CARBONIC ANHYDRASE POLYMORPHISM IN A NEW JERSEY POPULATION OF THE WHITE-FOOTED MOUSE PEROMYSCUS LEUCOPUS

PATRICK L. WILMOT AND DONALD K. UNDERHILL

Department of Zoology, Rutgers University, New Brunswick, New Jersey 08903

Manuscript received January 31, 1972

ABSTRACT

Two electrophoretic forms of erythrocytic carbonic anhydrase were found to be controlled by one autosomal locus with two codominant alleles, CA\textsuperscript{f} and CA\textsuperscript{s}. The gene frequencies for the CA\textsuperscript{f} and CA\textsuperscript{s} alleles were found to be .15 and .85, respectively, in a sample of 53 mice from Middlesex County, New Jersey. The observed genotypic frequencies indicated that the population was in Hardy-Weinberg equilibrium.

The identification of serum and tissue proteins by electrophoretic separation and histochemical staining has made available a number of genetic markers useful in population studies. Lush (1970) has listed many of these proteins for mammals in general. Recent additions to this list for the genus Peromyscus, in particular, would be the serum polymorphisms described in P. polionotus by Biggers and Dawson (1971) and the survey of serum esterases by Rasmussen and Jensen (1971). This paper describes the genetic variation found in the erythrocytic enzyme, carbonic anhydrase, among individuals sampled from a New Jersey population of white-footed mice, P. leucopus.

Carbonic anhydrase is of physiological importance in red cell, kidney and secretory organ acid-base balance (Maren 1967). The enzyme is normally of low molecular weight (~ 34,000), monomeric and contains one atom of zinc per molecule. Tashian, Shreffler and Shows (1968) identified the erythrocytic carbonic anhydrase in 115 P. maniculatus and 15 Mus musculus. Two genetic loci, CA\textsuperscript{L} and CA\textsuperscript{II}, were proposed to account for 2 electrophoretic forms. Neither species exhibited any variation.

MATERIALS AND METHODS

Two groups of mice were used in this study. The inheritance studies were done on wild mice collected in the vicinity of New Brunswick, New Jersey, and their lab-bred offspring. The population data is from a sample of 53 mice live trapped between 27 October and 5 November, 1970, at Kendall Park, South Brunswick Township, Middlesex County, New Jersey.

Blood was collected from the suborbital sinus by capillary tube. The red cells were washed twice in saline and lysed in distilled water. A volume of 0.15 ml of a 1 : 10 lysate, with sucrose added for viscosity, was separated by acrylamide column electrophoresis, with a 0.375 M Tris HCl gel buffer, pH 8.9, and a 0.3 M sodium borate electrode buffer, pH 8.0. Electrophoresis was carried out at 4 milliamperes per gel in a 5% gel, until the bromthymol blue dye marker migrated 50 mm from the origin in individual gels.

The staining method for carbonic anhydrase followed Tashian (1969). The substrate used was β-napthyl acetate, there was no staining reaction with α-napthyl acetate. The substrate was dissolved in acetone and mixed with the diazonium salt, Fast Blue RR in a 0.5 M Tris HCl buffer, pH 7.0. Confirmation of the bands as carbonic anhydrase was by inhibition of the enzyme by $10^{-4}$ M acetazolamide before adding the staining solution.

Removal of the hemoglobin by precipitation in ethanol and chloroform adapted from Rickli et al. (1964) did not reveal any carbonic anhydrase bands covered by hemoglobin in the gel.

RESULTS

The migration patterns of carbonic anhydrase are shown in Figure 1 as well as the results of acetazolamide inhibition. The enzyme is found in two molecular forms, a fast migrating form (F) and a slow migrating form (S). The genetic

![Figure 1](image)
The inheritance of carbonic anhydrase phenotypes from laboratory matings

<table>
<thead>
<tr>
<th>Genetic cross</th>
<th>Number of pairs</th>
<th>Offspring phenotypes</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>F × F</td>
<td>1</td>
<td>F 10 FS</td>
<td></td>
</tr>
<tr>
<td>F × FS</td>
<td>1</td>
<td>F 17 FS 15 S</td>
<td>0.13 1 &gt;0.50</td>
</tr>
<tr>
<td>F × S</td>
<td>1</td>
<td>F 37</td>
<td></td>
</tr>
<tr>
<td>S × S</td>
<td>7</td>
<td>S 76</td>
<td></td>
</tr>
<tr>
<td>S × FS</td>
<td>5</td>
<td>S 27 28</td>
<td>0.02 1 &gt;0.75</td>
</tr>
<tr>
<td>FS × FS</td>
<td>3</td>
<td>FS 28 21</td>
<td>0.44 2 &gt;0.75</td>
</tr>
</tbody>
</table>

The frequencies of carbonic anhydrase alleles and the test for Hardy-Weinberg equilibrium for the Kendall Park sample

<table>
<thead>
<tr>
<th>Allele frequencies</th>
<th>CA'</th>
<th>CA*</th>
<th>Phenotypic counts</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>E</td>
<td>F 2 FS 12 S</td>
<td>x² df P</td>
</tr>
<tr>
<td>Obs.</td>
<td>15</td>
<td>.85</td>
<td>12</td>
<td>0.72 1 &gt;0.30</td>
</tr>
<tr>
<td>Exp.</td>
<td>±.035</td>
<td>±.035</td>
<td>13.6 38.2</td>
<td></td>
</tr>
</tbody>
</table>

Data listed in Table 1 is consistent with two codominant alleles, CA' and CA*, at one autosomal locus.

Table 2 is an analysis of 53 mice sampled from the Kendall Park population. The gene frequency for the CA' allele was found to be .15 ± .035 and the frequency for the CA* allele was .85 ± .035. The analysis of observed genotypes vs expected, showed the population to be in Hardy-Weinberg equilibrium (.50 > P > .30).

Discussion

The inheritance of carbonic anhydrase was found to be a one locus, two codominant allele system. The same model was shown in domestic cattle, while one locus with three codominant alleles was found in American buffalo (Sartore et al., 1969). Tashian et al. (1968) proposed two loci in P. maniculatus and M. musculus which were not variable in any individual examined of either species. No genetic data was listed for these mice and a two locus model is inconsistent with the genetic information reported here for P. leucopus.

Few population genetics studies have been done on Peromyscus. The analysis of the Kendall Park sample suggests that the population is in Hardy-Weinberg equilibrium. Blair (1947) found populations of P. m. blandus to be in Hardy-Weinberg equilibrium for the Buff (G) and gray (g) alleles. Biggers and Dawson (1971) found the same for populations of P. p. lucubrans using a transferrin model with three alleles. Rasmussen (1964) found a heterozygote deficiency for a blood group polymorphism in P. m. gracilis. This was attributed to inbreeding and the limited dispersal of mice from birth to mating sites.
RASMUSSEN (1970) has stated the need for more information on the neighborhood size of these mice as important in understanding their population genetics. This work on carbonic anhydrase is part of a continuing mark recapture program to measure the changes in gene frequencies, population structure and movements of individual mice.

The authors gratefully acknowledge the financial support of the Rutgers Research Council and the use of the animal housing facilities of the Rutgers Bureau of Biological Research.

LITERATURE CITED


