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HETEROSIS AT AN ENZYME LOCUS OF DROSOPHILA: EVIDENCE FROM EXPERIMENTAL POPULATIONS

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SUMMARY

An excess of heterozygotes over Hardy-Weinberg expectations at a protein locus has been observed in both natural and experimental populations of *Drosophila paulistorum*. Data are presented which rule out the possibility of the excess being caused by association of alleles with heterotic karyotypes. A spurious excess of heterozygotes owing to directional selection is also ruled out. Arguments are put forth which makes associative overdominance unlikely. It is likely that heterosis is acting at this locus to maintain the polymorphism.

1. INTRODUCTION

THE question, what mechanisms are acting to maintain genetic polymorphism in populations, remains insufficiently answered. Heterosis (superior fitness of heterozygotes) is thought to be a major factor in the maintenance of genetic variability. One way of detecting heterosis acting at polymorphic loci is to observe excesses of heterozygotes over Hardy-Weinberg expectations. This is not always easy for three reasons. First, sampling must be done after the selection has taken place. Second, if the locus is undergoing directional selection, the observed excess of heterozygotes may be spurious (Wallace, 1958). Third, unless the selection coefficients are quite high, very large samples are needed to detect significant departures from Hardy-Weinberg expectations (Lewontin and Cockerham, 1959).

Even if the above difficulties are overcome, one further doubt arises before heterosis can be invoked. If the locus being studied is linked to an overdominant locus or loci, then the observed excess may not be due to the locus under study but to its association with the overdominant loci. This was first called pseudooverdominance by Lerner (1958) and later associative overdominance by Frydenberg (1963). It has recently been shown that selectively neutral alleles may display linkage disequilibrium with overdominant loci and thus appear to be overdominant themselves (Ohta, 1971). Associative overdominance may also be caused by linkage disequilibrium with recessive lethals.

Richmond and Powell (1970) reported an excess of heterozygotes over Hardy-Weinberg expectations at the tetrazolium oxidase locus (*To*) of *Drosophila paulistorum* (Andean-Brazilian semispecies). This was found in a natural population based on a sample of 106 females. *To* is a sex-linked gene with two alleles, designated *S* and *F*, segregating in this population from Mirassol, Brazil. Tests were made for segregation distortion and association with inversions. No segregation distortion was observed, no one-to-one correlation of alleles with gene arrangements was observed and it seemed unlikely that the locus was in a state of disequilibrium due to continuing

selection. The tentative conclusion was reached that heterosis was probably acting at the locus. Data presented below are a development of the original work of Richmond and Powell. The accompanying paper (Powell, 1974) presents theoretical considerations pertinent to the data presented below.

2. Materials and methods

The 106 single female lines of *D. paulistorum* (Andean-Brazilian semispecies) studied by Richmond and Powell were combined and used to start two cage populations. These were maintained in cages described by Ayala (1968) on Spassky's medium (Spassky, 1943). Periodic samples of adult females were obtained by etherising the population and randomly picking about 100 females. These were then assayed on starch gels for their *To* genotypes. Details of the nature of this protein are given by Brewer (1967) and details of electrophoresis procedure are given by Richmond and Powell (1970).

Lines homozygous for each allele were extracted from the populations for cytological examination of gene arrangements in the X chromosome. Females were extracted from the cages and placed singly into vials and allowed to lay eggs. After one week the females were tested for their *To* genotype. If the female was a homozygote, six of her female progeny were also tested. If all of these were homozygotes then it was assumed the line was homozygous. Six instead of one F_1 was analysed to check for double inseminations; none was detected. A total of 100 independently derived lines was studied for each allele. Several of these lines were studied cytologically and one strain homokaryotypic for the X chromosome was arbitrarily chosen as a standard tester strain. All other strains were crossed to this tester strain and one resulting F_1 female larva was examined for X chromosome karyotype. Thus each strain contributes a sample of one chromosome. This tester strain technique was employed because of the difficulty in distinguishing between homokaryotypes in *D. paulistorum*. The right arm of the X chromosome (XR) was found to have a double inversion which always occurred together, and the XL have sometimes a single inversion.

3. Results

Table 1 gives the results of the sampling of genotypes at the *To* locus. Since the populations appeared to be at equilibrium at this locus, the four samples from each cage can be pooled and treated as a single sample. The overall significance, combining both cages, was calculated by using Fisher's formula, $\chi^2 = -2\Sigma \ln p$, p being the probability of random deviation in each cage.

In every sample from both cages there is a statistically insignificant excess of heterozygotes. When the samples were combined the excess in cage I is nearly significant and in cage II is highly significant. The overall deviation obtained by combining both cages is highly significant.

Table 2 reports the results of the cytological tests for association between the alleles at the *To* locus and gene arrangements in the X chromosome. Obviously there is no significant association between *To* alleles and the gene arrangements. Lakovaara and Saura (1972) report that the *To* locus is in the XR. In this arm the frequency of gene arrangements in each set of lines is nearly identical. The analytical considerations of Powell (1974) indicate

that even if one assumes a large sampling error, the association of the alleles with gene arrangements cannot account for the observed heterozygote excess.

TABLE 1

Number of each genotype at the To *locus in repeated samples in two population cages and* χ^2 *values for deviations from Hardy-Weinberg expectations. Cages were begun with frequency of* F *allele,* f(F), *equal to 0·475. The time is measured in days from starting of cages. All deviations from H-W expectations involve excesses of heterozygotes. Upper figures are for cage I and lower figures cage II*

Time (days)	*F/F*	*F/S*	*S/S*	*f*(*F*)	χ_1^2
111	35	38	8	0·667	0·27
	32	43	6	0·660	3·42
202	34	41	6	0·673	1·54
	38	39	4	0·710	2·28
224	42	44	10	0·667	0·29
	43	43	5	0·709	1·02
284	45	52	7	0·683	2·45
	42	52	10	0·654	1·15
Total cage I observed:	156	175	31	0·673	3·47
expected:	164·0	159·3	38·7		$P \simeq 0{\cdot}06$
Total cage II observed:	155	177	25	0·682	7·34
expected:	166·1	154·8	36·1		$P \simeq 0{\cdot}007$

Combining cage I and cage II to give overall significant differences:
$\chi_2^2 \simeq 16$; $P < 0{\cdot}001$

TABLE 2

Frequency of X chromosome gene arrangements in two sets of strains homozygous for different alleles at the To *locus. ST indicates the "standard" gene arrangement, the gene arrangement in the arbitrarily chosen tester strain. INV indicates a chromosome with an inversion (or double inversion) with respect to ST. XR is the right arm of the X chromosome, XL the left.* To *is probably in the XR*

Homozygous strains	XR		XL	
	ST	INV	ST	INV
F/F	54/100	46/100	76/100	24/100
S/S	50/100	50/100	81/100	19/100

4. DISCUSSION

The results reported here lend support to the conclusion that heterosis is acting at the *To* locus of *D. paulistorum*. The data here rule out two alternative explanations. First, table 1 indicates that in these cages the alleles at this locus were at equilibrium, yet the heterozygote excess was observed. This excludes the possibility of the spurious excess of heterozygotes due to directional selection (Wallace, 1958). Second, the possibility that the enzyme alleles are associated with heterotic gene arrangements to an extent great enough to account for the data is remote.

One alternative explanation still not ruled out is a tight linkage of *To* alleles to a heterotic locus or loci. It is possible, as shown above, to test for associative overdominance due to inclusion in a gene arrangement. However to test for overdominance due to tight linkage to another locus is practically impossible. Nevertheless, arguments can be proffered which indicate that associative overdominance is unlikely.

Ohta and Kimura (1971) have shown that associative overdominance can be an important phenomenon which retards the loss of neutral genetic variability. The neutral alleles may be maintained for several generations due to tight linkage with overdominant loci. After several generations, depending on the tightness of linkage, the neutral alleles will eventually drift to either fixation or elimination. Thus associative overdominance can only account for relatively short-lived polymorphisms. The *To* polymorphism in *D. paulistorum* is very widespread occurring in every studied population of the Andean-Brazilian semispecies, from Colombia to Southern Brazil (Richmond, 1972). It is highly unlikely that all these populations would be experiencing the same transient polymorphism simultaneously. The evidence suggests rather that this is a stable, relatively old polymorphism. There probably has been sufficient time to eliminate associative overdominance due to chance events. Thus, the fact that the heterozygote excess was found in a natural population argues against chance associative overdominance.

This argument does not dismiss the possibility of associative overdominance due to fitness interactions. Lewontin and Kojima (1960) have shown that if there is quite tight linkage and strong fitness interactions between alleles at two loci, linkage disequilibrium may persist. If there are such strong interactions occurring in the present study, it then becomes a semantic question whether heterosis is acting at the *To* locus or a closely linked locus. The *To* heterozygote has a superior fitness for whatever reason.

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