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Studies of linkage in populations. X. Altitude and autosomal gene arrangements in *Drosophila robusta*

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Summary

Data are presented concerning the gene arrangements in the second and third chromosomes of *Drosophila robusta* in eight altitudinal transects. A consistent change is the increase in the arrangement 2L-3, particularly in the linkage combination *2L-3.2R*, with increasing altitude. The reciprocal decrease with increasing altitude affects several different 2-left arrangements, most consistently 2L-1. The arrangements of 2-right show no significant variation with altitude, and those of 3-right do so only in a few samples of the two northern transects studied, none in any of the southern ones. These results confirm previous evidence for the significant role of the arrangements of the left arm of the second chromosome in the adaptations of this species to altitude and suggest further that interactions of linked arrangements are involved in these adaptations. The data also indicate that the factors responsible for the altitudinal adaptations of this species are in many cases not the same ones that are responsible for variations in its gene arrangements with latitude.

1. Introduction

Drosophila robusta Sturtevant 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 & Stalker, 1947; Carson, 1958; Levitan, 1982, 1992). In an investigation of the gene arrangements on the left and right arms of the species' metacentric chromosomes, Levitan (1954, 1955, 1958*a*) noted significant linkage disequilibria in the populations inhabiting several woods in southwest Virginia. Subsequently, he has engaged in a long-term study to determine whether such disequilibria were widespread and, if so, whether they were significant factors in the geographic gradients (reviewed by Levitan, 1982, 1992) that characterize many gene arrangements of the species. Some of these data have shown that selection for certain *combinations* of X-chromosomal arrangements were important factors in cyclic temporal variations (Levitan, 1973*a*, *b*) and altitudinal gradients (Levitan, 1978). The data presented here demonstrate that the altitudinal transects are characterized by significant gradients in some *autosomal* arrangements and arrangement combinations as well. However, these results do not always parallel the ones that might be expected from other geographic data. A small portion of the data was mentioned in the aforementioned reviews (Levitan, 1982, 1992).

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2. Materials and methods

(i) *Chromosome morphology and terminology*

The *D. robusta* haploid number is 4. Chromosome 2, the largest autosome, is nearly metacentric. The other autosome of concern here, chromosome 3, is smaller and somewhat less metacentric, the euchromatin of the more polymorphic right arm being about two thirds the length of the left arm.

Carson & Stalker (1947) designated the band arrangements of the 'Standard' arrangements of *D. robusta* and named them for the respective arm: XL, XR, 2L, etc. The other arrangements encountered in this study resulted from one-step inversions from these Standards. These authors also introduced the practice of naming non-Standard arrangements by numbering them for each arm in the order of their discovery (e.g. 2L-1, 2L-2, 3R-1). Levitan (1964*b*)

divided the genome into 100 units, designated by successive capital letter from the centromere distalward on each arm, so that each reflects 1% of the euchromatin (Levitan, 1964*b*, 1992).

The data of this report concern 2L, 2R, 3R, and the three earliest found and most widespread non-Standard arrangements of the 2-left arm, plus one each on 2-right and 3-right. 2L-1 differs from 2L by inversion of the euchromatin from G through Q, 10·0% of the total genome, 42·9% of the arm. 2L-2 differs from 2L by inversion of the proximal three-quarters of the arm, from an indeterminate point in the centromeric heterochromatin to R, 17·6% of the euchromatic genome, 75·5% of the arm. 2L-3 also has its proximal breakpoint in the centromeric heterochromatin, apparently closer to the centromere than that of 2L-2 (so close, indeed, that Carson & Stalker (1947) and Levitan (1950) thought it to be pericentric). The distal break is in unit L, so the underlying inversion encompassed about 11·8% of the total euchromatin, slightly more than half of the arm. 2R-1 arose from a subterminal – indeed to the eye it appears to be terminal – inversion whose proximal break is in unit I, therefore spanning 9·4% of the total euchromatin, 51·4% of the arm. Similarly, 3R-1 differs from 3R by a subterminal inversion, in this case of most of the arm, from B through H, 5·6% of the total genome, 70·0% of the arm. Figures illustrating these arrangements may be found in Carson (1958) and Levitan (1992).

As in Levitan (1978), to save space the tables use the short-hand notation for the gene arrangements introduced by Carson (1953). In this notation the karyotype *2L/2L-1, 2R-1/2R-1* (*2L.2R-1/2L-1.2R-1* in linkage form), for example, would be written S/1, 1/1, or S1/11.

(ii) *Collection analysis*

The collection sites and the methods of collection and analysis are the same ones described in Levitan (1978). Generally the data were derived from the analysis of collected adults mated to laboratory flies of known chromosomal constitution. In the few instances that the collections were very small (chromosome count less than 100), females that did not survive the initial desperming periods were represented in the data by an 'egg sample' offspring, if available; usually this was the first sacrificed female larva resulting from the inseminations of its mother in the wild. Fortunately, no instance of ambiguous linkage was encountered in these samples.

(iii) *Statistical analysis*

RxC G-tests of independence were used to test for associations between chromosome arrangement, or chromosome combination, and altitude for each chromosome arm in each transect. The recommended Williams correction was used (Sokal & Rohlf, 1981). Analyses were carried out by the RxC program of the BIOM statistical package (Rohlf, 1987).

In cases in which data were collected from the same transect in different months or years, a log-linear model was used to test for an effect of month (or year) on the association between chromosome arrangement, or combination, and altitude. In a three-way analysis the log-linear model we use is:

$$\ln f_{ijk} = u + A_i + C_j + M_k + AC_{ij} + AM_{ik} + CM_{jk} + ACM_{ijk},$$

where, analogous to an anova, u represents the mean, and the letters A, C and M represent the main effects, altitude, chromosome arrangement (or combination), and month (or year), respectively.

We are interested in the interaction terms because we wish to test for associations. For example, the AC term represents the association between altitude and chromosome arrangement (or combination). In our two-way G-tests we have already tested the data from each collection date for this association, but in this analysis the AC association is for all the data pooled with regard to season. The AM term will be present in all our models because it was fixed by the experimenter (Sokal & Rohlf, 1981, p. 762), but it is not biologically meaningful. The CM term represents the association between chromosome arrangement (or combination) and month pooled across all altitudes. This term tells whether the frequencies of various chromosome arrangements differ across months. The term of greatest interest to us is the three-way interaction term, ACM. If this term is significant, the relationship between any two factors depends on the level of the third. Specifically, in this case, it would mean that the pattern of association between altitude and chromosome arrangement changes with month.

To test the significance of the terms in a log-linear model, first the entire model is fitted; then the terms are dropped one at a time, starting with the most complex. At each step the significance of the model is assessed, and when significance is found, no more terms are dropped. If a term is removed and the difference in G-values between the model with the term and the model without the term is itself significant, the term is placed back in the model. Analyses of this sort were conducted for Tables 1 and 2 (with the 1000 ft altitudes removed) and Table 10.

Log-linear analyses for the interaction of altitude, chromosome arrangement, and location were also performed. Interpretation is the same as that described above except that location replaces month. These analyses show whether the associations between chromosome arrangement and altitude differ across transect locations. Analyses include all data from all transects having four altitudes. (Note that these analyses were possible only if we consider the altitudes

Table 1. *Frequency (%) of autosomal arrangements in an altitudinal transect of the Great Smoky Mountains near Gatlinburg, Tennessee*

Altitude in ft (m)	N_2	2L	2L-1	2L-2	2L-3	2R	2R-1	N_3	3R	3R-1
A. April 16–21, 1958 and May 5–11, 1959										
1000 (303 m)	79	35·44	22·78	15·19	26·58	89·87	10·13	77	29·87	70·13
1400 (424 m)	170	23·53	15·29	7·06	54·12	91·76	8·24	170	35·88	64·12
2000 (606 m)	205	10·24	11·22	7·32	71·22	87·80	12·20	215	31·16	68·84
3000 (909 m)	20	10·00	5·00	0	85·00	95·00	5·00	20	40·00	60·00
G-tests:	2-left: 59·018 D.F. = 9 $P < 0·01$					2-right: 2·228 D.F. = 3 $0·5 < P < 0·7$		3-right: 1·714 D.F. = 3 $0·5 < P < 0·7$		
B. August 1–10, 1958 and 9–13, 1959										
1400 (424 m)	58	25·86	25·86	6·90	41·38	91·38	8·62	57	24·56	75·44
2000 (606 m)	977	12·38	17·20	6·96	63·46	89·25	10·75	983	25·23	74·77
3000 (909 m)	95	16·84	16·84	8·42	57·89	88·42	11·58	93	31·18	68·82
G-tests:	2-left: 13·953 D.F. = 6 $0·02 < P < 0·05$					2-right: 0·349 D.F. = 2 $0·8 < P < 0·9$		3-right: 1·542 D.F. = 2 $0·3 < P < 0·5$		

in each transect to be low, medium–low, medium–high, and high rather than their measured values; for example, the low class includes data for 439 m of Table 3 and 36 m of Table 8.) For the chromosome arms this analysis included Tables 1 (July only), 3, 4, 5, and 8. For arrangement combinations this included Tables 2 (July only), 3, 4, 5, and 9. Analyses were carried out by the Loglin program of the BIOM statistical package.

3. Results

Tables 1 through 11 present the altitudinal comparisons of this study. Tables 1, 8, and 10, and the upper parts of Tables 3-7 show the frequencies, in percent, of the 2-left, 2-right, and 3-right gene arrangements. Tables 2, 9, and 11, and the lower parts of Tables 3-7 show the frequencies of the left- and right-arm combinations of the second chromosome arrangements.

The first transect studied was in the Great Smoky Mountains of Tennessee, a southwestward extension of the Blue Ridge division of the Appalachian chain. Because of seasonal heterogeneity, these data (Tables 1 and 2) must be treated separately for spring and summer.

At each of the altitudes that were sampled in both seasons 2L-1 and, to a lesser extent, 2L are more frequent in the summer than in the spring, whereas 2L-3 has decreased. Table 2 indicates that these seasonal differences are larger and more consistent for the combinations of these left-arm arrangements with 2R than with 2R-1.

The RxC G-tests of the data in Table 1 show that in both seasons the frequencies of the 2-left arrangements varied significantly with altitude, but not those of 2-right or 3-right. The differences in 2-left are greatest in the spring data, with 2L-3 almost doubling between 1000 and 1400 ft, then increasing at a lower rate with further increases in elevation. The other three arrangements tend to decrease, though less regularly than the concomitant changes in 2L-3. In the summer the largest difference is again an increase in 2L-3, this time between 1400 and 2000 ft, with 2L and 2L-1 showing concomitant decreases. Although there was no significant 3-way interaction, i.e. the pattern of variation with altitude did not depend on the month, the smaller G value in the summer data is partly attributable to the absence of a sample at 1000 ft (omitted in order to concentrate greater effort at obtaining a larger one at 3000 ft) and partly due to the apparent lack of significant differences in these data between the populations at 2000 and 3000 ft.

The G-tests for the variation of second chromosome arrangement *combinations* with altitude in the Smokies are highly significant in the spring data. Although the summer data as a whole do not reach statistical significance, the difference between 1400 ft and 2000 ft follow the same patterns as the April results. Examination of Table 2 shows that the variations are primarily in the combinations of 2-left arrangements with 2R, *2L-3.2R* exhibiting the large increases with altitude, the other three combinations with 2R tending to decrease. The combinations with 2R-1 do not exhibit the same consistent patterns. Thus, for example, in both spring and summer *2L-1.2R* decreases with altitude between 1400 and 2000 ft, whereas *2L-1.2R-1* does not (it even appears to increase). The numbers of the 2R-1 combinations in most of the samples were small, however, so that the statistical significance of their variations is in doubt.

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Table 2. *The Table 1 second chromosome data classified according to linked combinations of the left and right arm arrangements*

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
A. April and May									
1000 (303 m)	79	30·38	17·72	15·19	26·58	5·06	5·06	0	0
1400 (424 m)	170	22·35	14·12	5·29	50·00	1·18	1·18	1·76	4·12
2000 (606 m)	205	7·80	9·27	6·34	64·39	2·43	1·95	0·98	6·83
3000 (909 m)	20	10·00	0	0	85·00	0	5·00	0	0
G-test: 73·611 D.F. = 21 $P < 0·01$									
B. August									
1400 (424 m)	58	20·69	24·14	6·90	39·66	5·17	1·72	0	1·72
2000 (606 m)	977	10·24	14·84	6·35	57·83	2·15	2·35	0·61	5·63
3000 (909 m)	95	13·68	12·63	6·32	55·79	3·16	4·21	2·11	2·11
G-test: 19·974 D.F. = 14 $0·10 < P < 0·20$									

Table 3. *Frequency (%) of autosomal arrangements and second chromosome arrangement combinations in an altitudinal transect of the Highlands of North Carolina, 27 July–29 August 1962*

A. Arrangements

Altitude in ft (m)	N_2	2L	2L-1	2L-2	2L-3	2R	2R-1	N_3	3R	3R-1
1450 (439 m)	214	17·29	24·30	15·89	42·52	91·59	8·41	208	25·00	75·00
2500 (758 m)	44	9·09	20·45	15·91	54·55	95·45	4·55	44	29·55	70·45
3800 (1152 m)	205	11·22	15·61	9·27	63·90	90·73	9·27	201	25·87	74·13
4120 (1248 m)	51	7·84	19·61	11·76	60·78	86·27	13·73	48	10·42	89·58
G-tests:	2-left: 22·213 D.F. = 9 $P < 0·01$					2-right: 2·528 D.F. = 3 $0·3 < P < 0·5$		3-right: 6·874 D.F. = 3 $0·05 < P < 0·1$		

B. Second chromosome arrangement combinations

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
1450 (439 m)	214	16·36	21·03	14·02	40·19	0·93	3·27	1·87	2·34
2500 (758 m)	44	9·09	20·45	15·91	50·00	0	0	0	4·55
3800 (1152 m)	205	10·73	13·17	8·29	58·54	0·49	2·44	0·98	5·37
4120 (1248 m)	51	7·84	15·69	7·84	54·90	0	3·92	3·92	5·88
G-test: 27·631 D.F. = 21 $0·10 < P < 0·20$									

A similar pattern is evident in the Highlands of North Carolina (Table 3), to the east of the Smokies: significant variation with altitude in 2-left arrangements, but not in those of 2-right and 3-right. Again the increases with altitude occur essentially in 2L-3, particularly *2L-3.2R*, with smaller concomitant decreases in the other 2-left arrangements and their combinations with 2R.

In the Blue Ridge of northeastern Georgia (Table 4), to the south of the Smokies, *2L-3.2R* at the highest altitude is again double its frequency at the lower elevations, whereas *2L-3.2R-1* is actually lower. *2L-2.2R* drops with increasing altitude, but *2L-2.2R-1* increases. However, the samples are too small for these variations, as well as those of the chromosomal arms, to be statistically significant.

In the larger samples from the Blue Ridge of Virginia (Table 5), northeast of the Smokies, the

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Table 4. *Frequency (%) of autosomal arrangements and second chromosome arrangement combinations in an altitudinal transect in Northeastern Georgia, 12–15 May 1959*

A. Arrangements

Altitude in ft (m)	N_2	2L	2L-1	2L-2	2L-3	2R	2R-1	N_3	3R	3R-1
1100 (333 m)	67	16·42	38·81	20·90	23·88	88·06	11·94	68	20·59	79·41
1400 (424 m)	63	33·33	33·33	14·29	19·05	90·48	9·52	61	13·11	86·89
1800 (545 m)	32	12·50	40·63	21·88	25·00	96·88	3·13	32	28·13	71·87
2200 (667 m)	26	7·69	34·62	15·38	42·31	96·15	3·85	26	26·92	73·08

G-tests: 2-left: 13·532, D.F. = 9, $0{\cdot}10 < P < 0{\cdot}20$; 2-right: 3·228, D.F. = 3, $0{\cdot}3 < P < 0{\cdot}5$; 3-right: 3·829, D.F. = 3, $0{\cdot}2 < P < 0{\cdot}3$

B. Second chromosome arrangement combinations

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
1100 (333 m)	67	13·43	32·84	19·40	22·39	2·99	5·97	1·49	1·49
1400 (424 m)	63	31·75	30·16	9·52	19·05	1·59	3·17	4·76	0
1800 (545 m)	32	12·50	37·50	21·88	25·00	0	3·13	0	0
2200 (667 m)	26	7·69	34·62	11·54	42·31	0	0	3·85	0

G-test: 21·240, D.F. = 21, $0{\cdot}3 < P < 0{\cdot}5$

Table 5. *Frequency (%) of autosomal arrangements and second chromosome arrangement combinations in an altitudinal transect of the Blue Ridge Mountains of Virginia extending from Charlottesville to stations along the Skyline Drive near Afton and Waynesboro, 5–13 August 1963*

A. Arrangements

Altitude in ft (m)	N_2	2L	2L-1	2L-2	2L-3	2R	2R-1	N_3	3R	3R-1
500 (152 m)	123	34·96	26·83	11·38	26·83	86·99	13·01	119	47·90	52·10
1400 (424 m)	475	30·74	24·21	14·11	30·95	91·58	8·42	469	47·97	52·03
2200 (667 m)	283	30·04	15·55	6·01	48·41	88·69	11·31	279	55·20	44·80
3100 (939 m)	40	25·00	7·50	12·50	55·00	95·00	5·00	37	48·65	51·35

G-tests: 2-left: 46·115, D.F. = 9, $P < 0{\cdot}01$; 2-right: 4·233, D.F. = 3, $0{\cdot}2 < P < 0{\cdot}3$; 3-right: 3·983, D.F. = 3, $0{\cdot}2 < P < 0{\cdot}3$

B. Second chromosome arrangement combinations

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
500 (152 m)	123	32·52	21·14	9·76	23·58	2·44	5·69	1·63	3·25
1400 (424 m)	475	26·95	21·26	13·68	29·68	3·79	2·95	0·42	1·26
2200 (667 m)	283	27·21	12·72	6·01	42·76	2·83	2·83	0	5·65
3100 (939 m)	40	25·00	7·50	10·00	52·50	0	0	2·50	2·50

G-test: 60·209, D.F. = 21, $P < 0{\cdot}01$

variations in both 2-left arrangements and in the arrangement combinations are highly significant. Note the consistent increases of *2L-3.2R* with increasing elevation as contrasted to irregular variation in *2L-3.2R-1*. Both linkage forms move synchronously, however, between 1400 and 2200 ft. The most consistent concomitant decreases with elevation are in *2L-1.2R* and *2L-1.1R-1*, albeit each in a somewhat different pattern from the other.

To the west of this transect are two smaller ones, in

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Table 6. *Frequency (%) of autosomal arrangements and second chromosome arrangement combinations in coincident collections at two elevations of the Allegheny Plateau at approximately 37° 15′ N latitude, 23–26 August 1962*

A. Arrangements

Altitude in ft (m)	N_2	2L	2L-1	2L-2	2L-3	2R	2R-1	N_3	3R	3R-1
1450 (439 m)	64	23·44	20·31	21·88	34·38	93·75	6·25	62	50·00	50·00
2100 (636 m)	90	27·78	14·44	10·00	47·78	92·22	7·78	90	58·89	41·11

G-tests: 2-left: 5·999, D.F. = 3, $0{\cdot}1 < P < 0{\cdot}2$; 2-right: 0·127, D.F. = 1, $0{\cdot}7 < P < 0{\cdot}8$; 3-right: 1·160, D.F. = 1, $0{\cdot}2 < P < 0{\cdot}3$

B. Second chromosome arrangement combinations

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
1450 (439 m)	64	23·44	17·19	20·31	32·81	0	3·13	1·56	1·56
2100 (636 m)	90	25·56	13·33	8·89	44·44	2·22	1·11	1·11	3·33

G-test: 7·727, D.F. = 7, $0{\cdot}3 < P < 0{\cdot}5$

Table 7. *Frequency (%) of autosomal arrangements and second chromosome arrangement combinations at two elevations on the eastern edge of the Allegheny Mountains at approximately 37° 50′ N latitude 19–24 June 1964*

A. Arrangements

Altitude in ft (m)	N_2	2L	2L-1	2L-2	2L-3	2R	2R-1	N_3	3R	3R-1
1150 (348 m)	222	37·39	15·77	14·86	31·98	91·89	8·11	218	53·67	46·33
1920 (582 m)	281	20·64	9·25	10·32	59·79	93·95	6·05	278	62·23	37·77

G-tests: 2-left: 39·358, D.F. = 3, $P < 0{\cdot}01$; 2-right: 0·794, D.F. = 1, $0{\cdot}3 < P < 0{\cdot}5$; 3-right: 3·672, D.F. = 1, $0{\cdot}05 < P < 0{\cdot}1$

B. Second chromosome arrangement combinations

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
1150 (348 m)	222	34·23	15·77	13·06	28·83	3·15	0	1·80	3·15
1920 (582 m)	281	20·28	7·12	10·32	56·23	0·36	2·14	0	3·56

G-test: 57·759, D.F. = 7, $P < 0{\cdot}01$

the Allegheny plateau (Table 6) and at the eastern edge of the Allegheny Mountains (Table 7). The data are not statistically significant over the small altitudinal differences of the plateau, though again 2L-3 appears to be more abundant at the higher elevation than at the lower one. Both *2L-3.2R* and *2L-3.2R-1* seem to move in the same way here. The similar changes in the mountain transect (Table 7), on the other hand, achieve some of the largest observed deviations from chance, for both 2-left arrangements and combinations. Most noteworthy are two contrasts: (1) between *2L-3.2R*, which doubles with elevation, and *2L-3.2R-1*, which increases only slightly, and (2) between *2L-1.2R*, which decreases by 50% with elevation, and *2L-1.2R-1*, which seems to *increase*.

The two northern transects (Tables 8–11) involve smaller differences in altitude than the southern ones. In southeastern Pennsylvania significant heterogeneity between the Swarthmore and Philadelphia samples (not encountered in the X-chromosome data of Levitan, 1978) forces the results from these low elevation stations to be presented separately.

As in the southern transects the most consistent change in Tables 8 and 10 is the increase in 2L-3 with increased elevation. Here, however, the concomitant changes are almost entirely in 2L-1. Indeed, 2L-2 tends to be *higher* at the higher elevations in every

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Table 8. *Frequency (%) of autosomal arrangements in an altitudinal transect in southeastern Pennsylvania*

Altitude in ft (m)	N_2	2L	2L-1	2L-2	2L-3	2R	2R-1	N_3	3R	3R-1
A. July										
120 (36 m)[a]	740	56·35	33·78	2·30	7·57	96·22	3·78	724	85·08	14·92
180 (55 m)[b]	279	44·80	36·20	3·94	15·05	97·13	2·87	276	76·81	23·19
400 (121 m)[c]	94	48·93	18·08	7·45	25·53	96·81	3·19	88	85·23	14·77
1200 (364 m)[d]	81	44·44	7·41	3·70	44·44	96·30	3·70	80	83·75	16·25
G-tests:	2-left: 108·805 D.F. = 9 $P << 0·01$					2-right: 0·537 D.F. = 3 $0·90 < P < 0·95$		3-right: 9·448 D.F. = 3 $0·02 < P < 0·05$		
B. August										
120 (36 m)[a]	758	47·76	43·01	2·77	6·46	96·44	3·56	744	83·20	16·80
400 (121 m)[c]	199	45·23	23·62	4·52	26·63	95·98	4·02	193	86·53	13·47
1200 (364 m)[d]	53	50·94	15·09	3·77	30·19	94·34	5·66	49	79·59	20·41
G-tests:	2-left: 85·836 D.F. = 6 $P << 0·01$					2-right: 0·550 D.F. = 2 $0·7 < P < 0·8$		3-right: 1·869 D.F. = 2 $0·3 < P < 0·5$		

[a] Philadelphia (July 10–31/63, 16–31/64, & 10–31/65; August 1–8/64, 13–15/69, & 27–30/73).
[b] Swarthmore (July 14–16/61).
[c] Allentown (July 17–25/65; August 13–30/64, 26–28/69).
[d] Carbon County, near Jim Thorpe (July 19–24/64, 17–25/65; August 13–29/64, 26–27/69).

Table 9. *The Table 8 second chromosome data classified according to linked combinations of the left and right arm arrangements*

A. July

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
120 (36 m)	740	54·73	31·89	2·30	7·30	1·62	1·89	0	0·27
180 (55 m)	279	43·01	35·48	3·58	15·05	1·79	0·72	0·36	0
400 (121 m)	94	46·81	18·08	7·45	24·47	2·13	0	0	1·06
1200 (364 m)	81	41·98	7·41	3·70	43·21	2·47	0	0	1·23

G-test: 89·757
D.F. = 21
$P << 0·01$

B. August

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
120 (36 m)	758	46·04	41·42	2·64	6·33	1·72	1·58	0·13	0·13
400 (121 m)	199	44·22	21·61	4·52	25·63	1·01	2·01	0	1·01
1200 (364 m)	53	50·94	9·43	3·77	30·19	0	5·66	0	0

G-test: 71·615
D.F. = 14
$P << 0·01$

season in New Jersey (Table 10), and it also increases between Philadelphia and Allentown in Table 8.

Although the frequencies of 2R-1 in the northern areas are generally small, the G-tests of the data in Tables 9 and 11 are consistently significant, in most instances at a very high level. A particularly interesting contrast appears in the fairly large August Pennsylvania samples (Table 9), in which 2R-1 is quite abundant: *2L-1.2R* drops 11 percentage units between Allentown (325 ft) and Jim Thorpe (1200 ft), whereas *2L-1.2R-1* seems to *increase*.

Taking the data of Tables 1, 3, 4, 5, and 8 as a whole – 2, 3, 4, 5, and 8 for the combinations –, significant interaction is present between arrangement (or combination), altitude, and location for 2-left, indicating that the altitudinal changes, albeit generally highly significant, did not follow quite the same pattern in the different transects. There were no such 3-way

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Table 10. *Frequency (%) of autosomal arrangements in an altitudinal transect in northeastern New Jersey*

Altitude in ft (m)	N_2	2L	2L-1	2L-2	2L-3	2R	2R-1	N_3	3R	3R-1
A. Late April–Early May, 1972[a]										
150 (45 m)	298	52·35	30·87	1·68	15·10	99·66	0·34	297	96·30	3·70
900 (273 m)	168	41·67	20·83	3·57	33·93	98·21	1·79	167	91·62	8·38
Chi-squares:	2-left: 25·667					2-right: 2·837		3-right: 4·587		
	D.F. = 3					D.F. = 1		D.F. = 1		
	$P << 0·01$					$0·05 < P < 0·1$		$0·02 < P < 0·05$		
B. Late May–Early July, 1972 and 1973[b]										
150 (43 m)	519	50·87	31·02	0·58	17·53	98·46	1·54	517	94·58	5·42
900 (273 m)	545	44·95	17·61	1·10	36·33	98·17	1·83	543	90·61	9·39
Chi-squares:	2-left: 29·275					2-right: 0·145		3-right: 6·036		
	D.F. = 3					D.F. = 1		D.F. = 1		
	$P << 0·01$					$0·7 < P < 0·8$		$0·01 < P < 0·02$		
C. Early–Mid July, 1970[c]										
150 (45 m)	286	54·55	31·12	0·35	13·99	99·65	0·35	281	95·37	4·63
900 (300 m)	82	43·90	10·98	3·66	41·46	98·78	1·22	82	90·24	9·76
Chi-squares:	2-left: 41·265					2-right: 1·131		3-right: 3·167		
	D.F. = 3					D.F. = 1		D.F. = 1		
	$P << 0·01$					$0·2 < P < 0·3$		$0·05 < P < 0·1$		
D. Late August–Early September, 1970										
150 (45 m)	78	37·18	44·87	1·28	16·67	97·44	2·56	78	94·87	5·13
900 (300 m)	156	37·82	22·44	3·85	35·90	99·36	0·64	156	89·74	10·26
Chi-squares:	2-left: 16·427					2-right: 1·519		3-right: 1·790		
	D.F. = 3					D.F. = 1		D.F. = 1		
	$P < 0·01$					$0·2 < P < 0·3$		$0·1 < P < 0·2$		

[a] Englewood: April 30–May 5; Ledgewood: May 6–14.
[b] Englewood: June 16–July 3, 1972; May 30–June 14, 1973; Ledgewood: June 9–July 3, 1972; June 23–27, 1973.
Heterogeneity Chi-Squares: Englewood, 8·796 (D.F. = 5). Ledgewood, 4·941 (D.F. = 5).
[c] Englewood: July 5–12; Ledgewood: July 18–19.
[d] Englewood: Aug. 26–Sept. 9; Ledgewood: Aug. 31–Sept. 2.

interactions for the 2-right and 3-right data, a reflection of 1) the consistent absence of variation between chromosome and altitude in 2-right from location to location and 2) the generally low level of altitudinal variation of the 3-right arrangements (only one of seven RxC G-tests significant in these tables).

4. Discussion

Five *D. robusta* autosomal gene arrangements found in more than one state, 2L, 2L-3, 2R, 3R, and 3L-R, tend to be more common in the northern latitudes than in the South (Carson, 1958; Levitan, 1982, 1992). Concomitantly 2L-1, 2L-2, 2R-1, and 3R-1 have their highest frequencies in the south. The data indicate that the altitudinal relationships of these arrangements are in some instances very similar to the ones that one might expect from their relation to latitude, that is, that those with higher frequencies in the north be also those that are more frequent at higher altitudes, and in other instances they are very different.

For the 'Standard' arrangement of the left arm of the largest autosome, 2L, the altitudinal characteristics are very different from its latitudinal variation. 2L is generally more common in northern latitudes than in southern ones, and in some regions the changes in its frequencies even seem clinal. The latitudinal patterns of this arrangement are rather erratic, however, and its rise or fall seems to depend more on the increase and decrease in other arrangements of the arm than in its own qualities. Although its relations to altitude also contain many irregularities, its frequencies exhibit a real tendency to be highest in the lower altitudes of the transects – the opposite of what might expect from its higher frequencies in the north than in the south. In the Spring data from Great Smoky Mountains in Table 1, for example, it drops from about 35% at 1000 ft to about 10% at 2000 ft, with an intermediate frequency of about 24% at 1400 ft; and the frequencies at 1400 and 2000 ft are very similar to these in the August data. In the data of Etges (1984) from the same area the percentages of 2L tend to be lower than those of the writer's samples, but again the highest

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Table 11. *The Table 10 second chromosome data classified according to linked combinations of the left and right arm arrangements*

Altitude in ft (m)	N	SS	1S	2S	3S	S1	11	21	31
A. Late April–Early May, 1972									
150 (45 m)	298	52·01	30·87	1·68	15·10	0·34	0	0	0
900 (273 m)	168	41·07	20·83	2·98	33·33	0·60	0	0·60	0·60
	$\chi^2 = 27{\cdot}009$ D.F. = 6 $P < 0{\cdot}01$								
B. Late May–Early July, 1972 and 1973									
150 (45 m)	519	50·10	30·64	0·58	17·15	0·77	0·39	0	0·39
900 (273 m)	545	44·59	17·25	0·92	35·41	0·37	0·37	0·18	0·92
Independence	$\chi^2 = 58{\cdot}602$ D.F. = 7 $P < < 0{\cdot}01$		Year to Year Heterogeneity: Englewood: $\chi^2 = 9{\cdot}888$ (D.F. = 6 or 7) Ledgewood: $\chi^2 = 4{\cdot}544$ (D.F. = 7)						
C. Early–Mid July, 1970									
150 (45 m)	286	54·20	31·12	0·35	13·99	0·35	0	0	0
900 (273 m)	82	43·90	10·98	3·66	40·24	0	0	0	1·22
Independence	$\chi^2 = 43{\cdot}582$ D.F. = 5 or 7 $P < < 0{\cdot}01$								
D. Late August–Early September, 1970									
150 (45 m)	78	35·90	44·87	1·28	15·38	1·28	0	0	1·28
900 (273 m)	156	37·82	21·87	3·85	35·90	0	0·64	0	0
	$\chi^2 = 23{\cdot}203$ D.F. = 6 $P < 0{\cdot}01$								

one, 17·2, is at the lowest altitude studied, 1360 ft, with fairly regular decreases with increasing altitude until 3040 ft (5·8%), some apparent increases till 4520 ft (11·8%), then the lowest recorded amount, 2·1%, at the highest altitudes (combining his statistically homogeneous samples at 4680 and 4840 ft). In their lower elevation samples of the earliest Great Smokies transect Stalker and Carson (1948) also observed a steady decrease of 2L: from 26·9% at 1000 ft to 18·6% 2000 ft, but they found no significant change from 2000 ft (or 1400 ft, for that matter) to 3000 and 4000 ft. Their data showed, however, that the variation in 2L was independent of the profound changes, discussed below, in other arrangements of the arm.

The tendency of 2L to be more frequent at the lowest elevation of a transect than at the highest elevation is repeated in six of the other seven transects of this study. The sole exception is in Table 6, where the small amount of data cause the 2-left variation to be statistically nonsignificant.

Unlike 2L, both 2L-1 and 2L-2 have their highest frequencies at low altitudes in the south, albeit with different patterns. That of 2L-1 is essentially clinal. Along with 2R-1 it accounts for 100% of the second chromosomes in the southernmost collections. It then decreases as one moves northward but continues to be the majority arrangement of the arm till at least the 35th parallel. Between 35 and 40° N latitude its frequency tends to be in the 25–40% range (e.g. the lowest elevation data in Tables 8 and 10), and it falls below 10% around 42°, finally to zero in many of the northernmost samples.

In contrast to 2L-1's 100% frequencies in some areas, those of 2L-2 are nowhere greater than the 30–33% in some small samples from northern Alabama, in good sized samples never more than the 23% in Buckingham County, Virginia. The tendency for its numbers to decrease in all directions from these apices, especially to the north and west, led Carson (1969) to suggest that it had a radiate, rather than a simple north–south, distribution. In any case the highest frequencies are clearly in the south.

In keeping with its latitudinal tendencies, 2L-1 decreases with increasing elevation in every transect of this study. The same tendency was observed by Etges (1984) and Stalker and Carson (1948) in their Great Smoky Mountain transects. (There was an increase between 1400 and 2000 ft in the Stalker and Carson data, but it was not statistically significant.) 2L-2, too, tends to decrease with increased elevation in most studies. It does not do so, however, in the northernmost transects (Tables 8 and 10); indeed, in most samples in those areas it is more frequent at higher

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elevations than at lower ones. Although the small numbers of this arrangement here cast some doubt on their statistical significance, their consistency in regard to elevation is impressive.

Perhaps the clearest autosomal correlation with both latitude and altitude is exhibited by 2L-3. It is absent below the 35th parallel at low elevations. Indeed, it rarely reaches frequencies of 10% or more at these altitudes below 39° N latitude. As one moves northward from that point, however, it increases consistently, until it reaches 90% or more in many northern populations. Similarly, the frequency of 2L-3 tends to increase with increased altitude in all eight transects of this study. Although in some of them it seems to level off at one of the higher altitudes, it is universally more frequent at the highest altitude studied than at the lowest. In most instances the frequency has doubled or tripled in the altitudinal span.

Similar results were obtained by Stalker & Carson (1948) and Etges (1984) in their Great Smoky Mountain transects. Etges noted that at several elevations the frequency of 2L-3 in 1981 was significantly greater than Stalker and Carson had found in 1948. Comparison of their data with that of Table 1 of this study shows that the apparent increase at 2000 ft was already present in 1958–1959, when our samples were collected. At 1400 and 3000 ft, on the other hand, our data are closer to those of Stalker and Carson than to those of Etges. These variations may represent real changes over time that have taken place in this area, although it is not certain that all the collections at a given altitude were made in the same locations. The data of Armentrout (1963) exhibit considerable heterogeneity between stations at similar altitudes in the nearby Unaka Mountains. On the other hand, the writer experienced an apparently significant increase in the woods at 2100 ft of Table 6. In 1952–1953 collections the frequency of 2L-3 was 32·24% (n = 580); in the 1962 data shown in Table 6, it was 47·78%. The difference is statistically highly significant despite the relatively small size of the 1962 sample.

The 'Standards' of the other two variable autosomal arms, 2R and 3R, are northern in mirror image to the way that 2R-1 and 3R-1, respectively, are southern, inasmuch as they are their only allelic arrangements over most of the species range. The other northern autosomal arrangement found in more than one state, the pericentric inversion 3L-R, is completely absent in the altitudinal transects.

At low altitudes, 2R, like 2L-3, is absent from the southernmost collections in Florida. It becomes quite frequent earlier than 2L-3, however, as one moves north, accounting for nearly 15% of the population even in northern Florida and soon overtaking its allelic arrangement, 2R-1, in Georgia and Alabama. Moreover, in the western longitudes it is the most common 2-right arrangement even at latitudes comparable to those of northern Florida. In all latitudinal transects it increases steadily until it reaches a plateau of about 80–90%, generally at around 38° N latitude, attaining 100% in some of the same northernmost samples that 2L-3 attains its highest frequencies. There is some evidence that two relatively new arrangements, 2R-4 and 2R-5 (Levitan, 1992), which were absent from earlier collections in the area (Carson, 1958), are increasing at the expense of 2R in some localities in the northwestern corner of the species range.

As expected from its northern distribution, 2R is the predominant 2-right arrangement in every altitudinal table. Indeed its frequency is 88% or more in the lowest elevations of these tables even in northeastern Georgia (Table 4), at about 34° N latitude, and southern North Carolina (Table 3), at about 35°. In no table does it show any significant change with increasing altitude, however. The same is true in the data of Stalker & Carson (1948) and Etges (1984).

3R also starts out at zero in the southernmost latitudes and increases northward, but its increase is much slower than that of 2R. Thus it remains at zero, or very close to it, in low elevation areas of Georgia and Alabama where 2R has already attained substantial frequencies. Typically also its frequencies are about 30–40% where those of 2R are already about 80–90. 3R generally reaches the latter figures, where there are substantial samples, at about 40° N latitude.

Although it exhibits more variation than 2R in the altitudinal data – indeed its relation to altitude are significant in parts of Tables 8 and 10 –, it resembles 2R in that there appear to be no consistent changes with altitude, certainly none in the southern part of the range (Tables 1 and 3–7).

In view of the absence of significant altitudinal variation in 2R and 2R-1, it is surprising that their linkages to the left arm arrangements do not share equally in the altitudinal changes. One would expect, for instance, that both *3S* and *31* would show similar increases, and *1S* and *11* similar decreases, with elevation. We have seen that very often this does not happen. In both parts of Table 2, for example, *1S* decreases consistently with altitude; *11*, on the other hand, decreases between 1000 and 1400 ft in part A, then increases, and in part B it *increases* with every increase in altitude. In part B of the same table *3S* does not change significantly between 2000 and 3000 ft, but *31* decreases, the opposite of expectation. In Table 7, while *3S* increases by almost 100% *31* increases by less than 14%; concomitantly *11* increases while *1S* decreases. In Table 5 the changes in *1S* and *11* correlate better, but *3S* steadily increases with elevation while *31* changes irregularly, decreasing in two of the three increases in altitude. Similar irregularities occur in parts of Tables 9 and 11.

Levitan (1954, 1955, 1958*a*, 1961*a*, 1961*b*, and 1964) found that the gene arrangements of *D. robusta* are often present in natural populations in linkage

disequilibrium. This is surprising because population genetic theory holds that, if they have been together long enough, linked as well as unlinked variants with any recombination at all between them should be present at equilibrium frequencies that are the products of the frequencies of the component variants. If 2L, for example, is present at frequency *a*, and 2R-1 at frequency *b*, the expected equilibrium frequency of the combination *2L.2R-1* (*S1*, in the short-hand notation) is *ab*. The widespread gene arrangements of *D. robusta* have probably been together at least since the recession of the last Pleistocene ice age (Carson & Stalker, 1947). Furthermore, the detection of almost all possible linkage combinations indicates that crossing over does occur between them, as has been confirmed by laboratory studies (Carson, 1953; Levitan, 1953*a*, 1953*b*, 1958*b*; Massie & Levitan, 1956).

Disequilibria of X-chromosome arrangements occur in at least four different forms (Levitan, 1973*b*) and have been detected in many localities. They are especially prominent in the altitudinal transects (Levitan, 1978; Etges, 1984).

Disequilibria of second chromosome arrangements are more difficult to detect because, as stated earlier, the frequency of 2R-1 is quite low in most parts of the species range, especially in those areas with substantial frequencies of all the widespread arrangements of the left arm. Levitan (1955, 1958*a*, 1964*a*) found that the major cause of linkage disequilibrium of the second chromosome gene arrangements of *D. robusta* stemmed primarily from an excess of *3S* and a corresponding deficiency of *31* in heterozygotes where they are expected to be equal under linkage equilibrium. In many cases there was also a significant excess of *11* and deficiency of *1S* in these heterozygotes.

In populations with low frequencies of 2R-1 such as those described in this study, this arrangement is carried almost entirely by heterozygotes, where its linkages to the same left arm arrangement are expected to be equal. Hence the proportions of these linkages in the total data is a reflection of their relative proportions in the heterozygotes. When the pertinent data available is small, a handle on the probable existence of second chromosome disequilibria can also be gained by lumping the data from several localities, in much the same way that deviations from equality among the double heterozygotes may be combined (Levitan, 1964*a*, 1992).

Most of the samples of this study exhibit such an excess of *3S* and diminution of *31* from random expectation. In the total data from the Great Smoky Mountains (Tables 1 and 2), for example, 60·79% of the second chromosomes are 2L-3 and 10·54% 2R-1. On a random basis one would expect 6·41% to be *31* and 54·38% to be *3S*; in the total sample of 1604 chromosomes there would be 102·8 *31*, 872·2 *3S*, 166·2 non3-1, and 562·8 non3-S. Actually there are 79 *31*, 896 *3S*, 90 non3-1, and 539 non3-S. The difference is highly significant (G-test, after Williams's correction, 15·062, for 1 D.F.). (Very similar results are obtained by calculating separately, and then combining, the random expectations from each of the seven subsamples of Tables 1 and 2: 871·5 *3S*: 103·4 *31*; corrected G-test = 16·108 for 1 D.F.).

Looking at all the data in this study, in the eight transects there are 2586 *3S*, 166 *31*, 5160 non3-S, and 308 non3-1. On a random basis one would expect 2548·1 *3S* and 203·9 *31*. The corrected G-test of the deviation from expectation = 13·433, significant at the 0·1% level for 1 D.F. The deviation would be even greater if all the data from the Allegheny plateau (Levitan, 1955, 1958*a*, 1964*a*, 1992) were included.

From another point of view, on a random basis one would expect an excess of *31* as often as a deficiency. In the 38 samples of this study 30 have a deficiency of *2L-3.2R-1* (and excess of *2L-3.2R*) and 8 have an excess of *31* (and deficiency of *3S*). The deviation from equality is highly significant.

Similarly, in the total data there are 1890 *1S*, 138 *11*, 5856 non1-S, and 336 non1-1. On a random basis 1925·9 *1S* and 102·1 *11* are expected. The excess of *11* and deficiency of *1S* is significant at the 0·1% level.

A closer look at the data reveals a dichotomy between the northern (Pennsylvania, New Jersey) and the southern samples. In the northern transects (Tables 9 and 11) the ratio of *3S*:*31* is 793:17, hardly different from the 792·3:17·6 expected on a random basis, whereas in the southern transects (Tables 2–7) the same ratio is 1793:149, significantly different from the random expectation of 1755·8:186·3.

In the *1S, 11* data the dichotomy between northern and southern samples is even greater: corrected G = 1·647 with one D.F. for the northern data (1267 *1S*:40 *11*, on a random expectancy of 1273·2:33·8), whereas G = 75·664 with 1 D.F. for the southern data (623 *1S*:98 *11* on a random expectancy of 652·7:68·3). Only six of the 15 northern samples have an excess of *11* and deficiency of *1S*, as compared to 15 of the 23 southern samples. (The total deviation is so large in the southern data because seven of the eight samples that deviate from pattern do so by 0·4 or less.)

A dichotomy between northern and southern transects was also noted in the X-chromosome disequilibria (Levitan, 1978). There, however, all the transects exhibited significant disequilibria, but in different form, and those of the Allegheny plateau and Virginia Blue Ridge (southern) transects were similar to the Pennsylvania and New Jersey forms.

Although clinal variation can sometimes be simulated by genetic drift and gene flow (Endler, 1977), it is difficult to conceive of any factor other than natural selection to be responsible for the consistent variations in as many different altitudinal transects as were observed in this study and in the X-chromosome data reported earlier (Levitan, 1978; Etges, 1984). Some support for this conclusion has come from perturbation studies in nature (Levitan, 1992) and from

laboratory experiments. For example, the carriers of 2L-1 tend to be favoured at higher temperatures in population cages (Levitan, 1951). Etges (1989) found that 2L-1 and 2L-2 from the Great Smoky Mountains were associated with shorter developmental time under warm temperature conditions, whereas 2L-3 from the same collections had shorter developmental time at under low temperature conditions. However a number of his other findings were not in accord with the expectations from the altitudinal data. For example, third chromosome arrangements also varied in developmental time at different temperatures even though these exhibitied no significant altitudinal variation. Further studies of specific life-cycle effects of the gene arrangements and arrangement combinations are clearly desirable.

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