EFFECT OF YEASTS ON POLYMORPHISM AT THE *Adh* LOCUS IN *DROSOPHILA MELANOGASTER*

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SUMMARY

Thirty-six populations were founded from equal numbers of females and males from ten pairs of isofemale lines of *Drosophila melanogaster* and maintained on standard medium. They were divided into four sets of 9, each set being provided with a different yeast. Six enzyme polymorphisms and the In(3R)P inversion polymorphism were scored. No effect of yeast type was evident except for the *alcohol dehydrogenase* locus. This showed evidence for strong balancing selection in populations maintained with *Metschnikowia pulcherrima*, but not with *Saccharomyces cerevisiae*.

1. INTRODUCTION

YEAST is one of the more important components of the environments of *Drosophilids*. Indeed Starmer (1981) provides evidence that the spectrum of yeast species is a major component determining the distribution of *Drosophila* species. Further, natural populations may grow on a variety of yeasts which differ in their capacity to support the flies. For instance, Ali and El-Helw (1974) demonstrated that larvae of Egyptian populations of *D. melanogaster* and *D. simulans* preferentially chose different yeast species from a mixture of species in their natural habitats. They also showed that the larvae of wild type and a *vg* laboratory strain of *D. melanogaster* differed in yeast preference, and that the relative productivity of females of the two strains also differed with yeast. Preference and productivity were not correlated. A striking feature of their results was that of the yeasts used, species of *Candida* and *Nadsonia* supported their wild type *Drosophila* better than the standard laboratory yeast, *Saccharomyces cerevisiae* even though the larvae never chose *Nadsonia* when offered a choice.

It therefore seemed worthwhile testing whether these particular yeasts differentially affect the maintenance of protein polymorphisms, for most laboratory experiments on polymorphism have been made using *S. cerevisiae* to supplement the medium and other yeasts might give different results.

2. MATERIALS AND METHODS

(i) *Origin of flies*

The populations were founded from the OK lines. These lines were established from 10 females collected together by J. N. Thompson in Oklahoma, the first progeny of each female being split to set up a pair of isofemale lines. The allele and inversion frequencies in these lines are given in table 1.

TABLE 1
Allele and inversion frequencies in the OK lines

Line	α -Gpdh ^F	Adh ^F	Est-6 ^F	Allele Est-C ^F	Odh ^F	Acph-1 ^F	In(2R)OK	Inversion In(2R)NS	In(2L)t	In(3R)P
1a	1.00	1.00	0.75	1.00	1.00	1.00	0.0	0.2	0.0	0.7
1b	1.00	1.00	0.88	1.00	1.00	1.00	0.0	0.0	0.0	0.4
2a	1.00	0.63	1.00	1.00	1.00	0.15	0.0	0.1	0.0	0.0
2b	1.00	0.93	1.00	1.00	1.00	0.94	0.0	0.5	0.0	0.0
3a	1.00	0.00	0.86	1.00	1.00	1.00	0.0	0.6	0.0	0.2
3b	1.00	0.42	0.80	1.00	1.00	1.00	0.2	0.0	0.0	0.7
4a	1.00	0.13	0.00	1.00	1.00	0.63	0.0	0.0	0.0	0.4
4b	0.70	0.72	0.00	0.70	0.73	0.42	0.0	0.0	0.0	0.7
5a	0.92	0.38	1.00	1.00	1.00	1.00	0.0	0.0	0.0	0.4
5b	0.96	0.72	1.00	1.00	1.00	1.00	0.0	0.1	0.2	0.2
6a	0.66	0.35	0.00	1.00	1.00	0.02	0.0	0.0	0.0	0.0
6b	1.00	0.00	0.00	1.00	1.00	0.00	0.0	0.0	0.0	0.0
7a	1.00	0.00	0.00	1.00	1.00	0.00	0.0	0.0	0.0	0.0
7b	1.00	0.62	0.00	1.00	1.00	0.38	0.0	0.0	0.2	0.0
8a	1.00	0.05	0.00	1.00	0.78	1.00	0.0	0.0	0.1	0.5
8b	1.00	0.00	0.00	1.00	0.33	1.00	0.0	0.0	0.4	0.7
9a	1.00	0.00	0.00	1.00	1.00	0.28	0.0	0.0	0.0	0.1
9b	1.00	0.00	0.00	1.00	1.00	0.52	0.0	0.0	0.0	0.1
10a	1.00	0.27	0.00	1.00	1.00	1.00	0.0	0.0	0.0	0.4
10b	1.00	0.27	0.25	0.95	0.76	1.00	0.0	0.0	0.0	0.2
Mean:	0.96	0.38	0.38	0.99	0.93	0.67	0.01	0.07	0.05	0.29

Notes: (1) There are two alleles (fast and slow) at each polymorphic locus with the exception of Est-C, where there are three, an ultrafast found exclusively in OK10B and a slow found exclusively in OK4B. (2) Each allele frequency is based on a sample size of at least 15 males and 15 females. (3) Each inversion frequency is based on a sample of ten haploid genomes.

(ii) *Establishment and maintenance of populations*

The experimental lines were established as follows: 10 bottle populations (OKY1-10) were set up with ten of each sex from each of the OK lines. At the next generation each bottle was used to found four bottle populations one on each of the following yeasts, obtained from the National Collection of Yeast Cultures: (Stocks 166 and 471 are synonymous with two of the "*Candida*" species of Ali and El-Helw).

NCYC Stock No.

166	<i>Metschnikowia pulcherrima</i>
378	<i>Nadsonia elongata</i> var. <i>anomala</i>
471	<i>Hansenula fulvescens</i>
1335	<i>Saccharomyces cerevisiae</i>

The experiment started in early 1974, and the populations were maintained by mass transfer thereafter. The populations were maintained at 25°C with transfer every two weeks, except that for a period of one year they were maintained at 18°C with transfer every three weeks. When the populations were returned to 25°C a replicate set was kept at 18°C. (Tests of these replicates gave similar gene frequencies to those from the main lines so that this 18°C period does not seem to have influenced the results.) Oatmeal-molasses-agar medium was used for the first two years. Since then Harris instant medium has been used. Of course, other microorganisms occur in the culture medium as well as the desired species of yeast. Nevertheless, inoculation with the required fresh yeast every two weeks must have ensured that the desired yeast was predominant, or at least frequent, in the culture media. One population, OKY10-166, was lost early in the experiment. Analyses have therefore been restricted to 36 populations, nine supplemented with each type of yeast.

(iii) *Assays*

After approximately 120 generations (April 1979) ten adults of each sex from each population were each assayed for six loci (α -*Gpdh*, *Adh*, *Est-6*, *Est-C*, *Odh* and *Acph-1*). The sampling was repeated after approximately 145 generations (March 1980).

Forty chromosomes from every population were assayed for In(3R)P between late March and early July 1980. Presence or absence of the inversion was ascertained from the progeny of individual F₁ females, one only from any OKY parent, backcrossed to a homozygous *red e* stock (description of mutants in Lindsley and Grell, 1968). In(3R)P almost completely eliminates recombinants between these loci.

3. RESULTS

Allele and inversion frequencies in the OK lines provide one estimate of the initial frequencies in the OKY populations, though it should be borne in mind that the OK lines were sampled over four years after the OKY populations were founded, so that the estimates may not be accurate if some loci in the OK lines changed frequency during that period. However, a comparison of inbreeding coefficients (see Cavalli-Sforza, 1966) gives no

TABLE 2

Genotype totals at the six enzyme loci sampled in the OKY populations. Sex and sampling occasions have been combined as no significant differences were apparent

Population	α -Gpdh			Adh			Est-6			Est-C			Odh			AcpH-1		
	FF	FS	SS	FF	FS	SS	FF	FS	SS	FF	FS	SS	FF	FS	SS	FF	FS	SS
OKY1-166	40	0	0	5	20	15	0	0	40	40	0	0	40	0	0	8	25	7
OKY2-166	35	5	0	14	19	7	17	18	5	40	0	0	40	0	0	1	14	25
OKY3-166	10	18	2	10	22	8	14	17	9	37	3	0	40	0	0	37	3	0
OKY4-166	40	0	0	7	18	15	0	8	32	40	0	0	40	0	0	12	19	9
OKY5-166	40	0	0	8	21	11	13	22	5	40	0	0	39	1	0	13	23	4
OKY6-166	40	0	0	15	19	6	6	15	19	37	3	0	40	0	0	11	22	7
OKY7-166	40	0	0	4	24	12	4	16	20	40	0	0	40	0	0	40	0	0
OKY8-166	40	0	0	12	22	16	0	5	35	40	0	0	40	0	0	9	16	15
OKY9-166	40	0	0	21	16	3	0	0	40	40	0	0	40	0	0	18	14	8
OKY1-379	23	16	1	1	16	23	0	0	40	40	0	0	40	0	0	40	0	0
OKY2-379	40	0	0	30	8	2	0	0	40	18	22	0	31	9	0	12	23	5
OKY3-379	20	19	1	40	0	0	2	15	23	27	9	4	39	1	0	20	19	1
OKY4-379	40	0	0	7	16	17	0	0	40	40	0	0	40	0	0	6	26	8
OKY5-379	40	0	0	3	15	22	8	19	13	40	0	0	40	0	0	29	11	0
OKY6-379	26	13	1	0	0	40	1	20	19	40	0	0	30	10	0	11	20	9
OKY7-379	6	17	17	0	3	37	0	5	35	40	0	0	40	0	0	11	19	10
OKY8-379	40	0	0	16	17	7	6	16	18	40	0	0	40	0	0	20	16	4
OKY9-379	40	0	0	33	7	0	22	17	1	40	0	0	22	14	4	12	21	7

OKY1-471	39	1	0	7	21	12	0	1	39	40	0	40	0	39	1	0
OKY2-471	36	4	0	9	16	15	0	0	40	40	0	40	0	17	18	5
OKY3-471	26	12	2	19	17	4	0	12	28	20	18	2	0	22	17	1
OKY4-471	40	0	0	0	4	36	12	16	12	40	0	0	0	6	22	12
OKY5-471	33	7	0	7	14	19	1	3	36	40	0	0	2	2	24	14
OKY6-471	40	0	0	24	13	3	0	5	35	40	0	0	0	8	25	7
OKY7-471	27	9	4	10	21	9	0	3	37	40	0	0	0	9	28	3
OKY8-471	30	10	0	3	14	23	5	17	18	40	0	0	0	11	22	7
OKY9-471	40	0	0	4	19	17	0	3	37	40	0	0	0	29	9	2
OKY1-1335	40	0	0	10	17	13	0	0	40	40	0	40	0	12	24	4
OKY2-1335	25	10	5	0	7	33	5	9	26	40	0	40	0	19	14	7
OKY3-1335	29	10	1	1	7	32	21	15	4	40	0	0	0	25	15	0
OKY4-1335	40	0	0	40	0	0	1	7	32	40	0	0	0	40	0	0
OKY5-1335	28	11	1	3	19	18	3	17	20	40	0	32	7	22	18	0
OKY6-1335	28	11	1	1	3	36	2	21	17	40	0	33	7	24	12	4
OKY7-1335	22	17	1	15	18	7	0	0	40	40	0	30	10	25	13	2
OKY8-1335	6	20	14	0	10	30	0	14	26	40	0	40	0	19	16	5
OKY9-1335	20	17	3	0	4	36	5	18	17	34	6	0	0	31	8	1

indication that selection affected the loci differentially in these OK isofemale lines. The genotype frequencies for the experimental lines are listed in table 2 and the gene frequencies, mean gene frequencies, σ_p^2 and F computed from these are given in table 3.

TABLE 3

Allele frequencies at the six enzyme loci and In(3R)P frequency in the OKY populations. Sexes and sampling occasions have been combined

Population	α -Gpdh ^F	Adh ^F	Est-6 ^F	Est-C ^F	Odh ^F	Acph-1 ^F	In(3R)P
OKY1-166	1.0000	0.3750	0.0000	1.0000	1.0000	0.5125	0.150
OKY2-166	0.9375	0.5875	0.6500	1.0000	1.0000	0.2000	0.150
OKY3-166	0.4750	0.5250	0.5625	0.9625	1.0000	0.9625	0.175
OKY4-166	1.0000	0.4000	0.1000	1.0000	1.0000	0.5375	0.675
OKY5-166	1.0000	0.4625	0.6000	1.0000	0.9875	0.6125	0.650
OKY6-166	1.0000	0.6125	0.3375	0.9625	1.0000	0.5500	0.700
OKY7-166	1.0000	0.4000	0.3000	1.0000	1.0000	1.0000	0.650
OKY8-166	1.0000	0.5750	0.0625	1.0000	1.0000	0.4250	0.225
OKY9-166	1.0000	0.7250	0.0000	1.0000	1.0000	0.6250	0.475
$\bar{p}$	0.9347	0.5181	0.2903	0.9917	0.9986	0.6028	0.4278
σ_p^2	0.0268	0.0124	0.0620	0.0002	0.0000	0.0552	0.0551
F	0.4391	0.0497	0.3009	0.0243	—	0.2305	0.2251
OKY1-379	0.7750	0.2250	0.0000	1.0000	1.0000	1.0000	1.000
OKY2-379	1.0000	0.8500	0.0000	0.7250	0.8875	0.5875	0.500
OKY3-379	0.7375	1.0000	0.2375	0.7875	0.9875	0.7375	0.700
OKY4-379	1.0000	0.3750	0.0000	1.0000	1.0000	0.4750	0.575
OKY5-379	1.0000	0.2625	0.4375	1.0000	0.7875	0.8625	0.225
OKY6-379	0.8125	0.0000	0.2750	1.0000	0.8750	0.5250	0.125
OKY7-379	0.3625	0.0375	0.0625	1.0000	1.0000	0.5125	0.400
OKY8-379	1.0000	0.6125	0.3500	1.0000	1.0000	0.7000	0.500
OKY9-379	1.0000	0.9125	0.7625	1.0000	0.7000	0.5625	0.500
$\bar{p}$	0.8542	0.4750	0.2361	0.9458	0.9153	0.6625	0.5028
σ_p^2	0.0411	0.1290	0.0588	0.0105	0.0110	0.0281	0.0580
F	0.3300	0.5173	0.3260	0.2048	0.1419	0.1257	0.2320
OKY1-471	0.9875	0.4375	0.0125	1.0000	1.0000	0.9875	1.000
OKY2-471	0.9500	0.4250	0.0000	1.0000	1.0000	0.6500	0.300
OKY3-471	0.8000	0.6875	0.1500	0.7250	1.0000	0.7625	0.500
OKY4-471	1.0000	0.0500	0.5000	1.0000	0.9750	0.4250	0.275
OKY5-471	0.9125	0.3500	0.0625	1.0000	1.0000	0.3500	0.550
OKY6-471	1.0000	0.7625	0.0625	1.0000	1.0000	0.5125	0.675
OKY7-471	0.7875	0.5125	0.0375	1.0000	0.9125	0.5750	0.500
OKY8-471	0.8750	0.2500	0.3375	1.0000	0.7625	0.5500	0.075
OKY9-471	1.0000	0.3375	0.0375	1.0000	1.0000	0.8375	0.550
$\bar{p}$	0.9236	0.4236	0.1333	0.9694	0.9611	0.6275	0.4917
σ_p^2	0.0065	0.0417	0.0264	0.0075	0.0057	0.0369	0.0618
F	0.0921	0.1708	0.2285	0.2528	0.1525	0.1579	0.2473
OKY1-1355	1.0000	0.4625	0.0000	1.0000	1.0000	0.6000	0.600
OKY2-1355	0.7500	0.0875	0.2375	1.0000	1.0000	0.6500	0.300
OKY3-1355	0.8500	0.1125	0.7125	1.0000	0.9625	0.8125	0.700
OKY4-1355	1.0000	1.0000	0.1125	1.0000	1.0000	1.0000	0.675
OKY5-1355	0.8375	0.3125	0.2875	1.0000	0.8875	0.7750	0.350
OKY6-1355	0.8375	0.0625	0.3125	1.0000	0.9125	0.7500	0.275
OKY7-1355	0.7625	0.6000	0.0000	1.0000	0.8750	0.7875	0.225
OKY8-1355	0.4000	0.1250	0.1750	1.0000	1.0000	0.6750	0.275
OKY9-1355	0.7125	0.0500	0.3500	0.9250	1.0000	0.8750	0.400
$\bar{p}$	0.7944	0.3125	0.2431	0.9917	0.9597	0.7694	0.4222
σ_p^2	0.0285	0.0928	0.0421	0.0006	0.0025	0.0132	0.0306
F	0.1745	0.4319	0.2288	0.0729	0.0646	0.0744	0.1254

FIG. 1.—Genotype frequencies at the (a) *Adh*, (b) *Est-6* and (c) *Acph-1* loci in OKY populations.

FIG. 1.—continued

The *Adh*, *Est-6* and *Acph-1* loci each started with two alleles at appreciable frequencies and so provide more information than the other loci. The data from table 2 for these loci have accordingly been plotted on de Finetti diagrams (Cannings and Edwards, 1968). The pattern of variation at the *Adh* locus among populations maintained with 166 yeast is strikingly different from the *Adh* pattern on other yeasts, and from the *Est-6* and *Acph-1* patterns (fig. 1). These 166 populations cluster around the (0.25, 0.50, 0.25) point for *Adh*, reflecting gene frequencies close to 0.5 and a low variance of genotype and allele frequencies.

To determine whether the variances of genotype frequencies differ, a χ^2 may be calculated for the nine populations maintained on medium supplemented with any yeast, thus measuring the heterogeneity of genotype frequencies at any locus. These χ^2 values, calculated by combining the two least frequent genotypic classes if necessary, and the associated mean squares are presented in table 4. The mean squares for *Adh* genotypes among populations maintained on medium supplemented with the four yeasts are compared in table 5. The variance among populations maintained on 166 is significantly lower than that of populations maintained on medium supplemented with the other yeasts. The variance for populations maintained on 471 is significantly lower than that of those maintained on medium supplemented with 379 or 1335, confirming the impression given by fig. 1 that the pattern of variation among the 471 populations is intermediate

TABLE 4

χ^2 values measuring the heterogeneity of genotype numbers for all possible combinations of locus and yeast

Locus	Yeast	df	χ^2	M.S.
<i>α-Gpdh</i>	166	—	—	—
	379	8	171.6295	21.4537
	471	8	56.1769	7.0221
	1335	8	95.9884	11.9986
<i>Adh</i>	166	16	40.0228	2.5014
	379	16	299.2229	18.7014
	471	16	120.0871	7.5054
	1335	16	285.5238	17.8452
<i>Est-6</i>	166	16	184.5966	11.5373
	379	8	170.8857	21.3607
	471	8	117.2504	14.6563
	1335	8	115.6992	14.4624
<i>Acph-1</i>	166	16	182.7843	11.4240
	379	16	102.9435	6.4340
	471	16	138.5260	8.6579
	1335	8	53.0879	6.6360

TABLE 5

Variance ratios comparing the heterogeneity of genotype numbers at the *Adh* locus among populations maintained on the four types of yeast. Degrees of freedom are given in table 4

		larger mean square		
		471	1335	379
smaller	166	3.0005*	7.1340***	7.4763***
mean	471	—	2.3776*	2.4917*
square	1335	—	—	1.0480 ^{n.s.}

* $0.05 > P > 0.01$.

** $0.01 > P > 0.001$.

*** $P < 0.001$.

n.s. not significant.

between that for the 166 populations and those for the 379 and 1335 populations. The mean squares for loci among populations maintained on medium supplemented with 166 yeast have been compared in table 6. The variance of genotype numbers at the *Adh* locus is significantly lower than that of those of the *Est-6* or *Acph-1* loci. Moreover, the variance of genotype numbers at the *Adh* locus among populations maintained on

TABLE 6

Variance ratios comparing the heterogeneity of genotype numbers at three loci among populations maintained on medium supplemented with 166 yeast. Degrees of freedom are given in table 4

		larger mean square	
		<i>Acph-1</i>	<i>Est-6</i>
Smaller	<i>Adh</i>	4.5670**	4.6123**
mean			
square	<i>Acph-1</i>		1.0099 ^{n.s.}

** $0.01 > P > 0.001$.

n.s. not significant.

medium supplemented with 166 yeast is significantly lower than any other combination of yeast and locus. The *Adh* locus has clearly been less affected by inbreeding in the 166 treated populations than it has been in populations maintained on medium supplemented with other yeasts, and also than have other loci in the 166 treated populations. This can only be the result of some kind of selection, and since the mean *Adh*^F frequencies are similar among populations maintained on medium supplemented with each of the yeasts, it must be some form of balancing selection. The variance provides no evidence whether such selection affected other loci or affected *Adh* in populations on the yeasts 379 or 1335; the alleles could be effectively neutral in these conditions or, if they were affected by balancing selection, it must have been much weaker than that affecting *Adh* on 166 (and 471).

In addition to this balancing selection there is a suggestion that *Est-6* has been affected by directional selection, for the *Est-6*^F frequency is lower in populations maintained on all four yeasts than the initial frequency estimated from the OK lines. There are no striking differences at the α -*Gpdh*, *Est-C* and *Odh* loci, but with allele frequencies initially so close to fixation the evidence is weak.

The estimates of In(3R)P frequency among populations maintained on medium supplemented with each of the four yeasts are very similar, and higher than the initial frequency estimate from the OK lines, which estimate is based on examination of only ten chromosomes in each OK line. However, the calculated variances of inversion frequency in the OKY lines do not suggest that inbreeding has affected the inversion less than it has affected allozyme polymorphisms, other than the *Adh* polymorphism on 166. The balancing selection for *Adh* on yeast 166 must have been stronger than any there was affecting inversion 3R Payne. We had expected that this inversion, which is so common in natural populations, might have given lower inbreeding coefficients than the allozyme polymorphisms. That it has not, could be regarded as evidence that *Est-6* and *Acph-1* as well as *Adh* on 379 and 1335 are also affected by balancing selection.

4. POSSIBLE NATURE OF BALANCE SELECTION FOR *ADH*

The low between-population variance of *Adh* frequency on 166 could result either from heterozygous advantage or frequency-dependent selection or a combination of the two. Although five populations are above the Hardy-Weinberg parabola in the Finetti diagram (fig. 1) indicating an excess of heterozygotes, none of the eight possible χ^2 tests of goodness of fit with Hardy-Weinberg genotype frequencies are significant. An increased sample of 150 individuals was assayed from OKY5-166, but no excess of heterozygotes was detected. If heterozygous advantage were the mechanism responsible for, or contributing to, the maintenance of the polymorphism, selection must be too weak to detect in this way, at least at the gene frequencies in the lines.

The experimental design of Kojima and Tobari (1969) was used to test for frequency-dependent selection. Two populations descended from a common founder, OKY5-166 and OKY5-1335 were chosen, and ten homozygous lines, five of each of *Adh*^{FF} and *Adh*^{SS}, were extracted from each population by pair-mating males and virgin females. To extract *Adh*^{FF} lines from OKY5-1335, two rounds of pair-matings had to be made.

These lines were used to produce mixed progenies at two levels of egg input: the high (H) with 60 *Adh*^{FF}:30 *Adh*^{FS}:10 *Adh*^{SS} females, and the low (L) with 10 *Adh*^{FF}:30 *Adh*^{FS}:60 *Adh*^{SS} females per culture. The heterozygous eggs were laid by equal numbers of females from the two reciprocal crosses. 16 experimental cultures were set up, each population being tested at each input level on each yeast, the whole being replicated twice. While the parents were being collected they were kept on medium supplemented with the yeast that was to be used in the experimental culture, so a population to be tested on a novel yeast could become to some extent free of its routine yeast. The eggs were laid in standard culture bottles supplemented with either 166 or 1335 yeast, the females being allowed to lay for two days. Twelve days later a sample of 200 adults was taken. The results are presented in table 7.

TABLE 7
Results of the frequency-dependent selection experiment

(i) <i>H</i> input						
Population	Yeast	Replicate number	Mortality	<i>Adh</i> ^{FF}	output <i>Adh</i> ^{FS}	<i>Adh</i> ^{SS}
166	166	1	2	114	62	24
166	166	2	0	90	84	26
166	1335	1	1	114	62	24
166	1335	2	2	112	64	24
1335	166	1	8	90	68	42
1335	166	2	5	103	63	34
1335	1335	1	7	126	50	24
1335	1335	2	5	122	59	19
Totals				871	512	217
(ii) <i>L</i> input						
166	166	1	0	19	72	109
166	166	2	5	14	74	112
166	1335	1	0	23	100	77
166	1335	2	2	34	71	95
1335	166	1	6	28	91	81
1335	166	2	3	24	79	97
1335	1335	1	4	23	65	112
1335	1335	2	0	25	72	103
Totals				190	624	786

The ratio of output of adults of each genotype to its expected input in each population on each yeast, combining replicates, is shown in fig. 2. The results of a variance test of χ^2 values, for each input level, are given in table 8. The major source of heterogeneity at the high input is associated with the different yeasts. The output from cultures supplemented with 1335 yeast is close to the expected input, but for the cultures supplemented with 166 yeast there is a clear excess of the rarer classes.

When *Adh*^F is at the low input level, the between-yeasts source of variation is not significant but there is a significant flies $\times$ yeast item, indicating that the populations have responded differently to the two yeasts. The 166 population shows evidence of frequency-dependent selection when tested on 1335 yeast but not on 166 yeast, whereas the reverse is true for the 1335 population (fig. 2). There appears to be an excess of heterozygotes

FIG. 2.—Results of the test for frequency-dependent selection at the *Adh* locus in OKY5 at egg inputs of (a) 60FF:30FS:10SS, and (b) 10FF:30FS:60SS.

TABLE 8
Analysis of χ^2 for the Adh frequency-dependent experiment results

(i) <i>H</i>					
Source of variation	df	χ^2	MS	F	P
Populations	2	4.17	2.09	1.46	>0.05n.s.
Yeasts	2	15.90	7.95	5.56	0.05-0.01*
Populations $\times$ Yeasts	2	4.14	2.07	1.45	>0.05n.s.
Replicates	8	11.41	1.43		
Total	14	35.62			
(ii) <i>L</i>					
Populations	2	0.69	0.35	<1	>0.05n.s.
Yeasts	2	2.39	1.20	<1	>0.05n.s.
Populations $\times$ Yeasts	2	18.93	9.47	5.85	0.05-0.01*
Replicates	8	12.97	1.62		
Total	14	34.98			

at this input level. The results suggest that frequency-dependent selection may be important at high *Adh*^F frequencies, whereas at low *Adh*^F frequencies heterozygous advantage is more important.

5. DISCUSSION

Evidence for the selective effects of yeast on protein polymorphisms has been rather indirect. Powell (1971) and McDonald and Ayala (1974) found that cage populations provided with a variety of environmental factors, including yeasts, maintained a higher degree of heterozygosity than cage populations in more uniform environments. However, the design and analysis of these experiments did not resolve the specific selective mechanisms responsible for the observed correlations. In a more thoroughly designed population cage experiment, Oakeshott (1979) found that various yeasts differed in their selective effects on two loci, *Amy* and *Idh-NADP* in *D. melanogaster*.

The present results show that the maintenance of the *Adh* polymorphism is affected by yeast. The results do not provide evidence that selection is not contributing to the maintenance of the *Adh* polymorphism on other yeasts, nor to that of the other enzyme and inversion polymorphisms. But selection must be invoked to explain the results obtained for the *Adh* locus in populations maintained on medium supplemented with the yeast *Metschnikowia pulcherrima*.

We cannot of course be sure that the selection we have demonstrated acts on the *Adh* locus itself, but three lines of evidence suggest that the *Adh* locus may well be the target for selection: (a) ten genomes from OKY5-166 were free of inversions so there is no evidence that selection via In(2L)t can explain the results; (b) the experiment had run for 120 generations before the first assays so any initial disequilibria should have decayed to a considerable degree; (c) there appear to be selective effects at the *Adh* locus detectable in one generation in a population previously exposed to 1335 yeast when tested on 166 yeast (table 8).

We are aware that the tests for frequency-dependent selection we have done are open to some criticism (see Prout, 1971) and that the fact that the two input frequencies gave quite different results indicates that it would

be unwise to conclude much about relative roles of heterosis and frequency-dependent selection in maintaining both *Adh* alleles on yeast 166.

But our results clearly indicate that conclusions based on experiments using *Saccharomyces cerevisiae* only must be of limited generality. And it should be noted the yeasts used in the experiment are of species which are common in the ecology of an Egyptian natural population (Ali and El-Helw, 1974), and that *Melschnikowia pulcherrima* (166) was one of those yeasts that gave higher productivity than *Saccharomyces cerevisiae* in their experiments.

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