

NULL ALLELE FREQUENCIES AT ALLOZYME LOCI IN
NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

We have sampled a London population of *Drosophila melanogaster* for null alleles at twenty-five allozyme loci. The same loci and biochemical techniques were used as in our previous survey of a North Carolina population (VOELKER *et al.* 1980). This second survey is completely concordant with the first. No nulls were detected among the five X-linked loci. The mean frequency of nulls at the twenty autosomal loci was 0.0023. Although there is significant interlocus heterogeneity, the two populations appear to have the same frequencies at each locus. This suggests that null alleles at these allozyme loci are in mutation-selection balance, and we estimate the average heterozygous effect of an allozyme null to be 0.0015. Consideration of allozyme null-allele frequencies, the effects of allozyme null alleles on viability and fertility and the generally greater amount of genetic variability at allozyme loci determined by electrophoresis lead us to doubt the validity of generalizing from allozyme data to the whole genome.

WE recently reported the frequencies of null alleles at 25 allozyme loci in a sample of *Drosophila melanogaster* from North Carolina (VOELKER *et al.* 1980). The average frequency of alleles coding for enzymes with reduced *in vitro* activity was 0.0025. There was significant variation among loci in null frequencies. In order to interpret these data, it is necessary to make some assumptions about the sampled population being at or near equilibrium. We reasoned that the simplest hypothesis was that these frequencies reflected mutation-selection balance at the individual loci. Furthermore, we hypothesized that the mutation rates and selective forces might be general to the species over its whole range. Many alternative hypotheses incorporating drift, variable mutation and/or selection would predict that a second sample from another population might have a similar sample mean frequency, but a considerably different distribution over loci. To exclude this simple hypothesis, we surveyed a sample from London and report here the frequencies of null alleles at the same 25 allozyme loci.

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MATERIALS AND METHODS

The details of the genetic and biochemical methods utilized can be found in VOELKER *et al.* (1980) and references therein. The twenty-five allozyme loci (see Table 1 and VOELKER *et al.* 1980) were chosen to meet the following two criteria: (1) sufficient enzyme activity to be scored from the starch gel electrophoresis of a single adult fly, and (2) available electrophoretically distinct alleles (only 1 of 30 appropriate enzyme loci was eliminated by this criterion). Several larval and pupal enzymes were discarded because of the inordinant amount of labor involved in their inclusion. These various allozymes were synthesized into "tester" stocks that were heteroallelic for sets of enzymes that could be scored conveniently from a minimum number of crosses and gels.

Individual wild-caught males were crossed according to the scheme in VOELKER *et al.* (1980). The assay for a null allele was the electrophoretic phenotype of the "wild-caught" allele heterozygous with the two different electromorphs of the "tester" stock. If the "wild-caught" allozyme failed to demonstrate normal activity as a monomer or homodimer, it was designated a putative null. The sample of males from Great Britain (GB) was collected over a two-week period in London in 1978. Another sample of males from Japan (1978) was also surveyed, but only for X-linked loci. Unlike the North Carolina sample (VOELKER *et al.* 1980), the X-chromosome stocks for the GB and Japan samples were maintained in attached-X stocks, and males from these stocks were electrophoresed directly.

Individual nulls were crossed to appropriate chromosome deficiencies and/or made homozygous to determine viability and fertility of hemi- or homozygotes, as well as electrophoretic phenotype. Nulls at the same locus were crossed *inter se* to determine allelism, viability, fertility and the electrophoretic phenotypes of heterozygotes. Salivary gland chromosomes were examined to determine if any aberrations were associated with the nulls. Allelism was demonstrated in every case by the failure of putative nulls for a given enzyme to complement fully (DICKSON *et al.*, in preparation). Evidence for identity by descent (*e.g.*, recovery of the same null more than once) was obtained by comparing electrophoretic phenotypes, associations with alleles at other allozyme loci, associations with inversions, recessive lethals and steriles.

RESULTS

Table 1 lists the numbers of alleles scored (n), numbers of nulls recovered (k) and the mean frequencies of null alleles at the 25 loci in the samples from Great Britain (GB), Japan and North Carolina (NC) (VOELKER *et al.* 1980). These new data confirm our impression from the NC survey that X-linked nulls are indeed rarer than autosomal nulls. The absence of a single null on the X-chromosome is statistically significant, even when the heterogeneity among autosomal loci (see below) is taken into account.

There is (as was observed in the NC sample) considerable variation among autosomal loci in null frequencies. The statistical significance of this heterogeneity is similar when tested in a $2 \times 20 \chi^2$ (χ_1^2 in Table 1) or in goodness-of-fit to a Poisson distribution. In comparing autosomal null frequencies in the two samples, it is striking how similar the distributions are. The means are 2.52 and 2.31×10^{-3} in NC and GB samples, respectively. Furthermore, the comparison of frequencies at the various loci are concordant. The χ^2 attributable to variation in frequencies within loci between the two samples is only 12.70, with 14 degrees of freedom (χ_2^2 in Table 1). The impression of small interpopulation variation in frequencies is further reinforced by the fact that not a single locus demonstrates a deviation close to statistical significance. These results indicate that the frequencies of null alleles at allozyme loci are very similar in populations that are spatially and temporally isolated.

TABLE 1

Null alleles

Autosomal locus	North Carolina			Great Britain			χ_2^2
	<i>n</i>	<i>k</i>	χ_1^2	<i>n</i>	<i>k</i>	χ_1^2	
<i>Got-2</i>	782	3	0.54	357	0	0.83	1.37
<i>Pgk</i>	702	0	1.77	429	0	0.99	—
α <i>Gpdh</i>	814	7	11.97	386	3	4.98	0.02
<i>cMdh</i>	815	2	0.00	408	0	0.95	1.00
<i>Adh</i>	808	1	0.53	362	0	0.84	0.45
<i>Dip-A</i>	767	2	0.00	380	3	5.13	1.64
<i>Pgi</i>	716	1	0.36	436	0	1.01	0.61
<i>Hex-C</i>	796	1	0.51	369	1	0.03	0.31
<i>Idh</i>	916	1	0.74	450	2	0.89	1.55
<i>Est-6</i>	806	0	2.03	418	0	0.97	—
<i>Pgm</i>	913	0	2.31	431	1	0.00	2.12
<i>Est-C</i>	758	4	2.29	408	2	1.19	0.01
<i>Odh</i>	769	1	0.46	404	0	0.94	0.53
<i>Men</i>	734	6	9.34	413	1	0.00	1.56
<i>ry</i>	575	0	1.45	401	0	0.93	—
<i>Aldox</i>	739	9	27.43	413	4	9.73	0.15
<i>mMdh</i>	723	0	1.83	450	0	1.04	—
<i>Ald</i>	912	0	2.30	438	0	1.02	—
<i>Acph-1</i>	799	1	0.51	418	2	1.11	1.39
<i>Tpi</i>	637	0	1.61	426	0	0.99	—
Total	15481	39	67.97*	8197	19	33.54†	12.70‡
Mean frequencies§		2.52×10^{-3}			2.31×10^{-3}		2.45×10^{-3}

X-linked loci

Numbers of alleles scored||

	<i>6Pgd</i>	<i>Fum</i>	<i>Hex-AB</i>	<i>Gpt</i>	<i>Zw</i>
North Carolina	731	740	716	678	666
Great Britain	496	502	497	493	491
Japan	438	421	426	472	442

* $P < 0.001$ with 19 degrees of freedom.† $P < 0.025$ with 14 degrees of freedom.‡ $P < 0.5$ with 14 degrees of freedom.

§ Weighted average null allele frequency at the 20 autosomal loci in the NC and GB samples and in the combined sample.

|| No null alleles were detected among these X-linked loci.

As expected, many of the nulls appear to be identical by descent. The three *Got-2* nulls in the NC sample could not be distinguished on the basis of their allozymic phenotypes, although the null-bearing chromosomes did differ in their *Adh* alleles (two were *Adh^s* and one *Adh^f*). The seven α *Gpdh* nulls from the NC sample could be placed into three groups, based on allozymic phenotypes (four showed weak α *Gpdh¹* activity over a chromosomal deficiency for the α *Gpdh* region; two showed some 2–4 heterodimer activity over α *Gpdh²* and one showed no activity in either case). In the GB sample, the three α *Gpdh* nulls fell into two allelic classes (two showed some 2–4 heterodimer activity when heterozygous with α *Gpdh²*; the third showed weak 2 activity when homo-

or hemizygous). The three *Dip-A* nulls from the GB sample can be divided into two classes (one allele completely inactive, while the other two show weak *Dip-A*² activity). The two *Idh* nulls from the GB sample differ (one shows some heterodimer activity, the other not). The four *Est-C* nulls from the NC sample can be separated into three classes (one completely null, another with marginally detectable activity and two with discernably more, yet still not wild-type, activity). *Est-C* nulls from the GB sample appear to be identical. The six *Men* nulls from NC fall into two classes (all but one with no detectable activity; the exception being a weak *Men*). The nine *Aldox* nulls in the NC sample appear phenotypically identical, although there are five distinct third chromosomes based on other polymorphic allozyme loci. The four *Aldox* nulls from the GB sample show three phenotypes (two are weak *Aldox*⁴, one is a weak *Aldox*⁸ and the fourth shows heterodimer activity characteristic of the *Aldox*⁴ allele). The two *AcpH-1* nulls from the GB sample differ in that one does demonstrate heterodimer activity of an *AcpH-1*⁴ allele, while the other appears completely null. Only one case of association between nulls at a locus and a particular recessive lethal or sterile was found. Two *cMdh* nulls from the NC sample were carried on chromosomes carrying an allelic female sterile mutation.

DISCUSSION

Before discussing possible population genetic implications of these results, it is important to address several technical questions that impinge on the quality of the data. An obvious question is whether the nulls actually occurred in the natural populations or were by-products of some poorly understood, but apparently ubiquitous, phenomena such as "hybrid dysgenesis" (STURTEVANT 1939; SVED 1979; WOODRUFF, SLATKO and THOMPSON 1981). The crosses made to establish the stocks were in fact similar to those thought to elicit increases in mutation rate. Of all the null-bearing stocks, only two showed any evidence of segregating nulls that would be expected if they arose during extraction or maintenance. Although some occurrence of "hybrid dysgenesis" was present, since six unique inversions were found, the lack of X-chromosome nulls and any significant incidence of segregating lines indicate that the recovered nulls reflect the situation in the natural populations sampled.

Although we expect, and suspect, some stockkeeping errors, these should be independent of the null phenotype. The error rate and consequences are sufficiently small to be neglected with respect to the results. All nulls reported were put into stock and retested many times in the further analysis. Some of the mutants have been examined in considerable detail by us and/or others. These studies will be reviewed in our next report.

The fact that the two samples are so similar in distribution suggests that the frequencies are spatially and temporally stable. Our simple hypothesis that the frequencies are determined by mutation-selection balance is clearly not rejected. Further, it would seem that these balances are similar in two different locations and times. Accepting this, we can attempt to estimate the parameters of this balance utilizing independent estimates of null mutation rates (VOELKER, SCHAFFER and MUKAI 1980). Their estimate of 3.86×10^{-6} is based on seven

chromosome 2 loci, six of which are included in our surveys (*Got-2*, *α Gpdh*, *cMdh*, *Adh*, *Dip-A*, and *Hex-C*). If we calculate the selective effects in null heterozygotes (HALDANE 1927) using only those six loci, we obtain an average of 0.0014 ($hs_6 = u_6/q_6$; $u_6 = 4.7 \times 10^{-6}$, $q_6 = 3.25 \times 10^{-3}$). If we calculate this selective disadvantage using the average rate found by VOELKER, SCHAFFER and MUKAI (1980) and our average frequency, 2.4×10^{-3} , *hs* is 0.0016. Since these two estimates do not differ significantly, we can accept an estimate of depression in heterozygote fitness of 0.0015. The lack of nulls at *X*-linked loci can be attributed to the hemizygous effects. Nulls at the *6Pgd* locus are known to be recessive and hemizygous lethals. Nulls at *Zw* are known not to be recessive or hemizygous lethals. The effects at the other *X*-linked loci are unknown. The 95% upper confidence bound on the overall frequency of *X*-linked nulls is 3.7×10^{-4} (or 4.9×10^{-4} excluding *6Pgd*). Thus the difference in null frequencies between autosomal and *X*-linked loci is at least five fold, perhaps even ten fold. This would indicate that the deleterious hemizygous effect of nulls at allozyme loci is greater than 0.015 (or 0.0075 excluding *6Pgd*, assuming the mutation rate cited above). These levels of hemizygous effects are certainly consistent with the general observation of mild homozygous and hemizygous effects of allozyme loci. It is little wonder that there has been so much difficulty measuring any selective differences between active allozymic alleles at some of these loci.

As we pointed out previously (VOELKER *et al.* 1980) the phenotypic effects of these nulls when made homozygous or hemizygous appear less severe than one might expect. Various lines of evidence suggest that 80% of the loci in *Drosophila melanogaster* are recessive-lethal-mutable (ABRAHAMSON *et al.* 1980). In contrast, only one (*Pgi*) of the 14 loci for which we found nulls is either lethal or sterile when homozygous or hemizygous. We can expect that loci with more severe effects will have fewer nulls segregating in natural populations. Even if all six remaining autosomal loci (at which nulls were not found) were recessive-lethal-mutable, the difference is important (two, *Est-6* and *ry*, are known to be null-viable and fertile). Note also that the per-locus frequency of nulls is perhaps ten-fold higher than that of recessive lethals. If we assume 1600 recessive-lethal-mutable loci per chromosome 2 (2000 bands times 80% recessive-lethal-mutable) and accept a proportion of lethal-bearing second chromosomes of less than 40% (MUKAI and YAMAGUCHI 1974), the per-locus frequency of recessive lethals is estimated to be 0.0003. We suggest that allozyme loci are less likely to mutate to recessive lethality or sterility because of the bias in ascertaining them as loci to study. The reason is simply that natural selection is less stringent on these loci (for many possible reasons; thus, electrophoretic variation is more common. Since communication of biochemical genetic techniques is inherently dependent on variation, those techniques for intrinsically variable enzymes were disseminated more rapidly. This interpretation is concordant with surveys of electrophoretic variability using two-dimensional gel electrophoresis (O'FARRELL 1975). This technique selects loci on the basis of protein abundance, as opposed to previously published reports of polymorphism in some populations. The two-dimensional electrophoretic surveys of *D. melano-*

gaster, *D. simulans*, *Mus musculus* and man all indicate less genic variability than allozyme surveys estimated (LEIGH BROWN and LANGLEY 1979; our unpublished results; RACINE and LANGLEY 1980; SMITH, RACINE and LANGLEY 1980, respectively).

It appears that we have a good estimate of the frequencies of null alleles at allozyme loci. Comparison of two populations suggests stable frequencies at each locus determined by mutation-selection balance. Based on the average frequencies (0.00244) and published allozyme null mutation rates, we estimate the average heterozygous deleterious effect to be approximately 0.0015. The lack of nulls at X-linked loci can be attributed to greater hemizygous effects. Despite this consistent picture, the applicability of this picture to the genome as a whole is compromised by evidence suggesting that allozyme loci may not be representative of gene loci as a whole.

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