Dynamics of a Transferrin Polymorphism in a Population of *Sylvilagus nuttallii*

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**Summary.** Frequencies of three codominant alleles at the transferrin (*Tf*) locus and four of the six possible genotypes in a population of Nuttall's cottontails *Sylvilagus nuttallii* on an 87-ha study area in central Oregon were determined for parental stocks, trappable offspring, and over-winter survivors in 1974 and 1975. One rare allele disappeared during winter 1974–75 and did not reappear during the study. The *Tf* genotype frequency shifted in favor of *Tf*-BB between parents and offspring in 1974, remained stable over winter, shifted in favor of *Tf*-BC (to near the original frequency) between parents and offspring in 1975 and remained stable over winter. Allele and genotype frequencies were significantly different between 1974 and 1975 offspring; differences in frequencies between other samples were not significant because of the small number of cottontails that survived to spring each year. We were unable to discount the possibility of non-random breeding being responsible for observed differences, but, because survival of juveniles was related to moisture available for plant growth (and presumably to the moisture content in forage) during the cottontail breeding season and because frequencies of *Tf* genotypes of the four litters produced each year seemed related to available moisture, we postulated that precipitation was the selective force responsible for shifts in allele and genotype frequencies. Although we were unable to ascertain the probability that the polymorphism was balanced, the stochastic precipitation pattern seemed adequate to prevent fixation of an allele by selection if the selective mechanism was as postulated.

**Introduction**

This is a report on an investigation of relationships between a polymorphism at the transferrin (*Tf*) locus in a population of Nuttall's cottontails *Sylvilagus nuttallii* and changes in various attributes of that population and in environmental conditions possibly responsible for those changes.

Tamarin and Krebs (1969) and Gaines et al. (1971) suggested that observed changes in the frequency of *Tf* alleles in fluctuating populations of voles *Microtus ochrogaster* and *M. pennsylvanicus*, whose density they considered regulated intrinsically, might be the result of density-dependent selection. Similarly, relationships between frequencies of different colormorphs and stages in the 10-year cycles of red foxes *Vulpes vulpes* were suggested (Calhoun 1950).

Merrell (1965) and Merrell and Rodell (1968) believed that shifts in selective pressures were responsible for temporal fluctuations in the dominant *burnsi* gene in the leopard frog *Rana pipiens*. Gullion and Marshall (1968: p. 157) reported that they had "...demonstrated significant differences in the predominance of one color phase over the other between expanding and declining Ruffed Grouse populations and [had] shown that these changes can be related to density-independent, physical factors." Berry (1978) found seasonal fluctuations in both allele and genotype frequencies at the *Hbb* locus in an insular population of house mice *Mus musculus*. He believed that temperature-regulated survival of the different morphs was responsible for winter to summer fluctuations in the frequency of *Hbb* alleles.

The characteristics of synchronous breeding and immediate postpartum estrus (Powers and Verts 1971), combined with large within- and between-year fluctuations in density and survival rates of various cohorts in response to extrinsic factors (McKay and Verts 1978a) made Nuttall's cottontails uniquely adapted for further elucidation of mechanisms by which seasonal fluctuations in allele and genotype frequencies occur. We wished to determine if seasonal differences in allele and genotype frequencies occurred in populations of Nuttall's cottontails, and, if so, to determine if observed differences were attributable to differential selection among the litter-group cohorts.

**Study Area**

The 87-ha study area 4.9 km west of Terrebonne, Deschutes County, Oregon, established by McKay and Verts (1978a, b) was used for this study. Briefly, the area was an Upper Sonoran, sagebrush-juniper scabland community with numerous lava hummocks. Predominant vegetation consisted of western juniper *Juniperus occidentalis*, big sagebrush *Artemisia tridentata*, rabbit brush *Chrysothamnus* sp., bitterbrush *Protocolea tridentata*, with an understory of cheatgrass *Bromus tectorum*, Idaho fescue *Festuca idahoensis*, squirreltail *Sitanion hystrix*, and Sandberg bluegrass *Poa sandbergii* (McKay and Verts 1978b). The climate is semiarid with precipitation averaging less than 22.9 cm annually (U.S. Department of Commerce 1979).

**Material and Methods**

A 9- × 13-trap grid of 15- × 18.7- × 58-cm wooden box traps set at approximately 90-m intervals was operated 3 days in alternate weeks 1 April–9 June 1975, and 3 days each week 14 June–31 July and daily 1–30 August 1974 and 1975. No bait was used.
Livetrapped cottontails were weighed, measured, and marked, and were assigned to sex and age cohorts (McKay and Verts 1978a). A 1- to 5-ml blood sample was obtained from each cottontail by excising the xylene-dilated medial ear vein and by allowing blood to drip into chilled paraffin-lined tubes. Blood samples were allowed to coagulate, then transported in the field in a vacuum bottle of ice water. Sera were separated after centrifugation and were stored at -20°C until analyzed. Cottontails were released at their respective points of capture.

Serum and plasma proteins were separated by starch-gel electrophoresis. Gels were prepared by heating 10.4 g Electrostarch per 100 ml buffer solution (0.3 M tris(tris(hydroxymethyl)) aminomethane), 0.005 M citric acid, and 1% electrode buffer at pH 8.0 to boiling, degassing with an aspirator, and pouring into 230-x 150-x 10-mm molds to cool (Ridgway et al. 1970). Electrode buffer was 0.06 M LiOH and 0.3 M H2BO3 at pH 8.3.

Pieces of Whatman chromatography MM paper (5 x 15 mm) were loaded with serum, blotted, and inserted into a slit in the gel. A 50-75 ma current at a maximum of 300 v was applied until the ion front migrated 10 cm across the gel, about 3.5-4.5 h. Gels were sliced into 1.6 mm layers; the top layer was discarded, the remainder were destained with a 1:4:5 solution of glacial acetic acid, methanol, and water (for 30 min. The gels were destained with a 1:4:5 solution of glacial acetic acid, methanol, and water until the background faded.

Densities of cottontail populations were estimated at about monthly intervals either by Bailey’s (1952) modification of the Lincoln Index (Petersen, 1896), by a relative-density estimator (McKay and Verts 1978a), or by the frequency of capture estimator (Edwards and Everhardt 1967). Justification for sequential employment of different estimators was provided by McKay and Verts (1978a).

The genotype frequency of the parental stock during the 1974 breeding season was based on adults caught during June-August trapping; we assumed that mortality among adults from onset of the breeding season was proportional among genotypes. In 1975, the genotype frequency of the parental stock was based on over-winter survivors captured in April and May combined with unmarked adults caught during June-August trapping periods. Allele and genotype frequencies of samples between seasons and between years were tested for differences by chi-square analyses; we accepted the p < 0.05 level as indicating significance.

Results

Two hundred ninety-one Nuttall’s cottontails were livetrapped 950 times during the study; sera from 275 were analyzed by electrophoresis. These analyses revealed a polymorphism at the Tf locus similar to those found in S. floridanus and S. transitionalis (Walkowiak 1967). The locus was characterized by three codominant alleles (labeled Tf-A, Tf-B, and Tf-C with relative mobilities of 1.04, 1.00, and 0.95, respectively) that potentially could produce six genotypes (phenotypes). However, we found no individuals with Tf-AA or Tf-AC genotypes among our samples, a result consistent with expectations based on Hardy-Weinberg frequencies. Inheritance of Tf polymorphisms was documented for voles (Maurer 1967; Guiness and Krebs 1971), deer mice Peromyscus maniculatus (Rasmussen and Koehn 1966), and rhesus monkeys Macaca mulatta (Goodman and Wolf 1963); we proceeded by assuming inheritance at the locus occurred in cottontails because we were unaware of reports indicating that Tf polymorphisms failed to follow Mendelian inheritance systems.

Tf Allele Frequencies and Tf Genotype Frequencies

No significant relationship was found between Tf genotype frequencies of Nuttall’s cottontails and their sex among samples collected during the study; consequently, we pooled data for the two sexes for subsequent analyses.

Frequencies of Tf genotypes for trappable progeny were significantly different between years (Fig. 1). In 1974, the Tf-BB genotype increased at the expense of all other genotypes between the parental stock and the trappable progeny they produced, whereas, in 1975, the Tf-BB genotype declined in frequency in favor of the remaining genotypes; the Tf-A allele disappeared during winter 1974–75 and did not reappear during the remainder of the study (Fig. 1). Genotypes of over-wintering survivors were proportional to observed frequencies of populations of trappable offspring; thus, parental stocks were produced with Tf genotype frequencies not significantly different from those in summer populations (Fig. 1). Therefore, during the course of our investigation, the genotype frequencies shifted in favor of Tf-BB, remained stable over winter, then shifted back to near the original frequencies and remained stable over winter.

Changes in Tf-B and Tf-C allele frequencies similar to those in genotype frequencies occurred between parent stocks, trappable offspring, and over-winter survivors each year (Fig. 1).

Significant differences in genotype and allele frequencies were noted only between trappable offspring between years; nevertheless, we believe that differences in the frequencies between parental stocks, trappable offspring, and over-winter survivors each year were real. We determined the genetic composition of a large proportion of the parental stock each spring (Fig. 1); however, differences in genotype and allele frequencies between pa-