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Clines in body dimensions in populations of *Drosophilia subobscura*

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1. INTRODUCTION

Body-size in *Drosophila* is much affected by the environmental conditions of growth, but little is known about the extent of either genetically or environmentally determined geographical variation is size and proportions of the many species which have been subject to genetical study. The main exception is the interesting work of Stalker & Carson (1947), who compared several dimensions of forty-five strains of *Drosophila robusta* taken from different parts of its American range. The samples measured were reared under standard laboratory conditions, so that any differences between them must be of genetic origin; and the dimensions measured were head width, thorax length, leg length, and wing length and width.

Mean annual temperature of the place of origin was found to be positively correlated with head and thorax size, but negatively correlated with wing size and leg length, so that genetically determined temperature (or north–south) clines existed for the various dimensions. These included a trend such that the northern populations had the largest ratio of wing length to thorax length.

Stalker & Carson (1948) found a generally similar trend in relation to height above sea level, among strains of *D. robusta* coming from sites at altitudes ranging from 1000 to 4000 ft. within a single district. Strains from the higher (colder) sites had longer legs and both longer and wider wings, while head and thorax size showed little or no correlation with altitude. Prevosti (1955) discovered a similar north–south cline in wing size among eight British populations of *D. subobscura*, the wings being largest in populations from the north, while all the British populations had larger wings than a population from Barcelona.

In view of these results, an analysis of the geographical trends in body dimensions of a number of species of *Drosophila* should prove of interest, and in this paper we examine the variation in body dimensions among twelve populations of *D. subobscura* coming from localities spread over the species' range from Scotland to Israel. The dimensions measured were essentially the same as those of Stalker & Carson (1947)—head width, thorax length, leg length, and wing length and width, with some minor differences in the method of measurement. The populations were reared for measurement under standard laboratory conditions, so that any differences discovered must be of genetic origin.

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The clinal pattern found in *D. subobscura* differed in several respects from that reported in *D. robusta*, and some detailed comparisons between the two species will be made. These comparisons were made possible through the kindness of Dr H. D. Stalker, who placed at our disposal additional computations on *D. robusta*, made on the data of Stalker & Carson (1947). It is hoped that the differences found between the two species will encourage study of other species of *Drosophila* along the same lines.

2. MATERIAL AND METHODS

A number of populations of *D. subobscura* had been maintained in this laboratory in mass culture at room temperature, for periods of eight to eleven generations, and twelve of these were selected for study so as to cover as wide a geographical range as possible. These populations were each started from thirty or more pair matings of wild flies, whose progeny were pooled to form the population, which was then continued by large-scale mass mating. These precautions were taken to ensure that a large and representative population from each site was obtained. The populations were maintained by Dr A. M. McFarquhar and Dr F. W. Robertson, who kindly made them available to us. The place of origin of each population and its latitude and mean annual temperature are listed in Table 1. The temperatures quoted are means of the average annual maxima and minima for thirty years (Meteorological Tables, 1958), and may in some cases be very approximate, since the nearest recording station was often far from the site of capture of the flies, and adjustments for altitude could not be made.

To obtain flies for measurement, eggs laid over a 24-hour period were placed, 50 per vial, in 3 × 1 in. vials containing about half an inch of the usual *Drosophila* culture medium (maize meal, molasses and agar, seeded with live yeast), and were incubated at 18° C. On emergence the flies were put, etherized, into small tubes which went immediately into a commercial deep-freeze, where they remained until measured. Here they remained fresh with pliable joints, and could be easily measured on the microscope special platform (Reeve & Robertson, 1949) without dissection. All dimensions except leg length were measured on the whole fly. The dimensions were:

Wing length: Tip of L IV to most anterior point inside the second basal cell.

Wing width: Distance between two lines drawn parallel to L III so as just to touch wing edges at outermost points.

Thorax length: Along mid-dorsal line from most anterior point to posterior edge of scutellum, as viewed from the dorsal aspect.

Head width: maximum distance between outer surfaces of eyes, as seen from dorsal aspect.

Tibia length: Total length of first tibia, measured after amputation and mounting in glycerine on a slide, with femur flexed.

Carson & Stalker (1947) measured the femur instead of the tibia (we found the femur was sometimes damaged during preparation), and they measured wing length

Table 1. *Origin and mean dimensions* ($\frac{1}{100}$ *mm*) *of populations of* D. subobscura

Place of origin	Lati- tude (N.)	Av. annual temp. (° F.)	Mean dimensions—deviations from constant									
			Wing length		Wing width		Thorax length		Head width		Tibia length	
			♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Aberdeen, Scotland	57° 10′	47	23·46	25·52	9·68	10·58	8·56	9·50	4·83	4·65	6·62	5·82*
Helensburgh, Scotland	56° 01′	47	20·69	22·50	9·46	12·01	6·82	6·71	2·73	1·99	6·31*	5·86*
Copenhagen, Denmark	55° 41′	46·5	18·86	19·52	7·64	9·05	6·73	7·45	3·79	3·93	4·56	4·17
London	52° 29′	50·5	17·29	16·95	7·25	7·87	6·48	5·22	3·83	2·64	5·01	3·63
Vienna, Austria	48° 15′	49	10·49	12·25	4·44	6·85	4·63	4·66	2·35	2·16	4·36	4·01
Zürich, Switzerland	47° 23′	48	6·97	9·14	2·75	1·47	5·12	4·60	2·94	2·33	4·31	3·17
Lunz, Austria	47° 06′	48·5	7·60	11·09	2·25	6·37	0·39	0·03	0·89	0·09	1·37	1·60
Chur, Switzerland	46° 52′	48	11·27	14·37	6·45	7·83	4·64	4·46	3·51	2·37	4·28	3·48
St. Etienne, France	45° 30′	52	11·27	17·33	5·05	8·07	4·49	5·65	3·30	3·40	4·15	3·76
Pavia, Italy	45° 12′	55	10·57	12·81	1·73	3·78	3·64	2·76	2·83	2·42	3·42	1·65
Formia, Italy	41° 16′	61	8·36	10·59	3·58	6·19	1·86	2·17	1·52	0·36	3·27*	2·26
Qiryat, Israel	31° 47′	63	0·83	0·99	0·39	0·72	2·27	2·44	1·48	1·24	0·29	0·76
Constant subtracted	—	—	216	238	101	110	104	118	90	97	59	61
S.E. of mean	—	—	±0·80	0·90	0·48	0·42	0·41	0·43	0·27	0·31	0·28	0·30
							S.E.s of items marked *:				0·39	0·42

from the anterior cross-vein instead of from the anterior end of the second basal cell, but otherwise their dimensions appear to have been virtually the same as ours. These differences are unlikely to affect any comparisons between the species except those between their means. All measurements were made by Misra.

The measurements were made in four batches, spread over six months, each population being measured twice. Ten males and ten females were measured per vial and five vials per population in each batch, except where insufficient flies were available. Most samples were complete, and an average of 9·44 males and 9·23 females were measured per vial and ninety-one flies of each sex per population for all dimensions except tibia length—in the third batch the material from two populations was accidentally destroyed before the legs had been amputated.

For the main analysis the vial means were treated as units of equal weight, and the variances were multiplied by the mean number of flies per vial to convert them to the basis of an individual fly.

A preliminary statistical analysis showed that, while mean body-size increased by $2\frac{1}{2}\%$ between the first and last batch measurements, there were no appreciable population × batch interactions. We can, therefore, assume that this size trend was the result of environmental factors—probably a seasonal effect on the temperature of the incubator—and will not affect any comparisons between populations. Batch effects were eliminated in the main analysis.

3. RESULTS

(i) *Correlations with latitude and temperature*

Table 1 lists the populations in order of decreasing latitude of their place of origin, and gives the mean dimensions for both sexes in each population. The dimensions are expressed in $\frac{1}{100}$ mm. as deviations from the constants at the foot of each column, and the last row gives the average standard error of each population mean.

Latitude has a range of 25° and mean annual temperature a range of 15° F., and it is at once apparent that all dimensions increase steadily with increase of latitude or decrease in mean temperature. Table 2 gives the correlation coefficients of each dimension with latitude and temperature, significance levels being shown at the foot of the table. The correlations with latitude are all positive and very high, those for males being generally a little higher than those for females, and there is no doubt about their statistical significance in spite of the small number (12) of populations examined. The correlations are higher for wing size and leg length than for head and thorax size. Temperature shows a similar picture, except that the correlations are uniformly negative and rather smaller than for latitude. The lower magnitude of the correlations with temperature may well reflect inaccuracies in the estimates of mean annual temperature for some of the localities, or the fact that this measure is not always a good index of the temperature at the most important period in the annual cycle of the population.

The general picture is clearly one of a very uniform geographical cline in all the dimensions measured, each increasing as we move northwards.

Table 2. *Correlations with latitude and temperature:* D. subobscura

	Latitude		Mean annual temperature	
Dimensions	Males	Females	Males	Females
Thorax length	0·774	0·729	−0·597	−0·546
Head width	0·672	0·604	−0·544	−0·500
Wing length	0·923	0·908	−0·650	−0·685
Wing width	0·851	0·796	−0·647	−0·593
Tibia length	0·855	0·856	−0·658	−0·712

Significance levels (10 degrees of freedom):
At $P = 0{\cdot}05$; 0·02; 0·01
$r = 0{\cdot}576$; 0·658; 0·708

The clinal patterns in *D. subobscura* and *D. robusta* are compared in Table 3, which gives the correlations of population means of females with latitude and temperature in both species, both for the five dimensions and for the ratio of wing length to thorax length.

Table 3. *Correlations with latitude and temperature (females)*

	Latitude		Temperature	
	D. subobscura	*D. robusta**	*D. subobscura*	*D. robusta*†
Thorax length	0·729	−0·370	−0·546	0·351
Head width	0·604	−0·638	−0·500	0·646
Wing length	0·908	0·544	−0·685	−0·539
Wing width	0·796	0·188	−0·593	−0·205
Leg length‡	0·856	0·369	−0·712	−0·333
W/T§	0·621	0·706	−0·368	−0·707
Latitude			−0·865	−0·988
5% significance levels	±0·576	±0·293	±0·576	±0·293

* From Dr H. D. Stalker (personal communication).
† From Stalker & Carson (1947).
‡ Tibia length for *D. subobscura*, femur length for *D. robusta*.
§ Ratio of wing length to thorax length.

Taking first the individual dimensions, the contrast between the two species is striking. While *subobscura* shows a virtually uniform cline in all dimensions, as we have seen, only the wing and leg dimensions follow a similar cline in *robusta*, and the cline appears to be less definite in the latter species since the correlation coefficients are much lower. Head and thorax size follow opposite trends in the two species, since they increase in *subobscura* but decrease in *robusta* as latitude increases. Head width alone of the dimensions shows correlations as high in *robusta* as in *subobscura*, though of opposite sign, the correlations being much lower in *robusta* for the other four dimensions.

The ratio of wing length to thorax length unexpectedly shows the lowest correlations of all in *subobscura* and the highest of all in *robusta*, its correlation with temperature being twice as high in the latter species (–0·707 compared with –0·368). Stalker & Carson (1947), using a wing length index adjusted to constant thorax length, also noted the high negative correlation with temperature in *robusta*.

In both species the correlations with temperature are all opposite in sign to those with latitude, and Table 3 brings out the interesting fact that latitude is more highly correlated with temperature for the places of origin of the *robusta* populations (–0·988) than for those of the *subobscura* populations (–0·865). In consequence, the correlations with any dimension or index are numerically equal for temperature and latitude in *robusta*, but higher for latitude than temperature in *subobscura*. The significance of this difference is not clear.

(ii) *Further analysis of the variance between populations*

The high correlations with latitude in *subobscura* suggest the possibility that the cline in dimensions is entirely a function of latitude. This is tested in Table 4, which gives the regression variance of each dimension on latitude, the remaining variance

Table 4. *Analysis of variance between populations of* D. subobscura

		Mean squares in ($\frac{1}{100}$ mm.)^2 per fly					
MALES	D.F.	Thorax length	Head width	Wing length	Wing width	Tibia length	
Between populations	11	500	117	3,850	890	293	
Regression on latitude	1	3,300	582	36,010	6,910	2,360	
Remainder	10	220	71	630	262	87	
Error	92	15·4	6·6	58·0	21·4	7·8	(84)*
FEMALES							
Between populations	11	600	161	3,940	1,040	228	
Regression on latitude	1	3,520	647	35,780	7,250	1,840	
Remainder	10	310	112	760	420	67	
Error	93	17·3	8·9	74·8	16·0	8·4	(85)*
INTERACTION							
Population × Sex	11	24·6	11·7	131·2	98·0	15·3	
Population × Batch (pooled)	18	23·0	6·1	88·7	47·3	13·2	(14)*

* Degrees of freedom for tibia length.

between populations, and the error variance (between replicate vials within populations) for each sex. While latitude accounts for a major part of the variance of each dimension, when its effect is removed the remaining variance between populations is still about ten times the within-population variance in all cases, so that some local differentiation between the populations, not attributable to geographical position, must have occurred. The last two rows of the table show that no appreciable population × sex interactions are present, indicating that sex differences in the five dimensions do not vary noticeably between the populations.

Stalker & Carson (1947) gave a partial analysis of the variances between strains for *robusta* in their Table 3, and we have extended this for comparison with *subobscura* in Table 5. Stalker & Carson drew attention to the large variance between strains from the same locality for each dimension (shown in row *d*), but this item must be interpreted with caution in view of the nature of their material. Their strains mostly started from single wild females or pair matings, measured in the F_2, so that each strain probably consisted of a family founded on full sibs. If all strains from the same locality came from a single interbreeding population, we should still expect a large variance between strains within localities, calculated as in Table 5. This may be seen by treating rows *d* and *e* as a progeny test giving the mean squares between and within families of full sibs, the expectations of their mean squares being, approximately,

Table 5. *Analysis of variance between localities in* D. robusta *females*

		Mean squares in $(\frac{1}{100}\text{ mm.})^2$ per fly				
Source	D.F.	Thorax length	Head width	Wing length	Wing width	Femur length
(*a*) Between localities	21	161	171**	1014**	405*	50
(*b*) Regression on temperature†	1	808*	2053**	8506**	517	166
(*c*) Remainder between localities	20	129	77	640	401*	44*
(*d*) Between strains within localities	23	92*	57**	346**	167**	20**
(*e*) Within strains	1530	6·57	4·20	22·0	9·80	2·31
(*f*) h^2% within localities (see text)		54	53	59	62	36

For significance, (*a*) and (*c*) are tested against (*d*), (*b*) against (*c*), (*d*) against (*e*).

$* \; P < 0{\cdot}05$

$** \; P < 0{\cdot}01$

† The regression on latitude would give virtually the same variances.

$$\text{row } d: \quad E + \tfrac{1}{2}(1+35)G$$

$$\text{row } e: \quad E + \tfrac{1}{2}G$$

where E and G are the variances due to environmental and genetic causes affecting a single individual, and 35 is the number of flies measured per strain. The corresponding heritabilities, calculated as $100G/(E+G)$, are given in row *f*, and vary from

36 to 62% for the various dimensions. These are rather higher than is usually found in tests on wing or thorax length in laboratory populations of *D. melanogaster*, but may not be excessive for a wild population. The important point here is that the large within-locality variances of Table 5 row *d* do not necessarily imply that there is any local differentiation in dimensions of the *robusta* populations within the localities from which Stalker & Carson collected their samples. A single pair mating, as the authors realized, gives a rather poor estimate of the mean characteristics of a particular locality, and the small number of samples per locality is doubtless partly responsible for the low correlations with geographical position in *robusta* compared with *subobscura*. Rows *a* and *c* in Table 5 suggest that there are significant differences between localities for all dimensions, though these are really large only in the case of wing length and head width. The regressions on temperature of wing width and femur length are not significant, but both dimensions show significant variation between localities uncorrelated with temperature.

Tables 4 and 5 allow a final rough comparison of some interest to be made, as shown in Table 6. Here, after eliminating the regression on latitude, we have calculated the residual component of variation between localities and expressed it as per cent of the variance component within localities ($E+G$ as defined above).

Table 6. 100 × *ratio of adjusted variance component between localities to variance component within localities (females)*

	Thorax length	Head width	Wing length	Wing width	Leg length
D. subobscura	170	110	91	250	70
D. robusta	6	10	14	24	13

Methods of computation

	Component between localities	Variance within localities
D. subobscura (from Table 4)	(Remainder—Error)/10	Error
D. robusta (from Table 5)	$(c-d)/35 \times 1{\cdot}9$	$e+(d-e)/35$

The ratios are all very much larger for *subobscura* than for *robusta*, indicating that the amount of variation between localities which is uncorrelated with geographical position is much greater in the former species in spite of the fact that its dimensions are much more closely correlated with latitude. This quite unexpected result is found consistently with all dimensions, and appears to be well established in spite of the crudity of the comparisons.

(iii) *The rates of change of dimensions with latitude and temperature*

Table 7 gives the regression coefficients of each dimension on latitude and temperature in both species, expressed as per cent change for unit change in geographical index, and enables us to compare the steepness of the clines in each species. Standard errors for these coefficients are given in the lower half of the table, and show that some of them are somewhat inaccurately determined. The rates of change for individual dimensions are generally more rapid in *subobscura*, which makes about 1% change in both wing dimensions and in femur length for every three degrees change of latitude or temperature. *D. robusta* requires about five degrees change in latitude or seven degrees in temperature to produce 1% change in wing length or head width. On the other hand, *W/T* changes more than twice as fast in *robusta* as in *subobscura*, for unit change in latitude, and is the character with the steepest cline in *robusta*.

Table 7. *Regressions on latitude and temperature as percentage of mean dimensions (females)*

	Thorax length	Head width	Wing length	Wing width	Femur length	*W/T**
Percentage regressions on latitude						
D. subobscura	0·219	0·115	0·348	0·330	0·300	0·138
D. robusta	−0·11	−0·22	0·24	0·09	0·10	0·357
Percentage regressions on temperature						
D. subobscura	−0·206	−0·120	−0·321	−0·318	−0·314	−0·100
D. robusta	+0·066	+0·129	−0·143	−0·053	−0·058	−0·210
Standard errors for above regression coefficients						
On latitude:						
D. subobscura	±0·066	±0·047	±0·049	±0·080	±0·058	±0·052
D. robusta	±0·044	±0·043	±0·067	±0·080	±0·051	±0·054
On temperature:						
D. subobscura	±0·10	±0·065	±0·11	±0·13	±0·098	±0·081
D. robusta	±0·026	±0·025	±0·039	±0·047	±0·030	±0·034

Note: The standard errors are based on the variances between localities, and the error degrees of freedom in applying a *t*-test should be 10 and 20, for *subobscura* and *robusta*.

* *W/T* is ratio of wing length to thorax length.

A point of great interest suggested by these comparisons is the question how far the effects of changing the temperature of rearing flies in the laboratory mimic the effects of the temperature clines. In the data so far discussed, *subobscura* was reared at 18° C. and *robusta* at 25·5° C., but Stalker & Carson (1949, Table 3) compared five of their strains from widely different localities at 25·5° and 17·6° C., and their data enable us to make the comparisons shown in Table 8. Rows 2 and 3 summarize their data and row 1 gives the means for *subobscura* at 18° C. In comparing the two species we must remember that wing length and leg length were different dimensions in each.

Row 7 shows that *subobscura* is about 20% smaller in thorax length and head width and 26% smaller in wing width than *robusta* when both species are reared at 18° C. This may not be an entirely fair comparison, since the natural temperature ranges of the two species are certainly different and *subobscura* can barely survive at 25° C. But *subobscura* at 18° C. is still some 16% smaller than *robusta* at 25° C.

Row 4 gives the percent reduction in each dimension when *robusta* is reared at 25·5° C. instead of 17·6° C., an increase of 14° F., and shows that wing length is reduced most (by 9%) and head width least (by 5%) of the dimensions, while *W*/*T* declines 3%. Comparable figures for *subobscura* would be of considerable interest, but we have at present only an estimate for thorax length, based on McFarquhar & Robertson (1963, Table 4), where it is shown that two of our *subobscura* strains differed on the average by 0·28 in $\log_e(\text{thorax length})^3$, when reared at 18° and 25° C. This represents a reduction in thorax length of 8·9%, compared with 6·2% for a slightly bigger temperature difference in *robusta*. So size appears to be reduced rather more in *subobscura* than in *robusta* by raising the temperature of rearing the larvae from 18° to 25° C., a result which may well be due to the fact, pointed out by McFarquhar & Robertson (*loc. cit.*), that 25° C. is too high for normal growth and survival of *subobscura*.

Table 8. *Comparison of means in the two species (females:* $\frac{1}{100}$ *mm.)*

	Temp. reared	Thorax length	Head width	Wing length	Wing width	Leg length	*W*/*T*
1. *D. subobscura*	18° C.	123	100	241†	116	64†	—
2. *D. robusta**	17·6° C.	156	125	239	157	81	1·526
3. *D. robusta**	25·5° C.	147	119	217	144	76	1·481
4. *D. robusta* % reduction at	25·5° C.	6·2	5·0	9·0	8·3	6·6	3·0
5. *D. robusta* % regression on	temp. °F.	−0·44	−0·35	−0·63	−0·58	−0·46	−0·21
6. Corresponding regression between populations on temp. °F.							
(*a*) *D. robusta*		+0·07	+0·13	−0·14	−0·05	−0·06	−0·21
(*b*) *D. subobscura*		−0·21	−0·12	−0·32	−0·31	−0·31	−0·10
7. *D. subobscura*/*D. robusta* % at	18° C.	79	80	—	74	—	—

* Calculated from Stalker & Carson (1949), Table 3.

† Different dimensions measured in the two species (see text).

Table 8, row 5, gives the per cent 'environmental' regressions on temperature for *robusta*, obtained by dividing row 4 by the difference in °F. These figures may be compared with the corresponding 'genetic' regressions for the two species in rows 6, *a* and *b*, copied from Table 7. This brings out the striking fact that, while the environmental regressions are several times as large as the genetic regressions for each dimension and different in sign for two of them, in *robusta*, the two regressions are equal for the wing/thorax ratio (*W*/*T*), both being −0·21%. It should perhaps be emphasized that there is no environmental component in the genetic regressions, since they are computed from differences between strains reared all at the same temperature. The similarity of the two regression coefficients for *W*/*T* in *robusta* has arisen from a combination of different inter-relationships in the two cases. Environmentally, both wing length and thorax length decrease as temperature increases, the former more rapidly (coefficients −0·63% and −0·44%), while the genetic trends are such that wing length decreases but thorax length increases with rise of temperature (coefficients −0·14% and +0·07%). The net result of both is a coefficient of −0·21% for W/T.

The genetic regressions for *subobscura* (Table 8, row 6*b*) are all very close to half the corresponding environmental regressions for *robusta*. So, assuming that *robusta* and *subobscura* would show similar changes in size and proportions when the temperature of rearing is changed, it appears that the geographical cline in body dimensions mimics rather closely the effects of changing the temperature of rearing the larvae in *subobscura* but not in *robusta*.

(iv) *The basic variability of dimensions in the two species*

Another comparison of interest is that between the basic within-strain variabilities of the two species for each dimension. Table 9 gives these variances expressed as squared coefficients of variation, based on the within-culture variances of *subobscura* (row 1), of *robusta* compared at 17·6° and 25·5° C. (rows 2 and 3) and of the full *robusta* data at 25·5° C. Clearly the variances in *robusta* are not appreciably altered by changing the temperature of rearing. The *subobscura* variances are all rather uniformly about twice as large as those for *robusta*. Part of this difference may be due to the narrower base of the *robusta* populations, referred to earlier, which could have reduced their variances by 20–30%, though some loss of genetic variance may also have occurred in the *subobscura* populations during their period of maintenance in the laboratory. Since *D. melanogaster* under the same culture conditions generally has a within-culture variance of between 3 and 5 for wing and thorax length, i.e. very similar to those of *D. robusta* (e.g. Reeve & Robertson, 1953; Robertson, 1955), it appears that *subobscura* is, under favourable laboratory conditions, 50–100% more variable in body dimensions than *robusta* and *melanogaster*. Clearly, similar data on other species would be of great interest.

Table 9. *Comparison of within-culture variances in the two species*

		Squared coefficients of variation (females)					
	Temp. reared (° C.)	Thorax length	Head width	Wing length	Wing width	Leg length	Degrees of freedom
1. *D. subobscura*	18	7·7	5·1	8·9	9·7	9·5	970
2. *D. robusta**	17·6	3·9	3·4	4·4	4·8	4·7	245
3. *D. robusta**	25·5	2·7	2·1	4·9	5·3	4·0	170
4. *D. robusta*	25·5	3·0	3·1	4·5	4·7	4·0	1530

* Calculated from Stalker & Carson (1949), Table 3.

4. DISCUSSION

We have been concerned in this paper with the genetically determined geographical varation in body dimensions which is found when populations from different localities are reared under standard conditions in the laboratory. Comparing our data on *D. subobscura* with that of Stalker & Carson (1947) on *D. robusta*, it is evident that gene-controlled clines exist in both species, such that wing and leg size and wing length/thorax length increase with latitude in both species while body-size, as judged by head width or thorax length, follows the same trend in *subobscura* but not in *robusta*—in the latter species the cline for body-size runs in the opposite direction so that the more northern populations are genetically the smaller. Another striking difference between the two species is that the correlations with latitude and the steepness of the clines for the various dimensions are much greater in the case of *subobscura*, with the exception of the wing length/thorax length ratio which shows a steeper cline for *robusta* than for *subobscura*.

An altitudinal transect of *D. robusta*, taken at sites within a single district by Stalker & Carson (1948), showed that the strains from the higher (colder) sites had genetically the largest wings and longest legs, while head and thorax size showed little or no correlation with altitude. So the altitudinal and latitudinal clines were very similar for wing size and leg size in *D. robusta*, and also bring out the difference in the clinal pattern between these characters and head and thorax size: the latter dimensions followed a cline in the opposite direction in relation to latitude, and no cline at all in relation to altitude. In another interesting study, Stalker & Carson (1949) sampled a single population of *D. robusta* at different times of the year, and found significant seasonal variations in all five dimensions. These were such that in the spring the population tended to resemble the northern and high altitude populations in body proportions, while during the summer it gradually changed towards the proportions of the southern and low altitude populations. It is possible to doubt the validity of this interpretation, since the trends are rather slight and the numbers sampled small, and further data on seasonal variation of this kind are clearly needed. The authors suggest that the differences in body proportions are adaptive responses to climatic differences over the species range, both geographically and in altitude, and that natural selection is causing changes of the same kind in response to seasonal variation in temperature or related factors within a single population. If this interesting hypothesis is true, then our data suggest that different species do not all react in the same way to the selective agencies responsible for the clines in body dimensions.

Since all our observations were made on flies reared under standard conditions in the laboratory, the important question arises of the relation of body dimensions in flies reared in their natural habitat to those of the same flies reared in the laboratory. McFarquhar & Robertson (1963) give a most instructive comparison of the frequency distributions of log (thorax length)^3 in wild and laboratory-reared *D. subobscura* of the Scottish (Helensburgh) population, which shows that wild flies are smaller and very much more variable than those reared under favourable laboratory conditions.

In units of thorax length, the wild flies averaged about 8% smaller, with variance (in squared coefficient of variation) 60 compared with 7 for flies reared in the laboratory. The Scottish wild flies were, in fact, about the same size as flies of the Israel population reared in the laboratory. Data are obviously needed on the size and proportions of wild flies from other places, so that we can relate the dimensions of wild and laboratory-reared flies from different populations. No data of this kind appear to be available for any other species.

Measurements on several populations of *robusta* reared at both 17·6° C. and 25·5° C. by Stalker & Carson (1948) demonstrated the existence of a strong negative correlation between all dimensions and temperature of rearing, so that these 'environmental' correlations are similar to the genetic correlations with temperature for all dimensions in *subobscura*, and to those for wing and leg length only in *robusta*. These contrasts raise interesting problems on which we can do little more than speculate at present, in view of our ignorance of the ecology of each species and of the actual range of body-size and proportions which the various populations show in their natural habitats.

The more northern populations of each species may be assumed to grow in their natural habitat at a lower temperature than the southern populations do, and will therefore be larger. However, this direct environmental effect does not seem to meet the size requirements of the various habitats, since the northern populations of *subobscura* are also genetically larger in all dimensions, while the northern *robusta* populations are genetically larger in wing and leg size, but genetically smaller in head and thorax size. Natural selection appears to have acted on the many genes affecting the various body dimensions so as to magnify the environmental temperature effects on all of them in *subobscura* and those on wing and leg size only in *robusta*.

Laboratory experiments in which populations were maintained for many generations at different temperatures should produce genetic changes of the same kind as those between the northern and southern populations, if temperature is the main selective agency, particularly in view of the rapid seasonal changes observed by Stalker & Carson (1948). No such tests have been reported on *subobscura* or *robusta*, but Dr F. W. Robertson (personal communication) maintained two strains of *D. melanogaster* both at 18° C. and at 25° C. in mass culture for about two years, and then compared wing and thorax length of the four populations when reared at each temperature. No difference was found in either dimension as a result the long period of maintaining the strains at different temperatures. If we can assume that the three species would react in the same way, this result would suggest that the selective agency in the wild populations is not simply temperature. It is more likely to be some other ecological factor, such as humidity or the composition of the food, which is itself correlated with temperature under natural but not under laboratory conditions.

A final problem of interest is the inter-relationship of genetical changes in the different dimensions. Both the latitudinal and the altitudinal clines in *robusta* suggest a strong tendency for wings and legs to change size together, and for head and thorax size to run together, the second pair of characters showing no correlation with the first pair. In *subobscura* one can detect less clear signs of the same pattern, in that head and thorax size are less closely correlated with latitude than are wing and leg size. The similarity of the two species in this respect is brought out more clearly on calculating the partial correlations of each dimension with latitude, holding thorax length constant (Table 10). The partial correlations of latitude with the wing and leg dimensions are positive and significant for both species, while those with head width are both negative, though only significantly so in *robusta*. In *subobscura*, it appears that head and thorax size are closely intercorrelated through genetical factors, and that the cline in each is due to change in frequency of genes which affect both, and which are probably general size factors. When the effects of these factors have been eliminated, there remains a high positive correlation between latitude and wing and leg size, which is probably due to genetic factors affecting both characters. Essentially the same pattern appears in the *robusta* correlations, with the difference that the relationship of latitude with the general size factors affecting head and thorax size runs in the opposite direction.

Table 10. *Partial correlations with latitude, holding thorax length constant* (*data on females*)

	D. subobscura	*D. robusta*
Wing length	+0·792	+0·650
Wing width	+0·634	+0·400
Leg length	+0·641	+0·453
Head width	−0·162	−0·560
5% significance level	0·602	0·295

Confirmation of this general picture comes from an interesting selection experiment by Robertson (1962), in which the ratio of wing length to thorax length was selected for increase and decrease, during ten generations, in *D. melanogaster*. The marked progress made in both directions was attributable entirely to the effects of the selection on wing length, since the up and down lines hardly differed in thorax length at the end of the experiment. Wing shape was unaltered by the selection, so that its width must have changed at the same rate as its length. More remarkable, tibia length of the three legs had increased in the high ratio line and decreased in the low ratio line, showing that a genetic correlation between wing and leg length existed which was independent of thorax length. Selection for a single dimension would, of course, bring into operation genes affecting general body-size, causing correlated changes in all dimensions.

The general picture emerging from these results is that there are at least two important groups of genes affecting the various dimensions:

(1) genes affecting general body-size, which change head and thorax size, and probably all other dimensions roughly in proportion, and
(2) genes affecting wing and leg size only.

In addition, there are doubtless genes affecting each dimension separately, which could be used to adjust their relative proportions.

The wing-leg size genes evidently follow a similar north–south cline in both *robusta* and *subobscura*, but the general size genes follow opposite clines, causing an increase in size northwards in *subobscura* and southwards in *robusta*. That the dimensional clines in *subobscura* are not simply the result of a single cline in general size is demonstrated by the fact that wing and leg size are still positively correlated with latitude when the partial correlations with thorax length are removed, although no such residual correlation of latitude with head width remains.

In *robusta*, there was no significant direct correlation between latitude and wing width, but the partial correlation became significantly positive when thorax length was held constant (Table 10), thus bringing the latitudinal cline more closely into line with the altitudinal cline in this species: the latter was such that thorax length and head width remained almost constant but both wing dimensions and leg length increased with altitude.

One of the most remarkable results established by Robertson's (1962) selection experiment and our correlation analysis is the fact that wing and leg size are genetically correlated independently of head and thorax size. Clearly the genetic intercorrelations of the different dimensions need further study, and it would be of great interest to know how far head width can be changed independently of thorax length, leg size independently of wing size, and wing width independently of wing length, in various species of *Drosophila*. On our hypothesis, we should expect selection for wing/thorax ratio to cause head width to change in the same direction as thorax length, but this has yet to be demonstrated. It is to be hoped that this discussion will stimulate further research into the many problems raised in our study.

SUMMARY

Five dimensions—wing length and width, thorax length, head width and tibia length—have been measured on samples of twelve populations of *Drosophila subobscura* taken from different parts of the species range, extending from Scotland to Israel. The populations had been started from thirty or more pairs of flies and maintained in the laboratory for eight to eleven generations. They were reared for measurement under standard conditions, so that any differences between them must be of genetic origin. The localities from which the populations came ranged over 25° of latitude and 15° F. in mean annual temperature.

The population means of the five dimensions all showed strong positive correlations with latitude and slightly lower negative correlations with mean annual temperature. There is, in consequence, a very uniform cline in the five dimensions, each increasing as we move northwards. This pattern differs from that found for essentially the same dimensions of *D. robusta* by Stalker & Carson (1947), where all the correlations with latitude are lower and those for head and thorax size are of opposite sign in the two species.

A partial correlation analysis and reference to selection experiments suggests that there are two distinct groups of genes involved in these clines: (1) a group of genes

causing increase in relative wing and leg size, and responsible for the positive correlation of these dimensions with latitude in both species, and (2) a group of general size genes causing correlated changes in all dimensions, which have been selected in opposite directions in the two species, with the result that head and thorax size increase in *subobscura* but decrease in *robusta* as latitude increases. The ratio of wing length to thorax length has a high positive correlation with latitude in both species.

Further analysis of the data available on both species brings out the following points:

1. In *subobscura* there is considerable variation between localities remaining for all dimensions when the correlations with latitude are eliminated. This component of variance is much larger in proportion to the within-locality variance in *subobscura* than in *robusta*.
2. Comparison of the percentage regression coefficients of dimensions on latitude or temperature shows that individual dimensions change more rapidly in proportion to both geographical indices in *subobscura*, but the ratio of wing to thorax length changes more rapidly in *robusta*.
3. The 'environmental' regression of dimension on temperature of rearing in the laboratory, measured only for *robusta*, is very much higher than the corresponding genetic regression between populations on mean annual temperature for all the *robusta* dimensions and different in sign for head and thorax size. But this environmental regression is uniformly only about twice as high as the corresponding genetic regression for each character in *subobscura*. Unexpectedly, the environmental and genetic regressions on temperature of the wing/thorax length ratio are equal in *robusta*.
4. *D. subobscura* is about 20% smaller in head and thorax size, and 26% less in wing width, than *robusta*, when both species are reared at 18° C.
5. The basic within-culture variances of the five dimensions are 50–100% greater in *subobscura* than in *robusta* for all dimensions. Variability in *D. melanogaster* is about the same as in *D. robusta*.

Some of the possible implications of these results are discussed.

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