SOME GENETIC MARKERS FOR COTTONTAIL RABBITS (*SYLVILAGUS FLORIDANUS*)

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ABSTRACT

Fifteen cottontail rabbit blood protein loci were electrophoretically examined to identify polymorphic loci suitable for utilization as genetic markers. Nine loci (albumin, alkaline phosphatase-1, esterases 6 and 7, alpha and beta hemoglobin, leucine aminopeptidase, transferrin, and lactate dehydrogenase B) were monomorphic. Two polymorphic loci (lactate dehydrogenase A and esterase 1) which exhibited characteristics appropriate for utilization as genetic markers were identified; four other loci (esterases 2-5) were polymorphic but would be less applicable as markers due to their densitometric requirement for Rf determination.

INTRODUCTION

Numerous genetic markers have been electrophoretically identified for many mammalian species, although with a few notable exceptions, e.g., man and white-tailed deer (*Odocoileus virginianus*), most of this work has been conducted on small mammals. There is little or no such information for many other species, such as cottontail rabbits (*Sylvilagus floridanus*; Chapman and Morgan 1973; Walkowiak 1967). The cottontail, a ubiquitous and highly adaptable species, possesses attributes which make it well suited for many types of ecological genetic studies; however, baseline data (e.g., identification of polymorphic loci, and documentation of genetic variation) must be established before large-scale investigations of this nature can be undertaken.

A number of cottontail blood protein loci were electrophoretically examined in the course of an investigation of the population biology of rabbits in southern Illinois. The purpose of this paper is to describe the observed genetic variation, emphasizing the identification of those polymorphic loci which would be most useful as genetic markers in other cottontail research.

MATERIALS AND METHODS

Blood samples were collected from 63 Mearns cottontails (*S. f. mearnsii*; Schanda 1972) captured near Carbondale, Illinois between 18 November 1976 and 13 February 1977. Animals were taken within a 25 ha area exhibiting no marked variation of habitat types; thus, it was assumed that a single population was represented. Serum and hemolysate were prepared (Brewer 1970) and stored at $-25\,^{\circ}C$.

Polyacrylamide disc electrophoresis was utilized to analyze genetic variation at 15 presumptive loci: albumin (*Alb*); liver alkaline phosphatase (*Aph-1*); erythrocyte esterase (*Est-1*); serum esterases, six loci (*Est-2*, *3*, *4*, *5*, *6*, and *7*); hemoglobin, two loci (alpha and beta *Hb*); leucine aminopeptidase (*Lap*); lactate dehydrogenase, two loci (*Ldh-A*, *Ldh-B*); and transferrin (*Tf*). *Est-1* and *Hb* were examined in the hemolysate fraction; serum was used for all others. A tris-borate buffer system (Lee and Kenny 1975; 5 minute 2 ma/gel prerun, 50 minute 4 ma/gel run) was used for all runs except *Lap*, when borate buffer (Gelman 1975; 120 minutes 2 ma/gel) was employed. A stacking gel (Davis 1964) was utilized in combination with a 6.6% acrylamide separation gel for all proteins except *hb*, *Ldh-A* and *B*, and *Lap*, for which a 5.0% separation gel was superior. Staining procedures were those of Gelman (1975), except for *Hb* which required no staining for visualization, and *Aph* where the technique of Lee and Kenny (1975) was used. L-phenylalanine, L-homoarginine, and heat were utilized as inhibitors to attempt to identify the *Aph* loci; nitroso-R (Mueller et al. 1962) was employed to initially identify the *Tf* band.

Some serum esterase, *Ldh*, and *Aph* bands stained too faintly to permit direct Rf (mobility) determination; therefore, all serum esterase gels were scanned on a Gilford 240 spectrophotometer at 600 nm while *Ldh* and *Aph* gels were scanned at 560 nm on a Helena Flur-Vis densitometer. Mobilities were then determined from scanner printouts. In addition, a standard sample of known phenotype was run with each set of gels to aid scoring of phenotypes having bands with similar mobilities.

Presumptive polymorphic loci were first identified by observation of variation of protein bands in codominant fashion. Chi-square goodness of fit tests were subsequently utilized to examine the relationship of observed phenotypic frequencies to those expected by Hardy-Weinberg equilibrium. Nomenclature for loci and alleles was that of Ayala et al. (1972); the 1% criterion (Lewontin 1974) for designation of polymorphic loci was employed.

RESULTS AND DISCUSSION

This population was monomorphic at nine loci (mean Rf and SD in parentheses): *Alb* (0.894 ± 0.009), *Aph-1* (0.370 ± 0.007), *Est-6* (0.359 ± 0.008), *Est-7* (0.314 ± 0.007), *Hb* (both loci; 0.677 ± 0.005), *Lap* (0.538 ± 0.016), *Ldh-B* (origin), and Tf (0.562 ± 0.004). Chapman and Morgan (1973) also observed monomorphic *Tf* in small samples of *S. f. mearnsii* from Missouri and *S. f. chapmani* from Texas (n = 11 and 5, respectively), but noted polymorphism at the same locus in *S. f. alacer*. Walkowiak (1967) found *Tf* polymorphism in Connecticut cottontails; although the subspecies was not specified, distribution maps indicate the probability of *S. f. mallurus* (Hall and Kelson 1959). Further anaylsis of subspecific and geographic variation of *Tf* may support the utility of this locus as a taxonomic marker for some subspecies of the *S. floridanus* group.

A fast *Hb* band ($Rf = 0.749 \pm 0.008$) was separated from stored samples in addition to the primary *Hb* band. Absence of the fast band when fresh hemolysate was run indicated it was a storage artifact; thus, both *Hb* loci were monomorphic and contributed to the production of a single primary *Hb* band through tetramerization.

Two *Ldh* band patterns were observed, one four-banded (n = 61; Rf = 0.632 ± 0.003, 0.520 ± 0.001, 0.411 ± 0.003, 0.297 ± 0.005), and one nine-banded (n = 2; Rf = 0.605, 0.572, 0.522, 0.481, 0.410, 0.363, 0.329, 0.281). Lactate dehydrogenase is a tetramer under the control of two loci; therefore, an individual homozygous at both loci exhibits five bands (Boyer et al. 1963). The four-banded pattern was apparently a homozygote with LDH_5, the tetramer composed entired of *Ldh-B* subunits, remaining at the origin. The nine-banded pattern appeared a consequence of a polymorphic, heterozygous *Ldh-A* locus (Table 1). Although a heterozygote at one locus would produce 15 tetrameric combinations of subunits, overlapping bands and predictably low concentrations of some combinations would prevent visualization of all 15. Absence of a visible band corresponding to the LDH^1 band of the double homozygote indicated the heterozygosity must exist at the *Ldh-A* locus.

The liver *Aph* isozyme was identified by its susceptibility to the inhibitor L-homoarginine. A fast zone of *Aph* activity contained three bands (Rf = 0.632 ± 0.013, 0.598 ± 0.013, 0.569 ± 0.011); but these were not inhibited by L-homoarginine, L-phenylalanine, or heat, so their origin was not identified. Seven phenotypes were observed in this fast *Aph* zone; the bands occurred singly, in all combinations of two, and all together. The latter phenotype indicated involvement of more than one locus and prevented interpretation of the pattern of inheritance. In addition to this problem, these *Aph* bands would probably not be a suitable marker for most purposes due to the need for a scanner for Rf determination when using disc techniques. Slab gel electrophoresis would reduce that need if the pattern of inheritance could be elucidated.

Est-1 was the only erythrocyte esterase locus visualized and was polymorphic with two codominant alleles (Table 1). Each primary band was accompanied by a faster migrating storage artifact when frozen hemolysate was used; however, no ambiguity was caused by the extra bands. There were no significant deviations of observed from expected numbers of individuals of any phenotype for *Est-1* or for any other polymorphic locus (Table 2). Phenotypes of two adult females and their eight progeny (Table 3) from a separate confined population were utilized to examine the hypothesized patterns of inheritance for all polymorphic esterase loci. Although limited in quantity, these data exhibited no inconsistencies between maternal and progeny phenotypes which would indicate an error in any of the hypothesized inheritance patterns (Table 3). In addition, the presence of male and female heterozygotes at each locus indicated these were probably all autosomal loci.

Four presumptive serum esterase loci (*Est-2*, *3*, *4*, and *5*; Table 1) were investigated; six other esterase bands were present but could not be interpreted due to weak staining intensities. *Est-2* and *Est-5* appeared to be simple diallelic codominant loci (Table 2). Two alleles, one of which was null, were also observed at the *Est-3* locus. The three phenotypes seemed to be distinguishable as a result of the dosage effect, with null homozygotes producing no band, 1.00/1.00 homozygotes producing a relatively dark band, and heterozygotes producing a band of intermediate staining intensity. Six phenotypes for *Est-4* were apparently controlled by three codominant alleles (Tables 1 and 2). Some variation in staining intensity was evident in these bands, particularly for the 1.00 allele, although other possible interpretations of this variation, such as the presence of a

null allele(s) and/or involvement of two loci, were unsatisfactory (Yaich 1978). Modifying loci could be responsible for this variation, but could not be proven from the data available. Thus, the initial hypothesis of three alleles at a single locus appears most acceptable at this time.

SUMMARY

The *Est-1* and *Ldh-A* loci would be most useful as genetic markers in population studies, assuming frequencies of each allele were sufficiently high in the population under examination. Phenotypes observed at these loci were readily identified and scoring was unambiguous. The requirement of a densitometer for accurate Rf determination of all serum esterases except *Est-4* (where it would still be desirable if disc techniques were employed) makes these polymorphic loci less appropriate for utilization as genetic markers. Horizontal electrophoresis would reduce the necessity of a densitometer for *Est-2* and *Est-5* evaluation but might not fully eliminate that need; densitometry could not be avoided for interpretation of *Est-3*.

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Table 1. Allele frequencies and electrophoretic mobilities (Rf) of polymorphic blood proteins in a population of cottontail rabbits near Carbondale, Illinois.

Protein	Allele	Frequency	Electrophoretic mobility (Rf)
Ldh-A	1.00	0.984	0.632(0.003)*
		0.016	
Est-1	1.03	0.055	0.827(0.006)†
	1.00	0.945	0.805(0.007)
Est-2	1.04	0.333	0.830(0.008)
	1.00	0.667	0.799(0.005)
Est-3	1.00	0.190	0.771(0.008)
	null	0.810	
Est-4	1.11	0.270	0.740(0.011)
	1.04	0.254	0.694(0.012)
	1.00	0.476	0.666(0.010)
Est-5	1.06	0.246	0.481(0.011)
	1.00	0.754	0.454(0.012)

*Values in parentheses are standard deviations.
†The mobility of the less frequent allele could not be determined due to overlapping bands and predicably low staining intensity of some bands.

Table 2. Observed and expected numbers of rabbits exhibiting each phenotype at polymorphic esterase loci based on autosomal codominant pattern of inheritance.

Locus	Phenotype	Number of rabbits		X^2
		Observed	Expected	
Est-1	1.03/1.03	0	0.2	
	1.03/1.00	7	6.6	
	1.00/1.00	56	56.2	0.4531*
Est-2	1.04/1.04	8	7.0	
	1.04/1.00	26	28.0	
	1.04/1.00	29	28.0	0.0982
Est-3	1.00/1.00	4	2.3	
	1.00/null	16	19.4	
	null/null	43	41.3	1.0945
Est-4	1.11/1.11	1	4.6	
	1.11/1.04	8	8.6	
	1.11/1.00	24	16.2	
	1.04/1.04	6	4.1	
	1.04/1.00	12	15.2	
	1.00/1.00	12	14.3	8.5968
Est-5	1.06/1.06	2	3.8	
	1.06/1.00	27	23.4	
	1.00/1.00	34	35.8	0.9026

*All chi-square analyses had one df except for *Est-4* where df = 4; no X^2 values were significant.

Table 3. Phenotypes of polymorphic esterase loci for two mothers and eight progeny from a confined population.

		Est-1	*Est-2*	*Est-3*	*Est-4*	*Est-5*
Mother 1		1.00/1.00	1.00/1.00	1.00/null	1.11/1.00	1.06/1.00
Progeny	1A	1.00/1.00	1.04/1.00	1.00/null	1.11/1.04	1.00/1.00
	1B	1.00/1.00	1.04/1.00	1.00/null	1.11/1.04	1.00/1.00
	1C	1.00/1.00	1.04/1.00	1.00/null	1.11/1.04	1.06/1.00
	1D	1.00/1.00	1.00/1.00	null/null	1.11/1.04	1.00/1.00
Mother 2		1.03/1.00	1.04/1.00	null/null	1.04/1.00	1.00/1.00
Progeny	2A	1.00/1.00	1.04/1.04	null/null	1.00/1.00	1.00/1.00
	2B	1.00/1.00	1.00/1.00	1.00/null	1.11/1.00	1.00/1.00
	2C	1.00/1.00	1.04/1.00	1.00/null	1.11/1.00	1.00/1.00
	2D	1.00/1.00	1.04/1.04	null/null	1.04/1.00	1.06/1.00