Table I. Number of corpora lutea and embryos in *E. lutescens*.

Number of embryos or corpora lutea per individium	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	lim	*n*
Number of animals with given number of corpora lutea	0	0	0	1	2	2	1	0	0	2	0	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	4–25	17
Number of animals with given number of embryos	0	1	5	4	9	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2–11	24

Materials and methods. In April-May, 1969 we obtained 105 specimens of *E. lutescens* from near the village of Vokhchabert, 15 km N. Erevan, Armenian U.S.S.R. Embryo numbers were counted in 24 females out of 38 adults captured, and the number of corpora lutea in ovaries of 17 pregnant females was also recorded. (Identification of corpora lutea was obtained by examination of histological sections of ovaries).

Results. Table I shows that great differences exist between the number of corpora lutea, indicating ovulation, and the number of embryos. Comparison of data from *E. lutescens* with data on *Microtus oregoni* (Table II) shows that there are great differences in embryonic mortality between these two species. Our data confirm the concept[2,3,7–11] of 50% zygotic mortality in *Ellobius*, due to death of individuals with even number of chromosomes.

The existence of the unpaired sex chromosome in *E. lutescens* is connected with a specific mechanism that reduces the size of litters. However, it is known that all species of *Ellobius* have smaller litters than temperate zone terrestrial microtines. It is not clear which mechanisms regulate the decreased fertility of *Ellobius fuscocapillus* ($2n = 36$)[12] and forms of *Ellobius talpinus* with $2n = 32$[13], $2n = 52$[12,14] and $2n = 54$[12,15]. It would be of interest to compare the embryonic mortality of *E. lutescens* with other species of *Ellobius*, and with other terrestrial microtines[16].

Table II. Embryonic mortality in 17-chromosome *Microtinae*

	Ellobius lutescens[9] (M ± m)	*Microtus oregoni*[5] (M ± m)
Number of corpora lutea	12.9 ± 1.67	3.9 ± 0.22
Number of embryos	4.8 ± 0.38	3.82 ± 0.25

ВЫВОДЫ. Сопоставление числа эмбрионов и желтых тело показало существование у Ellobius lutescens близкой к 50% эмбриональной смертности, что подтверждает гипотезу Уайта–Кастро–Сиерра и Вольфа.

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Selection at the Alcoholdehydrogenase Locus in *Drosophila melanogaster*

A major controversy in population genetics at the moment concerns the great amount of genetic variation at the molecular level revealed by protein electrophoresis in populations of *Drosophila* species and most other animal species thus far studied (review by LEWONTIN[1]). Contrary to the view that natural selection is the principal force maintaining this protein variation is the conception that the observed variation is mainly a product of mutation and genetic drift of selectively neutral variants[2,3]. Up to now most experimental data on this disputed matter came from studies on geographical variation in natural populations. These studies, however, failed to provide

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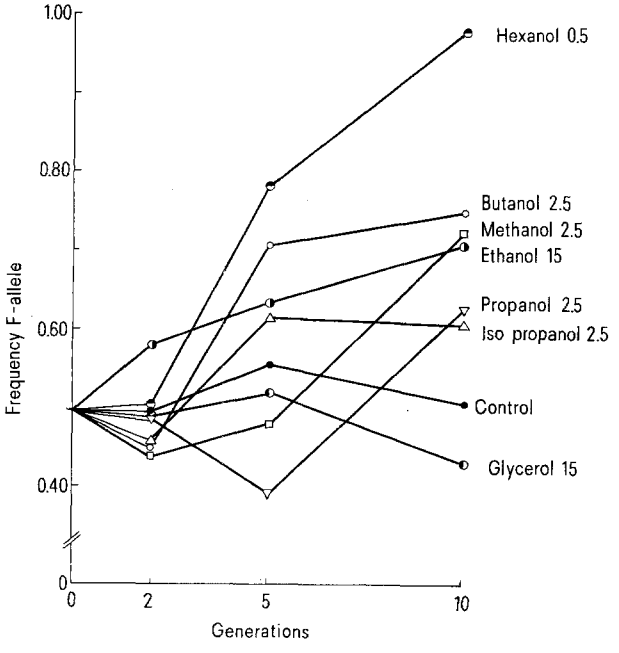
unambiguous evidence concerning the all-or-none existence of selection for allozyme variants, because of the confounding effects of even small amounts of migration[4]. In a recent study[5], however, it was shown that laboratory populations of *Drosophila melanogaster*, polymorphic for the alcoholdehydrogenase locus, when started with different allele frequencies, showed marked frequency changes in the course of time. The frequencies in all populations converged to a common equilibrium frequency irrespective of the initial frequency. It further appeared from this study that the frequency of the Adh^F-allele rapidly increased in populations kept on food supplemented with ethanol. These findings strongly pointed to the conclusion that selection was involved. As definite changes in allele frequencies in the presence of substrates for the enzyme would unambiguously prove the action of selection, it was decided to extend the former experiments by studying the effects of various alcohols, supplemented to the regular food medium.

Material and methods. 8 populations were set up with F_1 flies from crosses between 5 homozygous S- and 5 homozygous F-lines derived from the Groningen population (details in BIJLSMA-MEELES and VAN DELDEN[5]), the initial frequency therefore was $p_F = 0.50$. Each population consisted of 5 bottles with food of a particular composition concerning its alcohol contents. Parents were removed and discarded every generation, prior to the emergence of their offspring. The offspring of all 5 population bottles were thoroughly mixed each generation and thereafter divided in approximately equal numbers over 5 new bottles containing the appropriate food medium, except for a sample of about 150 flies, which were available for genotype identification by electrophoresis. The experiment was continued over 10 generations and populations were kept at 25 °C and 50–70% R.H.

The standard food (control medium) consisted of 1000 ml water; 19 g agar; 54 g sucrose; 32 g dead yeast and 13 ml nipagin solution (10 g nipagin in 100 ml ethanol 96%). The alcohol media consisted of regular food to which a certain volume of alcohol was added. The following alcohols were used (concentrations given as volume percentages): methanol 2.5%; ethanol 15%; propanol 2.5%; isopropanol 2.5%; butanol 2.5%; hexanol 0.5%; and glycerol 15%. Electrophoresis was carried out on whole fly homogenates on 5.5% polyacrylamide gels for 2.5 h at 300 V and 40 mA, in 0.041 *M* veronal -HCl buffer, supplemented with 0.001 *M* EDTA, pH 8.4. After electrophoresis the gels were transferred to a staining solution, consisting of 25 ml 0.041 *M* veronal-HCl/0.001 *M* EDTA buffer, pH 8.4; 7.5 mg MTT; 10.0 mg NAD$^+$; 0.5 mg PMS and 0.1 ml isopropanol. The nomenclature of GRELL et al.[6] was applied for the description of the S- and F-alleles. Alcoholdehydrogenase activity was assayed as follows: centrifuged whole fly homogenate was added to 0.05 *M* *tris*-HCl/0.001 *M* EDTA buffer, pH 8.5, with a final concentration of 0.1 *M* isopropanol and 0.001 *M* NAD$^+$. The increase in absorbance at 334 nm in the reaction mixture was followed in a Vitatron spectrophotometer (1 cm light path; 25 °C). Activity is then given as the initial change in absorbance per mg flyweight per min.



Change in the frequency of the Adh^F-allele on different substrates. The numbers following the alcohols refer to volume percentages.

Results. Genotypic frequencies, generally based on 75 ♀♀ and 75♂♂, were determined in generations 2, 5 and 10. The resulting allele frequencies are shown in the Figure. There is a clear tendency for an increase in the F-frequency on most alcohol media. In generation 10 allele frequencies on all alcohol media, except for glycerol medium, differ significantly from control medium as measured by a χ^2-test ($p < 0.025$ for isopropanol; $p < 0.01$ for the other alcohols). The most drastic change is observed on hexanol medium ($p_F = 0.78$ in generation 5, $p_F = 0.98$ in generation 10). On the alcohol media a consistent shift in genotype frequencies has occurred: the frequency of the SS homozygotes has decreased as the frequency of the FF homozygotes has increased. Comparison of the observed genotype numbers with the expected number based on the assumption of Hardy-Weinberg equilibrium only shows significant deviations for the methanol medium and the isopropanol medium. The actual number of SS homozygotes on methanol medium exceeds the expected number in generation 2, as the number of FF homozygotes is lower than expectation. In generations 5 and 10, however, an excess of heterozygotes and a deficiency of both kinds of homozygotes is observed on this medium. On isopropanol medium, an excess of heterozygotes and a shortage of homozygotes is observed in generations 2 and 10, as the opposite is found in generation 5.

Discussion. Though the addition of the alcohols to the food effected a decrease in population size, the concentrations were purposely chosen in such a way that no drastic reduction in population-size occurred. Consequently the changes in allele frequencies of the observed magnitude, cannot be ascribed to genetic drift. The unidirectional change in allele frequency on alcohol media then strongly points to the action of differential selection for the Adh-genotypes. The SS homozygotes apparently have a disadvantage compared to the FF homozygotes. Recent experiments (VAN DELDEN, unpublished) concerning the survival of Adh-genotypes on ethanol medium showed that both larval and adult survival varied in the following decreasing order: FF > FS > SS. The fitness differences must be considerable; crude estimates based on the observed rate of change, assuming intermediate fitness

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for the heterozygote, provide selection coefficients for the SS genotypes of approximately 0.50 (on hexanol), 0.25 (on butanol) and 0.20 (on ethanol).

In vitro activity assays show that the FF homozygotes of the Groningen population have a higher activity than the SS homozygotes (0.242 versus 0.069). Identical differences have been reported for other strains[7,8], where heterozygote activities were found to be intermediate. It is tempting to relate these differences in activity to the observed fitness differences. The fitness differences between FF and SS genotypes may then be brought about by a better conversion of the alcohols by FF homozygotes. The rise in F-frequency on methanol medium is quite unexpected, as we did not find alcoholdehydrogenase activity using methanol as a substrate. There are indications, however, that the heterozygote has a higher fitness than both homozygotes on methanol medium, leading to a equilibrium frequency of the F-allele around 0.70.

Concerning the ecological relevance of these experimental findings, it should be noticed that decaying fruit is a common feeding and oviposition site for *D. melanogaster* in nature. Yeasts generally flourish well on these fruits and ethanol is produced by fermentation in quantities, which can easily be detected by smell. A great variety of other alcohols are also present in fruits, though in smaller amounts[9].

Concluding, it may be stated that changes in substrate, relevant to the enzyme, induce determinate changes in allele frequencies at the Adh-locus. This proves the occurrence of selection at this locus and is not concordant with the neutrality hypothesis.

Résumé. Des populations de *Drosophila melanogaster*, élevées avec une nourriture contenant différents alcools, montrent une augmentation de la fréquence de l'allèle «fast» du locus alcool déshydrogénase. Ce phénomène est interprété comme étant le résultat d'une sélection et ne correspond pas à l'hypothèse de la neutralité des variants d'isoenzymes.

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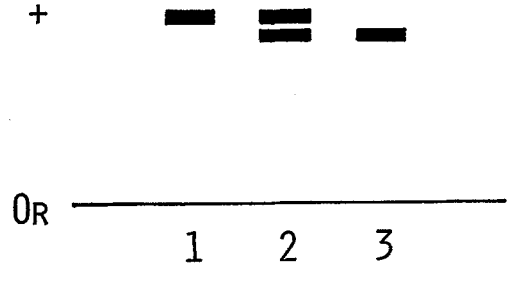
Genetic Control of Erythrocyte Esterase (Es-1) in the Pinon Mouse, *Peromyscus truei* (Shufeldt)

Recent use of techniques in electrophoresis for the study of enzyme polymorphism among vertebrate species has provided a wealth of information in attempting to elucidate the role of genetic variation in the evolutionary process[1]. Although it is accepted practice to consider multiple electrophoretic bands indicative of heterozygosity at a given protein locus, the Mendelian inheritance of most proteins is rarely examined experimentally. This report identifies a pair of codominant alleles segregating from a single autosomal locus controlling the electrophoretic mobility of a hemolysate esterase, Esterase-1. This esterase is commonly investigated by most workers studying vertebrates, especially mammals[1–8]. Furthermore, the species studied, *Peromyscus truei*, is a member of a common genus of North American rodents studied by vertebrate population biologists[1,2,4,7,8].

Materials and methods. The original stock of mice was collected from the vicinity of Canyon, Randall County, Texas, and their laboratory-bred offspring were used in inheritance studies. Blood was obtained from the suborbital canthal sinus, and a 4% sodium citrate solution was used to prevent clotting. Erythrocyte samples were washed 3 times with 5 volumes of buffered saline, lysed with approximately an equal volume of distilled water, and centrifuged at $1000 \times g$ for 10 min.

Starch gels were prepared using a 12% starch concentration and a 0.01 *M tris*-hydrochloric acid buffer (pH 8.5) according to SELANDER et al.[1]. The electrode buffer was 0.3 *M* sodium borate solution (pH 8.2). Horizontal electrophoresis was carried out at 3 °C at 250 V for 1.5 h. After electrophoresis, gels were sliced horizontally and incubated at 37 °C for 2 h in a solution of 25 mg Fast Garnet GBC salt, 24 ml 0.2 *M* monobasic sodium phosphate, 6 ml 0.2 *M* dibasic sodium phosphate, 20 ml distilled water and 1 ml 1% α-napthyl propionate. Alleles were numbered according to their mobilities relative to the fastest migrating allele (100) and calculated as a percentage of the 100 allele.

Results and discussion. Esterase-1 migrates anodally and in front of the hemoglobin on *tris*-hydrochloric acid gels. 3 electrophoretic patterns occur in *P. truei* as follows: a single fast migrating band designated 100/100; 2 bands a faster one designated 100, and a slower one designated 93; a single slow band designated 93/93 (Figure). The single banded patterns were considered homozygous for the 100 or 93 alleles, while the double banded pattern was



Zymogram of erythrocyte esterase-1 phenotypes of *Peromyscus truei*. Slot 1: genotype, 100/100. Slot 2: genotype, 100/93. Slot 3: genotype, 93/93.

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