SELECTION AT THE TRANSFERRIN LOCUS IN CROPPED VOLE POPULATIONS*

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SUMMARY

The transferrin polymorphism was studied during a cycle in numbers of two vole populations, one of *Microtus ochrogaster* and one of *M. pennsylvanicus*. The populations were both on a fenced 2-acre grid and were cropped of adults in an attempt to maintain the populations in a state of increase. The populations maintained a high rate of increase in spite of this cropping, reached high numbers, and declined. The transferrin polymorphism was balanced in both species but equilibrium gene frequencies were not constant. The possibility of directional selection existed. In *M. ochrogaster* the Tf^E allele went to fixation for 9 months during the population increase. The Tf^F allele also occurred in this population. In addition to these two, the *M. pennsylvanicus* population also had the Tf^C allele. The ecological factors of fencing and cropping greatly affected the selection regime and allow only limited comparison with unfenced, unmanipulated populations.

1. INTRODUCTION

THE 3-4 year cycle of abundance of microtine rodents might be caused by genetic changes in these populations (Chitty, 1967), and we are concerned here with testing this hypothesis. We have previously (Krebs, Keller and Tamarin, 1969) presented a demographic description of seven populations of two vole species, *Microtus ochrogaster* and *M. pennsylvanicus* in southern Indiana during a population fluctuation lasting from 1965 to 1967. Also, we have reported systematic changes in gene frequency at the serum transferrin locus correlated with demographic parameters in five of these seven populations (Tamarin and Krebs, 1969). In this paper we report the genetic analysis of the remaining two populations which were subjected to artificial cropping.

2. MATERIALS AND METHODS

The two populations reported here existed sympatrically on an enclosed 2-acre area of grassland in southern Indiana and are referred to as the Grid C populations (Krebs *et al.*, 1969). The populations were live-trapped bi-weekly, individuals were ear-tagged, and the majority of animals were bled by toe-clipping. The populations were periodically cropped of adults.

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Blood plasma samples were run by acrylamide electrophoresis, both vertical (*E-C Bulletin*, 1965) and disc (Ornstein, 1964; Davis, 1964). All analyses were done either on the CDC 3400-3600 computer at Indiana University or the IBM 360 computer at the University of Hawaii. For a more detailed account of the populations and procedures see Krebs *et al.* (1969) and Tamarin and Krebs (1969).

3. RESULTS

During this study, 301 *M. ochrogaster* were trapped of which 228 (75.8 per cent.) were bled, and 634 *M. pennsylvanicus* were trapped of which 446 (70.4 per cent.) were bled. Bleeding did not cause increased mortality in these voles. In the *M. ochrogaster* population, two transferrin alleles were found: Tf^E and Tf^F (Maurer, 1967). In the *M. pennsylvanicus* population, the Tf^C allele was also found. On an electrophoretic gel, the

TABLE 1

Genotypic comparison of voles removed with those not removed. Data summed over the entire cycle for the two populations on Grid C. Probability for M. ochrogaster data was calculated using the Fisher exact probability method (Siegel, 1956). For M. pennsylvanicus, chi-square contingency was used

	Males			Females		
	Removed	Not removed	Prob.	Removed	Not removed	Prob.
<i>M. ochrogaster</i>						
$Tf^E Tf^E$	74	29	—	81	23	—
$Tf^E Tf^F$	13	2	0.13	2	2	0.19
<i>M. pennsylvanicus</i>						
$Tf^C Tf^C$	18	9	—	20	32	—
$Tf^C Tf^E$	62	24	—	61	109	—
$Tf^C Tf^F$	11	10	—	14	25	—
$Tf^E Tf^E$	46	19	—	63	79	—
$Tf^E Tf^F$	13	11	$P > 0.2$	10	28	—
$Tf^F Tf^F$	—	—	—	2	3	$P > 0.3$

two bands produced by the Tf^C allele were the fastest migrating, the Tf^E intermediate, and the Tf^F slowest.

The genotypic randomness of the removal procedure was examined by comparing the genotypes of the removed animals with the genotypes of those animals which were not removed. Table 1 presents this comparison. There were no significant differences found by chi-square comparison, nor were there any significant differences found when the data were broken down into the five periods of the cycle (see fig. 1). Thus we conclude that removing of animals introduced no bias into subsequent analysis of gene or genotypic frequency changes.

Figs. 1 and 2 show changes in population density and transferrin allele frequencies during the course of this study. Both densities and gene frequencies were determined by bi-weekly enumeration of animals known to be alive. As is evident from fig. 1, the Tf^E allele in the *M. ochrogaster* population was maintained at a very high frequency in both sexes, and going to fixation for about 9 months. The three other *M. ochrogaster* populations studied (Tamarin and Krebs, 1969) also had high Tf^E frequencies with occasional fixation in one or the other sex.

The *M. pennsylvanicus* population on Grid C (fig. 2) was unique in that it was the only population of either species which had three alleles present at this locus. The frequency of the Tf^E allele in this population was approximately 50 per cent. as was the Tf^E frequency in the two other *M. pennsylvanicus* populations studied (Tamarin and Krebs, 1969).

FIG. 1.—*M. ochrogaster*, Grid C, population density (upper graph) and Tf^E frequency (lower graph). The four vertical lines divide the study into five periods on the basis of seasonal reproductive changes. The shaded areas are the four winter months (November to February). The histograms represent the numbers of cropped individuals.

(i) *The nature of the polymorphism*

To determine if the polymorphism was balanced (had a stable equilibrium), change in gene frequency, Δp , was regressed on gene frequency, p , for the bi-weekly data. If the polymorphism was balanced, the regression should be negative and should cross the $\Delta p = 0$ line (Li, 1955, fig. 1). We have used this technique to estimate equilibrium gene frequencies previously (see Tamarin and Krebs, 1969, for details).

A negative regression can be obtained under restricted circumstances

FIG. 2.—*M. pennsylvanicus*, Grid C. See legend for fig. 1. $\bigcirc$ — $\bigcirc$ = $\delta\delta$ Tf^E . $\bullet$ — $\bullet$ = ♀♀ Tf^E . $\square$ — $\square$ = $\delta\delta$ Tf^C . $\blacksquare$ — $\blacksquare$ = ♀♀ Tf^C . $\triangle$ — $\triangle$ = $\delta\delta$ Tf^F . $\blacktriangle$ — $\blacktriangle$ = ♀♀ Tf^F .

where the polymorphism is not balanced but is directionally heading towards an equilibrium that may or may not involve fixation. However, in all cases, except during fixation in *M. ochrogaster*, data points existed above and below the $\delta p = 0$ line. This argues in favour of the hypothesis that our calculated equilibrium values are truly equilibrium values and not arbitrary points in a unidirectional change.

Confidence limits were put on the equilibrium Tf values by inverting the regression of Δp on p to make p the dependent variable. These equilibrium gene frequencies and their confidence intervals are shown in fig. 3, which illustrates that equilibrium gene frequencies change significantly during the two years of the study and differ at times in the two sexes. This suggests that fitnesses are not constant. Non-constant fitnesses do not allow us to assume that the polymorphism is maintained by heterosis, nor at this point can we define the mechanism of maintenance. In this analysis we have not attempted to calculate fitnesses from a selection model because of the difficulty in fitting the data to any standard model. Also, there is doubt as to what new information might be obtained (Sokal and Sonleitner, 1968, p. 376).

(ii) *Analysis of gene and genotypic frequencies*

An analysis of observed and expected genotypic frequencies was initially attempted. Due to statistical difficulties of pooling animals of varying lifespan and the inherent difficulties of interpretation (Novitski and Dempster, 1958; Wallace, 1958; Lewontin and Cockerham, 1959), the results were inconclusive and another approach was taken.

In a study of this type where animals have overlapping generations and our sampling includes many of the same animals over several sampling periods, statistical treatment of gene frequency change is almost impossible due to the non-independence of our samples. To obtain statistically random samples, gene frequency was studied in new recruits. To determine if significant changes in the gene frequencies of recruits were occurring, the new genes brought into the populations each period were compared to all genes brought into the populations throughout the whole cycle. These comparisons are presented in table 2.

For the *M. ochrogaster* population, the Tf^F gene is recruited at the beginning and end of the cycle only. No Tf^F recruitment occurred in the middle due to the fixation of the Tf^E allele in the population during this period of time. For the *M. pennsylvanicus* population, in the males, the Tf^C allele is recruited at a higher than expected rate in the beginning and a lower than expected rate at the end of the cycle with the reverse effects found for the Tf^E allele. The Tf^F allele shows little of this effect. The females show the same trend, but with no statistical significance. If the data are treated as a contingency table (3 alleles by 5 time-periods), the males show a significance at the 0.01 level (chi-square, 8 degrees of freedom = 21.05). The females have a non-significant chi-square (8 degrees of freedom = 10.33). The data, however, are homogeneous (heterogeneity chi-square, 8 degrees of freedom = 5.70), and the pooled sexes show significance at the 0.01 level (chi-square, 8 degrees of freedom = 25.69).

Because of these apparent trends in gene frequency for the Tf^C and Tf^E alleles in the *M. pennsylvanicus* male recruits, allele frequency was regressed on time using weighted regression. The regression of Tf^C on time in males was very highly significant with a negative slope. The Tf^E in males had a highly significant positive regression and the Tf^F pooled sexes had a significant positive regression. For Tf^C and Tf^E , the regressions for the pooled sexes were also significant, but these were due primarily to the effects shown in the males. Thus, there is a consistent increase in recruitment of the Tf^E allele in males and consistent decrease in the Tf^C

FIG. 3.—Equilibrium *Tf* frequencies with 95 per cent. confidence intervals *v.* the phase of the cycle for the two populations on Grid C.

TABLE 2

Comparison of observed new genes brought into the populations on Grid C during the five periods with those expected if gene recruitment were occurring randomly throughout the cycle. (That is, expected values are in the ratio of the totals column.) See text for further explanation

	Period										Totals	
	I		II		III		IV		V			
	Summer 1965		Winter 65-66		Summer 1966		Winter 66-67		Summer 1967		Male	Female
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		
<i>M. ochrogaster</i>												
<i>Tf^E</i> Observed	64	61	42	34	86	82	4	12	25	23	221	212
Expected	67.4	60.9	39.3	33.4	80.5	80.5	3.7	13.7	30.0	23.6	—	—
<i>Tf^F</i> Observed	8	1	0	0	0	0	0	2	7	1	15	4
Expected	4.6	1.1	2.7	0.6	5.5	1.5	0.3	0.3	2.0	0.4	—	—
<i>M. pennsylvanicus</i>												
<i>Tf^C</i> Observed	37	37	49	31	53	66	16	17	6	1	161	152
Expected	23.7	28.8	46.7	35.6	58.2	67.8	19.4	15.7	12.9	4.1	—	—
<i>Tf^E</i> Observed	25	39	70	66	89	114	30	24	26	9	240	252
Expected	35.4	47.7	69.6	59.0	86.8	112.4	28.9	26.1	19.3	6.8	—	—
<i>Tf^F</i> Observed	4	8	11	7	20	18	8	5	4	2	47	40
Expected	6.9	7.6	13.6	9.4	17.0	17.8	5.7	4.1	3.8	1.1	—	—
Chi-square	11.71**	3.97	0.62	2.02	1.06	0.07	1.60	0.45	6.07*	3.84	—	—

* = Chi-square goodness-of-fit significant at the 0.05 level.

** = Significant at the 0.01 level.

allele in males throughout the study. The Tf^F allele shows a consistent increase with time.

(iii) *Components of fitness*

Some parameters, that were measurable on individuals and comparable genotypically, were looked at: reproductive differences, body weight distributions, growth rates and survival rates. The results of these analyses were similar to the results of our earlier paper (Tamarin and Krebs, 1969), and are summarised here. Neither survival rates, which were uniformly high, nor adjusted growth rates showed significant differences among genotypes. The only significant differences in breeding condition occurred in the *M. pennsylvanicus* females where the Tf^E/Tf^E types were in breeding condition to a greater than expected extent and the Tf^C/Tf^C females to a lesser than expected extent. Body weight distributions were lastly examined because high body weight is characteristic of peak density populations. Only males were looked at because of the confounding effects of pregnancy. In *M. ochrogaster*, all the heavy weight males were of the Tf^E/Tf^E genotype. In *M. pennsylvanicus*, the Tf^C/Tf^E and Tf^C/Tf^F males had an overabundance in the heavy weight category, and the Tf^C/Tf^C and Tf^E/Tf^F males showed a deficiency in this category.

4. DISCUSSION

In our previous analysis of other populations of these two vole species we have defined an "increase genotype" in both species. This genotype is favoured during the increase and peak phases and is selected against during the decline. This "increase genotype" was defined in unfenced populations. Manipulated and unmanipulated fenced populations showed varying amounts of agreement (Tamarin and Krebs, 1969; Gaines and Krebs, 1971; Gaines, Myers and Krebs, 1971). Fencing introduced some artifacts in this study; cropping of the two populations discussed here has further altered the "natural" ecological situation.

The aim of the cropping experiment was to test the hypothesis of Chitty (1960) that a cropped population would remain in a state of increase. The proper level at which to crop was underestimated and the populations reached typical peak numbers and then declined. They did, however, maintain a very high rate of population increase during the increase and peak phases (Krebs *et al.*, 1969). Thus we may consider the ecological regime similar to the unmanipulated fenced populations but having magnified increase and peak phases.

In the *M. ochrogaster* population, the Tf^E allele was fixed in both sexes for 9 months (mid-Nov. 1965 to mid-Aug. 1966), with continuous breeding throughout. The subsequent reappearance of the Tf^F allele is somewhat unexpected, especially since *M. ochrogaster* has a very high recapture rate (*c.* 94 per cent.). We must assume either that during this period a few Tf^E/Tf^F heterozygotes escaped capture at the periphery of the grid, or that re-introduction must have accounted for the return of the allele, either by migration or mutation. Although the grid was fenced, there were occasional migrants between fenced grids caused by tunnelling under the fence. None, however, were caught in either species on Grid C prior to the re-introduction of the Tf^F allele. We thus presume that heterozygotes

escaping capture at the periphery of the grid were responsible for the re-introduction.

The Tf^E allele went to fixation during the early increase period in both sexes (fig. 1; period I in females, period II in males). We presume a superior fitness of the Tf^E/Tf^E homozygote during this increase phase, and this homozygote is the "increase genotype" we have found in other populations of *M. ochrogaster* (Tamarin and Krebs, 1969). During the decline (period IV), the Tf^F allele increased in the females (Tf^E/Tf^F heterozygote), whereas this did not occur in the males until a partial recovery. Thus, the females agree with our previous finding of the Tf^E/Tf^F heterozygote being favoured during the decline.

The *M. pennsylvanicus* population was unique in that it was the only population studied that had all three transferrin alleles (Tf^C , Tf^E , Tf^F) and the six resulting phenotypes. Although the Tf^F allele (which was not found in other *M. pennsylvanicus* populations) occurred at a relatively low and constant frequency, it apparently had some effects on the fitness relationships of the various phenotypes.

The regression analysis of alleles in new recruits indicated a directional trend in the Tf^C and Tf^E genes in males and Tf^F in both sexes. This is somewhat contradictory to our analysis that indicated there were equilibrium gene frequencies for all three alleles. In light of data points above and below the $\Delta p = 0$ line, our interpretation is that stable equilibria existed that changed from period to period, in some cases in a directional manner. Only a continuation of this study in time, which was not done, could have determined whether fixation would have occurred.

In an earlier paper (Tamarin and Krebs, 1969) we have demonstrated ample room for selection in vole populations although at present we do not know exactly which components of reproduction, mortality or dispersal produce these changes in gene frequencies. On Grid C, the *M. ochrogaster* population had a 55 per cent. loss of young, and the *M. pennsylvanicus* population had a 69 per cent. loss of young before reaching the trappable population (computed from an index of early juvenile survival; Krebs *et al.*, 1969). If selection is acting through differential mortality of these small juveniles, fenced populations may show atypical changes. Differential reproduction may be of increased significance in these populations subjected to heavy cropping, and may explain some of the observed genetic changes.

We conclude that selective effects are measurable in both populations. The ecological regime of fencing and cropping apparently had a major effect on the selection regime. From the genetic standpoint, the *M. ochrogaster* population showed similar effects to the unfenced population of this species whereas the *M. pennsylvanicus* population shows little resemblance.

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