STUDIES OF LINKAGE IN POPULATIONS. IX. THE EFFECT OF ALTITUDE ON X-CHROMOSOMAL ARRANGEMENT COMBINATIONS IN DROSOPHILA ROBUSTA[1,2]

MAX LEVITAN

Department of Anatomy, Mount Sinai School of Medicine of the
City University of New York, New York, New York

ABSTRACT

Data are presented concerning the gene arrangements in both arms of the X-chromosome of Drosophila robusta in eight altitudinal transects. The major change appears to be the increase of gene arrangement XL-1 (and decrease in XL, sometimes also XL-2) with increasing altitude. In each transect only one combination of XL-1 with a right arm arrangement seems primarily responsible for the increases with altitudinal change, and only one combination of XL for the decreases. In four of the transects the increasing combination appears to be XL-1.XR, XL-1.XR-2 in the other four. A substantial decrease with elevation in XL-2.XR-2 occurs only in the four areas where XL-1.XR exhibits the major increase. These results indicate that interactions between arrangements play a significant role in the adaptation of this species to altitude. The interactions appear related to the X-chromosomal linkage equilibriums in these regions. The data also suggest that the species employs its inversions in different methods of adaptation in the Appalachian Mountains from those it uses in other parts of the range.

DROSOPHILA robusta inhabits the deciduous woods of North America east of the Rocky Mountains. Its chromosomes contain many gene arrangements, most of them differing by paracentric inversions (CARSON and STALKER 1947; CARSON 1958). In an investigation of the gene arrangements on the right and left arms of the species' metacentric chromosomes, I noted significant linkage disequilibria in the populations inhabiting several woods in southwest Virginia. The nonrandom associations were found on both the X chromosome (LEVITAN 1958) and chromosome 2 (LEVITAN 1954, 1955, 1958). Subsequently, I have engaged in a long-term study to determine whether such disequilibria were widespread and, if so, whether they were significant factors in the geographic clines (reviewed by CARSON 1958) that characterize many gene arrangements of the species. Some of these data have shown that in a number of localities cyclic temporal variations in X-chromosomal arrangement frequencies depend on selection for certain combinations of the arrangements (LEVITAN 1973a,b). The

data presented here demonstrate that selection for specific X-chromosomal arrangement combinations is probably also a factor in altitudinal variation in the species, but that the critical combinations are not always the same as ones involved in temporal changes.

MATERIALS AND METHODS

Collection sites: Comparisons are made in this study whenever the altitudes of collection sites at the extremes of a transect differ by 500 feet or more, but differ by less than one degree in latitude and longitude, and, with certain exceptions to be noted, the collections were made either simultaneously or on closely successive periods at the different altitudes. The transects studied were:

(1) The Great Smoky Mountains in the vicinity of Gatlinburg, Sevier County, Tennessee;

(2) The Blue Ridge Mountain Region of North Carolina (collections of MILLER and WEEKS 1964);

(3) The Appalachian Mountain Region of northeastern Georgia;

(4) Blue Ridge Mountains of Virginia at approximately 38°N latitude;

(5) The eastern edge of the Allegheny Mountains at approximately 37°50'N latitude;

(6) The Great Valley of Virginia just east of the Allegheny Plateau;

(7) Southeastern Pennsylvania, from the vicinity of Philadelphia to an area above Jim Thorpe in Carbon County;

(8) Northern New Jersey at approximately 41°N latitude.

Transects 5, 6, and 8 involved two altitudes each; the others three or more. Details of the location of collections are obtainable or request.

Collection and analysis techniques: In making these collections, my goal was a "satisfactory sample", arbitrarily set at fifty males and fifty females, in order to obtain data concerning at least 75 X chromosomes. This was not always attainable, however, due to limitations on the time that could be spent in a locality and occasional accidents in transit. In a few cases, notably in several of the Georgia stations, the area may have recently been sprayed with insecticides. If a larger sample was collected, an attempt was made to analyze every individual, male or female, caught.

The karyotypic constitution of a collected female was determined whenever possible by mating her to males of known constitution, after initial "desperming" transfers if she had been inseminated in nature; but X-chromosomal inferences could also be made from the karyotypes of the sons, larval or adult, from her matings in the wild. Generally, homozygosity was scored on the basis of ten progeny, with a minimum of six. As has been explained in previous papers (LEVITAN 1955, 1958) the rarity of crossovers on this chromosome make *D. robusta* adult analyses highly accurate for the X-chromosome arrangements, so that correction factors of the type discussed by DOBZHANSKY and LEVENE (1948) are not necessary. These techniques have been previously described (LEVITAN 1955, 1964.)

Notation: The gene arrangements encountered in this study are named and described by CARSON and STALKER (1947) and CARSON (1958). To save space, the tables will use the short-hand notations explained in previous publications (CARSON 1953, 1958; LEVITAN 1955, 1958). In this notation the karyotype XL/XL-1, XR-2/XR-2 (XL.XR/2/XL-1.XR-2, in linkage form), for example, would be written S/1, 2/2, or S2/12.

RESULTS

Tables 1 through 8 present the altitudinal comparisons of this study, showing the frequencies, in percent, of the combinations of left- and right-arm arrangements in each transect. The interested reader can thereby derive the frequencies of the individual gene arrangements if desired; the reverse would not be possible.

Because of seasonal heterogeneity (LEVITAN 1973a), my Gatlinburg data must be treated separately for spring and summer. Table 1 shows that in both seasons arrangement combination XL-1.XR(1S) increases, while XL.XR-2(S2) and XL-2.XR-2(22) decrease, with altitude.

A similar pattern obtains in the transect based on Highlands, North Carolina, to the east at nearly the same latitude (Table 2). Since several of the samples were very small, they were combined as follows for the calculations: 1100′ (16 X chromosomes) and 1500′ (120), with weighted average altitude of 1450′; 2000′ (10), 2400′ (6), and 3000′ (16), with weighted average 2500′; 3650′ (29) and 3840′ (120), with weighted average 3800′. Substantially identical results would have been obtained if the small samples had been distributed in other statistically homogeneous groupings. Again, clinal combinational differences are confined to XL-1.XR, increasing with increasing elevation, and to XL-2.XR-2 and, less regularly, XL.XR-2, for the opposite changes.

TABLE 1

Frequencies (in percent) of X-*chromosome gene arrangement combinations in an altitudinal transect in the Smoky Mountains near Gatlinburg, Tennessee*

Elevation	N	SS	S2	1S	12	22
A. LEVITAN (May)						
1000	50	14.0	30.0	4.0	24.0	28.0
1400	110	5.5	28.2	18.2	22.7	25.5
2000	56	1.8	10.7	28.6	41.1	17.9
3000	12	8.3	0.0	75.0	16.7	0.0
Total	228	6.58	22.81	20.61	27.19	22.81
χ^2 (12 $d.f.$) $=$ 50.6**						
B. LEVITAN (August)						
1400	51	7.8	29.4	7.8	15.7	39.2
2000	798	6.6	21.8	25.4	22.1	24.1
3000	58†	1.7	10.3	51.7	13.8	22.4
Total	907†	6.39	21.50	26.13	21.17	24.81
χ^2 (8 $d.f.$) $=$ 35.2**						
C. STALKER and CARSON (July)						
1000	53	47.2	37.7	0.0	7.5	7.5
1200	78†	33.3	25.6	6.4	7.7	26.9
1400	95†	11.6	34.8	10.5	18.9	24.2
2000	258‡	7.0	25.9	13.9	17.1	36.1
3000	40	0.0	15.0	27.5	17.5	40.0
4000	34	0.0	14.7	8.8	26.5	50.0
Total	458	15.94	27.29	11.14	15.50	30.13
χ^2 (20 $d.f.$) $=$ 122.1**						

Elevations in feet above sea level, to the nearest 100 ft. Parts (A) and (B) are collections of the author, (C) adult data inferred from protocols of STALKER and CARSON (1948).

 * $P < 0.05$.
 ** $P < 0.01$.
 † Also one XL-2.XR (2S), a rare combination, not included in the calculations.
 ‡ Also three XL-2.XR, not included in the calculations.

TABLE 2

Frequencies (in percent) of X-chromosome gene arrangement combinations in an altitudinal transect in the Blue Ridge Mountains of North Carolina

	Elevation	N	SS	S2	1S	12	22
	1450	135†	5.2	27.4	15.6	24.4	27.4
	2500	38	10.7	7.1	42.9	17.9	21.4
	3800	149	5.4	10.7	53.7	18.8	11.4
	4100	38	2.6	10.5	63.2	15.8	7.9
Total		350†	5.43	16.86	39.14	20.57	18.00
χ^2 (12 d.f.) =	63.8**						

Footnotes as in Table 1.

Altitudinal variation in *XL-1.XR* stands out also in the samples taken to the south of the Smokies in the Blue Ridge Mountains of Georgia (Table 3A), although firm conclusions are hampered by the small numbers and the deviation from pattern at 1800 feet. Coalescing the statistically homogeneous data from the three sites near Cornelia (part B of Table 3) strengthens the conclusion.

These basic patterns are repeated also in the two-locality transect of the Allegheny Mountains in eastern West Virginia and western Virginia (Table 4). With increasing altitude, there are again strong increases in *XL-1.XR* and smaller decreases in *XL.XR-2* and *XL-2.XR-2*. Comparing the data to those of the Smokies for the same season (August), it may be noted that at comparable altitudes the *XL-1* frequencies are generally higher, and *XL* and *XL-2* correspondingly lower, in the Allegheny sample, presumably because of the higher latitude, and this is mainly reflected in SS and 12.

Passing on to the transect taken in the Blue Ridge Mountains of Virginia and

TABLE 3

Frequencies (in percent) of X-chromosome gene arrangement combinations in an altitudinal transect in Northeastern Georgia

	Elevation	N	SS	S2	1S	12	22
(A). Raw data							
	1100	59	5.1	33.9	3.4	15.3	42.4
	1400	44†	9.1	45.5	9.1	11.4	25.0
	1800	26	0.0	34.6	3.8	3.8	57.7
	2200	18	11.1	16.7	22.2	16.7	33.3
χ^2 (12 d.f.) =	20.8						
(B) Coalescing Cornelia area data							
	1300‡	129†	5.4	38.0	5.4	11.6	39.5
	2200	18	11.1	16.7	22.2	16.7	33.3
χ^2 (4 d.f.) =	9.8*						

Footnote as in Table 1, except as below.
† Excludes one instance of *XL.XR-1* (S1), which is rare in the Southeast.
‡ Weighted average altitude; χ^2 testing heterogeneity $= 11.7$ ($P < 0.2$ for 8 d.f.).

TABLE 4

Frequencies (in percent) of X-chromosomal arrangement combinations two elevations on the eastern edge of the Allegheny Mountains at approximately 37° 50' N. latitude

Elevation	N	SS	S2	1S	12	22
1150	180	0.6	28.9	2.2	55.0	13.3
1920	206	1.5	17.0	23.8	54.9	2.9

χ^2 (4 d.f.) — 52.7**.

Footnotes as in Table 1.

its foothills (Table 5), once more substantial variation is evident in only one linkage of *XL-1*. Here, however, it is in *XL-1.XR-2*, which increases with altitude even though arrangement *XR-2* tends downward. The concomitant decrease with altitude involves mainly *XL.XR-2*. Though *XL-1.XR* apparently also increases on the first leg of the altitudinal increases, it levels off thereafter; *XL-2.XR-2* follows a similar pattern in the opposite changes.

The small samples comparing arrangement frequencies at two Allegheny Plateau woods in Montgomery County, Virginia (Table 6) are not particularly illuminating, the only case where the overall χ^2 is not significant. Interestingly, the largest change is an increase in *XL-1.XR-2* with altitude.

The transects studied in southeastern Pennsylvania (Table 7) and north-

TABLE 5

Frequencies (in percent) of X-chromosome gene arrangement combinations in an altitudinal transect of the Blue Ridge Mountains of Virginia extending from Charlottesville to stations alongside the Skyline Drive near Afton and Waynesboro

Elevation	N	SS	S2	1S	12	22
500	75	2.7	44.0	6.7	29.3	17.3
1400	344	9.6	32.3	11.0	37.5	9.6
2200	196	9.7	18.9	13.8	50.0	7.7
3100	225	8.9	18.2	13.8	50.2	8.9
Total	840	8.81	26.43	12.02	43.10	9.64

χ^2 (12 d.f.) = 46.2**.

Footnotes as in Table 1.

TABLE 6

X-chromosomal arangement combinations (in percent) in coincident collections in August, 1962 at two elevations of the Allegheny Plateau at approximately 37° 15' N. latitude

Elevation	N	SS	S2	1S	12	22
1450	43	14.0	34.9	11.6	30.2	9.3
2100	72	5.6	23.6	6.9	48.6	15.3
Total	115	8.70	27.83	8.70	41.74	13.04

χ^2 (4 d.f.) =   8.1

TABLE 7

Frequencies (in percent) of X-*chromosomal gene arrangement combinations in an altitudinal transect in southeastern Pennsylvania*

	Elevation	N	SS	S2	1S	12	S1	22
(A) July								
	120	514	59.1	8.8	12.6	14.8	1.9	2.7
	400	75	26.7	12.0	9.3	44.0	2.7	5.3
	1200	72	5.6	13.9	19.4	55.6	0.0	5.6
Total		661	49.62	9.68	13.01	22.54	1.82	3.33
χ^2 (10 *d.f.*) = 118.9**								
(B) August								
	120	517	73.5	8.1	9.5	5.2	1.9	1.7
	400	148	36.5	15.5	13.5	21.6	8.8	4.1
	1200	43	20.9	4.7	23.3	46.5	0.0	4.7
Total		708	62.57	9.46	11.16	11.16	3.25	2.40
χ^2 (10 *d.f.*) = 154.1**								

Footnotes as in Table 1.

eastern New Jersey (Table 8) involve relatively small changes in altitude, the one in Pennsylvania having a north-south alignment and the one in New Jersey an east-west one. However, these have sharply evident effects on the *D. robusta* chromosomal polymorphism. Here, the clearest trend is for *XL-1.XR-2* to increase, and *XL.XR* to decrease, with increasing altitude (note especially part A of Table 7 and part C of Table 8).

Pennsylvania and New Jersey are the first areas encountered in this study with more than sporadic quantities of *XR-1*, an arrangement that is very common west of Ohio and north of Arkansas and Oklahoma. It is almost invariably linked to *XL* along the east coast (LEVITAN 1973b), and the combination tends to be more frequent at higher altitudes even though the overall frequency of *XL* is usually diminished. It was absent, however, in the samples from the highest Pennsylvania elevation (Table 7).

DISCUSSION

Series of gradually changing contiguous populations—which Huxley has dubbed "clines"—play a very important role in the modern understanding of the evolutionary process (*cf.*, MAYR 1966). They underline particularly the significant role of natural selection in the adaptation of populations to variations in their environment, since random processes of mutation, drift, or migration could hardly account for such regular variations. The major problem has been to determine exactly what portion of the genetic material is involved in the clinal selection, and exactly what interplay of genetic material and ecological conditions determine its persistence.

The most consistent finding in the eight *Drosophila robusta* transects described here is that a linkage combination involving *XL-1*—albeit not always the same

TABLE 8

Frequencies (in percent) of X-chromosome gene arrangement combinations in an altitudinal transect in northeast New Jersey

	Elevation	N	SS	S2	1S	12	S1	22
(A) May, 1972								
	150	222	47.3	1.4	46.8	3.6	0.9	0.0
	900	112	22.3	3.6	58.0	13.4	2.7	0.0
Total		334	38.92	2.10	50.60	6.89	1.50	1.0
χ^2 (4 d.f.) = 27.5**								
(B) June, 1973								
	150	127	41.7	1.6	46.5	7.9	0.0	2.4
	900	209†	16.3	3.3	56.5	22.0	1.0	1.0
Total		336†	25.89	2.68	52.68	16.67	0.60	1.49
χ^2 (4 d.f.) = 34.1**								
(C) July, 1970								
	150	203	41.9	1.0	54.2	2.5	0.0	0.5
	900	66	28.8	1.5	48.5	16.7	4.5	0.0
Total		269	38.66	1.12	52.79	5.95	1.12	0.37
χ^2 (5 d.f.) = 29.9**								
(D) July, 1972								
	150	172	46.5	0.6	50.0	2.3	0.0	0.6
	900	260†	11.9	3.1	60.0	20.8	2.3	1.9
Total		432†	25.69	2.08	56.02	13.43	1.39	1.39
χ^2 (5 d.f.) = 84.7**								
(E) August, 1970								
	150	44	43.2	0.0	47.7	6.8	0.0	2.3
	900	93	16.1	4.3	52.7	21.5	2.2	3.2
Total		137	24.82	2.92	51.09	16.79	1.46	2.92
χ^2 (5 d.f.) = 15.7**								

Footnotes as in Table 1, except as noted.
† Excludes one *XL-1.XR-1*, which is very rare in the eastern United States (LEVITAN 1973b).

one—attains higher frequencies in clines of increasing elevation. If the data in the present study were analyzed by gene arrangement rather than by arrangement combination, the regular increase of *XL-1* with increased elevation is clearly evident in seven of the eight transects. The only apparent exception, Georgia (Table 3), involves some of the smallest samples of the study.

Concomitantly, there is a regular decrease in frequency of *XL* as elevation increases. This, too, is clear in all of the eight transects with the possible exception of the small Georgia samples (Table 3A). In three of the transects *XL-2* also decreases regularly (my 22 data of Tables 1, 2, and 4).

No *X*-right arrangement presents a consistent pattern.

The finding of altitudinal variation in *XL-1* and *XL* confirms a similar finding of STALKER and CARSON (1948) in their classic Great Smoky Mountain transect

(partially reproduced by Table 1C). It is interesting that the X-left clines in Tables 1A and 1B parallel closely those of STALKER and CARSON (1948), even though the frequencies of several arrangements are not the same in the two studies. For example, at 2,000′, where undoubtedly the same woods was used in both studies, they found about 34% XL, 32% XL-1, and 35% XL-2, whereas in my material, these frequencies for the same season were 28, 48, and 24%, respectively (data of Table 1B). The differences are substantially greater than would be expected from chance sampling error. Another difference is in the effect of elevation on XL-2 there: their adult data show a steady increase (Table 1C), and in their total data it increased between 1000′ and 1400′, remained stable between 1400′ and 3000′, and increased again between 3000′ and 4000′. It is not possible to determine, however, whether these differences arose from differences in collecting and sampling technique or from a real population shift during the eleven years intervening between the two studies. ARMENTROUT (1963) observed a similar increase in XL-1 for comparable altitudes on Unaka Mountain in Tennessee, though his data are hampered by heterogeneities and show a decline in this arrangement at some of the higher altitudes; the adult data of STALKER and CARSON (1948) and Table 1C suggest a similar effect. Altitude clines of gene arrangements in other Drosophila species are reviewed by CARSON (1967).

The data presented here indicate that in spite of the pervasiveness of the altitudinal changes in XL-1, they derive in each area from clinal changes in only one of the linkage combinations of XL-1 and a right arm arrangement. In the Tennessee-North Carolina-Georgia Blue Ridge area and in the Allegheny Mountains the combination that consistently increases with elevation is XL-1.XR, whereas in localities studied to the north and east of them the most consistent change is the direct relation of XL-1.XR-2 and altitude. In each case, the other XL-1 combination either shows a smaller increase, presents an inconsistent pattern (Tables 1, 3A, 5, 7, and 8), or actually decreases (Tables 2 and 6; nominally also Table 4). Note that at the same altitude range the portion of the data of STALKER and CARSON (1948) derived from adult samples (Table 1C) also shows a steady increase in XL-1.XR with elevation, but a much smaller, more irregular one in XL-1.XR-2.

The noted concomitant tendency of XL to dcrease with increasing altitude is based on less regularity in the underlying arrangement combinations. XL.XR-2 evidences such a decrease in all six of my Appalachian transects (Tables 1–6), though two of these (Tables 2 and 3) present some irregularities. In Pennsylvania and New Jersey (Tables 7 and 8), however, it increases with increased elevation. In these two areas the brunt of the decrease with increasing altitude is borne by XL.XR, but this combination decreases regularly in only two Appalachian transects (Tables 1B and 6), and increases in several others (Tables 3B, 5, and nominally, 4).

Arrangement XL-2 or combination XL-2.XR-2 shows an interesting consistency: regular decreases with altitude occur where the main increasing com-

bination is *XL-1.XR* (Tables 1A, 1B, 2, 3B, and 4), but not in the transects where the main increasing combination is *XL-1.XR-2*.

These relationships to changes in altitude, and particularly the tendency of one combination, *XL-1.XR*, to increase with increase in elevation in one part of the species range and a second combination, *XL-1.XR-2*, to do so in another part of the range, suggest once again a significant role of interactions of "independent" gene arrangements in the microevolution of *Drosophila robusta*.

One of these, *XL-1.XR-2*, also seems to play the major role in temporal changes (LEVITAN 1973a), even in an area (the Smokies) where it is probably not an altitudinal factor.

Despite the attractivenes of the interaction hypothesis to explain the observed results, there are at present no statistical methods available that would (1) test the significance of the changes in individual arrangements or combinations or (2) rule out the other possibility, namely, that the observed regularities stem from coincident changes in the gene arrangements *per se* of the left and right arms of the chromosome.

The interaction hypothesis is made more attractive, though it cannot be proved, by observation of an apparent relationship between the altitudinal changes and interarrangement interactions that manifest themselves as linkage disequilibriums. Of particular interest is the deviation from randomness among the four combinations of arrangements *XL*, *XL-1*, *XR*, and *XR-2* (not counting these arrangements when they are attached to *XL-2* or *XR-1*). I (LEVITAN 1973b) referred to this as the "*X*:S,1; S,2 coupling-repulsion association." As will be more apparent below, the association exists in some form in all the transects of Tables 1 through 8.

Since each transect consists of two or more samples that are heterogeneous because of elevation or seasonal factors, a check of their consistency with respect to these disequilibria may be obtained by performing a x test (SIMPSON, ROE and LEWONTIN 1960). This is done by adding their x values. each x being the square root of the disequilibrium x^2 of a sample in the transect. The significance of the test is measured by noting that the sum of the x values divided by the positive square root of the sum of the degrees of freedom in the samples is equal to t, the number of standard errors from the mean of a normal distribution.

For this test, x is considered positive if these is an excess of the "coupling" combinations (*XL.XR* and *XL-1.XR-2*), as was found earlier in the Blacksburg samples in the Virginia Plateau (LEVITAN 1958, 1961); a minus sign bespeaks an excess of the "repultion" combinations (*XL-1.XR* and *XL.XR-2*), as was reported for the total data from 2,000' in the Smoky Mountains by LEVITAN (1961). In the absence of consistency among the samples in this respect the x values would tend to cancel each other, and t would be small.

Table 9 summarizes the t values for this association in the data of Tables 1 through 8. All except one are significant at least the 5% level. Even the lone exception, the data of Table 5, contains a significant association in the larger samples, which were taken at elevations of 1400' or greater. Four of the t values

TABLE 9

t *values of a* χ *test for the coupling-repulsion association i nthe data of Tables 1 through 8*

Table	Transect	Assn. t†
1	Smoky Mts.	A,B) — 3.0**
		all) — 3.4**
2	N.C. Blue Ridge	— 4.8**
3	Ga. Blue Ridge	A) — 2.4*
		B) — 2.0*
4	Allegheny Mts.	— 2.1*
5	Va. Blue Ridge	+ 0.9§
6	Allegheny Plateau	+ 4.3**‡
7	SE Pennsylvania	+12.7**
8	No. New Jersey	+ 3.9**

* $P = 0.05$.
** $P = 0.01$.
† Testing for disequilibrium (see text); + shows excess of SS and 12; — an excess of S2 and
1S chromosomes.
‡ Table 6 plus LEVITAN (1958).
§ Above 1000′, $t = +1.98$*.

are negative, and four are positive. Interestingly, all four negative t values, indicating an excess of XL-$1.XR$ and $XL.XR$-2, are in the four transects where the major altitudinal changes seem to be in the frequencies of one of these, XL-$1.XR$. Similarly, all four positive t values indicating an excess of $XL.XR$ and XL-$1.XR$-2, are in the transects where XL-$1.XR$ remains relatively static, the major altitudinal changes seeming to be in XL-$1.XR$-2. Altitudinal selection for a given combination should not necessarily mean that it would be in disequilibrium excess in that region, as may be noted from the fact that the associations are not necessarily at their strongest (deviation from randomness greatest) at the higher elevations, where the critical combination reaches its maximum frequency. In the May Smoky Mountain data, for example, the negative association is significant only in the 1400′ sample, where the frequency of XL-$1.XR$ is but 18%, and not in two samples with higher frequencies of this repulsion combination; similarly the positive associations are almost invariably stronger in the lower elevation in Northern New Jersey, where XL-$1.XR$-2 is rarer, than in the higher elevation.

A possible relation between association and altitudinal change is strengthened by a further observation: In the Southern Appalachians at least, my data from different transects at similar latitudes and altitudes are invariably homogenous if the associations in these places are of the same type, but not if the associations are different. The three transects around 35° latitude, the data of Tables 1 through 3, for example, all have negative association χ values (S2 and 1S in excess). At each altitude for which there are comparable data, 1400′, 2000 to 2500′, and ≥ 3000′, pooled data from these transects are statistically homogenous at the 5% level of significance. Among the three transects at 37–38° latitude, two, Allegheny Plateau and Virginia Blue Ridge, have positive association χ

values (SS and 12, in excess); pooled data from these at two comparable altitudes, 1150 to 1450', and 1920 to 2100', are likewise statistically homogeneous. At this latitude there are, however, results from a transect where the association x values are negative (Allegheny Mts.). Pooling these with the rest of the data at this latitude leads to highly significant statistical heterogeneity at both altitudes.

Similar comparisons in the Pennsylvania-New Jersey region do not fit the point, apparently because of drastic shifts in arrangements frequencies over small distances that appear to be the rule in the Northeastern portion of the species range (LEVITAN, unpublished data). These seem mainly to involve gene arrangements XR and XR-2, whose frequencies in this region do not conform to the geographic pattern deduced by CARSON (1958).

WALLACE (1953a,b) points out that when triads of overlapping inversions coexist in a population, the resultant recombinations would create difficulties for co-adaptation of pairs of them. Hence, he argues, "no single population should possess high frequencies of all three members of a triad; at least one of the three should be rare." As a rule of thumb, he considers populations in which at least one member of the triad fails to attain a frequency of 5% as supporting his hypothesis, populations in which one or more members of the triad have a

TABLE 10

Fit of published samples of the frequencies of XL, XL-1, XL-2 *triad of overlapping arrangements to the hypothesis of* WALLACE *(1954a,b)—that one member of the triad of overlapping inversions should be present at a frequency of less than 5%*

Source	Support*	Leans to supporting†	Completely equivocal‡	Leans to contrary§	Contrary‖
A. Appalachian Mountains					
STALKER and CARSON (1948)	0	0	(1)	2	(3)
LEVITAN (1958)	0	0	0	2	0
Present study	0	1	7	5	8
TOTAL	0	1	7(+1?)	9	8(+3?)
B. Other parts of the range					
CARSON (1958)¶	6(+1?)	2	11	0	0
LEVITAN (1958)	0	1	0	0	0
Present study	3	1	0	0	0
TOTAL	9(+1?)	4	11	0	0

Populations in parentheses may duplicate samples shown later in the same list. All fits are based on the 5% level of significance.

* The frequency of rarest arrangement in triad does not fit H_0 that it is $> 5\%$.

† Fit equally well H_0 that rarest arrangement has frequency $< 5\%$ and H_0 that this frequency is $> 5\%$ but $< 10\%$, but do not fit H_0 that it is $> 10\%$.

‡ Fit equally well H_0 that frequency of rarest arrangement is $< 5\%$, between 5 and 10%, or $> 10\%$.

§ Fit equally well with H_0 that rarest arrangement is $> 10\%$ and H_0 that it is $< 10\%$, but do not fit H_0 that it is $< 5\%$.

‖ The frequency of the rarest arrangement does not fit H_0 that it is $< 10\%$.

¶ Excludes samples apparently monomorphic for this arm.

frequency between 5 and 10% as possibly supporting it, and populations in which all three have frequencies above 10% as contradicting it. LEVITAN, CARSON and STALKER (1954) noted that using these criteria many *D. robusta* populations do not fit well to this hypothesis. The present study adds further tests of the hypothesis, since *XL*, *XL-1*, and *XL-2* form such a triad of over-lapping inversions. Additional natural population data concerning this triad have been published by CARSON (1958) and LEVITAN (1958).

Upon analysis (Table 10), an interesting dichotomy emerges. The samples derived from the Appalachian Mountains either contradict WALLACE's (1953a,b) hypothesis for this triad or at best fall into the "possibly supporting" category. On the other hand the majority of samples derived from the rest of the range, tend to support the hypothesis. Some of the populations that support it are at 1000′ or more above sea level (in Nebraska), but these elevations are attained by gradual increments from the midwestern plains. Apparently the adaptation of this species to the sharp altitudinal changes characteristic of the Appalachian mountain range involves forms of co-adaptation different from those in other parts of its range. A similar conclusion is indicated by the large frequencies of arrangement *2L-3* encountered in the Appalachians by STALKER and CARSON (1948), ARMENTROUT (1963), and myself (LEVITAN 1955, 1958, and unpublished data), in sharp contrast to its virtual absence in the rest of the southern United States (CARSON 1958 and LEVITAN, unpublished data).

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Corresponding editor: R. C. LEWONTIN