

MORPHOLOGICAL VARIATION IN NATURAL POPULATIONS OF *DROSOPHILA ROBUSTA* STURTEVANT

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Received July 9, 1947

INTRODUCTION

For many years, the large size of populations, ease of collection, and economy in handling have made insects a favored group for the study of geographical variation. In those cases in which it has been possible to study the morphology, physiology, genetics and cytology of the same organism, studies of geographical variation have been particularly fruitful for the clarification of evolutionary relationships at the species and sub-specific levels. In the fruit-fly, *Drosophila robusta* Sturtevant, Carson and Stalker (1946, 1947) have made a survey of the geographical variation in the salivary gland chromosomes. The present paper deals with a study of the variation in the morphology of the same species.

HISTORICAL

Among the earliest outstanding studies of geographical variation in insects is that of Goldschmidt (review, 1934). This author studied strains of *Lymantria dispar*, the gypsy moth, from localities in Europe, Asia, and North America, and reared adults in the laboratory under uniform conditions. It was possible, therefore, to attribute the interstrain variability, which was observed and studied genetically, to strain differences in genotype, rather than to the direct effect of the environment. In Japan and Korea, Goldschmidt found geographical gradients or clines in regard to: "strength" of the sex races, chromosome size, incubation period of the eggs, and degree of larval pigmentation. Attempts to correlate these charac-

teristics with the meteorological conditions at the various collecting stations were unsuccessful, although the clines agreed in a general way with the climatic changes from northern to southwestern Japan.

Dobzhansky (1933) studied the geographical variation in the elytral pigmentation of several species of ladybird beetles (Coccinellidae) in which he followed both the general pattern of pigmentation and the size of the spots. Clines were found for both of these characters. In a general way, these findings agreed with Gloger's Rule which states that lightly pigmented forms occur in arid regions and more heavily pigmented forms in humid regions. There is considerable evidence that the major part of the observed variation was based on the genotype.

Alpatov (1929) made a biometrical study of geographical variation in the honey bee in Asia and North America. He found that in Asiatic populations absolute body size, relative size of wax-gland surface, color of abdomen, length of tongue and relative length of legs show clear north-south clines on the Russian plains. These clines tend to be reversed in the Caucasus for the first three characteristics mentioned. Colonies transplanted to different parts of North America retained their morphological identity under the new environmental conditions, and hence Alpatov concluded that the differences he had observed were genetic, rather than directly due to the environment.

Krumbiegel (1932) found that the European races of the beetle, *Carabus*

nemorialis, showed a correlation between observed temperature tolerance and average temperature of the source locality of the race.

The same author (1936) studied geographical variation in the morphology of several species of *Carabus* by measuring museum specimens. He found clines for the relative length of the legs and antennae, with both of these organs becoming relatively shorter in the north. The morphology of the individuals from various localities showed some correlation with the average annual temperature of the source locality. The specimens studied, however, developed in nature under what were probably very diverse environmental conditions, a fact that makes it impossible to say what part of the variation was due to genetic differences, and what part directly due to environment.

In the genus *Drosophila* Timofeeff-Ressovsky (1933) has measured the relative viability of different strains of *D. funebris* from Europe and Russia, and has shown that the strains from southern localities have a relatively high viability at high temperatures, and a lowered viability at low temperatures; while the strains from northern localities show just the reverse relationships. Dobzhansky (1935) has demonstrated a similar relationship between climate of origin and physiology in the two closely related species *D. pseudoobscura* and *D. persimilis*; in this case the physiological character studied was fecundity at various temperatures.

Few biometric studies on the morphology of wild populations of *Drosophila* have been made. This is partly due to the fact that most of the species are remarkably uniform throughout their range, thus making quantitative morphological comparisons particularly difficult. In the case of *D. pseudoobscura* and *D. persimilis* however, Mather and Dobzhansky (1939) have studied a number of strains of each species in an attempt to find morphological differences between the two. They found slight but statistically signifi-

cant differences in the number of teeth in the sex-combs of the males and in wing-size. Reed, Williams, and Chadwick (1942) demonstrated that the rate of wing-beat in flight was greater in *D. pseudoobscura* than in *D. persimilis*. This physiological difference was found to be correlated with differences in the ratio of wing-size to thorax-volume.

The brief review given above is by no means comprehensive, particularly in regard to the extra-drosophiline groups, but lists some characteristic examples of the types of geographical variation which have been found in the lower taxonomic categories of insects.

MATERIALS AND METHODS

Drosophila robusta was felt to have unusual recommendations for a study of geographical variation in morphology since it is easily bred in the laboratory, is fairly abundant in nature, has large salivary gland chromosomes facilitating comparison of cytological and morphological variation, and is a woods species, being rarely associated with human habitations. This last fact would tend to decrease the accidental admixture of populations by man, and should allow relatively discrete geographical races to be built up and maintained. *D. robusta* is found in eastern North America, south to the Gulf of Mexico, north to southern Canada, and as far west as Nebraska. Its distribution and habits are more fully discussed by Carson and Stalker (1947).

The 45 strains of *D. robusta* on which this report is based were collected from 22 widely separated localities which roughly cover the known range of the species except for the southern Atlantic states. In most cases, each strain was descended from a single wild female, either inseminated before capture, or inseminated in the laboratory by a male caught at the same locality and at approximately the same time. Fifteen to twenty pairs of adult flies from each strain were aged at 25.5° C. on several changes of standard cornmeal-agar food for approxi-

mately two weeks, and until the majority of the individuals were mature and breeding. The adults were then transferred to a half-pint culture bottle and allowed to oviposit on a slice of richly-yeasted culture medium on a glass slide. After three days, the food slice was examined under the microscope and larvae transferred from it to several half-pint bottles, each containing a sheet of cleansing tissue and 16 cc. of yeast suspension, 36 larvae being transferred to each rearing bottle. Under such conditions there appears to be little competition for food since the number of larvae may be increased to 60 per bottle before the emerging adults show any decrease in body size. The entire development took place at 25.5° C. When the adults emerged they were transferred to standard food medium and allowed to age for several days, after which they were killed with ether and preserved in alcohol for later measurement.

In the early part of the work morphology at all stages of development was studied in an attempt to find workable characters showing differences between the various localities. No clear differences were found in the eggs, larvae or pupae, nor were any marked differences noted in the time of development from egg to adult. In the adults, careful comparisons were made of: color of thorax, abdomen, testis, face and eyes; size and intensity of wing clouds; number of hairs on ventral abdominal plates; external genitalia of both sexes; length and color of eye pile; number and length of arisal branches; length and width of wings, as well as the usual wing-indices used by *Drosophila* taxonomists; length of femora and tibiae; number of rows of hairs on anterior tibiae; length of thorax and width of head and cheek. Of these characters the ones which showed workable inter-locality differences were length of wing, measured from anterior cross-vein to tip; width of wing, measured at right angles to the long axis through the posterior end of the posterior cross-vein; greatest width of the head; length of the thorax

from the most anterior point to the tip of the metascutellum, and length of the fore-femur. Although the length of the middle and hind femora showed clear inter-locality variation, they were highly correlated with the length of the fore-femur, and were therefore not included in the final measurements. There was some evidence that the testis color of southern strains was lighter than that of northern strains, but this difference was so affected by age and so difficult to measure quantitatively that it was not followed further. Preliminary measurements were made on both sexes, but since the male and female showed a high inter-locality correlation, final measurements were made on females only.

For measurement, the preserved adults were placed in water for a few minutes, then transferred to a microscope slide, there the head was removed and the rest of the body placed in such a position that the thorax could be seen directly from the side. After the surface film of water had evaporated, thorax-length and head-width were determined. The wing and fore-femora were then dissected off and mounted under a coverslip in water for measurement. One femur was measured on each fly. All measurements were made with the aid of an ocular micrometer in which one unit of measurement equals 483 micra. The mean measurements in the tables and graphs are expressed in these ocular micrometer units, and are based on 35 females from each of the 45 strains.

The strain means and standard errors for the five characteristics mentioned above are listed in table 1. Attempts were made to correlate these means with latitude, longitude, distance from supposed centers of distribution and general ecology, including temperature and humidity of the regions from which the strains originated. Of these, only latitude, average July temperature, and average annual temperature seemed to show suggestive correlations. Figures 1A, B, C; 2A, B show the five sets of strain

TABLE 1. Average annual temperature and strain means for 22 localities
Errors listed are standard errors of means

Locality and strain	Aver. annual temp. F.	Thorax length	Head width	Femur length	Wing length	Wing width	Wing length adjusted	Discriminant D_1^*	Discriminant D_2^*
Big Fish L., Minn.	40.0	2.996 ± 0.0071	2.443 ± 0.0060	1.543 ± 0.0049	4.721 ± 0.0120	3.066 ± 0.0072	4.803 ± 0.0260	0.242	0.648
Rechester, N. Y.	48.0	3.007 ± 0.0083	2.421 ± 0.0062	1.565 ± 0.0052	4.469 ± 0.0171	3.292 ± 0.0107	4.540 ± 0.0338	0.120	0.671
C. S. Harbor, N. Y.	51.0	3.098 ± 0.0093	2.484 ± 0.0053	1.598 ± 0.0048	4.652 ± 0.0173	3.071 ± 0.0091	4.636 ± 0.0130	0.109	0.704
Mt. Vernon, Iowa 1	48.2	3.105 ± 0.0062	2.483 ± 0.0065	1.595 ± 0.0055	4.649 ± 0.0139	3.091 ± 0.0090	4.626 ± 0.0151	0.089	0.676
Mt. Vernon, Iowa 5		3.043 ± 0.0080	2.451 ± 0.0074	1.567 ± 0.0045	4.543 ± 0.0184	3.021 ± 0.0100	4.580 ± 0.0208	0.082	0.676
Mt. Vernon, Iowa 8		3.101 ± 0.0099	2.497 ± 0.0085	1.603 ± 0.0043	4.711 ± 0.0139	3.123 ± 0.0120	4.693 ± 0.0119	0.135	0.704
Mt. Vernon, Iowa 10		3.096 ± 0.0084	2.425 ± 0.0108	1.545 ± 0.0056	4.529 ± 0.0147	2.996 ± 0.0084	4.600 ± 0.0228	0.111	0.679
Mt. Vernon, Iowa 11		3.093 ± 0.0080	2.476 ± 0.0070	1.578 ± 0.0052	4.624 ± 0.0133	3.017 ± 0.0092	4.613 ± 0.0131	0.141	0.679
Glenview, Ill.	49.0	3.024 ± 0.0079	2.412 ± 0.0053	1.573 ± 0.0047	4.361 ± 0.0143	2.993 ± 0.0099	4.616 ± 0.0141	0.151	0.726
New Wilmington, Pa.	50.0	3.026 ± 0.0092	2.432 ± 0.0062	1.571 ± 0.0040	4.495 ± 0.0147	2.944 ± 0.0093	4.548 ± 0.0195	0.100	0.677
Philadelphia, Pa.	52.6	3.097 ± 0.0076	2.481 ± 0.0057	1.594 ± 0.0038	4.675 ± 0.0128	3.039 ± 0.0108	4.660 ± 0.0118	0.070	0.696
Woster, Ohio 42	50.0	3.090 ± 0.0100	2.479 ± 0.0057	1.580 ± 0.0041	4.557 ± 0.0185	3.044 ± 0.0122	4.548 ± 0.0164	0.054	0.682
Woster, Ohio 47		3.018 ± 0.0086	2.473 ± 0.0066	1.580 ± 0.0051	4.389 ± 0.0132	3.036 ± 0.0095	4.611 ± 0.0137	0.096	0.671
Woster, Ohio 53		3.107 ± 0.0082	2.443 ± 0.0067	1.556 ± 0.0056	4.541 ± 0.0135	3.016 ± 0.0099	4.601 ± 0.0127	0.092	0.661
Cape May, N. J. 1	55.0	3.073 ± 0.0061	2.496 ± 0.0059	1.590 ± 0.0040	4.678 ± 0.0110	3.030 ± 0.0085	4.654 ± 0.0102	0.169	0.680
Cape May, N. J. 2		3.105 ± 0.0077	2.497 ± 0.0071	1.577 ± 0.0045	4.576 ± 0.0164	3.051 ± 0.0112	4.554 ± 0.0164	0.058	0.638
Gallinburg, Tenn. 1	55.0	3.036 ± 0.0105	2.434 ± 0.0075	1.550 ± 0.0044	4.612 ± 0.0152	3.063 ± 0.0114	4.636 ± 0.0127	0.098	0.652
Gallinburg, Tenn. 2		3.126 ± 0.0092	2.519 ± 0.0074	1.576 ± 0.0049	4.591 ± 0.0163	3.081 ± 0.0121	4.634 ± 0.0140	0.083	0.702
Gallinburg, Tenn. 3		3.101 ± 0.0067	2.525 ± 0.0070	1.571 ± 0.0037	4.641 ± 0.0120	3.031 ± 0.0095	4.570 ± 0.0169	0.083	0.642
St. Louis, Mo. 2	55.0	3.068 ± 0.0083	2.464 ± 0.0060	1.571 ± 0.0037	4.641 ± 0.0120	3.205 ± 0.0091	4.623 ± 0.0131	0.007	0.655
St. Louis, Mo. 550		3.089 ± 0.0097	2.498 ± 0.0079	1.597 ± 0.0047	4.579 ± 0.0145	3.060 ± 0.0097	4.592 ± 0.0095	0.080	0.684
St. Louis, Mo. 792		3.104 ± 0.0108	2.498 ± 0.0079	1.597 ± 0.0047	4.667 ± 0.0149	3.090 ± 0.0123	4.646 ± 0.0141	0.114	0.692
St. Clair, Mo.	55.0	3.051 ± 0.0105	2.493 ± 0.0081	1.581 ± 0.0049	4.655 ± 0.0150	3.084 ± 0.0115	4.648 ± 0.0123	0.113	0.673
Montauk, Mo. 1	57.5	3.113 ± 0.0077	2.503 ± 0.0097	1.582 ± 0.0040	4.541 ± 0.0173	3.090 ± 0.0168	4.571 ± 0.0186	0.078	0.684
Montauk, Mo. 8		3.077 ± 0.0097	2.475 ± 0.0112	1.595 ± 0.0061	4.744 ± 0.0111	3.095 ± 0.0104	4.541 ± 0.0169	0.017	0.675
Montauk, Mo. 13		3.106 ± 0.0069	2.501 ± 0.0057	1.595 ± 0.0040	4.755 ± 0.0185	3.158 ± 0.0092	4.748 ± 0.0092	0.155	0.707
Montauk, Mo. 17		3.136 ± 0.0080	2.527 ± 0.0067	1.606 ± 0.0064	4.676 ± 0.0166	3.158 ± 0.0092	4.732 ± 0.0210	0.050	0.702
Clinton, Ky.	60.0	3.114 ± 0.0077	2.518 ± 0.0058	1.593 ± 0.0051	4.640 ± 0.0192	3.116 ± 0.0083	4.610 ± 0.0177	0.051	0.673
Morrilton, Ark. 8	61.0	3.168 ± 0.0109	2.539 ± 0.0092	1.599 ± 0.0048	4.481 ± 0.0192	3.020 ± 0.0101	4.399 ± 0.0298	-0.063	0.658
Morrilton, Ark. 9		3.127 ± 0.0086	2.537 ± 0.0056	1.583 ± 0.0053	4.515 ± 0.0175	3.020 ± 0.0130	4.472 ± 0.0220	-0.024	0.625
Morrilton, Ark.	63.5	3.107 ± 0.0091	2.541 ± 0.0081	1.583 ± 0.0053	4.577 ± 0.0177	3.077 ± 0.0126	4.553 ± 0.0179	-0.003	0.617
Verbeia, Ala.	64.5	3.051 ± 0.0120	2.499 ± 0.0099	1.563 ± 0.0058	4.501 ± 0.0196	3.009 ± 0.0118	4.549 ± 0.0187	0.025	0.611
Abbeville, Ala. 19D	66.0	3.091 ± 0.0089	2.535 ± 0.0074	1.562 ± 0.0051	4.501 ± 0.0196	3.033 ± 0.0098	4.492 ± 0.0173	-0.040	0.591
Abbeville, Ala. 19H		3.095 ± 0.0089	2.508 ± 0.0060	1.544 ± 0.0059	4.417 ± 0.0185	2.995 ± 0.0130	4.404 ± 0.0126	-0.075	0.605
Abbeville, Ala. 19K		3.074 ± 0.0103	2.500 ± 0.0054	1.544 ± 0.0065	4.536 ± 0.0164	3.007 ± 0.0108	4.544 ± 0.0136	0.045	0.603
Abbeville, Ala. 19M		3.067 ± 0.0133	2.513 ± 0.0011	1.546 ± 0.0048	4.403 ± 0.0227	3.009 ± 0.0149	4.417 ± 0.0148	-0.100	0.585
Leary, Ga. 21G	66.3	3.114 ± 0.0079	2.523 ± 0.0050	1.575 ± 0.0045	4.459 ± 0.0157	3.077 ± 0.0118	4.428 ± 0.0148	-0.104	0.640
Leary, Ga. 21H		3.066 ± 0.0097	2.459 ± 0.0066	1.551 ± 0.0055	4.514 ± 0.0230	2.950 ± 0.0119	4.528 ± 0.0209	0.095	0.646
Leary, Ga. 21V		3.101 ± 0.0060	2.514 ± 0.0043	1.537 ± 0.0052	4.513 ± 0.0182	2.963 ± 0.0113	4.496 ± 0.0205	0.042	0.581
Thomasville, Ga.	67.5	3.069 ± 0.0072	2.548 ± 0.0062	1.573 ± 0.0054	4.565 ± 0.0199	3.008 ± 0.0119	4.577 ± 0.0173	0.065	0.565
Marshall, Texas	65.4	3.098 ± 0.0072	2.546 ± 0.0055	1.569 ± 0.0044	4.479 ± 0.0208	2.981 ± 0.0127	4.420 ± 0.0128	-0.086	0.577
Austin, Texas 9	69.0	3.026 ± 0.0076	2.477 ± 0.0033	1.537 ± 0.0054	4.389 ± 0.0197	2.941 ± 0.0147	4.442 ± 0.0164	-0.036	0.589
Austin, Texas 10		3.111 ± 0.0072	2.522 ± 0.0053	1.548 ± 0.0052	4.567 ± 0.0104	3.024 ± 0.0082	4.539 ± 0.0117	0.046	0.601

* See text.

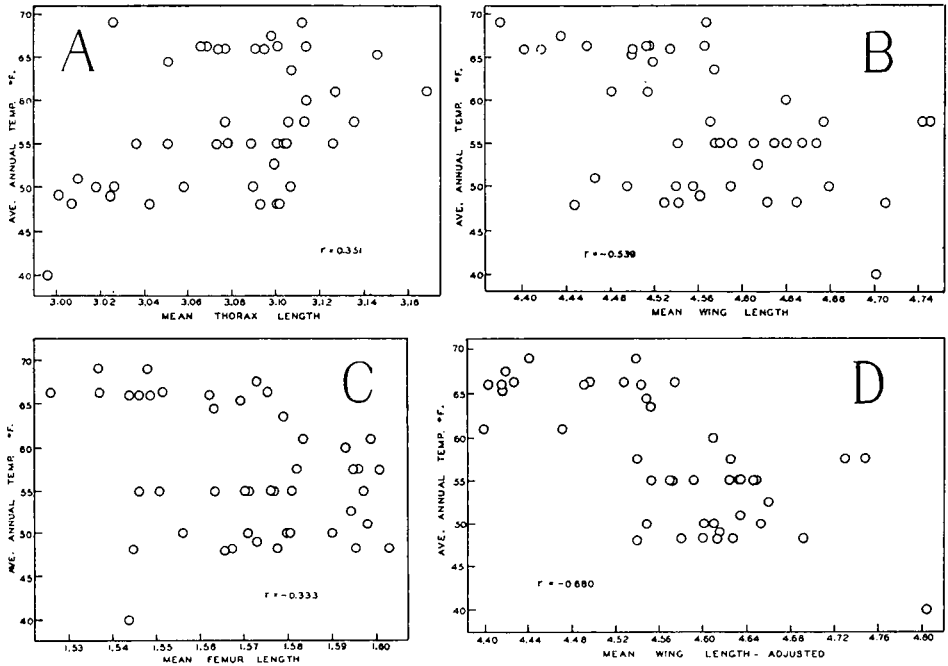


FIG. 1. Strain mean measurements plotted against average annual temperature of locality. A. Thorax-length. B. Wing-length. C. Femur-length. D. Wing-length adjusted to thorax-length. (See text.)

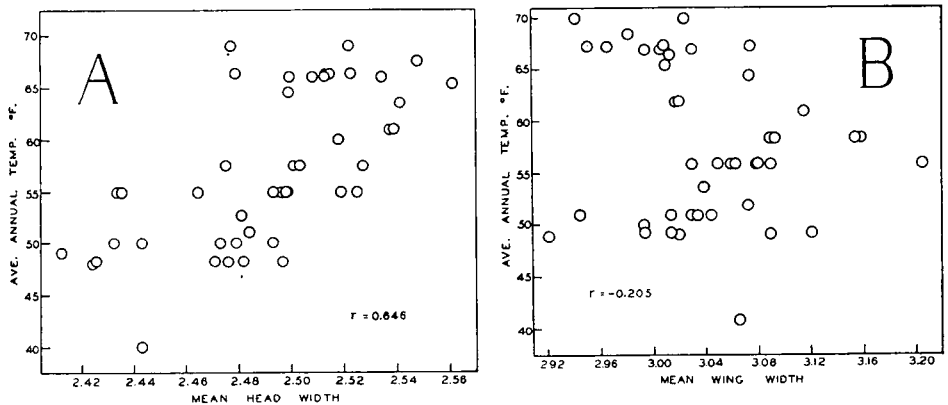


FIG. 2. Strain mean measurements plotted against average annual temperature of locality. A. Head-width. B. Wing-width.

means plotted against average annual temperature (Fahrenheit) of the strain locality. It should be emphasized that all flies measured were raised at the same temperature, 25.5°C . The temperatures used in these graphs are given in the second column of table 1 and come from the U.S.D.A. Yearbook (1941). Correlation

coefficients are given in each figure. Of the five characteristics, wing-width, for which $r = -0.205$, shows the lowest correlation with temperature. This is not a significant correlation since it is much smaller than the correlation coefficient at the 5 per cent level of significance, ($r = 0.301$). The coefficients for thorax-

length ($r = 0.351$) and for femur-length ($r = -0.333$) probably indicate a significant correlation of these characters with temperature, since both exceed the value 0.301 mentioned above. Head-width ($r = 0.646$) and wing-length ($r = -0.539$) show a very significant correlation with average annual temperature.

Thus, on the basis of these five measurements alone, it would appear that the strains studied do show graded differences in absolute dimensions correlated with the climate. Individuals from cold climates are characterized by longer wings, narrower heads, shorter thoraces and longer femora than those from warm climates.

The positive temperature correlation in the case of thorax-length, and the negative one in the case of wing-length are especially interesting. The opposite sign of these coefficients indicates a change in the wing/thorax ratio from north to south. This is more clearly shown by a statistical adjustment of strain mean wing-length to the over-all mean thorax-length (3.0818) for the total sample of 45 strains. The method used in making such an adjustment is described by Snedecor (1946). In our case the adjustment was made by the use of the formula: $Y = \bar{y} - 0.9506(\bar{x} - 3.0818)$, where Y is the adjusted strain mean wing-length, \bar{y} the unadjusted strain mean wing-length, \bar{x} the strain mean thorax-length, and 0.9506 is the coefficient of regression of wing-length on thorax-length, which represents an average of the wing-on-thorax regressions of the 45 strains. The use of such an average is probably valid, since an analysis of variance (table 2) indicates

that the regression coefficients of the 45 strains do not give clear evidence of significant heterogeneity. The adjusted strain mean wing-lengths and their standard errors are given in table 1. Figure 1D shows these adjusted strain means plotted against average annual temperature. They show a highly significant correlation, $r = -0.680$. Thus with an increasingly colder climate, the wing-length increases relative to the thorax-length.

Reed, Williams, and Chadwick (1942) in their study of wing-beat noted that low wing-beat frequencies were associated with species having a high value of the ratio between wing-area and thorax-volume. Conversely high wing-beat frequency was associated with a lower value of this ratio. These authors developed the formula:

$$(\text{wing-frequency})^2 = k \left[\frac{\text{muscle volume}}{(\text{wing area}) (\text{wing-length})^3} \right]$$

and tested the relationship by measurements on 11 species of *Drosophila*, using the product of thorax-length, width and depth as an index of muscle volume. The strains and species agreed very well with the theoretical thorax-wing dimension relationship. Frequency of wing-beat, moreover, was shown to differ significantly in the three species, *D. miranda*, *D. pseudoobscura*, and *D. persimilis*. Although these authors found no clear correlation between wing-beat and habitat within each of the three species, a correlation was found when the species were considered as units. Thus:

"... taking the averages for the three groups, we get a strong relationship between habitat

TABLE 2. Analysis of errors of estimate from average regression of wing-length on thorax-length within strains

Source of variation	d.f.	Error sum of squares	Mean square
Deviation from average regression within strains	1529	10.5309	
Deviation from individual strain regressions	1485	10.1361	0.006826
Differences among strain regressions	44	0.3948	0.008973

$$F = 0.008973/0.006826 = 1.31 \quad (F = 1.36 \text{ at } 5 \text{ per cent level of significance})$$

and wing-beat frequency. It is known from the early work of Dobzhansky (1937) that *D. miranda* prefers a colder climate than Race B (*D. persimilis*), and that Race B does best at a lower temperature than the optimum for Race A (*D. pseudoobscura*). This ecological relationship is in perfect agreement with the wing-beat frequency, which increases markedly with the optimum temperature progression from *D. miranda* through Race B through Race A."

Thus it appears that for these three closely related forms, species from colder habitats have a high wing/thorax ratio and a low wing-beat frequency, while those from warmer climates are just the reverse.

In our data, although we do not have direct measurements of wing-area, its general trend may be estimated from wing-length and -width data. It will be recalled that wing-length shows a graded increase from south to north. The correlation coefficient for wing-width of -0.205 (figure 2B) indicates that this characteristic either shows no climatic trend, or else shows a slight increase toward the north, similar to that of wing-length. Thus the south to north increase in the proportion of wing-length to thorax-length (figure 1D) must also involve a graded increase in the proportion of wing-area to thorax-length. To summarize, we may conclude that a gradient in the proportion of wing-length and area to thorax-length exists, with the wing relatively larger in the northern part of the range.

Any attempt to correlate our data with those of Reed, Williams, and Chadwick should take into account not only thorax-length, but also volume. Does the gradient indicated above for wing-size and thorax-length hold for thorax-volume as well? In the absence of differences in thorax-shape between northern and southern strains a change in any one linear dimension would represent a corresponding change in volume. Measurements of thorax-width and depth are difficult to make on preserved material, however careful observation has failed to reveal any differences in thorax-shape throughout the range of the species. Thus it

seems highly probable that we have here the same phenomenon which the above authors observed, although on a lower taxonomic level. Whereas they observed correlation between climate and wing/thorax proportions between species, we have observed it *within* a single species.

It would be expected that if the body-weight of wild flies from various localities were the same, then since for an individual fly the wing-beat frequency is lower at lower temperatures, the larger wing-size of the northern flies would be required to maintain them in the air. However, at present we know neither the average body-weight nor wing/thorax ratio of flies grown in nature, and such information is difficult to obtain since there is great variation in these characteristics both within and between seasons. In the absence of such data, the adaptive significance of the correlation of wing-beat, wing-size, and climate is not entirely clear.

COMPOUND MEASUREMENTS OF THE 45 STRAINS

It will be noted from table 1 and figures 1 and 2 that at least four of the five characters, plus a compound of two of them (wing-length and thorax-length), show significant differences, of an orderly sort, between the northern and southern strains. In order to get an over-all indication of the north-south cline in morphology, a compound measurement which includes all five basic characters is desirable. In devising such a compound two precautions should be taken. First, if the compound is devised to discriminate between certain groups, those characters which have a high intragroup correlation should be given relatively little weight, since they do not give independent evidence in making a separation between groups. Secondly, if the compound measurement is to be as efficient as possible, those characters in which the intergroup variance is large relative to the intra-group variance should be given most weight. The Discriminant Function, developed by Fisher

(1936), fulfills these requirements. It may take the form:

$$D = c_1x + c_2y + c_3z + c_4u + c_5v,$$

where D is the compound measurement, and x , y , z , u and v represent the measurements of the five characters mentioned above, with $c_1 \dots c_5$ chosen so as to maximize the inter-group variance relative to the intra-group variance. The calculation of such coefficients is discussed by Barnard (1935) and Fisher (1936). To obtain our first discriminant, coefficients were chosen so as to maximize the variance between strains relative to the variance within strains. The coefficients obtained were as follows:

thorax-length	$c_1 = + 0.0010$
head-width	$c_2 = - 0.8807$
femur-length	$c_3 = - 0.2045$
wing-width	$c_4 = - 0.6571$
wing-length	$c_5 = + 1.0000$

The mean compound measurement, or discriminant D_1 , was calculated for each strain by multiplying the strain means for the five characters by their corresponding coefficients, and summing the five products. The discriminants so calculated are listed in table 1 under the column heading D_1 .

Attempts were made to correlate the 45 discriminants with latitude, longitude, etc., as in the case of the five basic measurements. With this discriminant as with the basic measurements, the clearest correlation was shown with average annual temperature. The temperature correlation coefficient is -0.701 , which is higher than that obtained by considering any one of the five basic measurements or the wing-thorax compound independently.

The discriminant D_1 mentioned above was not chosen so as to discriminate most efficiently between temperature zones or localities, but rather to discriminate between *all* strains, even those in the same zone or locality. On the basis of the high correlation with temperature that this discriminant showed, it was decided to calcu-

late a new and more efficient discriminant, with coefficients so chosen that they would maximize the variance between temperature zones relative to that within temperature zones. For this purpose the 45 strains were arbitrarily assigned to seven zones. The zones contained from two to eleven strains each; the range of the average annual temperature within zones varied from one to eight degrees Fahrenheit. The most efficient coefficients for discriminating between these seven temperature zones turned out to be:

thorax-length	$c_1 = + 0.5030$
head-width	$c_2 = - 1.2630$
femur-length	$c_3 = + 1.0000$
wing-width	$c_4 = + 0.2193$
wing-length	$c_5 = + 0.0023$

Using these coefficients, and by the method outlined above, a new mean discriminant, D_2 , was calculated for each strain. These new discriminants are listed in the last column of table 1. Figure 3 shows D_2 plotted against average annual temperature. In this figure those localities from which more than one strain was measured are represented by the mean of the several strains. The small single circles represent localities from which only one strain was measured, the large single circles represent localities with two or three strains, the symbols consisting of two concentric circles indicate localities from which four or five strains were measured. The letters in the circles refer to the locality key at the bottom of the figure. It will be noted that when this new discriminant is employed the various localities show a striking correlation with average annual temperature. The coefficient of correlation (based on strain means rather than locality means) is now -0.753 . Although this is a higher correlation than was obtained by any other single character or combination of characters, it is interesting to note that the wing-thorax compound (with a coefficient of -0.680) shows almost as high a temperature correlation.

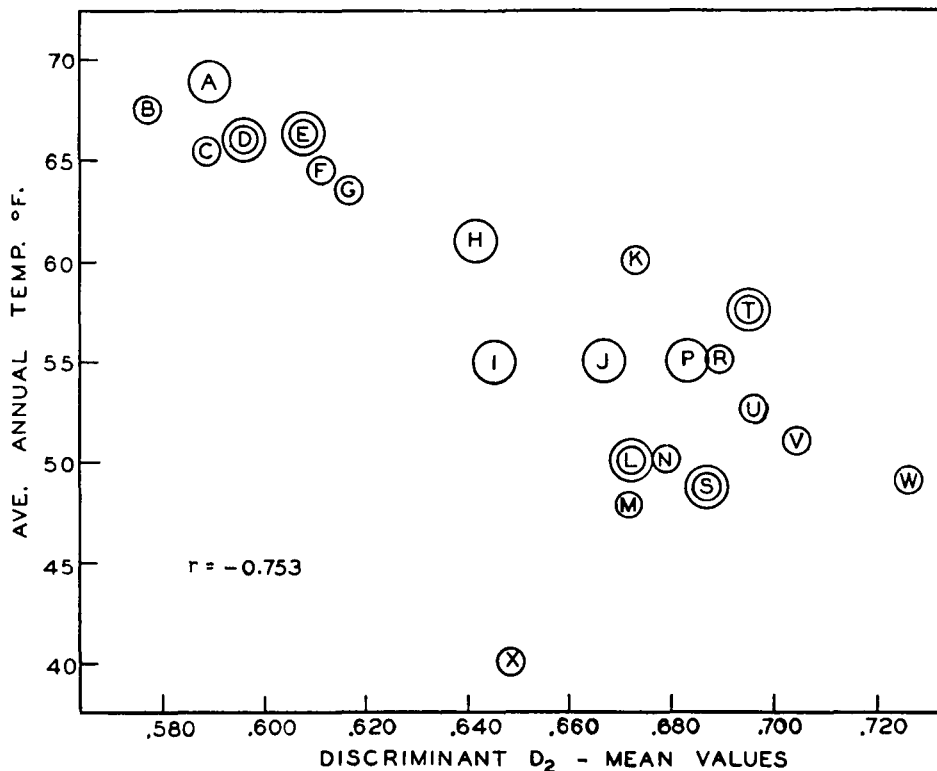


FIG. 3. Locality mean discriminant (D_2) plotted against average annual temperature of locality. Small single circles represent localities with only one strain, large single circles localities with 2-3 strains, two concentric circles represent localities with 4-5 strains. Letters in circles refer to locality key below.

A Austin, Tex.	L Wooster, Ohio
B Thomasville, Ga.	M Rochester, N. Y.
C Marshall, Tex.	N New Wilmington, Pa.
D Abbeville, Ala.	P St. Louis, Mo.
E Leary, Ga.	R St. Clair, Mo.
F Verbena, Ala.	S Mt. Vernon, Iowa
G University, Ala.	T Montauk, Mo.
H Morrillton, Ark.	U Philadelphia, Pa.
I Cape May, N. J.	V C. S. Harbor, N. Y.
J Gatlinburg, Tenn.	W Glenellyn, Ill.
K Clinton, Ky.	X Big Fish L., Minn.

Figure 4 shows the geographical variation of D_2 . In this figure the length of the bars is proportional to $D_2 - 0.52$. It will be noted from this figure and figure 3 that the gradient in over-all morphology, while quite regular in the southern parts of the range, shows a tendency to flatten out north of Missouri and Tennessee.

VARIABILITY BETWEEN STRAINS FROM THE SAME LOCALITY

Table 1 and figures 1, 2, and 3 show that although there is a distinct temperature trend in some of the five basic characters, the wing-thorax compound, and the discriminants D_1 and D_2 , there may be considerable variability in strain means

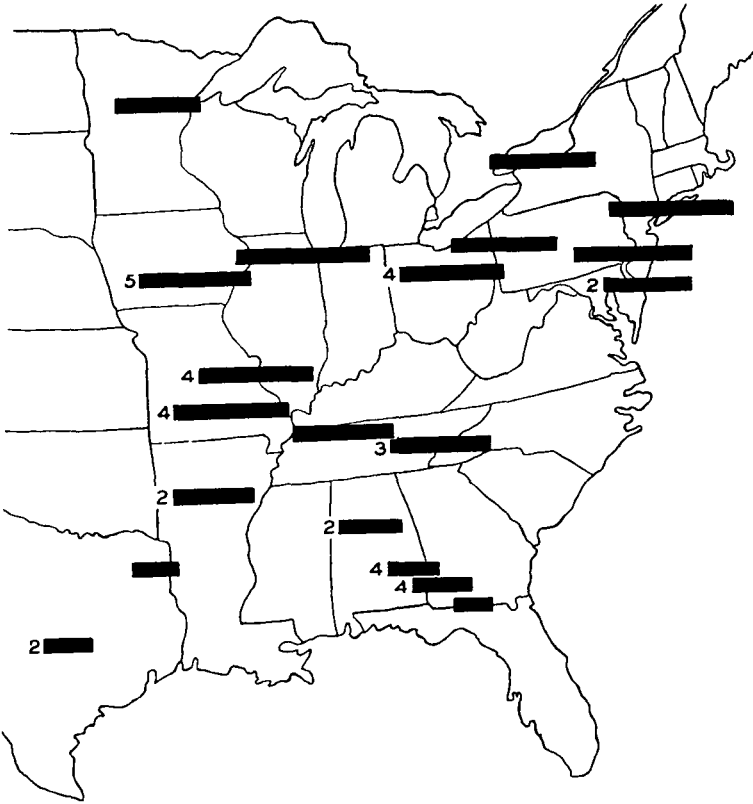


FIG. 4. Geographical variation in strain mean discriminant D_2 . Length of bars is proportional to strain mean $D_2 - 0.52$. (See text.)

within any one locality. In fact for all measurements, including the discriminants, there is much overlapping between localities and regions. We will next consider whether this observed variability within localities is significant, and thus due to genotypic differences in the strains,

or is merely the result of sampling errors. For such an analysis we have available ten widely separated localities with more than one strain each (table 1) and a total of 33 strains and 1155 flies. Table 3 shows the results of an analysis of variance for each of the five basic measure-

TABLE 3. *Analysis of variance of five basic measurements within localities.*
10 localities; 33 strains, 35 flies measured in each strain.

Source of variation	d.f.	Mean squares				
		Thorax length	Head width	Femur length	Wing length	Wing width
Within strains	1122	0.00285	0.00190	0.00100	0.00281	0.00435
Between strains within locality	23	0.03957	0.02446	0.05923	0.02540	0.07168
Variance ratio, F.		13.884	12.874	59.230	9.039	16.478

F value at 0.01 level of significance = < 1.89

ments. The variance ratios (F) given in the last line of the table are all much higher than the F value at the 5 per cent limit of significance, indicating that the strains within localities are significantly different in respect to each of the five basic measurements. Thus, in the case of these basic measurements, as well as the discriminants derived from them, it is clear that a single strain gives rather limited information on the average genotype of the locality from which it came. Such single strain measurements, while of limited value as representatives of particular localities, when lumped together do give information concerning the general region in which the various localities are found.

The unexpectedly low value of D_2 in the sample from Big Fish Lake, Minnesota, may well be due to the fact that this sample consists of a single strain.

COMPARISON OF MORPHOLOGICAL AND CYTOLOGICAL VARIATION

Carson and Stalker (1947) have shown that for many of the known gene arrangements in *D. robusta* there exist clear north-south frequency gradients similar to those found in the morphology. Since a chromosome inversion effectively reduces crossing-over in the heterozygote, the genes included in any one inversion tend to remain together, and may in time become different, as a group, from those genes in the homologous chromosome carrying a different gene arrangement. Approximately 50 per cent of the total length of the salivary gland chromosomes of *D. robusta* is involved in inversions. Thus it is reasonable to suppose that some of the genes which are responsible for the complex of morphological differences between northern and southern strains may be included in these inverted chromosome regions. The parallel changes of the gene arrangements and morphology would suggest that arrangements showing a high frequency in the north and a low frequency in the south would carry genes which are predominantly "northern-type," and would produce the large wing, long

legs, short thorax and narrow head characteristic of northern strains, while inversions with a reverse gradient would carry predominantly "southern-type" genes. At present this is only an hypothesis and further more exact comparisons between gene arrangements and morphology are needed to confirm or deny it. Such comparisons are in progress.

Although it seems probable that the geographical differences in morphology are the result of an adaptation to climate, this adaptation may be of a very indirect sort. For example, the morphological phenotype itself may be the by-product of some more fundamental physiological adaptation. While annual temperature is the most obvious *detectable* environmental factor which shows correlation with the morphology, the morphology and physiology may be primarily adapted to some other part of the environment, such as type of flora, which in its turn is correlated with temperature. Thus the apparent flattening out of the morphological gradient in the north may well be a reflection of a change in some environmental factor of which we have no knowledge.

SUMMARY

Studies of geographical variation in the morphology of *Drosophila robusta* Sturtevant are described. These studies are based on 45 strains from 22 widely separated localities in the eastern United States. Final measurements were made on 35 females from each strain, raised in the laboratory under uniform conditions. North-south morphological clines involving a significant correlation with average annual temperature of the source locality were found for the following absolute dimensions: length of thorax, width of head, length of fore-femur, and length of wing. The first two dimensions were greatest in the south, the last two greatest in the north. Absolute wing-width showed a tendency to be largest in the north, although its correlation with average annual temperature was not significant.

The relative size of wing to thorax was found to increase toward the north. This finding within one species is in agreement with the findings of Reed, Williams, and Chadwick concerning similar variation between different species of the *Drosophila pseudoobscura* group.

Compound measurements (Fisher's Discriminant Function) were calculated. The first set of discriminants, chosen so as to best differentiate between all strains, showed a highly significant correlation with average annual temperature. The second set, chosen so as to discriminate between seven temperature zones, showed a higher correlation with average annual temperature, and indicated that the morphological cline, while fairly regular from Missouri and Tennessee toward the south, showed a tendency to flatten out from Missouri and Tennessee northward.

The inter-locality differences became clear only when locality means were considered, since there was significant inter-strain variation within localities for all five of the absolute measurements mentioned above. The morphological gradient is paralleled in a general way by similar north-south gradients of inversion frequencies found in this species.

ACKNOWLEDGMENT

We are especially indebted to Dr. D. R. Charles for his helpful criticism and advice concerning the statistical procedures used in this investigation. Valuable collections and donations of material were made by the following: Drs. A. H. Sturtevant, W. P. Spencer, J. T. Patterson, G. B. Mainland, A. V. Beatty, C. P. Oliver, M. D. McCarthy, Lillian Nagel; Mr. Arthur Stupka and Mr. J. N. Stalker.

We are also indebted to Washington University for generous financial aid during the course of the investigation, and to Miss Florence Friedman for capable and painstaking technical assistance.

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