

Genetics of Natural Populations

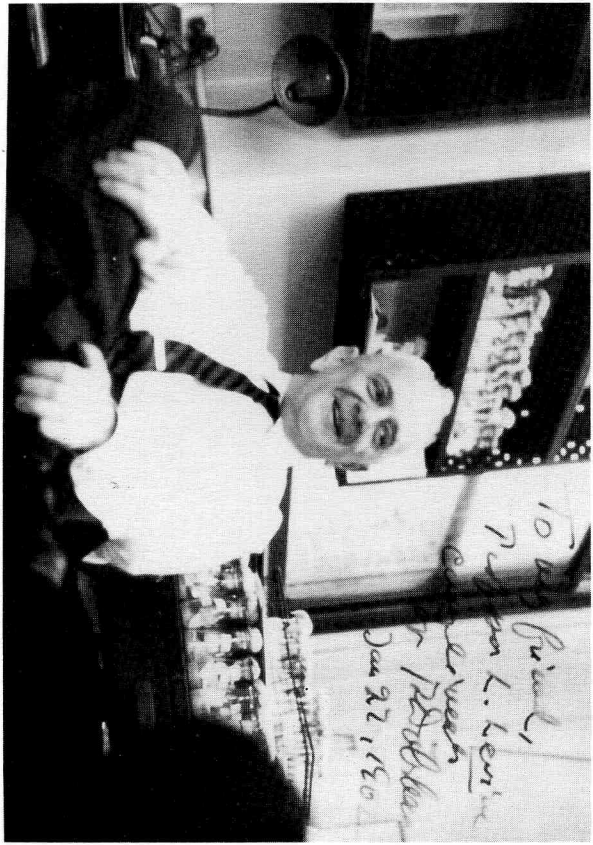
*The Continuing Importance
of Theodosius Dobzhansky*

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Professor Theodosius Dobzhansky in his Columbia University laboratory (January 22, 1962), smiling and relaxed, surrounded by his ever present fly-bottles.



Convergent Linkage Disequilibrium in Disparate Populations of *Drosophila robusta*

Max Levitan and William J. Erges

The title page of Levitan's paper (1970) carries the footnote, "Dedicated to a beloved teacher, Prof. Th. Dobzhansky, on his 70th birthday." Unfortunately, it was not possible to dedicate a similar work to him on the next major milestone, but the influence of this genius on us has continued, nevertheless, and probably on everyone in the world who studies the part played by genetics in evolution.

When M. L., the one of us who was his direct student, looks back, he realizes that this influence never consisted to any great extent of direct transmission of facts in lecture sessions, as is generally considered to be the role of the college teacher. Indeed, there was only one course with him: a seminar in which Dobzhansky shared the leadership with Dr. L. C. Dunn and almost all the "lecturing" was done by the students. And when it came time to choose a thesis problem, Dobzhansky did not, as was done by so many others in his position, dictate one; rather he asked, "Levitin (I believe he never learned to pronounce my name the way I do, perhaps because he thought I might be

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related to a great Russian painter with a similar patronymic), what would you like to do?" When the reply was, "I would like to do something similar to what you are doing, something that could combine fieldwork and laboratory studies," he did not assign *D. pseudoobscura* or one of its relatives, but without hesitation suggested that I might find interesting an eastern species, *D. robusta*, whose chromosomes H. L. Carson and H. D. Stalker were about to describe in the first volume of *Evolution* (Carson and Stalker 1947).

Thus he could not even teach me anything about this species, which he had probably never seen, and whose chromosomes he could not recognize. Yet he was the quintessential teacher, for he showed the way, he inspired by his example, in addition, of course, to his masterful ability to synthesize and organize the pieces of knowledge from many species and many sources into hypotheses and theories that continue to guide evolutionary study even when his physical presence is gone.

It is fitting, therefore, that the species he suggested should be the subject of this paper. Described by Sturtevant (1916, 1921), it is one of the more common species of the genus in the deciduous forest of North America east of the Rocky Mountains and north of 28° N latitude. Its haploid set of four consists of two metacentric chromosomes of nearly equal size, the X- and second chromosomes, a nearly metacentric third chromosome, and an acrocentric dot chromosome. Its most common gene arrangements were first described by Carson and Stalker (1947), and Carson (1958) and Levitan (1992) have described those that have been found subsequently in natural populations.

Levitan (reviewed by Levitan, 1982, 1992) found that the gene arrangements of *Drosophila robusta* are often present in natural populations in linkage disequilibrium. The data of this report show that deviations from linkage equilibrium of the arrangements of these chromosomes exist in populations of the Ozark and Ouachita Mountain regions, and many of these associations take forms characteristic of far distant populations rather than those of nearby areas.

Materials and Methods

Adult *D. robusta* were collected in the Ozark Mountain region in (1) June through July 1989 and 1992 in a wooded residential area in Fayetteville, Arkansas (elevation 1400 ft; Egges 1991); (2) June 1989 on the north side of Mount Magazine in the southern district of the Ozark National Forest (elevation 1375 ft); and (3) late July 1992 in the Ozark National Forest along Fane Creek in the White Rock Wildlife Management Area

(elevation 680 ft). A population from the Mill Creek Recreation Area in the Ouachita National Forest (elevation 880 ft) was collected in mid-July 1990. All populations were sampled by sweep-netting over aged, fermented bananas in buckets wired to trees located near flowing streams. Individual females were placed in vials and allowed to oviposit until they exhausted all stored sperm. Adults were shipped to one of us (M. L.) for analysis by the methods described by Levitan (1955), except for the Mill Creek sample, which was karyotyped by the second author (W. J. E.).

The Steelville collections were originally described by Carson (1958). The arrangement frequencies shown there were derived primarily from "egg sample" analyses, that is, from larval progeny of collected females that had been inseminated in the wild; in cases where more than one larva was reported from the same female, complete karyotypes could be inferred, especially for the X-chromosome. This was supplemented by data from especially for the X-chromosome. This was supplemented by data from analysis of some collected adults, in several ways: (1) complete analysis, seeing the salivaries from at least six larval offspring from crosses to flies of known constitution; and (2) crosses of collected males and females, leading in most cases to inferences of their linkage karyotypes but, in the case of the autosomes, not being able to determine which karyotype belonged to the male and which to the female. The arrangement combinations in these data were derived by one of us (M. L.) from the archival records of these collections, courtesy of Dr. H. L. Carson and the Library of the American Philosophical Society in Philadelphia, Pennsylvania. It was not possible to assign combination frequencies to thirty "egg sample" larvae that were doubly heterozygous in the X-chromosome and eighteen that were doubly heterozygous in the second chromosome.

Statistical significance of the data is based on log-likelihood, or G -, tests (also known as 2I tests), for these give a closer approximation to the theoretical chi-square distribution than the traditional chi-square tests (Sokal and Rohlf 1981; Lewontin 1992). For an even better fit, the correction of Williams (1976): $1 + (a+1)/6n$, where a = number of items of data, n = the total data in the sample, and v = degrees of freedom, was applied to all G -test results with more than one degree of freedom. For data with one degree of freedom, such as 2×2 tables, where the departures from continuity with the chi-square distribution are likely to be more severe, especially if the expected values in some cells are small, a more stringent Williams correction was applied (see Sokal and Rohlf 1981: 737). The data with more than one degree of freedom also often contain cells with small frequencies, but a number of writers (e.g., Cochran 1954; Lewontin and Felstein 1965; and Sokal and Rohlf 1981) have shown that drastic

adjustments, such as Yates's, tend to be overcorrections even for conventional chi-square tests and would certainly be so for G-tests that were adjusted with Williams's correction.

To save space, the text and tables will often use the shorthand notation for the arrangements introduced by Carson (1953): The Standard arrangement of each arm is dubbed "S." Each of the other arrangements is referred to by the Arabic numeral after the hyphen in its name. A fly with karyotype 2L/2L-3, 2R/2R-1, for example, would be S/3,S/1 in this notation. Depending on the linkage combination of the arrangements, it is also either SS/31 or S1/3S.

Results

Except for the smallest sample—Mt. Magazine, where $n = 22$ and one of the arrangements (XR-1) is absent in the karyotyped adults (but was present in the egg samples from 1 individual), all the Ozark and Ouachita region data deviate in a highly significant way from the numbers expected on the assumption of linkage equilibrium (table 10.1). If the Mt. Magazine sample is combined with the larger one from Fane Creek, with which it is statistically homogeneous, the deviations for the combined sample, too, are highly significant.

Table 10.2 examines the same data in more detail. Part A shows that in every population, except again the small Mt. Magazine sample, the combination of XL-2 and XR-2 appears more frequently, and the combinations of XL-2 with XR and XR-1 less frequently, than would be expected if the linkages were random. The deviations are highly significant at Steelville, in both Fayetteville samples, and in the Mill Creek sample, but they are not statistically significant at Fane Creek or in the combined Fane Creek and Mt. Magazine samples. Similar results are obtained if the linkage of XL-2 and XR-1 is omitted from the calculations. At Steelville, for example, the frequencies of XR and XR-2 are nearly equal, about 25:28. At equilibrium one would expect 14.2 of the 30 instances of XL-2 to be linked to XR, 15.8 to be linked to XR-2. Instead the ratio is 0 XL-2.XR:30 XL-2.XR-2, a highly significant deviation.

Part B of table 10.2 compares how often XR-1 is combined with the left-arm arrangements and the number expected if there were no association. In the absence of association, the numbers would reflect the relative frequencies of XL, XL-1, and XL-2 in each sample. This cannot be tested in the small Mt. Magazine adult sample, where XR-1 is absent. All the other data differ significantly at the 1 percent level from random expectation, in that

TABLE 10.1.
X-Chromosome Gene Arrangements in Adult Samples of Ozark and Ouachita Region Natural Populations of *Drosophila robusta*
Fayetteville 1989 Fayetteville 1992 Fane Creek Mt. Magazine FC & MM Mill Creek Steelville (FC) (MM)

Gene Arrangements	Fayetteville 1989		Fayetteville 1992		Fane Creek		Mt. Magazine		FC & MM		Mill Creek		Steelville	
	a	e	a	e	a	e	a	e	a	e	a	e	a	e
XL	77.45	53.57	16.38	9.09	15.22	26.62	77.88							
XL-1	17.65	34.82	53.45	40.91	51.45	5.46	77.88							
XL-2	4.90	11.61	30.17	50.00	33.33	67.92	15.35							
XR	30.39	18.75	1.72	9.09	2.90	11.95	25.28							
XR-1	50.98	31.25	3.45	*	2.90	3.07	46.73							
XR-2	18.63	50.00	94.83	90.91	94.20	84.98	27.99							
N	102	112	116	22	138	293	443							

Left-Right Combinations

Gene Arrangements	Fayetteville 1989		Fayetteville 1992		Fane Creek		Mt. Magazine		FC & MM		Mill Creek		Steelville	
	a	e	a	e	a	e	a	e	a	e	a	e	a	e
SS	25	24.0	18	11.3	1	0.3	1	0.2	2	0.6	35	9.3	99	75.4
S1	50	40.3	33	18.7	4	0.7	0	0.0	4	0.6	9	2.4	179	139.4
S2	4	14.7	9	30.0	14	18.0	1	1.8	15	19.8	34	66.3	37	83.5
1S	6	5.5	3	7.3	1	1.1	0	0.8	1	2.1	0	1.9	1	14.9
11	2	9.2	2	12.2	0	2.1	0	0.0	2	2.1	0	0.5	8	27.5
12	10	3.4	34	19.5	61	58.8	9	8.2	70	66.9	16	13.5	29	16.5
2S	0	1.5	0	2.4	0	0.6	1	1.0	1	1.3	0	23.8	0	6.5
21	0	2.5	0	4.1	0	1.2	0	0.0	0	1.3	0	23.8	0	6.5
22	5	0.9	13	6.5	35	33.2	10	10.0	45	43.3	199	169.1	30	12.1
Totals	102	102.0	112	112.0	116	116.0	22	22.0	138	138.0	293	293.0	383	383.0

G-tests: 45.684 36.821 16.604 3.284 19.004 139.566 174.856
df 4 4 4 2 4 4
P <<<0.01 <<0.01 <0.01 >0.15 <0.01 <<<0.01 <<<0.01

NOTE: Combinations are given in numbers, arrangements in percent; a = actual, e = expected on the assumption of random equilibrium; G-tests measure possible deviation of these data from linkage equilibrium.
*None in adult sample; present, however, in egg sample from one individual.

XL.XR-1 is more frequent, and XL-1.XR-1 and XL-2.XR-1 are less frequent, than expected. The association holds true even if XL-2.XR-1, which has never been found in nature, is eliminated from consideration. At Steelville, for example, the ratio of XL:XL-1 is about 5:1, but the ratio of XL.XR-1:XL-1.XR-1 is about 22:1.

Part C examines the data if the combinations involving XL-2 and those involving XR-1 are removed, leaving only the combinations of XL and XL-1 with XR and XR-2. This part tests, therefore, for a possible "S,1:S,2 coupling-repulsion association." In every Ozark and Ouachita region sample the coupling combinations, XL.XR and XL-1.XR-2, are more frequent

TABLE 10.2.
Tests for the Three Kinds of X-Chromosome Linkage Disequilibrium in the Table 10.1 Data

	Fayetteville 1989	Fayetteville 1992	Fane Creek (FC)	Mt. Magazine (MM)	FC & MM	Mill Creek	Steelville
A. The XL-2:XR-2 Association							
2S	0	1.5	0	2.4	0	0.6	1
21	0	2.5	0	4.1	0	1.2	0
22	5	4.0	13	6.5	35	33.2	10
Totals	5	4.0	13	13.0	35	35.0	11
G-tests:	15.131		17.143		3.627		0.000
df	2		2		2		2
P	<<<0.01		<<<0.01		>0.15		1
B. The XL:XR-1 Association							
S1	50	40.3	33	18.7	4	0.7	0
11	2	9.2	2	12.2	0	2.1	0
21	0	2.5	0	4.1	0	1.2	0
Totals	52	52.0	35	35.0	4	4.0	0
G-tests:	15.260		29.688		11.952		4
df	2		2		2		2
P	<<<0.01		<<<0.01		<0.01		<0.01
C. The S1:S2 Coupling-Repulsion Association							
SS	25	20.0	18	8.9	1	0.4	1
S2	4	9.0	9	18.1	14	14.6	1
1S	6	11.0	3	12.1	1	1.6	0
12	10	5.0	34	24.9	61	60.4	9
Totals	45	45.0	64	64.0	77	77.0	11
G-tests:	10.666		25.199		0.894		11.0
df	1		1		1		1
P	<0.01		<<<0.01		>0.30		>0.05

NOTE: The testing procedures are discussed in the text; a = actual, e = expected on the assumption of random equilibrium; G-tests measure possible deviation of these data from linkage equilibrium.

than would be expected if the combinations were random, and the repulsion forms, XL:XR-2 and XL-1:XR, less frequent. At Fayetteville, Mill Creek, and Steelville, the deviations are highly significant, and marginally so ($0.05 < p < 0.1$) in the Mt. Magazine and the combined Mt. Magazine and Fane Creek samples. The association is clearly absent, however, in the substantial Fane Creek data.

As a whole, the second chromosome combinations in the Fayetteville, Steelville, and Mill Creek samples do not deviate significantly from the

numbers expected on the assumption of linkage equilibrium (table 10.3). Significant deviations are encountered only in the Fane Creek and Mt. Magazine samples, especially in the statistically homogeneous combination of these two samples.

Table 10.4 shows a special portion of the second chromosome data of table 10.3, namely, the relative numbers of (1) double heterozygotes containing the same left- and right-arm arrangements and (2) arrangement combinations with the same left-arm arrangement in the double heterozygotes. Part A shows that in the Ozark samples the double heterozygotes containing the combination 2L-1.2R-1 (11) are consistently more frequent than the double heterozygotes containing 2L-1.2R (1S). The deviations from equality reach statistical significance in the Fayetteville, all Ozarks,

TABLE 10.3.
Chromosome 2-Gene Arrangements in Ozark and Ouachita Natural Populations of *D. robusta*

	Fayetteville 1989	Fayetteville 1992	Fane Creek (FC)	Mt. Magazine (MM)	FC & MM	Mill Creek	Steelville
Gene Arrangements							
2L	44.50	41.01	46.15	50.00	46.90	25.81	69.13
2L-1	38.50	46.76	37.36	34.09	36.73	49.87	22.18
2L-2	14.50	5.76	6.04	11.36	7.08	23.56	6.65
2L-3	2.50	6.47	10.44	4.55	9.29	0.75	2.03
2R	85.50	83.45	74.73	79.55	75.66	81.95	77.26
2R-1	14.50	16.55	25.27	20.45	24.34	18.05	22.74
N	200	139	182	44	226	399	541
Left-Right Combinations							
SS	73	76.1	48	47.6	63	62.8	20
1S	66	65.8	51	54.2	44	50.8	10
2S	27	24.8	8	6.7	10	8.2	3
3S	5	4.3	9	7.5	19	14.2	2
S1	16	12.9	9	9.4	21	21.2	2
11	11	11.2	14	10.8	24	17.2	5
21	2	4.2	0	1.3	1	2.8	2
31	0	0.7	0	1.5	0	4.8	0
Totals	200	200.0	139	139.0	182	182.0	44
G-tests:	3.886		7.022		16.015		44.0
df	3		3		3		3
P	>0.25		>0.05		<0.01		<0.025

NOTE: Combinations are given in numbers, arrangements in percent; a = actual, e = expected on the assumption of linkage equilibrium; G-tests measure possible deviation of these data from linkage equilibrium.

TABLE 10.4.

Double Heterozygotes (Part A) and the Included Gene Arrangements (Part B) in the Data of Table 10.3.

	Fayetteville 1989		Fayetteville 1992		Double Heterozygotes (Part A)				All Ozarks Creek		Mill Creek		Grand Total	
	Fayetteville	Fayetteville	All Fayetteville	Fane Creek	Mt Magazine	Streelville	Ozarks Creek	Ozarks Creek	Mill Creek	Grand Total				
SS/11	4	7	11	7	4	5	27	11	38					
S1/1S	2	2	4*	5	1	4	14*	6	20*					
SS/21	2	0	2	1	0	2	5	2	7					
S1/2S	1	0	1	0	0	2	3	3	6					
SS/31	0	0	0	0	0	0	0	0	0					
S1/3S	0	1	1	0	0	1	2	0	2					
1S/21	0	0	0	0	1	0	12	13						
11/2S	2	2	4	3	0	0	7*	6	13					
1S/31	0	0	0	0	0	0	0	0	0					
11/3S	0	0	0	2	0	0	2	0	2					
2S/31	0	0	0	0	0	0	0	0	0					
21/3S	0	0	0	0	0	0	0	0	0					
Totals	11	12	23	18	6	14	61	41	102					
The Included Combinations (Part B)														
SS	6	7	13	8	4	7	32	13	45					
S1	3	3	6	5	1	7	19	9	28*					
1S	2	2	4	5	2	4	15	18	33					
11	6	9*	15**	12*	4	5	36**	17	53					
2S	3	2	5	3	0	2	10	9	19					
21	2	0	2	1	1	2	6	15	21					
3S	0	1	1	2	0	1	4	1	5					
31	0	0	0	0	0	0	0	0	0					
Totals	22	24	46	36	12	28	122	82	204					

NOTE: Asterisks denote the pairs of karyotypes with the same gene arrangements (part A) and the pairs of combinations with the same left-arm arrangements (part B) that deviate significantly from the equality that would be expected in each case on the assumption of linkage equilibrium.
 * $p < 0.05$
 ** $p < 0.01$

and total data that compare the numbers of SS/11 and S1/1S. The consistency manifests itself in part B by the regular excess of 11 over 1S in the double heterozygotes in the Ozark region. That inequality is significant at the 1% level in the total Fayetteville and all Ozarks samples and at the 5% level in the 1992 Fayetteville and Fane Creek samples. Here the Oauidita Mountain sample differs markedly from the Ozark data, primarily because there

were 12 1S/21:6 11/2S at Mill Creek, whereas in the Ozarks the ratio of these karyotypes was 1:7. The opposite result, excess of the combinations containing 2R over the ones containing 2R-1, is found in the ratios of SS:S1, 2S:21 (but not at Mill Creek) and 3S:31, but only one of these inequalities achieves statistical significance, the 45 SS:28 S1 in the total sample.

In chromosome 3, only 3R and 3R-1 occur in this area. The frequencies of the karyotypes present are shown in table 10.5. With the exception of the small Mt. Magazine sample, the numbers of arrangement heterozygotes exceed, and the numbers of homozygotes fall short of, Hardy-Weinberg expectations. The discrepancy is significant at the 5% level at Fane Creek. In the other cases, including the combined Fane Creek and Mt. Magazine sample, the data do not contradict the assumption of random mating equilibrium.

Only a few years after Painter (1934) introduced the giant chromosomes of the larval salivary glands as a useful tool in *Drosophila* genetics, Dobzhansky, in collaboration with Alfred H. Sturtevant, used them to demonstrate chromosomal polymorphism in natural populations (Sturtevant and

TABLE 10.5.
 Chromosome 3-Gene Arrangements in Ozark and Ouachita Natural Populations of *D. robusta*

	Fayetteville 1989		Fayetteville 1992		Gene Arrangements								
	Fayetteville	Fayetteville	All Fayetteville	Fane Creek (FC)	Mt Magazine (MM)	FC & MM	Mill Creek	Streelville					
3R	41.00	43.75	42.15	15.08	11.36	14.35	1.75	60.51					
3R-1	59.00	56.25	57.85	85.00	88.64	85.65	98.25	39.49					
N	200	144	344	179	44	223	399	547					
Karyotypes													
	a	e	a	e	a	e	a	e	a	e	a	e	a
3R/3R	13	16.8	12	13.8	25	30.6	0	2.0	1	0.3	1	2.3	0
3R/3R-1	56	48.4	39	35.4	95	83.9	27	22.8	3	4.4	30	27.3	7
3R-1/3R-1	31	34.8	21	22.8	52	57.6	62	64.2	18	17.3	80	81.4	192
Totals	100	72	172	89	22	111	199	255					
G-tests:	2.466	0.732	2.842	4.735	1.450	1.203	0.125	0.615					
df	1	1	1	1	1	1	1	1					
p	>0.10	>0.30	>0.05	<0.05	>0.20	>0.20	>0.50	>0.30					

NOTE: Karyotypes are given in numbers, arrangements in percent; a = actual, e = expected on the assumption of Hardy-Weinberg equilibrium; G-tests measure possible deviation of these data from that equilibrium.

Dobzhansky 1936a). In a seminal publication Dobzhansky and Sturtevant (1938) showed that multiple inversions occurring on the same chromosome fall into three categories: (1) "independent," that is, separated inversions with recombination between them—dubbed a "linkage plexus" by Levitan (1958a); (2) "included," that is, having both breaks of one inversion between the breaks of a larger one; and (3) "overlapping," that is, having one break of an inversion fall *between* the breaks of a second one and one break *outside* the breaks of the second one. Although some of his earliest work on natural populations of *Drosophila* concerned what later proved to be an example of the first category, namely, the inversions of the SR ("sex ratio") complex of *D. pseudoobscura* (Sturtevant and Dobzhansky 1936b; Dobzhansky 1944), most of the studies by Dobzhansky and his students and collaborators in this period concerned inversions of the third category (reviewed by Powell 1992). The work with *D. robusta*, members of the *melanica* group, and many other species (see reviews by Levitan 1982 and Levitan and Fukutami 1993) has demonstrated, on the other hand, that multiple inversions of the first category also constitute major adaptations of Diptera to their environment. This report reinforces previous data that *D. robusta* populations especially develop complexes of the first category in its two largest chromosomes, and it shows that these complexes are often similar to those in geographically distant locations.

The association of arrangements XL-2 and XR-2, with concomitant virtual absence of any XL-2s linked to XI, occurs in every locality in which XL-2 exists (reviewed by Levitan 1992), and the Ozark and Ouachita populations described here maintain this pattern (table 10.2, part A). One instance of XL-2 linked to XR was found where more than 36 were expected under linkage equilibrium. The discrepancy of XL-2s linked to XR-1 is even more extreme. Indeed, to date, not a single instance of such a linkage has been found where the two arrangements coexist. In these data more than 27 such combinations would have been expected on the assumption of linkage equilibrium. It is interesting that the association of these two arrangements, like that of the SR complex of *D. pseudoobscura*, is maintained in this extreme fashion despite the large area of intervening euchromatin available for crossing-over. In both the *D. robusta* and *D. pseudoobscura* cases, recombination between X-chromosome inversions is very rare (Wallace 1948; Carson 1953; Levitan 1958b; Powell 1992).

In contrast, the nonrandom combinations of arrangement XR-1 with arrangements of the left arm observed in the Ozark and Ouachita populations (table 10.2, part B) are far from universal. Indeed, the presence of this disequilibrium in Fayetteville, Arkansas, and Steelville, Missouri, marks its

only instances in places with high frequencies of XR-1. The disequilibrium is strongest in southeastern Pennsylvania; nearby Princeton, New Jersey, and northeastern New Jersey, where XR-1 is quite rare. The XR-1 present is almost exclusively linked to XL, even though XL-1 is the most common left-arm arrangement in many of these localities (Levitan 1973).

The disequilibrium is absent, however, in substantial samples from Kentucky, Ohio, northern Indiana, southern Michigan, southern Minnesota, Iowa, east-central Missouri, and eastern Nebraska, where XR-1 attains some of its highest frequencies in the presence of both XL and XL-1 (as may be gleaned from the data in Levitan 1992). The disequilibrium is also absent in the areas to the east of the Ozark region and to the south of Pennsylvania, where XR-1 (and, in low elevations, also XL-1) are nearly or completely absent. The only place the disequilibrium has been encountered between the northeast and the areas of this report has been in the Bloomington, Indiana, area. There XR-1 is much rarer than expected on the basis of its typical tendency to increase from east to west: 209 XR-1 of 1388 X-chromosomes, about 15%, in contrast to frequencies of more than 50% in Dayton, Lima, and Columbus, Ohio, to the east of it (Levitan 1992); 185 of the 209 are linked to XL, 24 to XL-1, whereas on a random basis 168.1 would be expected to be XL.XR-1, 40.9 XL-1.XR-1, a highly significant deviation.

Levitan (1961) noted that the X-chromosome S₁S₂ coupling-repulsion association may take two forms: one in which the "coupling" combinations (SS and 12) are in excess of the numbers expected under equilibrium and one in which the "repulsion" combinations (S2 and 1S) are in excess. Highly significant associations of the first form characterize populations near Blacksburg, Virginia, in the Allegheny plateau; Lexington, Kentucky; southeastern Pennsylvania; northeastern New Jersey; and many midwestern localities (including the Bloomington area of Indiana), whereas the second form is characteristic of all studied elevations of the Great Smoky Mountains of Tennessee, the Blue Ridge mountains in North Carolina and Georgia, and the Allegheny Mountains in western Virginia and eastern West Virginia (reviewed by Levitan 1992; see also Etges 1984). It is interesting that the Ozark and Ouachita populations take the same form of this association as the populations of southeastern Pennsylvania, northeastern New Jersey, and the Bloomington, Indiana, area, just as the XR-1.XR association does.

Levitan (1955, 1958a, 1964) and Levitan and Scheffer (1993) found that the major features of second chromosome associations in *D. robusta* were the relative numbers of (1) double heterozygotes containing the same left-

and right-arm arrangements and (2) arrangement combinations with the same left-arm arrangement in the double heterozygotes. Equilibrium theory predicts equalities in each of these categories if the populations are in linkage equilibrium (Levitan 1964). Since the expectation of equalities is independent of the underlying gene or karyotype frequencies, the numbers in different samples may be added, and the sums for these categories are also expected to be equalities.

As noted earlier, the Ozark populations contain a number of significant departures from the random equilibrium equalities in the second chromosome data (table 10.4). Compilations of such data (Levitan 1964, 1982) have shown that the deviations from linkage equilibrium are not of the same intensity in all populations. Additional data published by Levitan (1992) strengthen the previous findings of very large deviations from equilibrium expectations in the areas with high frequencies of 2L-3, especially in the higher elevations of the South and in western localities of the North. In these populations heterozygotes combining 2L-3 and 2R with 2L.2R-1 or 2L-1.2R (S1/3S or 11/3S in the shorthand notation) tend to be significantly more abundant than the heterozygotes with the same arrangements in the opposite configurations, that is, SS/31 or 1S/31. The corollary result is that 3S is usually much more frequent than 31 in all forms of the double heterozygotes, accompanied by an excess of S1 or 11 (or both) over SS or 1S, respectively. The effect is much stronger in males than in females. Although the female data are often in the same direction, these are less likely to be statistically significant.

In areas where 2L-3 is rarer, such as the Ozark and Ouachita regions, 3S is again usually more frequent than 31, but the numbers are usually so small that they do not reach statistical significance; an exception is the cumulative total for the southern areas with 2L-3 less than 25%. Instead these areas tend to emphasize excesses of 2L-1.2R-1 (11) over 2L-1.2R (1S), or, less often, 2L.2R-1 (S1) over 2L.2R (SS). With respect to these arrangements, it is interesting that the Ozark region populations resemble more closely those of the southeastern lowlands (mainly Raleigh, North Carolina) than the results from nearby northcentral Missouri (St. Louis area), on the same side of the Mississippi River, where, contrary to pattern, 1S is significantly more frequent than 11. The two localities also differ in that in the St. Louis area S1 is, as usual, significantly more frequent than SS, whereas in the Ozark-Ouachita region, as in the southeastern lowlands (and the females of southern Indiana), the opposite is true.

Although based on a limited number of populations, a pattern of geographic variation in inversion polymorphism in the Arkansas-Missouri

highlands was apparent. Extending from the Ozark plateau (Steeleville and Fayetteville) down through the lowland transition (Fane Creek) to the Arkansas River valley, across to the Mt. Magazine escarpment, and farther south to the more isolated Ouachita highlands, the most western extension of the Appalachian chain, *D. robusta* populations exhibit considerable genetic differentiation across this complex landscape. Ozark plateau populations share similar inversion frequencies, particularly for the X-chromosome (table 10.1), although the matter may be complicated by evidence of year-to-year temporal variation in X-chromosome inversion frequencies (Erges 1991 and unpublished data; note also the difference between the two Fayetteville samples in table 10.1). Lowland Ozark populations on the north side of the Arkansas River are considerably distinct from the plateau populations, as shown by the large X-chromosome gene arrangement differences between Fayetteville and the two sites that have been sampled in the White Rock area, Fane Creek (this report, table 10.1) and Shores Lake (Erges 1991), located 10 km away. The similarity of the latter two populations to the Mt. Magazine population was surprising, given that they are separated from Mt. Magazine by the Arkansas River valley, more than 60 km.

No other data are known from the Ouachitas, but the Mill Creek sample suggests that XL-2 and the XL-2.XR-2 combination reach their highest frequencies here in the entire range. The previous high in substantial samples was the 53.7% at Emory, Georgia (Levitan 1992). This calls into question previous hypotheses about the location of the epicenter of the supposed "radiate" distribution of XL-2 (Carson 1958; Levitan 1992).

Second chromosome frequencies are much more indifferent to these geographical differences than the X-chromosome arrangements. This might suggest considerable gene flow if it were not for the X-chromosome variation. Such a large regional effect may be explained by the comparatively high frequencies of 2L-1 and 2L-2, "warm weather" arrangements that increase in frequency at lower latitudes and altitudes (Levitan 1992). These arrangements are associated with shorter egg-to-adult development times, particularly under warm temperature conditions (Erges 1989). This suggests a mechanism for maintaining the uniformity in second chromosome frequencies in these populations, where the summers are typically warm and dry. However, the nature of the selective forces shaping the extensive variation in X-chromosome arrangements and in the patterns of linkage disequilibrium in both chromosomes remain to be determined.

One of Dobzhansky's major accomplishments was to demonstrate the selectional basis of the overlapping inversion complexes of *D. pseudoobscura* and its relatives. The data of this paper further strengthen the hypothesis

that linkage complexes, too, have a selectional basis. The fact that some populations are at equilibrium for the linkage combinations that are in disequilibrium in other localities negates, for example, the possibility that the disequilibria represent merely a stage in the historical process toward equilibrium, that these populations just have not had enough time to accomplish sufficient recombination to reach the random proportions of equilibrium theory. This is further enhanced by the findings that several localities have developed (or marshaled selectional forces to *maintain*) the same disequilibrium even though the intervening populations have either developed a different disequilibrium of the same arrangements (Levitan 1961) or are at linkage equilibrium for them.

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