolution by natural selection and its sensitivity to environmental variation not seen in the laboratory. Measuring heritabilities and genetic correlations in nature also allows estimation of the rates and directions of short-term evolution (DICKERSON 1955), reconstruction of historical patterns of natural selection (LANDE 1979) and of the targets of natural selection in a set of evolving correlated characters (PRICE, GRANT and BOAG 1984), and determination of short-term evolutionary constraints (LANDE 1979). In addition, it is important to know whether heritable variation is as abundant in nature as in domesticated and laboratory populations (FALCONER 1981); perhaps environmental variation in the wild results in much lower heritability. Such "natural heritability" studies can be done by correlating either parental and offspring characters in nature (SMITH and DHONDT 1980; VAN NOORDWIJK, VAN BALEN and SCHARLOO 1980; BOAG 1983; GUSTAFFSON 1986) (cross-fostering is necessary to eliminate any effects of a common family environment) or correlating the characters of wild-caught individuals with those of their laboratory-reared offspring (HIGHTON 1960; UNDERHILL 1969). Estimates of "natural heritability" are at present limited to vertebrates.

The relative contributions of genes and environment to phenotypic variation of Drosophila in the laboratory have been widely studied, but little is known of their role in free-living populations. Estimation of heritability in nature requires rearing of family groups in the wild, a procedure not feasible with Drosophila. Such heritabilities might be approximated by correlating phenotypic characters of wild-caught parents with those of their laboratory-reared offspring, a method employed by PROUT (1958) in a study of wing length in *Drosophila melanogaster*. Using a half-sib analysis from wild-caught males mated to

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laboratory-reared females, he obtained a narrow-sense heritability of −0.41. This contrasts with reports of large positive wing length heritabilities from studies of laboratory-reared flies (ROBERTSON and REEVE 1952; REEVE and ROBERTSON 1953). PROUT (1958) concludes that "the wing length of a fly picked up in the field allows no prediction as to the wing length of his laboratory offspring." The possibility of very small heritabilities in nature is important in predicting the genetical implications of selection on wild populations, for example PARTRIDGE, HOFFMANN and JONES's (1987) observation that male wing length is correlated with mating probability in the wild.

There is also little information about the genetic component of *among*-population variation in nature. Although many characters such as body size, bristle number, or ovariole number very geographically, the measured flies are almost always reared in the laboratory (STALKER and CARSON 1947; PREVOSTI 1955; LEMEUNIER *et al.* 1986). Only the studies of SOKOLOFF (1965, 1966) allow the comparison of variation in both wild and laboratory-reared flies from different populations; he found a weak correlation (see DISCUSSION). LEVINS (1969) observed phenotypic differences between two natural populations of *D. melanogaster* that were opposite in direction to those of their laboratory-reared offspring. Such "countergradient variation" was also found in frogs (BERVEN *et al.* 1979).

Here we investigate the effect of the natural environment of *D. melanogaster* on phenotypic variation of wing length and abdominal bristle number, characters which have previously been studied almost exclusively in the laboratory. We determine "among population" heritability (SLATKIN 1981) by comparing the phenotypes of wild-caught flies with those of their offspring reared at several temperatures in the laboratory. This allows us to study clines in these characters, to test whether such clines are retained in flies developing under constant laboratory conditions, and to determine the extent of phenotypic variation in wild *vs.* laboratory-reared flies. We also estimate "natural" heritability within a population by correlating the morphology of parents trapped in nature with that of their offspring reared in the laboratory.

MATERIALS AND METHODS

The characters: Wing length, measured as the linear distance between the intersections of the third longitudinal vein with the wing tip and the anterior crossvein, is both genetically and phenotypically correlated with other measures of body size in *D. melanogaster* (REEVE and ROBERTSON 1953; DAVID, BOCQUET and DE SCHEEMAEKER-LOUIS 1977). The heritability of wing length in the laboratory varies between 0.2 and 0.6 (ROBERTSON and REEVE 1952; REEVE and ROBERTSON 1953). Flies raised under colder conditions have relatively longer wings and larger bodies (DAVID *et al.* 1983). Evolutionary divergence in wing length may occur when flies are reared for several years at different temperatures: when placed back at a single temperature, lines from the colder regime have longer wings (ANDERSON 1966, 1973; POWELL 1974). These results are consistent with latitudinal differentiation of wing length observed in several *Drosophila* species (STALKER and CARSON 1947; PREVOSTI 1955; HYYTIA *et al.* 1985; LEMEUNIER *et al.* 1986).

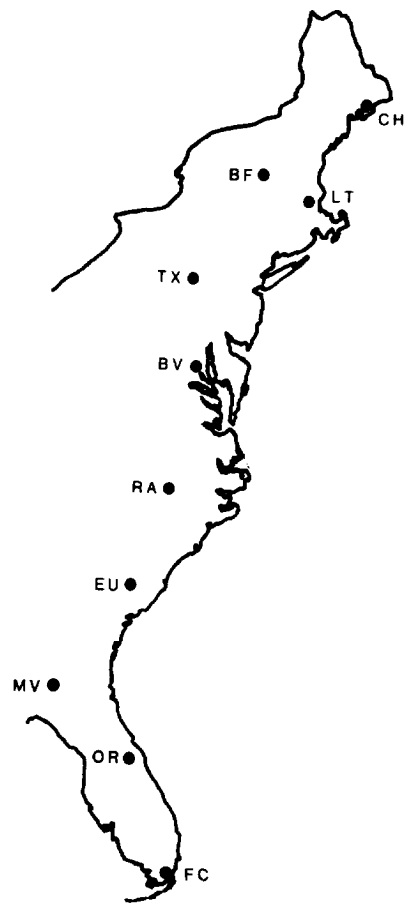

FIGURE 1.—Locations of sampled populations. Abbreviations correspond to sites described in the introduction.

Wing lengths in this study were determined from one randomly selected wing removed from each fly, mounted on a slide, and placed on a Nikon digital micrometer equipped with digital readout (COYNE 1983). Repeated measurements of single wings differ by less than 4 μm.

Abdominal bristles were counted on the fourth and fifth abdominal sternites. The genetic correlation between these counts is close to 1, so the genetic variation of the two sternites among individuals is apparently caused by the same loci (REEVE and ROBERTSON 1954). Bristle number is highly heritable in the laboratory, with values around 0.5 reported in *D. melanogaster* (CLAYTON, MORRIS and ROBERTSON 1956; MACKAY, 1981). Although these bristles may have a sensory function, the significance of differences among individuals or populations (LEMEUNIER *et al.* 1986) is unknown.

Variation among populations: Our survey included ten populations sampled over 2 yr on the east coast of North America from Maine to Florida (Figure 1). Northern and southern populations were collected each summer to even out any between-year variation. Our analysis was limited to males because wild-caught *D. melanogaster* females are difficult to distinguish from those of the sibling species *D. simulans*. Collection sites were as follows (the average yearly temperatures are from the most recent 4 yr recorded by the

nearest U.S. Weather Service station after 1976; all but two populations include 1976, 1979, and 1983):

Cherryfield, Maine (*CH* in Figure 1; 44°35′ N, 67°55′ W, elevation 56 m, average temperature 6.4°). Flies were collected at a blueberry dump on August 20, 1985).

Bellows Falls, Vermont (*BF*; 43°10′ N, 72°30′ W, el. 91 m, 7.8°), collected among bins of apples at a commercial cider press on August 14, 1984.

Littleton, Massachusetts (*LT*; 42°30′ N, 71°30′ W, el. 64 m, 8.9°), collected in a commercial peach orchard on August 23, 1985.

Trexlertown, Pennsylvania (*TX*; 40°35′ N, 75°45′ W, el. 117 m, 10.9°), collected at a commercial apple orchard on August 15, 1984.

Beltsville, Maryland (*BV*; 39°00′ N, 76°55′ W, el. 37 m, 12.2°), collected in a peach orchard at the National Agricultural Research Center on September 2, 1984.

Raleigh, North Carolina (*RA*; 53°45′ N, 78°40′ W, el. 115 m, 14.8°), collected around fruit piles at the Raleigh Farmers' Market on July 14, 1984.

Eutawville, South Carolina (*EU*; 33°20′ N, 81°25′ W, el. 99 m, 17.7°), collected in a commercial peach orchard on July 16, 1984.

Morven, Georgia (*MV*; 30°55′ N, 83°30′ W, el. 81 m, 19.8°), collected in a commercial peach orchard on June 5, 1984.

Orlando, Florida (*OR*; 28°30′ N, 81°20′ W, el. 26 m, 22.2°), collected at produce stands between Minneola and Orlando on June 3 and 4, 1985.

Florida City, Florida (*FC*; 25°30′ N, 80°30′ W, el. 5 m, 23.4°), collected in a wholesale fruit market on June 1, 1985.

Wild-caught *D. melanogaster* males were preserved in 70% ethanol, and females returned alive to the laboratory. Four males and four females from each of 30 isofemale lines from each population were randomly mixed and distributed into six bottles of Instant Drosophila Food (Carolina Biological Supply Company). Offspring from these bottles were again mixed and placed on colored food to lay eggs. Sixty eggs were placed in each vial to be reared at either 18°, 24°, or 30°. Male offspring from these vials were preserved in 70% ethanol, and ten measured per vial from ten vials reared at each of the three temperatures, giving a sample of 300 males per population. These males were the great-grandsons of wild-caught flies.

Variation within a population: In two experiments we obtained a total of one "laboratory" and two "natural" heritability estimates for each character. Experiment 1 involved collecting males and females that developed as late pupae in nature, and correlating their phenotypes with those of their offspring reared in the laboratory (experiment 1A). This natural heritability estimate was then compared to that derived from parents and offspring from the same population, but raised entirely in the laboratory (experiment 1B).

On August 29, 1985, we collected about 8 kg of decayed, fallen apples in an orchard in the National Agricultural Research Center in Beltsville, Maryland. Many pupae were visible on and beneath the fallen apples. We placed these apples in jars in the laboratory and collected eclosing flies during the next 2 days (these must have pupated 2 or 3 days before we collected the apples). Males and females were each divided by eye into two groups of large and small flies. An equal number of pair matings were made randomly within each group, and these pairs placed in vials at 24°. This provides a small degree of assorative mating for wing length (phenotypic correlation between parents = 0.25, P = 0.0003) but not for bristle number (correlation = 0.12, P = 0.15). Such assortative mating increases the variance among parental pairs, allowing more precise measurement of heritability (FALCONER 1981; but see GIMELFARB 1985). We removed parents from the vials after 5 days and preserved them and their progeny in 70% ethanol. Two randomly chosen offspring of each sex were scored for wing length and bristle number in each of 142 families.

To determine if either character was affected by the laboratory environment in the period between collection of apples and hatching of parents, we subjected two batches of late pupae to different temperatures. Vials containing 60 eggs from descendants of 30 combined isofemale lines from Beltsville were stored at 24°. Pupae formed on flexible plastic cards, which were removed after 48 hr and stored at 24° for 24 hr. The pupae-containing cards were divided among two groups of ten vials, one kept at 22° and the other at 26°. This treatment corresponds roughly to the age at which pupae were brought from the orchard into the laboratory. We scored five males and five females from each vial for wing length and abdominal bristle number.

A determination of "laboratory" heritability (experiment 1B) was made on the same population by pair-mating male and female relatives of the flies from the above experiment. Flies eclosing from apples (but not used in the natural heritability measurements) were mass mated and single females placed in vials. We combined thirty of these isofemale lines to produce offspring reared at the constant density of 60 eggs per vial. Virgin offspring eclosing from these vials were mated assortatively as described above, giving again a significant phenotypic correlation for wing length (0.29, P = 0.0002) but not for bristle number (0.01, P = 0.87). The protocol for collecting and measuring adults and offspring was identical to that given above. We obtained measurements from 159 families.

Experiment 2 produced estimates of natural heritability from a design similar to that of PROUT (1958): wild-caught males were mated to laboratory-reared females and characters correlated between father and offspring. Males were collected from the Beltsville orchard on November 10, 1985, and these were mated individually to randomly selected virgin females from the Beltsville laboratory stock used in experiment 1B. After 5 days we removed parents from the vial and preserved the male and later two adult offspring of each sex. Heritability among these 121 families is estimated as twice the slope of the regression of offspring means against paternal values. We did not estimate laboratory heritability from this sample.

Analysis of the variation between a natural and a laboratory environment is formally similar to genetic analysis of two correlated characters, for we can consider the phenotype in each environment as a distinct character. We have assumed that the regression of laboratory-reared offspring against their wild-caught parents approximates the natural heritability (wild offspring against wild parents). This is only true when certain conditions are satisfied (see APPENDIX), in particular that the additive genetic variances of the character are approximately equal in nature and in the laboratory, and that there is little genotype-environment interaction between the two environments. Extreme forms of genotype-environment interaction that produce nonconstancy of genotype ranks between the two environments will violate this assumption, causing our experimental estimate to be too low. If, on the other hand, the norms of reaction of genotypes between the two environments do not cross and the additive variance is greater in the laboratory than in nature, our experimental estimates may be too high.

TABLE 1

Wing length and abdominal bristle number (sum of segments 4 and 5) in wild-caught males

Population	N	Wing length			Abdominal bristles		
		Mean	SE	Rank	Mean	SE	Rank
Cherryfield, ME	96	1.288	0.006	B	38.95	0.51	A
Bellows Falls, VT	200	1.261	0.005	C	34.58	0.26	B
Littleton, MA	200	1.360	0.004	A	35.31	0.25	B
Trexlertown, PA	200	1.286	0.005	B	33.03	0.26	C
Beltsville, MD	200	1.207	0.006	D	30.91	0.32	D
Raleigh, NC	162	1.199	0.008	D,E	31.40	0.36	D
Eutawville, SC	200	1.194	0.005	E	32.04	0.30	D
Morven, GA	200	1.281	0.005	B	34.69	0.25	B
Orlando, FL	108	1.208	0.007	D	34.50	0.37	B
Florida City, FL	200	1.183	0.005	E	31.54	0.28	D

Ranks determined from Student-Newman-Keuls test on log-transformed data. Populations with the same letter are not significantly different.

RESULTS

Variation among populations: Tables 1 and 2 give the wing lengths and bristle numbers (sum of fourth and fifth segments) on wild and laboratory-reared flies from each population, and Figures 2 and 3 show the norms of reaction for laboratory-raised flies of these characters with respect to temperature. Table 3 summarizes the analysis of variance for both wild-caught and laboratory flies (data were log-transformed to eliminate an observed correlation between means and variances).

Populations of wild-caught flies differ significantly in both wing length and bristle number: the values of both characters decrease from north to south. The regression of mean population wing length of wild-caught flies on latitude is barely significant ($R = 0.64$, $F_{1,8} = 5.62$, $P = 0.045$), and that of bristle number on latitude is nonsignificant ($R = 0.46$, $F_{1,8} = 2.16$, $P = 0.18$).

Analyses of variance for laboratory-reared flies show significant main effects of population and temperature on each character, as well as significant vial effects and temperature by population interactions (Table 3). (ANOVAS within temperature, which are not presented, show a significant effect of geographic population on each character at each temperature.) Wing length decreases markedly with increasing temperature. The relative order of population mean wing length against rearing temperature is not completely preserved from one temperature to another (Figure 2), but is roughly similar among temperatures. There is no latitudinal trend to this interaction, as might be expected if the norm of reaction was adaptively differentiated among populations.

In most populations, bristle number is highest at intermediate rearing temperatures, so there is no phenotypic correlation between the two characters when all temperatures are considered. The two characters are, however, highly correlated within a temperature: 20 out of the 30 phenotypic correlations between wing length and bristle number among individuals within a temperature are positive and significant, and nine of the remainder are positive but nonsignificant. The population by temperature interaction of bristle number (Table 3, Figure 3) again produces a change in ranking of populations among temperatures, with Littleton and Florida City showing the most divergence. There is no obvious latitudinal trend to the interaction. Both wing length and bristle number, then, are genetically differentiated among populations for mean values and the pattern of response to temperature.

Regressions of phenotypes of laboratory-reared flies against latitude and mean locality temperature are significant for both characters (the rank correlation between latitude and temperature is 1). Northern populations have larger wings and more bristles. Analysis of covariance shows that the regression slopes of the ten population means against latitude or locality temperature are homogeneous among the three rearing temperatures. Figure 4 gives a combined latitudinal regression of population means averaged over all three temperatures. The slopes of these least-squares regressions differ significantly from zero (wing length: R (regression coefficient) $= 0.89$, $F_{1,8} = 30.0$, $P < 0.0005$; bristle number: $R = 0.78$, $F_{1,8} = 12.25$, $P = 0.008$). These results confirm that the clines previously reported in European and African populations (LEMEUNIER *et al.* 1986) are also found in the western hemisphere.

Both characters show significant product-moment correlations between the population means of wild-caught and laboratory-reared males when the latter are averaged over all temperatures (wing length: $r = 0.82$, $P < 0.005$; bristle number: $r = 0.72$, $P < 0.02$). Despite this correlation, the latitudinal regressions of wing length and bristle number in laboratory-reared flies are far more significant than those of the same characters in wild-caught flies, perhaps because environmental differences affect the means of local populations. It is likely that had the sample of populations been smaller, we would have detected an association between character and latitude only in laboratory-reared flies.

The among-population regression of phenotypes of laboratory-raised flies (averaged over all temperatures) against those of wild flies from same location provides an estimate of among-population heritability. This is similar to the heritability of family means used by quantitative geneticists, and predicts the response to selection on population means of wild-caught flies (SLATKIN 1981). Regression slopes are significant for both wing length (0.49 ± 0.12, $R^2 = 0.67$) and bristle number (0.43 ± 0.14, $R^2 = 0.53$). More than half the

TABLE 2

Wing length and abdominal bristle number in laboratory-reared males (N = 100 for each value)

	Wing length							Abdominal bristles						
	18°		24°		30°			18°		24°		30°		
Population	Mean	SE	Mean	SE	Mean	SE	Rank	Mean	SE	Mean	SE	Mean	SE	Rank
Cherryfield, ME	1.529	0.005	1.357	0.004	1.218	0.004	C	37.62	0.34	39.35	0.38	38.06	0.40	A
Bellows Falls, VT	1.522	0.006	1.391	0.004	1.224	0.004	B	36.43	0.34	37.97	0.31	36.32	0.37	B
Littleton, MA	1.559	0.005	1.365	0.004	1.254	0.003	A	34.39	0.33	36.96	0.40	36.05	0.37	C
Trexlertown, PA	1.532	0.006	1.377	0.006	1.234	0.004	B	36.18	0.33	36.78	0.37	34.20	0.30	C
Beltsville, MD	1.506	0.005	1.347	0.004	1.210	0.003	D	34.23	0.33	36.21	0.35	34.23	0.33	C,D
Raleigh, NC	1.484	0.006	1.331	0.004	1.199	0.004	E	34.28	0.30	35.56	0.32	33.00	0.36	E
Eutawville, SC	1.468	0.005	1.302	0.004	1.162	0.004	F	34.42	0.30	34.62	0.35	32.64	0.32	E
Morven, GA	1.502	0.006	1.323	0.004	1.210	0.004	E	33.47	0.35	34.48	0.31	33.28	0.30	E
Orlando, FL	1.446	0.006	1.264	0.006	1.177	0.004	G	35.04	0.29	35.40	0.36	34.53	0.36	D
Florida City, FL	1.467	0.006	1.280	0.004	1.168	0.005	F	34.38	0.31	34.25	0.30	34.47	0.38	E

Ranks determined from Student-Newman-Keuls test on log-transformed data averaged over all temperatures for each population. Populations with the same letter are not significantly different.

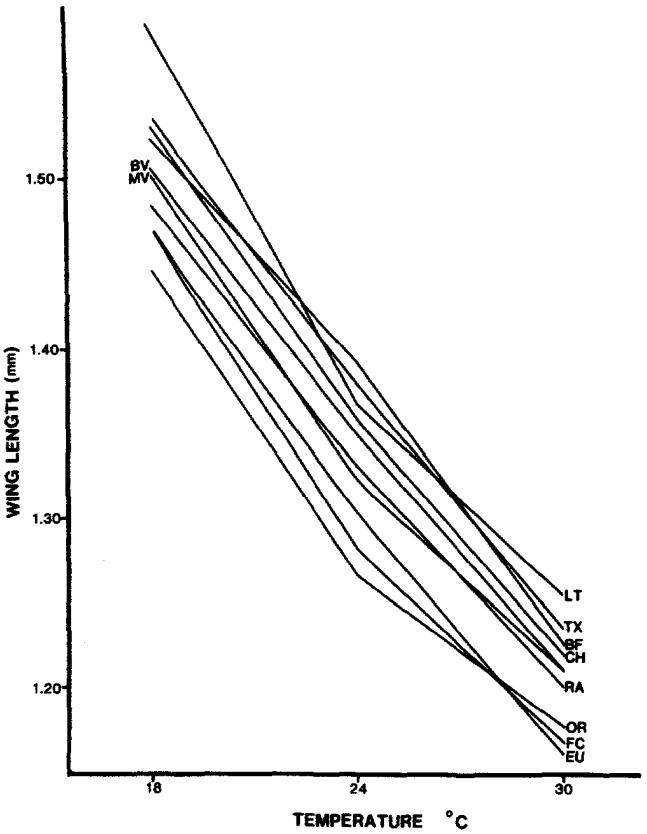

FIGURE 2.—Norms of reaction of wing length with respect to temperature for flies reared in the laboratory. Values are means of all individuals at a given temperature.

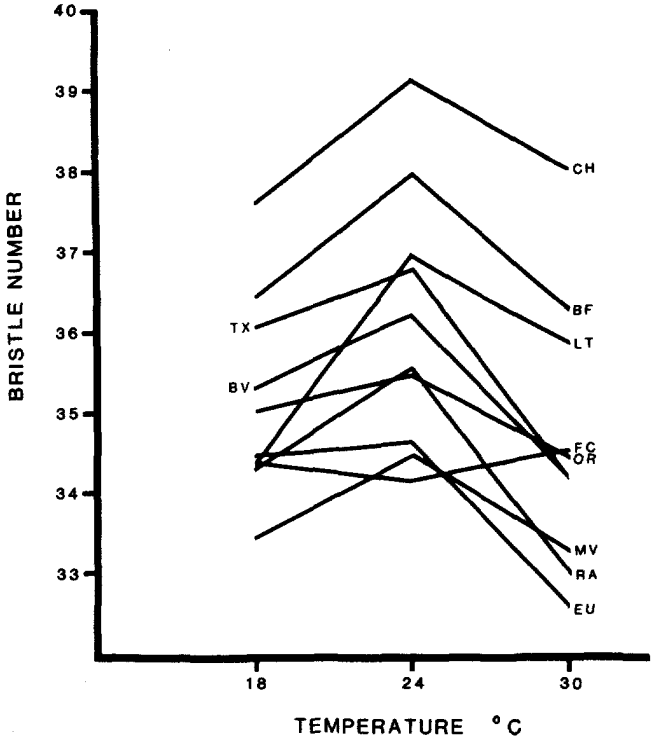

FIGURE 3.—Norms of reaction of bristle number (sum of segments 4 and 5) with respect to temperature for flies reared in the laboratory. Values are means of all individuals at a given temperature.

variance among the means of laboratory-reared populations is explained by the means of their wild-caught ancestors. We conclude that although values of the two characters in nature parallel population differences seen under constant laboratory conditions, an important part of variation among populations in nature arises from direct response to the environment. Clinal variation is hence much more obvious in laboratory-reared than in the wild-reared flies.

Phenotypic variances among individuals from nature are about twice as large as those from individuals reared under constant laboratory conditions. The average within-population and within-temperature variance of wing length is 0.0025 for laboratory-reared flies and 0.0052 for wild-caught flies, while for bristle number it is 11.6 for laboratory-reared and 16.7 for wild-caught flies.

Variation within a population: Table 4 gives her-

TABLE 3

Summaries of analyses of variance for log-transformed data on wild-caught and laboratory-reared males

Flies and source of variation	d.f.	Wing length MS	Wing length F[a]	Bristle number MS	Bristle number F[a]
Wild-caught					
Populations	9	0.393	109.0	0.744	47.1
Error	1756	0.003		0.016	
Laboratory-reared					
Populations	9	0.197	175.3	0.484	52.6
Temperature	2	12.050	10,755.0	0.471	51.2
Populations × temperature	18	0.009	8.4	0.035	3.8
Vial	270	0.002	1.7	0.014	1.5
Error	2700	0.001		0.009	

The model explains 35% of the variance for wing length and 20% of the variance for bristle number in laboratory reared flies, and 90% of the variance for wing length and 28% of the variance for bristle number in laboratory-reared flies.

[a] All probabilities less than 0.001.

itabilities and variance components from midparent-offspring regressions in experiments 1A (pair-mated virgin flies from wild collected pupae compared to their laboratory-reared offspring), 1B (same population as 1A, but parents and offspring all reared in the laboratory), and 2 (wild-caught fathers compared to their laboratory-reared offspring).

Experiments 1A and 1B yield significant heritabilities for both characters, which in the laboratory study 1B (0.58 for wing length and 0.57 for bristle number) are comparable to those found in other laboratory populations (ROBERTSON and REEVE 1952; REEVE and ROBERTSON 1953, 1954; MACKAY 1981). Comparison of the midparent-offspring heritabilities of studies 1A and 1B using analysis of covariance shows that the estimate for bristle number are homogeneous ($F_{1,297} = 0.34$, $P = 0.55$), but those for wing length differ significantly (heritability is lower when flies from nature were used as parents: $F_{1,297} = 10.3$, $P < 0.005$). This difference in wing length heritability cannot be explained by the amount of assortative mating, which was almost equal in the two studies (GIMELFARB 1985). Inspection of the variance components shows that the reduction of "natural" heritability compared to its laboratory counterpart is due largely to the higher phenotypic variance in nature. Additive and phenotypic variances for bristle number are, however, nearly equal among the three experiments, yielding similar heritabilities.

We conclude that in this population, natural heritabilities are somewhat lower than laboratory estimates for wing length, but for bristle number do not differ greatly. The APPENDIX describes the conditions under which our cross-environment estimates of natural heritability accurately reflect the true heritability in nature. Our values of "natural" wing length heritability probably *underestimate* true heritabilities in nature, but the estimates for bristle number may be accurate.

Regression of offspring against midparent may yield inaccurate heritabilities when phenotypic variances differ between the sexes, as is the case for parental wing length (but not bristle number) in experiments 1A and 1B. Table 5 gives the parent-offspring regressions broken down by sex and corrected for differences in variance between the sexes (FALCONER 1981). The estimates for wing length may be slightly inflated by assortative mating, but they allow us to compare the contributions of the two sexes to the heritability differences observed between studies 1A and 1B. Analysis of covariance shows that none of the regres-

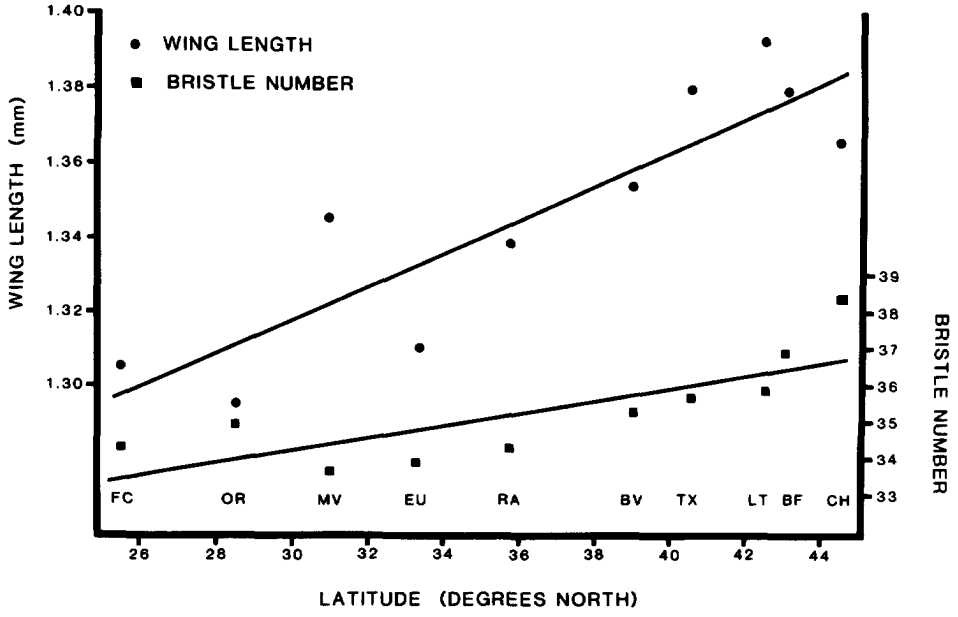

FIGURE 4.—Regressions of characters in laboratory-reared flies (means among all three temperatures) versus latitude of collecting location. Least squares regression of wing length: $y = 0.0046x + 1.1796$; regression of bristle number: $y = 0.169x + 29.172$.

TABLE 4

Heritabilities, additive and phenotypic variances, and genetic correlations (with standard errors) in samples from Beltsville, Maryland

Heritabilities and correlations	Experiment 1A (parents-nature; offspring-lab)	Experiment 1B (parents and offspring-lab)	Experiment 2 (father-nature; mother and offspring-lab)
Heritabilities			
Wing length	0.22 ± 0.08**	0.58 ± 0.08***	0.31 ± 0.14*
(σ_A^2:σ_P^2)	(0.000977:0.00444)	(0.00120:0.00271)	(0.00162:0.00521)
Bristle number	0.47 ± 0.14***	0.57 ± 0.12***	0.57 ± 0.14***
(σ_A^2:σ_P^2)	(7.39:15.73)	(7.58:13.30)	(8.40:14.72)
Genetic correlations			
Wing length/bristle number	0.53 ± 0.16**	0.42 ± 0.10***	0.15 ± 0.23
Bristles, segment 4/segment 5	0.93 ± 0.03***	1.11	1.07

The heritabilities are from midparent-offspring regressions in experiments 1A and 1B, and from father-offspring regression in experiment 2. Standard errors of genetic correlations between bristle number on the two segments are not determinable in experiments 1B and 2 because the values exceed 1 (FALCONER 1981).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 5

Slopes of parent-offspring regressions broken down by sex and corrected for differences in variances between the sexes

Character and regression	Experiment 1A (parents-nature; offspring-lab)	Experiment 1B (parent and offspring-lab)	Experiment 2 (father-nature; mother and offspring-lab)
Wing length			
Father-son	0.22 ± 0.06***	0.38 ± 0.08***	0.10 ± 0.05
Father-daughter	0.39 ± 0.11***	0.40 ± 0.08***	0.32 ± 0.09**
Mother-son	0.04 ± 0.06	0.38 ± 0.08***	
Mother-daughter	0.09 ± 0.07	0.32 ± 0.08***	
Bristle number			
Father-son	0.19 ± 0.11	0.13 ± 0.10	0.29 ± 0.08***
Father-daughter	0.24 ± 0.12*	0.40 ± 0.08***	0.23 ± 0.08***
Mother-son	0.32 ± 0.11**	0.39 ± 0.08***	
Mother-daughter	0.28 ± 0.11*	0.32 ± 0.08***	

Heritabilities and their standard errors can be obtained by doubling these values.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$.

sions of offspring of either sex on parental bristle number and neither of the offspring/father regressions for wing length are heterogeneous among experiments. Regressions of wing length of both sexes against the mother are, however, significantly lower in experiment 1A than in 1B (mother-son $F_{1,297} = 11.1$, $P = 0.001$; mother-daughter $F_{1,297} = 4.3$, $P = 0.04$), perhaps because larval crowding affects the body size of females more than that of males (ASHBURNER and THOMPSON 1978).

Table 4 gives the genetic correlations between wing length and bristle number (sum of fourth and fifth segments), as well as between bristle numbers on the two abdominal segments (FALCONER 1981; calculated using the arithmetic mean of the two midparent-offspring covariances). Correlations between bristles on the two segments are close to one, confirming previous reports that the same loci are responsible for genetic variation of bristle count on separate sternites (REEVE and ROBERTSON 1954). These genetic correlations are based on the assumption of random mating, but it is unlikely that the assortative mating in experiment 1 inflates the genetic correlations by more than 2% (GIANOLA 1982).

The validity of assuming that parental phenotypes in experiment 1A accurately reflect those in nature depends on whether the characters are affected by up to 2 days of pupal rearing in the laboratory. We collected late pupae and subjected them to two temperatures. A nested analysis of variance showed no effect of temperature on bristle number of either males or females (males: $F_{1,18} = 1.01$, females: $F_{1,18} = 0.58$, $P > 0.25$). The higher temperature significantly reduced wing length in females ($F_{1,18} = 4.6$; $0.05 > P > 0.025$) but had no significant effect in males ($F_{1,18} = 3.6$; $0.1 > P > 0.05$). The change in wing length is,

however, very small, amounting in females to an increase of only 1.5% at the lower temperature (1.515 mm at 22° and 1.493 mm at 26°). This is much smaller than the increase of 10–15% caused by a 6° reduction of temperature during the whole of the development period. Temperatures over this range, then, have no effect on bristle number of flies treated as late pupae, and only a small effect on wing length. The size of this effect is unlikely to seriously alter heritabilities based on wing lengths of adults hatched from wild-caught pupae.

Experiment 2 correlates characters of wild-caught fathers with those of their laboratory-reared progeny, and demonstrates significant positive heritabilities for both characters except in the father-son regression of wing length (Tables 4 and 5). The rather low wing-length heritabilities are comparable to those from experiment 1A, and the high heritability of bristle number comparable to those from studies 1A and 1B. In this experiment the genetic correlation between wing length and bristle number is not significantly different from zero, but the correlation between bristle counts on the two segments is again very high.

A comparison of heritabilities from all three experimental regimes can be undertaken for father-offspring regressions, although this is subject to some uncertainty because of assortative mating for wing length in experiments 1A and 1B. Analysis of covariance shows that father-son regression slopes of regime 1A, 1B, and 2 are not heterogeneous for bristle number ($F_{2,416} = 0.09$, $P = 0.92$), but are significantly so for wing length ($F_{2,416} = 3.91$, $P = 0.02$). The latter comparison becomes homogeneous when the estimate from experiment 1B is dropped from the comparison ($F_{1,259} = 1.23$, $P = 0.27$), but remains heterogeneous when this laboratory estimate is compared with the "natural" estimate of experiment 2 ($F_{1,276} = 7.0$, $P = 0.007$). Father-offspring regressions are almost heterogeneous between studies 1A and 1B ($F_{1,297} = 3.29$, $P = 0.07$), but are significantly so for heritabilities based on midparent-offspring regression.

We conclude that natural and laboratory heritabilities of bristle number are similar, but that wing length is less heritable in nature than in the laboratory. Wing length is nevertheless significantly heritable when wild-reared flies are used as parents, so our results do not support those of PROUT (1958).

DISCUSSION

Phenotypic variation for wing length and abdominal bristle number among individuals and populations in nature has a substantial genetic component, and environmental variation within and among populations does not completely obfuscate a fly's genetic endowment.

Phenotypes of wild-caught *vs.* laboratory-reared flies are correlated among populations, but local environmental differences affecting wild-caught flies obscure the clines seen in laboratory-reared flies. This is surprising for wing length, as we expected the effects of local temperature on development to produce an even steeper cline for wild-caught than for laboratory-reared flies. Perhaps habitat selection or local food abundance influence body size in nature (JONES, COYNE and PARTRIDGE 1987). The phenotypic and genetic correlation between wild-caught and laboratory-reared populations would allow the clinal variation to result from selection on the characters themselves.

SOKOLOFF (1965, 1966) compared wing characters in wild-caught and laboratory-reared populations of *D. pseudoobscura*. In ten populations there was a significant among-population correlation of wing length between females caught in nature and their daughters ($r = 0.64$, $P < 0.05$), and a nonsignificant correlation for males ($r = 0.50$, $P > 0.1$). There was no obvious geographic pattern to the variation.

Although the interaction between population and temperature found for both characters indicates genetic differentiation for the norm of reaction to temperature, the rank ordering of populations does not differ markedly among the three temperatures. Similar results are reported for bristle number among chromosomal heterozygotes of *D. pseudoobscura* (GUPTA and LEWONTIN 1982, Figures 1 and 2) and for wing length among European and African populations of *D. melanogaster* and *Drosophila simulans* (TANTAWY and MALLAH 1961, Figure 2).

One possible problem with these results is that the direction of selection in a population might change over the year, so that our clines might be an artifact of sampling populations at different parts of the yearly cycle. We think this unlikely because our temporal sampling scheme would then tend to obscure and not produce a cline (northern populations were sampled slightly later in the summer, when any temperature-induced selection should favor shorter wings) (STALKER and CARSON 1949).

Clines can result from either geographically varying natural selection or genetic drift in isolated populations that later regain contact. The latter possibility is an unlikely explanation of our data because identical clines are found in European and African populations, as well as in the sibling *D. simulans* and three other *Drosophila* species (STALKER and CARSON 1947; PREVOSTI 1955; DAVID and KITAGAWA 1982; HYYTIA *et al.* 1985; LEMEUNIER *et al.* 1986). An altitudinal increase in *D. melanogaster* wing length was also described by LOUIS *et al.* (1982). Natural selection almost certainly causes the geographic variation of these characters.

These two clines have developed over a relatively

short distance on a single continent, even though *D. melanogaster* is regularly transported by humans, and individuals can move substantial distances under their own power (COYNE and MILSTEAD 1987; COYNE, BRYANT and TURELLI 1987). It is possible that *D. melanogaster* did not arrive in North America until 1870 (JOHNSON 1913), so that selection has been strong enough to overcome substantial migration and perhaps recent colonization.

The extent of clinal variation in wing length is similar to that found in other species of Drosophila. MISRA and REEVE (1964) calculated the regression of percent change of wing length against latitude and temperature for *Drosophila subobscura* and *Drosophila robusta*. The slopes of the latitudinal regressions are 0.35 for *D. subobscura*, 0.24 for *D. robusta*, and 0.34 for our data on *D. melanogaster*. The respective slopes against temperature (°F) are −0.32, −0.14, and −0.21.

How might selection cause such clines? It could act directly on either wing length, bristle number or characters genetically correlated with them. Alternatively, the observed genetic correlation between wing length and bristle number could produce clinal variation of one as a correlated response to selection on the other. The expected slope of a latitudinal cline based only on genetic correlation with another character is equal to the slope of the selected character against latitude multiplied by the genetic regression of the correlated character on the selected character (REEVE and ROBERTSON 1953; LANDE 1982). Using the arithmetic means of a genetic regressions from offspring-midparent comparisons of experiments 1A and 1B, we find that any latitudinal slope of a bristle cline based solely on its genetic correlation with wing length would be 0.16, while that of wing length based solely on correlation with bristle number would be 0.0011. The observed slopes are 0.17 ± 0.05 for bristle number and 0.0046 ± 0.0008 for wing length. (The genetic correlations in experiments 1A and 1B do not differ significantly, indicating that genotype-environment interaction between nature and the laboratory would probably not invalidate them as estimates of genetic correlations in nature.) It is thus possible that the cline in bristle number arose as a correlated response to selection on wing length (or body size), but not vice versa.

Because wing length is genetically correlated with body size, its cline may reflect selection for larger flies in colder places. This increase in body size with latitude (Bergmann's rule) is well known in homeotherms (MAYR 1963). Larger animals, with a lower ratio of surface area to body volume, retain more metabolic heat. It is a mystery why poikilothermic flies obey this rule. Body size clines in Drosophila nevertheless appear to be caused by temperature-induced natural selection, because both ANDERSON (1966, 1973) and POWELL (1974) observed evolutionary changes in wing length when flies were reared at different temperatures in the laboratory. Body size and wing length also increase genetically with altitude in *D. melanogaster* and *D. robusta*, and with cooler weather in *D. robusta* (STALKER and CARSON 1948, 1949; LEMEUNIER *et al.* 1986).

It is not clear what type of selection is involved here. Flies might obey Bergmann's rule because larger individuals retain more heat absorbed from the environment or generated by flight, although small variations of body size in animals as small as drosophilids probably have no effect on heat balance (STEVENSON 1985). Alternatively, the negative genetic correlation between body size and development time (ROBERTSON 1957, 1963; L. PARTRIDGE, personal communication) may cause selection for more rapid development and hence for smaller flies in the warmer climates and more ephemeral resources of the south.

The increase in phenotypic variation of our wild-caught flies compared to their laboratory-reared descendants is smaller than that described in other *Drosophila* species. SOKOLOFF (1965, 1966) found that the variance of wing length in wild flies was eight times as large as that of laboratory-reared flies, while reanalysis of the data of MCFARQUAHAR and ROBERTSON (1963) on thorax length with the formula of WRIGHT (1968) shows that the variance of thorax length is 5.6 times larger in wild-caught flies than in laboratory-reared flies. In our experiments these ratios are between 1.5 and 3.0 for wing length and 1.3 to 1.5 for bristle number, depending on rearing temperature. Perhaps the more uniform and abundant distribution of food in orchards and fruit markets reduces the variance of body size in our wild fly populations.

We find positive heritabilities for both bristle number and wing length in both natural and laboratory analyses. Although heritability of wing length is significantly reduced when wild-caught parents are used, this value probably underestimates the true heritability in nature. On the other hand, our high estimates of natural heritability for bristle number may be accurate. Heritabilities in all cases are significantly larger than zero, so we have no support for PROUT's (1958) observation of negative correlation between wing lengths of wild-caught fathers and their laboratory-raised offspring. There is obviously the opportunity for rapid response to selection on these characters in nature. For example, males with large wings mate more often in the wild (PARTRIDGE, HOFFMANN and JONES 1987), which would cause rapid directional selection for longer wings and larger bodies. Any temporal stasis of these characters must reflect some counteracting force of selection.

We have shown that for these two characters, phe-

notypic differences among individuals and populations in nature have a sizeable genetic component. This accords with substantial estimates of phenotypic heritability usually found in vertebrates (*e.g.*, BOAG and GRANT 1978; SMITH and ZACH 1979; SMITH and DHONDT 1980; VAN NOORDWIJK, VAN BALEN and SCHARLOO 1980; BOAG 1983; GUSTAFSSON 1986). The data therefore support the prevailing neo-Darwinian view that the rate of evolution in the short term is limited more by the strength of selection than by the appearance of new mutations.

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APPENDIX

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To derive conditions under which the regression of laboratory-reared offspring on wild-caught parents approximates the heritability of a trait in nature, we can use the approach of FALCONER (1952; 1981, Ch. 19) in which the expression of a trait in two different environments is considered as two distinct characters that may be genetically correlated. Let z_n be the character expressed by individuals that develop in nature and z_l be the character expressed by individuals developing in a particular laboratory environment, with phenotypic variances σ^2_{Pn} and σ^2_{Pl} and additive genetic variances σ^2_{An} and σ^2_{Al}, respectively, in each environment. Denoting the additive genetic correlation between the two characters across environments as γ, the slope of the regression of laboratory-reared offspring on midparent values for wild-caught parents is $\sigma_{An}\,\gamma\sigma_{Al}/\sigma^2_{Pn}$. Comparing this to the theoretical heritability of the character from the offspring-midparent regression in the natural environment, $h^2_n = \sigma^2_{An}/\sigma^2_{Pn}$, the condition for the cross-environment regression to equal the heritability in nature is $\gamma\sigma_{Al} = \sigma_{An}$. This will be satisfied if there is no genotype-environment interaction (parallel norms of reaction), so that $\gamma = 1$ and the additive genetic variance is the same in both environments. Extreme genotype-environment interaction involving extensive crossing of the norms of reaction, with γ being low or negative, is likely to cause the cross-environment offspring-midparent regression to underestimate the heritability in nature. However, if the norms of reaction are fan-like, with similar rank ordering of genotypes in both environments so that γ is near 1.0, and the additive genetic variance is larger in the laboratory than in nature, then the cross-environment offspring-midparent regression will overestimate the heritability in nature.

We have no information about σ_{An}, but we can make some inferences about whether our estimates accurately reflect natural heritability in the following way. We can estimate both σ^2_{Al} and $\sigma_{Al}\gamma\sigma_{An}$ from our experiments; the former quantity is the product of the laboratory heritability and the phenotypic variance of laboratory-reared parents; the latter is the product of the heritability across both environments (regression slope of laboratory-reared offspring on wild-caught parents) and the phenotypic variance of wild-caught parents. If $\sigma_{An}\gamma\sigma_{Al} > \sigma^2_{Al}$, then $\sigma_{Al}/\gamma\sigma_{An} < 1$. Because $\gamma \leq 1$, it follows that $\sigma_{Al} < \sigma_{An}$ and therefore that $\gamma\sigma_{Al}/\sigma_{An} < 1$. This implies that our value of natural heritability from offspring-parent regressions across the two environments underestimates the true natural heritability. If, on the other hand, $\sigma_{An}\gamma\sigma_{Al} < \sigma^2_{Al}$, no conclusion can be drawn about the accuracy of the estimated heritability.

The following table gives these quantities from the three measurements of heritability:

	σ^2_{Al}	$\sigma_{Al}\gamma\sigma_{An}$	
Wing	0.000977	0.00157	(0.00162)
Bristle	7.580	7.393	(8.396)

The numbers under the headings are for similar genotypes tested in both environments (experiments 1A and 1B). The numbers in parentheses are from experiment 2, measuring the regression of offspring against wild-caught fathers. Phenotypic variances from experiment 1 are the average of those of the separate sexes.

For the wing measurements, the genetic covariance exceeds the genetic variance in the laboratory, so the heritability from wild parents and their laboratory-reared offspring underestimates that in nature by an unknown amount. For bristle counts, the near-equality of the two quantities is consistent with the absence of genotype-environment interaction (although it does not prove this), so that the estimated heritability may accurately indicate that in nature.