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Contribution of recombinants produced by female flies heterozygous for *Est-α* alleles to genic variation of *Drosophila virilis*

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Summary

With 12 alleles at the *Est-α* locus of *Drosophila virilis*, 44 genotypes of females heterozygous for a pair of them were constructed, and about 4×10^4 of their F_1 progenies per genotype were electrophoretically examined. Eighty-three variants different from both of their parental alleles were obtained from a total of $2{\cdot}0 \times 10^6$ progenies. Statistical analysis suggested most of their variants to be recombinants. And no null or inactive allele, of which frequency is several percent in natural populations, appeared in $11{\cdot}6 \times 10^4$ progenies from females heterozygous for active alleles. These results suggest that the high variation of the *Est-α* locus in this species is generated by recombinants from female heterozygotes.

1. Introduction

The number of alleles found at a genetic locus of an organism is affected by various genetic accidents: mutation, selection, genetic random drift, and so on. Some loci of *Drosophila* species contain a large number of alleles (e.g. see Ayala *et al.* 1974; Singh, Lewontin & Felton, 1976; Fukatami, 1977), while the *Adh* locus of *D. melanogaster* shows the presence of many silent nucleotide polymorphisms in exons and introns but no amino acid replacement polymorphism within each electromorph (Kreitman, 1983). The *Est-α* locus of *D. virilis*, as a representative of the former, is highly polymorphic and has high genetic heterozygosity (Tsuno, 1975; Tsuno, Aotsuka & Ohba, 1984). Alleles of this locus, moreover, can produce mutants different from the parental allele types by a putative intragenic recombination in female heterozygotes (Tsuno, 1985), but neither in homozygotes nor in male heterozygotes. So, we shall hereafter refer to these 'mutants' as 'recombinants'. The average recombination frequency is as high as $4{\cdot}5 \times 10^{-5}$ per locus per generation in heterozygous females. If the intragenic recombination frequency in natural populations is higher than the point mutation rate, its effect on allele frequency would be possibly large. Since recombinant types are limited by the genotype of female heterozygotes and their occurrence would be under the Poisson distribution, it is meaningful to examine the relationships among these parameters. Since the molecular weight of the α-esterase is 51000 Da (Sasaki & Narise, 1978), it is very interesting to know the relationship between the recombination frequency and the size of the locus.

Null alleles, which do not show any band of enzyme activity on a gel plate, are involved in some loci of *Drosophila* species, though their gene frequencies in general are very low and explainable by mutation-selection balance (for example, 0·002 in *D. melanogaster* (Voelker *et al.* 1980)). In *D. virilis*, the frequency of the *Est-α* null allele often amounts to several percent in natural populations (Tsuno, 1975). So, two points to be clarified are whether the occurrence frequency of the null allele is high or low and whether the null plays any role in recombinant production.

In this paper, the mode of the appearance of recombinants from female heterozygotes and their contribution to the *Est-α* allelic variation are described in the light of the present as well as previous data.

2. Materials and methods

In accordance with the presence of heterozygosity for polymorphic alleles in flies of natural populations, female flies of *D. virilis* heterozygous for a pair of 12 alleles of the *Est-α* locus, that is, α^0, $\alpha^{0{\cdot}66}$, $\alpha^{0{\cdot}79}$, $\alpha^{0{\cdot}87}$, $\alpha^{0{\cdot}93}$, $\alpha^{0{\cdot}98}$, $\alpha^{1{\cdot}00}$, $\alpha^{1{\cdot}09}$, $\alpha^{1{\cdot}14}$, $\alpha^{1{\cdot}16}$, $\alpha^{1{\cdot}23}$, and $\alpha^{1{\cdot}36}$ were established for the experiment, among which α^0 or null allele had been obtained by Professor S. Ohba of Tokyo Metropolitan University.

Alleles other than the above-mentioned are actually seldom found in natural populations and were discarded from the present experiment since it is

Table 1. *Old and new nomenclature for several alleles at the* Est-α *locus of* Drosophila virilis

Old	New	Old	New	Old	New
α^0	α^0	α^4	$\alpha^{0.93}$	α^7	$\alpha^{1.14}$ / $\alpha^{1.16}$
α^1	$\alpha^{0.66}$	α^5	$\alpha^{0.98}$ / $\alpha^{1.00}$		
α^2	$\alpha^{0.79}$			α^8	$\alpha^{1.23}$
α^3	$\alpha^{0.87}$	α^6	$\alpha^{1.09}$	α^9	$\alpha^{1.36}$

expected that their contribution, due to heterozygous combination with other alleles, to genetic variability of populations, if any, would be practically negligible. In Table 1, the old and a new nomenclature for several alleles of the *Est-α* locus are shown together (see Tsuno (1975) for the allele frequencies in natural populations).

Female flies heterozygous for each of the 12 alleles just mentioned were crossed with *Est-*α^0 males and their progenies were electrophoresed according to the procedure described earlier (Tsuno, 1981, 1985). Since most electrophoreses in my experiments were carried out on homogenates each comprising two progenies in order to accelerate the experimental procedure, the appearance of the null allele could not be detected inevitably in such a case. So, in the experiment for the production of the null recombinant (α^0) or mutant, the two strains *Est-*$\alpha^{1.14}$ *Est-*$\beta^{1.00}$ and *Est-*$\alpha^{1.23}$ *Est-*$\beta^{1.00}$ were used. Their heterozygous females were crossed with *Est-*α^0 males and the progenies were electrophoresed individually. In order to avoid taking a fly with denatured enzymes as a true α^0 one, only the case of appearance of one active β-band and no active α-band on the electrophoregram of a progeny was classified as an occurrence of the null allele. The number of examined progenies was about 4×10^4 flies in each cross, because this number had proved to give a reliable statistical discrimination of recombination frequency in such a study (see Tsuno, 1985). Then additional experiments were carried out for previous crosses that had yielded fewer than 4×10^4 progenies, that is, Cross Nos. F17, F21, and F22 in Tsuno (1985), because of the difficulty in statistical analysis. Unfortunately, some of those previously used strains had been lost, so newly established strains $\alpha^{1.14}$ and $\alpha^{1.16}$, having the same electrophoretic mobility as the previous ones, were tested. The progenies from homozygotes for the null allele (α^0/α^0) were also examined similarly.

3. Results

Genotypes of female heterozygotes examined were as follows: $\alpha^{0.66}/\alpha^{1.16}$, $\alpha^{0.66}/\alpha^{1.09}$, $\alpha^{1.14}/\alpha^{1.16}$, $\alpha^{0.98}/\alpha^{1.00}$, $\alpha^0/\alpha^{1.16}$, $\alpha^0/\alpha^{0.98}$, $\alpha^{0.98}/\alpha^{1.16}$, $\alpha^{1.00}/\alpha^{1.14}$, $\alpha^{0.98}/\alpha^{1.14}$, $\alpha^{0.87}/\alpha^{1.00}$, $\alpha^{0.93}/\alpha^{1.00}$, and $\alpha^{1.14}/\alpha^{1.23}$, from each of which the progenies amounted to about 4×10^4 per cross. In the additional experiments, progenies from female heterozygotes of $\alpha^{1.14}/\alpha^{0.79}$, $\alpha^{1.16}/\alpha^{0.79}$, and $\alpha^{1.16}/\alpha^{1.09}$ numbered 19080, 18000, and 10080, respectively. The genotype of $\alpha^{1.16}/\alpha^{0.79}$, which showed an extremely high occurrence rate of recombinants in the previous test (Cross No. 21 of Tsuno (1982)), gave rise to no recombinants with new electrophoretic mobility among 18000 offspring, while $\alpha^{1.16}/\alpha^{1.09}$ females produced two recombinants of $\alpha^{1.36}$.

As a result of the above crosses, 18 recombinants were detected from among a total of 529410 progenies, 16 of which were already well known to occur in natural populations. The remaining two recombinants $\alpha^{1.18}$ and $\alpha^{1.21}$, however, were quite new ones, first obtained in the present experiment. In the experiments dealing with females heterozygous for alleles showing minor mobility differences, the $\alpha^{0.98}/\alpha^{1.00}$ female genotype, belonging to the 'α^5' group, produced three recombinants among about 40000 progenies; while recombinants were found neither among the progenies of $\alpha^{1.14}/\alpha^{1.16}$ females of the 'α^7' group nor among those of such heterozygous females as $\alpha^{0.98}/\alpha^{1.14}$, $\alpha^{0.98}/\alpha^{1.16}$, $\alpha^{1.00}/\alpha^{1.14}$, and $\alpha^{1.00}/\alpha^{1.16}$ (Cross No. F-11 in Tsuno, 1981). For the null allele (α^0), two genotypes, $\alpha^0/\alpha^{0.98}$ and $\alpha^0/\alpha^{1.16}$, which were comparatively high in frequency in natural populations, were examined. Each of these crosses yielded one recombinant ($\alpha^{1.16}$ or $\alpha^{1.18}$) among about 40000 progenies.

Among 40320 F_1 progenies, i.e. 80640 alleles, from the null homozygous strain, no active alleles were obtained. In order to examine the production of the null allele from females heterozygous for active alleles, offspring from $\alpha^{1.14}/\alpha^{1.23}$ females were examined individually. No recombinant of the null allele, however, appeared among 4×10^4 progenies, though four active recombinants were obtained in the cross. So the present results and the previous one (Cross No. F-15, Tsuno, 1982) indicated no appearance of the null allele among $11{\cdot}6 \times 10^4$ progenies.

Table 2 gives a gross total of the present data and previous ones, showing the relationships between genotypes of parental female heterozygotes and newly produced recombinants. The rightmost column in the table indicates adjusted numbers of recombinants per 4×10^4 progenies in order to examine a fit of the observed frequencies to that expected from the Poisson distribution.

Since I reported earlier the detection of 65 recombinants from among about $1{\cdot}47 \times 10^6$ progenies (Tsuno, 1985), the addition of the present data mean that a total of $2{\cdot}0 \times 10^6$ alleles at the *Est-α* locus of *D. virilis* has been examined, from which 83 recombinants in all were obtained.

Those combinations of alleles given in Table 2 involve almost all genotypes of heterozygotes observed so far in flies from natural populations. The rest of heterozygotes are seldom found in natural populations because of their rare occurrence. The relationships between the occurrence frequencies of recombinants and the heterozygosity of the *Est-α* alleles in natural populations will be described elsewhere. Since the

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Table 2. *Relationships between genotypes of parental females heterozygous for the* Est-α *alleles and the recombinants obtained from them*

Genotypes of heterozygous females	Recombinant																	Total	No. of flies counted (× 1000)	Adjusted number of recombinants per 4 × 10^4 flies
	066	*079*	*083*	*085*	*087*	*093*	*100*	*104*	*109*	*114*	*116*	*118*	*121*	*123*	*127*	*130*	*136*			
066/098	.	1	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	3	41	2·9
109	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
116	.	2	.	.	2	.	1	.	.	.	.	.	.	.	.	.	.	5	40	5·0
136	.	1	.	.	.	2	.	.	2	.	.	.	.	2	.	.	.	7	87	3·2
079/087	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	41	0
093	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	42	0
109	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	2	41	2·0
114	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1	40	1·0
116	.	.	.	.	.	4	2	.	.	.	.	.	.	.	.	.	.	6	40	6·0
123	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	2	42	1·9
136	.	.	.	.	.	1	.	.	.	.	.	.	.	2	.	.	.	3	42	2·9
087/093	.	1	.	.	.	.	3	.	.	.	1	.	.	.	.	.	.	5	43	4·7
098	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
109	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	62	0
114	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
123	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	1	51	0·8
136	.	.	.	.	.	.	1	.	.	1	.	.	.	.	.	.	.	2	41	2·0
093/098	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
100	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	2	40	2·0
109	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	50	1·6
116	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	52	0
123	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	41	1·0
136	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	2	.	4	44	3·6
098/100	.	.	.	.	.	.	.	.	.	.	2	.	1	.	.	.	.	3	40	3·0
109	.	.	1	.	.	.	.	.	.	.	.	.	.	2	.	.	.	3	48	2·5
114	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
116	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
100/114	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
116	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	76	0
123	.	.	.	.	.	.	.	.	1	.	2	.	.	.	.	.	1	4	48	3·3
127	.	.	.	.	.	.	.	1	1	.	2	.	.	.	.	.	.	4	40	4·0
136	.	.	.	.	.	.	.	.	.	.	1	.	.	1	.	.	.	2	41	2·0
109/116	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	3	4	40	4·0
123	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
136	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	4	41	3·9
114/116	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	40	0
123	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	2	4	40	4·0
116/123	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	4	7	73	3·8
136	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	46	0
123/136	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	63	0
000/098	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1	40	1·0
116	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	1	40	1·0
Total	1	6	2	2	4	9	13	1	5	1	11	1	1	14	0	2	10	83	1996	.
Adjusted total	0·8	5·2	1·8	1·8	4·0	7·8	11·4	1·0	3·5	1·0	10·6	1·0	1·0	12·4	0	1·8	8·0	.	.	73·1

sample sizes of progenies counted in each cross were rather variable, the observed frequencies of these recombinants were adjusted by scaling each test to 4×10^4 progenies and are shown in the rightmost column of Table 2. In order to carry out statistical analysis, each adjusted number was rounded off to the nearest whole number and compared with the expected ones of the Poisson distribution (Table 3). With 75 recombinants and mean numbers of 1·70, the χ^2 test showed a highly significant difference between the observed and expected numbers ($\chi^2 = 26{\cdot}182$, D.F. = 4, $P < 0{\cdot}001$). A conspicuous point in the table is in the rank of no recombinants (in the leftmost column); that is, the observed frequency, or 18 crosses, was remarkably greater than the expected one, 18·1 % or 8·0 crosses, and the agreement was not very good in the rest of the table. If only two crosses instead of 18 had yielded no recombinants, however, the observed numbers in the table would fit satisfactorily with the Poisson expected ones ($\chi^2 = 0{\cdot}277$, D.F. = 3, $P > 0{\cdot}95$).

Table 3. *Poisson distribution of recombinant frequencies. All samples were scaled to 40000 progenies (see Table 2) and the number of recombinants then taken to the nearest whole number. Samples (crosses) were grouped according to number of recombinants appearing in them (Mean = 1·70)*

No. of recombinants	No. of samples (crosses)	Poisson number	Expected number (%)
0	18	8·0	18·1
1	5	13·6	31·0
2	6	11·6	26·4
3	6	6·6	15·0
4	6	2·8	6·4
5	2	1·0	2·2
6	1	0·4	0·9
Total	44	44·0	100·0

4. Discussion

1. *Intragenic recombination at the* Est-α locus

A few years ago I proposed that the variation of the *Est-α* locus in *D. virilis* may be produced by putative intragenic recombination within the locus, on the basis of the appearance of 'mutants' in female heterozygotes, but neither in male heterozygotes nor in female homozygotes (Tsuno, 1981). With the experiments revealing 83 'mutants' among about 2×10^6 progenies, it is reasonable at present to think that most of their 'mutants' are recombinants.

In Table 3, the numbers of crosses yielding given numbers of recombinants are compared with those expected according to a Poisson distribution. A highly significant difference ($\chi^2 = 26{\cdot}2$, D.F. = 4, $P < 0{\cdot}001$) was obtained. Most of the difference, however, results from the disparity in the 'no recombinants' category. This would possibly come from both chance and the molecular structure of the locus.

First, since the average recombination frequency between the ranks except for the no recombinant one was somewhere about 3 ($= 75/(44-18)$) per 4×10^4, the 95% confidence limits after Fisher & Yates' table (1963) are from 0·6 to 8·8 per 4×10^4. This would give a much better fit to the actual frequencies of 1–6 recombinants. Meanwhile, the comparison of the observed frequencies with the expected ones of a Poisson distribution shows that recombination could not occur between many of the pairs of alleles tested, perhaps as many as 10 (or possibly the recombination frequencies between these pairs would have been very small). The number of 4×10^4 progenies examined per cross, as a result, might be too small to minimize the overestimation of the number of no recombinant crosses. This might make the number of no recombinant crosses increase.

Second, in order for a new recombinant different from the parental alleles to appear, more than one site of the bases of a DNA coding for charged amino acids must differ between the parental alleles. And yet the difference of a single charged amino acid results in different electrophoretical mobility of proteins on a gel plate. Thus female heterozygotes for alleles different in a single codon coding for such an amino acid cannot produce any new alleles by recombination. Such cases of crosses might be included in the rank of no recombinants. If they are excluded from the data, the present data would satisfactorily fit with the expected numbers. For example, if the number of 'no recombinants' crosses is assumed as only two instead of the 18 observed, the χ^2 value decreases from 26·184 to 0·277.

The genome size of *D. virilis* is twice as large as that of *D. melanogaster*, being about $1{\cdot}5 \times 10^9$ base pairs (bp) (Laird, 1973, Rasch *et al.* 1971, Fristrom & Yund, 1973); and the total length of a haploid chromosome of *D. virilis* is 890 map units (Chino, 1936). So the number of base pairs per map unit is $3{\cdot}7 \times 10^5$. This value is in good agreement with the average frequency of intragenic recombination estimated for *D. melanogaster*, i.e. $5{\cdot}9 \times 10^5$ per map unit (Thuriaux, 1977) or $3{\cdot}7$–$3{\cdot}8 \times 10^5$ per map unit (Lefevre, 1971). The molecular weight of *D. virilis* α-esterase is 51000 Da (Sasaki & Narise, 1978), corresponding to about 425 amino acids, thus, 1275 bp. The *Adh* locus of *D. melanogaster*, which is constructed with four exons and three introns of which three exons (including 765 bp) and two short introns (including 135 bp) are transcribed into m-RNA, is translated into a dimeric ADH protein composed of two subunits each having a molecular weight of 27400 Da (Kreitman, 1983; Thatcher, 1980). If the size of the *Est-α* locus of *D. virilis* is proportional to that of the *Adh* of *D. melanogaster*, it would correspond to about 1700 bp of the DNA region coding for the α-esterase including the introns, i.e. 0·0046 map unit. Since the

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adjusted total number of 176×10^4 progenies (= 44 crosses $\times 4 \times 10^4$ progenies) were examined, roughly 81 recombinants would be expected, a number larger than that observed, 73·1 (see Table 2). The real number of the expected recombinants is possibly more than 81, because the length of introns in eukaryotes is in general longer than that of exons, that is, the *Est-α* of *D. virilis* might have more than 1700 bp calculated on the basis of the *Adh* of *D. melanogaster*. Thus, the conclusion mentioned before is not incompatible also with the expectation from the size of the locus.

In this experimental system, there are two possibilities which might obscure the present conclusion and which cannot be completely ruled out in understanding a total of 83 recombinants. One is the occurrence of point mutations; and the other, the suspicion of contamination of materials. If any, however, these two agents would be expected to be practically negligible. I have examined about $2{\cdot}8 \times 10^5$ genes in flies of double homozygotes for the *Est-α* and the *Est-β* locus and also $2{\cdot}1 \times 10^5$ in males double-heterozygous for these loci (Table 1 in Tsuno 1981 and the present study). Neither mutants nor contaminants were found in any of the experiments. Moreover, nine unique alleles have been discovered (see Table 2). These facts may give an assurance that most of recombinants obtained would not be affected by such disturbing agents.

2. *Null allele of the* Est-α

In natural populations of *Drosophila*, various isozyme loci contain null alleles, though their frequencies are in general low and explainable by mutation-selection balance (Prakash, 1974; Coyne & Felton, 1977; Voelker *et al.* 1980). Frequencies of esterase null alleles, however, are rather variable among species or among polymorphic loci of a species from zero (at the *Est-6* locus of *D. melanogaster*, see Voelker *et al.* 1980) to more than ten percent (at the *Est-α* and *Est-β* locus of *D. lutescens*; see Fukatami, 1977). In *D. virilis*, the frequency of the *Est-α* null allele often amounts to several percent in natural populations (Tsuno, 1975).

The mutation rate and recombination frequency from active alleles into a null allele were examined among $11{\cdot}6 \times 10^4$ progenies and the null allele was not detected. This suggests that the null allele is not produced by recombination between active ones. Possibly the point mutation rate of the *Est-α* null allele is not so different from the average rate of $1{\cdot}03 \times 10^{-5}$ per locus per generation obtained in *D. melanogaster* (Mukai & Cockerham, 1977). Since the null allele, reversely, has the ability to produce some recombinants, it may have a peculiar property hardly found in the other active alleles.

Experiments to test whether the null allele is under any selection forces or not were carried out by Ebitani (1976), who examined fitness components relating to egg-to-adult viability and concluded that homozygotes for the null allele did not differ from homozygotes for active ones in fitness. And segregation tests of the null allele in my laboratory also showed at least no indication that the null allele is under any selection forces (unpublished data).

Electrophoregrams of the null allele show no activity bands, while some enzyme activity was recognized at the origin or as a tailing zone midway down the gel. It probably resulted from esterases that had combined with the membranes or other particles. Thus, the normal fitness of null homozygotes might be supported by these esterases.

The author has demonstrated great variation at the *Est-α* locus of *D. virilis*. The actual mechanism producing it remains to be clarified by analysis at the molecular level.

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