ASSOCIATIONS OF ALLELES OF THE ESTERASE-1 LOCUS WITH GENE ARRANGEMENTS OF THE LEFT ARM OF THE SECOND CHROMOSOME IN *DROSOPHILA ROBUSTA*[1]

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ABSTRACT

Evidence of strong associations of *Est-1* alleles with the 2L, 2L1 and 2L3 gene arrangements of the left arm of the second chromosome in *D. robusta* is presented. Each gene arrangement is polymorphic for three to four *Est-1* alleles. The allele frequencies differ in the 2L3 and 2L arrangements; the allele $Est\text{-}1^{.92}$ is 8% in the 2L3 arrangement (n=203)—this allele is 82% in the 2L arrangement (n=203); the allele $Est\text{-}1^{1.0}$ is 66% and 14.8% in the 2L3 and 2L arrangements, respectively. There are no differences in allele frequencies in 2L3 arrangements from any of the widely separated seven different populations; similarly the allele frequencies in the 2L arrangement are alike in all five widely separated populations studied. The allele frequencies in the 2L1 arrangement are intermediate to those observed in the 2L3 and the 2L arrangements and show north-south clinal change. These associations between *Est-1* alleles and gene arrangements of the left arm of the second chromosome are due to natural selection favoring different allele frequencies in different gene arrangements, as a result of epistatic interactions between the *Est-1* locus and the loci on the gene arrangements. As expected, we observe that the proportion of heterozygotes is greater in the inversion heterokaryotypes than in the homokaryotypes.

POPULATIONS of *Drosophila robusta* are highly polymorphic for inversions in five of the six arms of the three major V-shaped chromosomes XL, XR, 2L, 2R and 3R, and in some regions 3L is involved as well. A total of fifteen different gene arrangements are found extensively in natural populations of this species (CARSON 1958). The left arm of the second chromosome is highly polymorphic for the 2L, 2L1, 2L2, and 2L3 gene arrangements. The 2L1, 2L2, and 2L3 arrangements differ from the 2L arrangement by a single inversion. The right arm of the second chromosome has two common alternate gene arrangements, 2R and 2R1, which also differ from each other by a single inversion.

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Different arrangements of *D. robusta* must differ genetically because: (1) Inversions of different chromosome arms show clinal geographic distribution (CARSON 1958). The 2L3 inversion, for example, occurs in high frequency in the northern populations, and its frequency decreases clinally in the southern populations. The 2L1 arrangement shows north-south clines in the opposite direction. (2) Seasonal cyclic changes in frequencies of gene arrangements occur in some populations. The frequency of the 2L3 arrangement, which is found in high frequency in the northern populations and is absent from the southern populations, is high in early spring collections and declines in fall collections (LEVITAN 1951a and unpublished data). (3) Population cages started with the same initial frequencies of gene arrangements show systematic directional changes in gene arrangement frequencies (CARSON 1958; LEVITAN 1951b). (4) Different karyotypes of *D. robusta* have different mating speeds (PRAKASH 1967, 1968). In addition, LEVITAN (1955, 1958a, 1964) has observed widespread nonrandom associations of gene arrangements of the left and right arms of the second chromosome and has evidence for temporal variation of different inversion linkages in the left and right arm of the X chromosome (LEVITAN 1973).

The purpose of this paper is to provide direct evidence of genetic differentiation of various gene arrangements of the left arm of the second chromosome at the Esterase-1 (*Est-1*) locus in *D. robusta*. Each of the gene arrangements is polymorphic for alleles at the *Est-1* locus, but the allele frequencies in different gene arrangements are very different.

MATERIALS AND METHODS

Wild flies from Williamstown, Mass.; Rockville, Neb.; Ledgewood, N.J.; Englewood, N.J. and Philadelphia, Pa. were mated to flies of opposite sex from the standard stock, which is homozygous for all standard gene arrangements. Wild females were fully despermed before mating to standard stock flies (PRAKASH 1967). Usually ten but at least six F_1 larvae were studied for karyotype determination of the wild flies. The wild flies were then electrophoretically analyzed to determine their Esterase-1 genotypes. The karyotype and *Est-1* genotype determinations of Raleigh, N. C. were done on larvae from freshly collected strains; the karyotype and the genotype of the same larva were determined simultaneously.

In samples from Chadron, Neb., *Est-1* genotypes of F_1 progeny from wild females were determined; electrophoresis of a single F_1 fly from each strain was done providing information on two wild genomes. Twenty strains from Myrtle Beach, S.C. and 25 strains from Astor, Fla. were studied; three to four individuals were studied from each strain of Myrtle Beach—each strain was assumed to provide information on two wild genomes for *Est-1* genotype and the karyotype. Up to eight individuals were studied from Astor, Fla. strains in F_3. Each strain then provided information on up to four wild genomes for the *Est-1* genotype and the karyotype.

Enzyme assay: 5% acrylamide gels were made in 0.1 M Trisborate disodium Edta buffer pH 8.3; this buffer was also used in the buffer compartments. Electrophoresis was performed at 360 volts for 1 hour and 25 minutes. The gels were incubated in 0.5 M boric acid at room temperature for 15 minutes. The gels were then stained as described in HUBBY and LEWONTIN (1966).

RESULTS

Table 1 gives the results of a cross made for determining the location of the *Est-1* gene. The parental second chromosome karyotypes show associations of the locus with the second chromosome. The linkage order of the *Est-1* gene and the

TABLE 1

Karyotype and Est-1 *genotype of maternal gamete examined in BC_1 larvae obtained from a cross of 2L1 2R1* Est-$1^{.92}$/*2L3 2R* Est-$1^{1.0}$ F_1 ♀ ♀ * × *2L3 2R* Est-$1^{1.0}$/*2L3 2R* Est-$1^{1.0}$ ♂ ♂. *F_1 females were obtained by crossing XL XR2 2L1 2R1* Est-$1^{.92}$ *3R1/XL XR2 2L1 2R1* Est-$1^{.92}$ *3R1* ♀ ♀ × *XL1 XR 2L3 2R* Est-$1^{1.0}$ *3R/XL1 XR 2L3 2R* Est-$1^{1.0}$ *3R* ♂ ♂

	Maternal gamete			
	2L karyotype	2R karyotype	Esterase-1 genotype	Observed no. offspring
Parentals	2L1	2R1	.92	34
	2L3	2R	1.00	25
Singles	2L1	2R	.92	15
	2L3	2R1	1.00	6
	2L1	2R1	1.00	7
	2L3	2R	.92	0
Doubles	2L1	2R	1.00	2
	2L3	2R1	.92	0
				89

"Gene order" *Est-1*, 2L, 2R by comparison of doubles to parentals

$$R_{2L,2R} = \frac{21+2}{89} = \frac{23}{89} = .258 \quad R_{Est\text{-}1,2L} = \frac{7+2}{89} = \frac{9}{89} = .10 \quad R_{Est\text{-}1,2R} = \frac{21+7}{89} = \frac{28}{89} = .315$$

Note the strong suggestion of viability differences between $\frac{2L1}{2L3}$ and $\frac{2L3}{2L3}$.

* F_1 ♀ ♀s were heterokaryotypic in the XL, XR and 3R chromosome arms also.

2L and the 2R gene arrangements is *Est-1*, 2L, 2R. The *Est-1* locus is outside the limits of the 2L1 and 2L3 inversions. Recombination in females, which are heterokaryotypic in five chromosome arms, between the *Est-1* locus and the 2L1, 2L3 inversions is 10%. Since the 2L1 and 2L3 arrangements span the proximal 72% of the left arm of the second chromosome, the *Est-1* locus must be located outside the inversion limits, in the distal 28% of the left arm of the second chromosome.

Table 2 presents the allele frequencies at the *Est-1* locus and gene arrangement frequencies in the second left chromosome in different *D. robusta* populations. Chadron, Neb. represents the northernmost, and Astor, Fla. the southernmost, population studied. Each population is highly polymorphic for the *Est-1* alleles; the frequencies of *Est-1* alleles in different populations are different, however, and vary clinally. The *Est-$1^{.92}$* allele shows a gradual north to south increase from 14% in the northernmost Chadron, Neb. population to 70–80% in the southernmost populations of Myrtle Beach, S.C. and Astor, Fla. The allele *1.0* shows differentiation in frequencies according to latitude as well as longitude. In the eastern coastal plain populations of Williamstown, Ledgewood, Englewood, Philadelphia, Raleigh, Myrtle Beach and Astor we observe a north to south decrease in the frequency of *Est-$1^{1.0}$*. This allele is in higher frequencies in the western Nebraska populations of Chadron and Rockville than the eastern population of Williamstown, Mass. Similar differentiation is apparent at the *Est-$1^{.86}$* allele.

TABLE 2

Frequencies of alleles at the Est-1 *locus and of gene arrangements in the left arm of the second chromosome in* D. robusta *populations. Populations are arranged from left to right according to their latitude. Chadron is the northernmost and Florida the southernmost population studied.* n *refers to the number of genomes examined.*

Alleles	State: Locality: Latitude:	Nebraska Chadron 42°49.9'N	Massachusetts Williamstown 42°42.7'N	Nebraska Rockville 41°07.2'N	New Jersey Ledgewood 40°53.9'N	New Jersey Englewood 40°53.6'N	Pennsylvania Philadelphia 39°57.0'N	No. Carolina Raleigh 35°36.3'N	So. Carolina Myrtle Beach 33°42.1'N	Florida Astor 29°09.2'N
.86		.23	.096	.122	.066	.045	.11	.03	.02	.01
.92		.14	.288	.244	.54	.524	.56	.60	.78	.70
1.0		.59	.48	.622	.37	.396	.296	.30	.15	.29
1.07		.03	.135	.012	.027	.035	.03	.07	.05	—
n		91	104	82	256	802	142	182	41	66
Gene arrangements										
2L		—	.310	.23	.435	.487	.446	.20	.02	—
2L1		—	.028	.12	.173	.338	.478	.53	.98	1.0
2L2		—	.014	.006	.029	.012	.019	.12	—	—
2L3		1.0	.648	.64	.362	.163	.056	.16	—	—
n		100	210	153	411	545	318	76	40	100

TABLE 3

Associations of alleles at the Est-1 *locus with gene arrangements of the left arm of the second chromosome* in various populations of* D. robusta. n *is the number of genomes studied*

Gene arrangement	*Est-1* alleles	Nebraska Chadron	Massachusetts Williamstown	Nebraska Rockville	New Jersey Ledgewood	New Jersey Englewood	Pennsylvania Philadelphia	North Carolina Raleigh	South Carolina Myrtle Beach	Florida Astor	χ^2 heterogeneity test	Allele frequencies in the pooled data
2L3	*.86*	.23	.184	.133	.30	.214	.167	—	..	..		.21
	.92	.14	—	.067	.10	—	—	—	..	..	$\chi^2(18)=21.80$ p $>$ .25	.08
	1.0	.59	.684	.767	.60	.714	.833	1.0	..	..		.66
	1.07	.03	.132	.033	—	.071	—	—	..	..		.05
	n	91	38	30	20	14	6	4	..	..		203
2L	*.86*	..	.142	..	—	—	.055	—	..	..		.015
	.92	..	.714	..	.795	.84	.945	.73	..	..	$\chi^2(4)=4.46$ p $>$ .25†	.822
	1.0	..	.142	..	.205	.14	—	.20	..	..		.148
	1.07	..	—	..	—	.02	—	.07	..	..		.015
	n	..	14	..	44	112	18	15	..	..		203
2L1	*.86*	..	..	..	..	.069	.15	.03	.02	.01		..
	.92	..	..	..	..	.362	.55	.47	.78	.70	$\chi^2(4)=23.89$ p $=.0001$†	..
	1.0	..	..	..	..	.552	.30	.45	.15	.29		..
	1.07	..	..	..	..	.017	—	.05	.05	—		..
	n	..	..	..	..	58	20	66	41	66		..

* All the populations studied, with the exception of Raleigh, Myrtle Beach and Astor, have the 2R arrangement fixed or in frequency greater than 95% in the second right chromosome. In Raleigh the arrangements 2R and 2R1 occur in 0.70 and 0.30 frequencies and in Myrtle Beach and Astor populations the 2R and 2R1 arrangements have .10:.90 and 0.0:1.0 frequencies. In the Raleigh and other populations no differences in *Est-1* associations were observed between 2L 2R and 2L 2R1; and 2L1 2R and 2L1 2R1 gene arrangements. The second right chromosome karyotype thus had no effect on *Est-1* associations in the second left chromosome.

† Only alleles *.92* and *1.0* were considered.

The gene arrangements of the left arm of the second chromosome show north-south clines in frequencies (Table 2). The 2L3 arrangement is highest in the northern populations and is absent from the southern (South Carolina and Florida) populations. The 2L1 arrangement, on the other hand, increases in the southern populations and is either absent or present in low frequencies in the northern populations. The 2L arrangement is in highest frequency in geographically central populations and declines radially from there.

Table 3 presents the associations of *Est-1* alleles with different gene arrangements of the left arm of the second chromosome. *The allele frequencies in the 2L3, 2L1, and 2L gene arrangements were calculated only from the flies which were homokaryotypes in the left arm of the second chromosome.* The important facts to be noted are as follows: First, inversions in *D. robusta* are highly polymorphic, each inversion having three to four alleles in some of the populations. Second, marked genetic differentiation is observed between the 2L3, 2L, and 2L1 gene arrangements. The 2L3 gene arrangement from all seven populations has a low frequency of the allele $Est\text{-}1^{.92}$ and a high frequency of the allele $Est\text{-}1^{1.0}$. The allele *.92* varies from 0–14% and the allele *1.0* has a frequency of 59% or higher in the 2L3 gene arrangement from various populations. The *Est-1* allele frequencies in the 2L3 gene arrangement are similar in the various populations ($\chi^2(18) = 21.8$; $p > .25$).

Allele frequencies in the 2L gene arrangement are almost opposite in direction to those in the 2L3 arrangements. The 2L gene arrangement has high frequency of the allele *.92* = 71% or higher and low frequency of the allele *1.0*. The frequencies of the alleles *.92* and *1.0* in the 2L gene arrangement from various populations are similar ($\chi^2(4) = 4.46$; $p > .25$). Because the χ^2 heterogeneity tests show no differences in allele frequencies for either the 2L3 or the 2L gene arrangement from various populations we have pooled these data (last column of Table 3) to obtain an overall estimate of allele frequencies in each of these arrangements.

In the populations of Englewood, Philadelphia, and Raleigh, where data for all three gene arrangements, 2L1, 2L, and 2L3 were available, we observe that the 2L1 gene arrangement differs from the 2L3 and the 2L arrangements in frequencies of the alleles *.92* and *1.0*. In all three populations the frequency of the allele *.92* in the 2L1 arrangement is higher than in the 2L3 arrangement but lower than in the 2L arrangement. Conversely, the frequency of $Est\text{-}1^{1.0}$ allele in the 2L1 arrangement is lower than in the 2L3 arrangement but higher than in the 2L arrangement. Table 4 gives the χ^2 values, degrees of freedom and levels of significance of the differences in allele frequencies between different gene arrangements. Most of the comparisons give very highly significant differences.

Third, in contrast to the 2L3 and the 2L gene arrangements which show similar allele frequencies in all populations, the 2L1 gene arrangement shows clinal variation in allele frequencies. The frequency of the allele *.92* increases from 36% in a northern (Englewood) population to 70–78% in southern (South Carolina and Florida) populations. χ^2 heterogeneity test gives highly significant differences in frequencies of alleles *.92* and *1.0* in 2L1 gene arrangements from different populations ($\chi^2(4) = 23.89$; $p < .0001$, Table 3).

TABLE 4

χ^2 tests for associations of Est-1 *alleles with gene arrangements of the left arm of the second chromosome in various populations*

Populations:	Williamstown Massachusetts	Ledgewood New Jersey	Englewood New Jersey	Philadelphia Pennsylvania	Raleigh North Carolina
χ^2 values with degrees of freedom and probabilities	$\chi^2_{(3)} = 34.69$	$\chi^2_{(2)} = 31.21$	$\chi^2_{(2)} = 54.77$†	$\chi^2_{(2)} = 19.84$†	$\chi^2_{(1)} = 3.30$‡
	$p \ll .0001$	$p \ll .0001$	$p \ll .0001$	$p < .0001$	$p > .05$

† Only alleles *.92* and *1.0* were considered.
‡ Only alleles *.92* and *1.0* and gene arrangements 2L and 2L1 were considered.

DISCUSSION

Our results clearly show considerable differentiation in allele frequencies at the *Est-1* locus between different gene arrangements of the left arm of the second chromosome in *D. robusta*. The *Est-1* allele frequencies are similar in all populations in the 2L3 arrangement and in the 2L arrangement; however the 2L1 gene arrangement shows a north-south clinal change in allele frequencies. The north-south allele frequency changes in the 2L1 arrangement from different populations are in the same direction as those observed in different populations in Table 2; i.e., a north-south clinal increase in *Est-1*$^{.92}$ frequency is observed in *D. robusta* populations (Table 2) and also in the 2L1 arrangement (Table 3).

The observed associations of the *Est-1* alleles with the gene arrangements cannot be due to random differentiation of different gene arrangements at the *Est-1* locus. If the associations of alleles with different inversions were due to chance fixation of different neutral alleles in different inversions, then we would expect these differences to disappear because of recombination between the *Est-1* gene and the inversions, since the *Est-1* gene is outside the limits of inversions and recombination can occur between the *Est-1* locus and the inversions. It may, however, be argued that enough time has not yet elapsed for recombination to even out the differences at the *Est-1* locus between different inversions. We would still expect the degree of divergence between different inversions in different populations to be a function of recombination between the *Est-1* gene and the inversions in these populations. In *D. robusta*, CARSON (1953) and LEVITAN (1958b) have shown that recombination is greatly increased in the second chromosome when the XL, XR and 3R chromosomes are heterokaryotypic. In females heterokaryotypic for both arms of the X chromosome and the 3R chromosome, recombination between two arms of the second chromosome may reach almost 50% (LEVITAN 1958b). Table 5 gives the frequency of inversion heterokaryotypes in different chromosome arms in different populations. As can be seen in the last column of Table 5 the extent of inversion heterozygosity in the XL, XR, 2L and 3R chromosomes in an individual is different in different populations; thus the percent recombination between the *Est-1* locus and the different 2L gene arrangements will be of different magnitude in different populations. We observe that the degree of differentiation between the 2L and 2L3 arrangements in different

TABLE 5

Proportion of heterokaryotypes in different chromosome arms in different D. robusta *populations*

	XL	XR	2L	2R	3R	Probability of inversion heterozygosity in XL, XR, 2L and 3R
Williamstown, Mass.	0.0	.215	.476	0.0	0.0	0.0
Rockville, Neb.	.473	.188	.523	.012	.026	0.0012
Ledgewood, N. J.	.38	.448	.649	.04	.18	0.0199
Englewood, N. J.	.506	.15	.622	.02	.077	0.0036
Philadelphia, Pa.	.234	.235	.569	.055	.28	0.0088
Raleigh, N. C.	.416	0.0	.64	.47	.354	0.0

populations is the same regardless of the expected recombination between the *Est-1* gene and the gene arrangements. The *Est-1* associations with the 2L1 arrangement provide further support to the argument that the differentiation of various gene arrangements is not due to fixation of different neutral alleles in different gene arrangements. The 2L1 arrangement differs most from the 2L arrangement in the Englewood, N.J. population; in this population we expect more recombination between the *Est-1* locus and the gene arrangements of the left arm of the second chromosome than in the Myrtle Beach and Astor populations, which have little or no inversion polymorphism. The 2L1 arrangement from Myrtle Beach and Astor populations has about the same allele frequencies as does the 2L arrangement. There is then no relationship between expected recombination of *Est-1* locus with gene arrangements and the extent of differentiation of gene arrangements in different populations. The associations of *Est-1* alleles with gene arrangements of the left arm of the second chromosome must be maintained due to natural selection which favors different allele frequencies in different gene arrangements, presumably because of epistatic interactions between the alleles of the *Est-1* locus and the genes on the left arm of the second chromosome in different gene arrangements. The clinal variation of the *Est-1* locus in the 2L1 gene arrangement can be explained due to adaptation of the 2L1 arrangement to latitudinal variation. In *Drosophila pseudoobscura*, associations of alleles at three different loci—*Pt10*, *Amy*, and *Pt12*—with the third chromosome inversions (PRAKASH and LEWONTIN 1968, 1971) and at two loci—*Est 5* and adult *A-P-6*—with the X chromosome arrangements (PRAKASH and MERRITT 1972) have also been explained due to coadaptation.

Due to the observed differentiation of the 2L, 2L3, and 2L1 gene arrangements in *Est-1* allele frequencies, we expect the heterokaryotypes of these arrangements to be more heterozygous than the homokaryotypes. Table 6 presents the observed proportion of heterozygotes in the homokaryotypes and the heterokaryotypes of these arrangements from different populations. The amount of heterozygosity is highest in the 2L3/2L heterokaryotypes followed by the 2L3/2L1 and the 2L/2L1 heterokaryotypes. Much lower proportion of heterozygotes was observed among the 2L3/2L3, 2L/2L and 2L1/2L1 homokaryotypes than in the heterokaryotypes.

TABLE 6

Observed proportion of heterozygotes at the Est-1 *locus in the homokaryotypes and the heterokaryotypes of 2L arrangements in five of the populations. In parenthesis, the numerator gives the number of heterozygous individuals and the denominator gives the number of total individuals*

Karyotype	State: Massachusetts Locality: Williamstown	Nebraska Rockville	New Jersey Ledgewood	New Jersey Englewood	Pennsylvania Philadelphia	Total
A. Homokaryotypes						
2L3/2L3	.47(9/19)	.27(4/15)	.50(5/10)	.285(2/7)	.33(1/3)	.39(21/54)
2L/2L	.29(2/17)	——	.36(8/22)	.285(16/56)	.30(3/10)	.30(29/95)
2L1/2L1	——	——	——	.567(17/30)	.30(3/10)	.50(20/40)
B. Heterokaryotypes						
2L3/2L	.88(15/17)	.57(8/14)	.77(30/39)	.744(32/43)	.875(7/8)	.76(92/121)
2L3/2L1	——	.70(7/10)	.57(12/21)	.543(19/35)	.670(4/6)	.58(42/72)
2L/2L1	——	——	.45(8/18)	.602(59/98)	.48(13/27)	.56(80/143)

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