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Founder Effects and Linkage Disequilibria in Experimental Populations of *Drosophila*

(allozymes/polymorphisms)

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ABSTRACT **Six laboratory populations of *Drosophila paulistorum* were examined for changes in gene frequencies at an enzyme locus, *tetrazolium oxidase* (*To*). In some of the populations, the alleles were introduced on over 100 independently derived chromosomes. These populations showed considerable stability in gene frequencies although they were at widely different starting frequencies. Other populations were begun with only a few (about 6) independently derived chromosomes. These populations showed significant and somewhat erratic changes in *To* gene frequencies. The difference in behavior of the two sets of populations was almost certainly caused by linkage effects due to sample size. The implication of these studies in understanding the role of the founder effect in natural populations is briefly discussed.**

L'Hértier and Tessier (1) were the first geneticists to use population cages to maintain large laboratory populations of Drosophila and to follow changes in gene frequencies due to selection. Since then this technique has been used to study selection on a variety of morphological markers, chromosomal polymorphisms and, most recently, biochemical polymorphisms. An important assumption in the studies with single gene markers is that the changes observed are due to selection at the locus being observed and not due to selection at a linked gene or block of genes. One way to partially avoid this complication is to introduce the marker into the laboratory populations on as many independently derived chromosomes as possible. This decreases the probability that the marker will be in linkage disequilibrium with other genes. Ohta and Kimura (2) have shown that the variance in linkage disequilibrium is equal to $1/(n-1)$ where n is the number of chromosomes extracted from a population in linkage equilibrium.

The study presented in this paper illustrates the variable outcomes that occur in laboratory populations that have been started with different numbers of independently derived chromosomes. This study is similar to that of Dobzhansky and Pavlovsky (3) in which they showed the effect of drift on the outcome of changes in gene arrangement frequencies. In the *Discussion* we mention the implications of these results for understanding the genetics of natural populations, especially in relation to the founder effect.

MATERIALS AND METHODS

A collection of *Drosophila paulistorum*, Andean semispecies, from Mirasol near São José do Rio Prêto, Brazil, was made by Dr. J. Gallo on 21 March 1970 and was sent to us in New York. One hundred six isofemale lines (lines started by one inseminated female from nature) were initiated and several enzyme polymorphisms were studied (4). One sex-linked locus, *tetrazolium oxidase* (*To*), was of particular interest because a substantial excess of heterozygotes over Hardy–Weinberg expectations was noted (5). The method used to study the allozymes of *To* is given in Richmond and Powell (5) and Richmond (4), and the biochemical nature of the protein coded by *To* has been studied by Brewer (6). Two electrophoretically detectable alleles exist at this locus in the Mirasol population; these are designated F (fast) and S (slow) to signify their relative electrophoretic mobilities in our procedure. F_1s from the 106 isofemale lines, representing at least 318 X-chromosomes from nature, were combined into a composite population. Two isofemale lines homozygous for the alternative *To* alleles were maintained separately. After about 14 generations, single females were removed from the composite population, allowed to lay eggs, and subjected to electrophoresis. Thirty-six of these reisolated strains were started from mothers homozygous for the S allele; these strains were combined and used to start a population designated MS. Sixty of the reisolated strains were from F/F mothers; these were combined and used to start a population designated MF. Thus, these two populations, MF and MS, were started with more than 100 X-chromosomes each, from a population that had been started with over 300 chromosomes from nature. These populations were maintained at 25°.

Four other populations were begun with the two homozygous isofemale lines from the original collection. Two populations with 80% F allele were started and maintained at 25° and 18°; these are designated IF-25 and IF-18, respectively. Two other populations were begun with 80% of the S allele and maintained at 25° and 18°; these are designated IS-25 and IS-18, respectively. Thus, these populations were begun with six X-chromosomes from nature, or possibly a few more if, as rarely occurs, the female was doubly inseminated.

These populations were maintained on a medium of corn meal–molasses–agar in cages that allow an adult population size of well over 1000 flies. Periodically, samples of adult flies were taken from the cages and the frequencies of alleles at *To* were determined.

RESULTS

Table 1 shows the starting frequencies and subsequent changes in gene frequencies in populations MF and MS. Figure 1*A*

Abbreviation: *To*, *tetrazolium oxidase* locus.

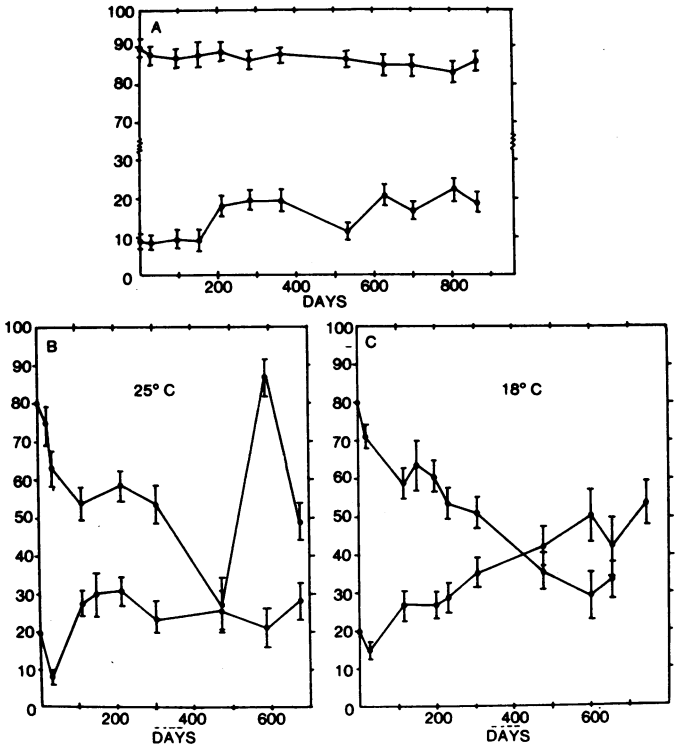

FIG. 1. Frequency of the F allele of *To* in 6 laboratory populations of *D. paulistorum*. (*A*) Data from MF and MS; (*B*) data from IF-25 and IS-25; (*C*) data from IF-18 and IS-18. The *ordinate* is in percent; the *confidence bars* are ±1 SEM.

gives a graphical picture of these data. Except for a shift at about 200 days in MS, there are no significant changes in gene frequencies in either of these populations over 900 days (approximately 45 generations). In these populations the alleles were introduced on many independently derived chromosomes.

In contrast, the populations started by introducing the alleles on only a few chromosomes showed significant and occasionally erratic changes in gene frequencies. Tables 2 and 3 and Fig. 1*B* and *C* give the data. Although the populations at both temperatures appeared, at first, to be approaching equilibrium frequencies, considerable fluctuations are still occurring even after about 700 days. The behavior of the gene frequencies in these populations is in sharp contrast with what occurred in the populations in which the alleles were introduced on many independently derived chromosomes.

TABLE 1. *Changes in To gene frequencies in populations MF and MS of Drosophila paulistorum*

	MF		MS	
Day	Percent F	Sample size	Percent F	Sample size
0	90	204	9	208
31	88	208	8	202
97	87	166	9	148
151	88	100	9	100
207	89	216	18	216
280	86	162	19	216
361	88	216	19	204
536	87	214	12	216
645	86	216	20	216
700	86	216	17	216
807	84	214	22	216
901	86	216	20	216

Sample sizes are the number of genes sampled.

TABLE 2. *Changes in To gene frequencies in populations IF-18 and IS-18 of D. paulistorum*

	IF-18		IS-18	
Days	Percent F	Sample size	Percent F	Sample size
0	80	800	20	800
22–30	71	182	15	226
116	58	144	27	110
150	63	60	—	—
198	60	128	27	134
229	53	126	29	128
302	51	144	35	144
474	35	97	42	96
601	30	54	50	54
656	33	90	43	40
746	—	—	53	72

Sample sizes are the number of genes sampled.

DISCUSSION

The difference in behavior of the gene frequencies in these populations was almost certainly due to linkage effects caused by sampling error. The probability of significant linkage disequilibrium is inversely proportional to the number of independently derived chromosomes used to start a population. Populations MF and MS have a much less chance of being influenced by linkage disequilibria than the other four populations. The former two populations had the alleles introduced on over 100 chromosomes (most of which are presumed to be independently derived), while the latter four populations were started with about six independently derived chromosomes.

It is possible that the linkage effects are due to chromosomal inversions. This population is known to be polymorphic for gene arrangements in both arms of the X-chromosome (7). According to Lakovaara and Saura (8), *To* is in the right arm of the X-chromosome in the closely related sibling species *D. willistoni*. In the large composite population made up of all the isofemale lines, no significant linkage disequilibrium was found between the alleles at *To* and X-chromosome gene arrangements (7). However, if one took a small sample of this population, as was done in starting populations IF-25,

TABLE 3. *Changes in To allele frequencies in populations IF-25 and IS-25 of D. paulistorum*

	IF-25		IS-25	
Day	Percent F	Sample size	Percent F	Sample size
0	80	800	20	800
22–33	68	194	8	200
116	54	144	28	152
150	—	—	30	60
218	58	144	31	138
302	54	110	24	144
478	27	41	26	70
593	87	54	20	54
677	49	94	29	100

Sample sizes are number of genes sampled.

IS-25, IF-18, and IS-18, then an association between alleles at the locus and gene arrangements is not unlikely. One argument against the involvement of the inversion polymorphism is the erratic course of the changes and apparent lack of stable equilibria. In laboratory populations, gene arrangement frequencies usually show consistent changes, with equilibria being attained in 10–15 generations (9).

In the original composite population formed by combining the 106 isofemales lines, the *To* gene frequencies remained stable for 284 days (7). Likewise, the two populations (MF and MS, which were derived from this presumed equilibrium population) showed stable *To* gene frequencies over about 900 days even though their frequencies were considerably different from each other as well as well as different from the original composite "equilibrium" frequency. The frequency of the F allele in the composite population was 0.67. Either no selection or very weak selection was acting to return the gene frequencies to a stable equilibrium. Only when populations were started with a few independently derived chromosomes were there rapid gene frequency changes.

The results of these studies may be important in understanding the genetics of natural populations. The establishment of a new population by a single (or few) individual has long been recognized as an important event in determining the genetic make-up of natural populations (10). This founder effect and subsequent isolation of the new colony may well lead to drastic reorganization of the gene pool or, as Mayr terms it, a genetic revolution. Carson (11) has elaborated on this observation, concluding that the founder effect may precede species formation, especially in insular situations. The experimental situation here mimics in a sense this founding event. Considerably different genetic constitutions can evolve depending on the number of founders that start a population.

The similarity of the results reported here and those of Dobzhansky and Pavlovsky (3) should be noted. They found a considerable variance in the outcome of selection experiments for gene arrangements of *D. pseudoobscura* depending on the number of founders used to start the populations. Besides the obvious difference that they were experimenting with blocks of genes and we are observing a single locus, there is another important difference between these experiments—Dobzhansky and Pavlovsky used interracial hybrids as founders of their populations while our experiments were done with a single population.

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