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GENETIC VARIATION BETWEEN WOODCHUCK POPULATIONS WITH HIGH AND LOW PREVALENCE RATES OF WOODCHUCK HEPATITIS VIRUS INFECTION

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ABSTRACT: Woodchuck hepatitis virus (WHV) infection is known to be endemic in areas of the mid-Atlantic states but is apparently absent from populations in New York and much of New England. Blood samples of 40 woodchucks (Marmota monax) from New York and from Delaware were examined by starch gel electrophoresis, and 18 monomorphic and six polymorphic protein-coding genetic systems were identified. Mendelian inheritance of variants of the six polymorphic systems was confirmed in 52 laboratory offspring of the original samples. Average heterozygosity of 0.066 in New York woodchucks and 0.039 in Delaware woodchucks were high values for mammals, although similar to those of other sciurids. Significant heterogeneity between samples from New York and Delaware woodchucks was observed at two loci (peptidase with glycyll leucine-4 and phosphogluconate dehydrogenase), suggesting that these populations were genetically distinct. Whether there are genetically determined differences in response to WHV infection remains to be determined experimentally.

Key words: Woodchuck hepatitis virus, Marmota monax, genetics, population genetics, starch gel electrophoresis, experimental, survey.

INTRODUCTION

In the original report describing woodchuck hepatitis virus (WHV), Summers et al. (1978) observed a chronic carrier rate in colony woodchucks (Marmota monax) of approximately 15%. An even higher rate of infection subsequently was reported in woodchuck populations of the mid-Atlantic states (Tyler et al., 1981). It has been shown that woodchucks from Tompkins County and adjacent counties of central New York have virtually no serologic evidence of WHV infection (Wong et al., 1982), and woodchucks from Massachusetts, New Hampshire, and Vermont have a similarly low prevalence of serologic markers of WHV infection (Tennant et al., unpubl. data). Because of the remarkable difference in prevalence of WHV infection in the Northeast compared to that of the mid-Atlantic states, it has been proposed that genetic differentiation between woodchuck populations from these geographic areas might explain the observed differences in the rate of WHV infection (Tyler et al., 1981).

Studies of electrophoretic variation in the protein products coded by structural genes have been useful for indicating the amount of genetic variation in populations of animals including other members of the family Sciuridae (Nevo, 1978). Using starch gel electrophoresis, we assayed enzyme variability in a sample of woodchucks from Delaware, an area in which WHV infection is hyperendemic, and one from central New York. Our purpose was to determine if there was sufficient genetic variability to make differentiation between populations on this geographic scale detectable, and to identify protein variants that might serve as genetic markers in epidemiologic studies of WHV infection.

MATERIALS AND METHODS

An initial survey was conducted to determine the tissue type (plasma or erythrocyte fraction of blood) and buffer system that would be op-
timum for electrophoretic separation. Heparin-
ized blood samples were obtained from the fem-
oral vein or by cardiac puncture from six normal
adult woodchucks. Blood was centrifuged within
30 min, and plasma and erythrocyte fractions
were placed in cryostat tubes, frozen immedi-
ately in crushed dry ice, then stored at −70 C
for 2 to 7 days. Horizontal starch gel electropo-
oretic methods were those described by May
et al. (1979), and staining procedures were
adapted from those of Harris and Hopkinson
(1976) and Allendorf et al. (1977). Plasma and
erythrocyte fractions were assayed for 49 en-
zymes or tissue proteins under each of four stan-
dard buffer systems (references in Table 1). The
survey determined which proteins, tissue frac-
tions, and buffer systems would produce scor-
able results in subsequent population studies.

Following the initial survey, blood samples
were taken from a population of woodchucks
from central New York and one from Delaware.
The New York group was composed of 22 un-
related woodchucks maintained in a WHV neg-
ative breeding colony at Cornell University that
had been trapped in Tompkins County, New
York. The Delaware sample included 18 wood-
chucks trapped during the summer of 1983 in
northern Delaware and purchased from a com-
mercial source (Hazleton-Dutchland Labora-
tories, Denver, Pennsylvania). Although these
individuals were woodchuck hepatitis surface
antigen negative (Cote et al., 1984) when blood
samples were obtained, the area from which
they were trapped regularly produced WHV
infected woodchucks. Plasma and erythrocyte
hemolysates from both groups were scored for
the specific enzymes and plasma globulin frac-
tion that had been resolved in the initial survey.
For each specific protein stain, bands of differ-
ing electrophoretic mobility were assumed to
represent separate allelic variants and scored as
such by standard methods and terminology (May
et al., 1979).

Genotype and allele frequencies in each of
the two samples were determined for each pro-
tein-coding locus. Expected heterozygosity (\(\bar{H} = \Sigma [1 - p^2] / [\text{number of loci}]\), where \(p\) is fre-
quency of allele \(j\) at each locus, and a chi-square
statistic for conformance of genotype frequen-
cies to Hardy-Weinberg expectation, were calcu-
lated for each population. Overall genetic
similarity between the two populations was cal-
culated as Nei’s (1972) genetic identity using all
loci. For each polymorphic locus, we tested sta-
tistically significant differentiation between the
New York and Delaware samples measured by
chi-square test of heterogeneity of allele fre-
cuencies (Workman and Niswander, 1970). For
all statistical tests, \(p < 0.05\) was accepted as
indicating significance.

To establish that the observed enzyme vari-
ants were genetically determined, we per-
formed electrophoresis on blood taken in Oc-
tober–November 1985 from 52 laboratory-born
descendants of 19 of the New York and Dela-
ware woodchucks. For each of six established
polymorphic loci, the phenotype of each descen-
dant was compared with the phenotypes of
its parents and examined for congruence to a
Mendelian model of inheritance. Where possi-
ble, we compared the genotypes of offspring to
expected Mendelian ratios by chi-square anal-
ysis.

RESULTS

Table 1 summarizes the specific plasma
protein and erythrocyte enzymes exam-
ined in the initial screening procedure and
the buffer systems identified as optimal for
their resolution. Of the 49 specific enzymes
and plasma protein fractions examined,
there was sufficient activity and resolution
to score 20 of these. Since four of these
enzymes (DIA, LDH, NP, and MUP; see
Table 1 for enzyme abbreviations) ap-
ppeared on the gels as two separate systems
apparently encoded by separate genetic
loci, we identified a total of 24 scorable
structural genes for Marmota monax.

In samples from New York and Dela-
ware, protein products of the following 18
loci were observed to be monomorphic:
Aat; Dia-2; Gapdh; Gpi; Idh; Lap; Ldh-1;
Mdh; Me; Mup-1 and -2; Np-1 and -2; Pep-
LA-1; Pep-LLL-2; Pep-PAP-3; Pgd; and
Sod. Polymorphisms were observed in the
protein products coded by Dia-1, Ldh-2,
Mpi, Pep-GL-4, Pgd, and Pro-4 (Table 2).
Genotype frequencies at all loci were in
Hardy-Weinberg equilibrium. Average
expected heterozygosity (\(\bar{H}\)), the index of
genetic variability, was 0.066 in the New
York sample and 0.039 in the Delaware
sample for the 24 loci (Table 2). Nei’s ge-
netic identity between the two samples was
0.992. To estimate overall genetic identity
we assumed that the 18 loci observed to
be monomorphic in the initial survey were
<table>
<thead>
<tr>
<th>Enzyme name</th>
<th>Abbreviation</th>
<th>Optimal (and alternate) buffer system*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>ACP</td>
<td>--</td>
</tr>
<tr>
<td>Aconitase</td>
<td>AC</td>
<td>--</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>ADA</td>
<td>--</td>
</tr>
<tr>
<td>Adenylylate kinase</td>
<td>AK</td>
<td>--</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>ADH</td>
<td>--</td>
</tr>
<tr>
<td>Aldolase</td>
<td>ALD</td>
<td>--</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>AKP</td>
<td>--</td>
</tr>
<tr>
<td>Alphaglycerophosphate dehydrogenase</td>
<td>AGP</td>
<td>--</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>AAT</td>
<td>CT</td>
</tr>
<tr>
<td>Catalase</td>
<td>CAT</td>
<td>--</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>CK</td>
<td>--</td>
</tr>
<tr>
<td>Diaphorase</td>
<td>DIA</td>
<td>CT (4)</td>
</tr>
<tr>
<td>Esterase</td>
<td>EST</td>
<td>--</td>
</tr>
<tr>
<td>Fructose diphosphatase</td>
<td>FDP</td>
<td>--</td>
</tr>
<tr>
<td>Fumarase</td>
<td>FUM</td>
<td>--</td>
</tr>
<tr>
<td>Galactosaminidase</td>
<td>GAM</td>
<td>--</td>
</tr>
<tr>
<td>Glucokinase</td>
<td>GK</td>
<td>--</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>G6PDH</td>
<td>--</td>
</tr>
<tr>
<td>Glucose-1-phosphate transferase</td>
<td>G1PT</td>
<td>--</td>
</tr>
<tr>
<td>Gluconeosephosphate isomerase</td>
<td>GPI</td>
<td>RSL (CT, 4)</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>BGLU</td>
<td>--</td>
</tr>
<tr>
<td>Glutamic dehydrogenase</td>
<td>GDH</td>
<td>--</td>
</tr>
<tr>
<td>Glutamic pyruvic transaminase</td>
<td>GPT</td>
<td>--</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>GR</td>
<td>--</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>GAPDH</td>
<td>CT</td>
</tr>
<tr>
<td>Guanine deaminase</td>
<td>GDA</td>
<td>--</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>IDH</td>
<td>CT (4)</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>LDH</td>
<td>CT (4)</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>LAP</td>
<td>MF (CT, 4)</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>MDH</td>
<td>CT</td>
</tr>
<tr>
<td>Malic enzyme</td>
<td>ME</td>
<td>CT</td>
</tr>
<tr>
<td>Mannosephosphate isomerase</td>
<td>MSI</td>
<td>RSL (MF, 4)</td>
</tr>
<tr>
<td>Menadione reductase</td>
<td>MR</td>
<td>--</td>
</tr>
<tr>
<td>Methylumbelliferyl phosphatase</td>
<td>MUP</td>
<td>CT (4)</td>
</tr>
<tr>
<td>Nucleoside phosphorylase</td>
<td>NP</td>
<td>RSL (CT)</td>
</tr>
<tr>
<td>Octanol dehydrogenase</td>
<td>ODH</td>
<td>--</td>
</tr>
<tr>
<td>Peptidase with glycyI-leucine</td>
<td>PEP-GL</td>
<td>4 (CT)</td>
</tr>
<tr>
<td>Peptidase with leucyl-alanine</td>
<td>PEP-LA</td>
<td>RSL</td>
</tr>
<tr>
<td>Peptidase with leucyl-leucyl-leucine</td>
<td>PEP-LLL</td>
<td>4</td>
</tr>
<tr>
<td>Peptidase with phenyl-alanyl-proline</td>
<td>PEP-PAP</td>
<td>4</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>PGM</td>
<td>--</td>
</tr>
<tr>
<td>Phosphogluconate dehydrogenase</td>
<td>PGD</td>
<td>4</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>PGK</td>
<td>CT (4)</td>
</tr>
<tr>
<td>Pyruvic kinase</td>
<td>PK</td>
<td>--</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase</td>
<td>SDH</td>
<td>--</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>SOD</td>
<td>MF</td>
</tr>
<tr>
<td>Triosephosphate isomerase</td>
<td>TPI</td>
<td>--</td>
</tr>
<tr>
<td>Xanthine dehydrogenase</td>
<td>XDH</td>
<td>--</td>
</tr>
<tr>
<td>Protein</td>
<td>PRO</td>
<td>RSL</td>
</tr>
</tbody>
</table>

* Buffer systems CT, MF and RSL are described in May et al. (1979). Buffer 4 is from Selander et al. (1971). A single minus (−) designates enzymes that were too poorly resolved to score reliably. A double minus (−−) indicates enzymes for which there was no activity in either erythrocyte or plasma fractions. For those enzymes that were resolved successfully, the erythrocyte fraction was the optimal tissue except for PRO and PEP-LA, for which the plasma fraction gave better resolution.
also monomorphic in all individuals from both samples. Slight deviations from this assumption would have had little effect on the genetic distance measure.

Between-sample chi-square heterogeneity tests of allele frequencies showed the New York and Delaware samples to be statistically indistinguishable at four of the six loci polymorphic loci (Dia-1, Ldh-2, Mpi, and Pro-4). The remaining two loci (Pep-GL-4 and Pgd), however, had allele frequencies that differed significantly between New York and Delaware samples ($P < 0.01$; Table 2).

The patterns of protein variation in descendants of the New York and Delaware woodchucks conformed to genetic inheritance expectation. We analyzed a total of 96 crosses (16 pairs producing 52 offspring, each scored at all six polymorphic enzyme systems) in which the enzyme phenotype of both parents and their offspring were determined. All the enzyme variants seen in the New York and Delaware samples were represented in the crosses, with the exception of the “3” variant of Pro-4. In all the crosses, the resulting enzyme phenotypes of offspring were what could be expected if they had been determined by Mendelian inheritance of allelic enzyme variants. Crosses between apparently homozygous parents of identical phenotype (54 crosses) always (177 cases) produced homozygote offspring of the same phenotype. Crosses between apparent homozygotes of different phenotype always produced heterozygote offspring (six crosses, 21 cases). Crosses between apparent homozygote and heterozygote parents produced roughly equal proportions of homozygote and heterozygote offspring (28 crosses, 51 homozygote and 35 heterozygote cases; deviation from 1:1 ratio not significant at any locus), and crosses between apparent heterozygotes produced heterozygote and both types of homozygote offspring (eight crosses, 16 homozygote and 13 heterozygote cases; data insufficient to test against the Mendelian expectation of 1:2:1 ratio at any individual locus).

**TABLE 2.** Allele frequencies at seven loci in samples of two populations of woodchucks (*Marmota monax*). $n = 18$ for Delaware samples; $n = 22$ for New York samples except Pro-4, where $n = 19$

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>New York</td>
</tr>
<tr>
<td>Dia-1</td>
<td>1</td>
<td>0.789</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.211</td>
</tr>
<tr>
<td>Ldh-2</td>
<td>1</td>
<td>0.909</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.091</td>
</tr>
<tr>
<td>Mpi</td>
<td>1</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.886</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.045</td>
</tr>
<tr>
<td>Pep-GL-4</td>
<td>1</td>
<td>0.432</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.568</td>
</tr>
<tr>
<td>Pgd+</td>
<td>1</td>
<td>0.795</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.205</td>
</tr>
<tr>
<td>Pro-4</td>
<td>1</td>
<td>0.947</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.000</td>
</tr>
<tr>
<td>Heterozygosity ($H$) over 24 loci</td>
<td>0.066</td>
<td>0.039</td>
</tr>
</tbody>
</table>

* Differences in allele frequency between populations significant (chi-square, $P < 0.01$).

**DISCUSSION**

As far as we can determine, these data are the first to be reported for electrophoretically detectable enzyme variation in *Marmota monax*. It is commonly assumed in population genetics studies that observed electrophoretic variants are genetically determined. Because woodchucks as seasonal hibernators are known to undergo extreme physiological shifts that could conceivably affect enzyme expression, we took the extra precaution of analyzing breeding crosses to verify that the enzyme variants we observed followed the inheritance patterns seen in other species (Bowen and Yang, 1978). Since all the variants conformed to simple Mendelian expectation, our working assumption that the electromorphs we observed were true genetic variants was upheld.

Average heterozygosity in the New York and Delaware samples was higher than that
for most mammalian species (Nevo, 1978), but was comparable to other sciurids such as Belding’s ground squirrel, *Spermophilus beldingi* (H = 0.107) (Hanken and Sherman, 1981), black-tailed prairie dog, *Cynomys ludovicianus* (H = 0.066) (Foltz and Hoogland, 1983), and yellow-bellied marmot, *Marmota flaviventris* (H = 0.075) (Schwartz and Armitage, 1981). High amounts of electrophoretic variation have made it possible to characterize small social groups (Chesser, 1983) and even to assign paternity (Hanken and Sherman, 1981), so the potential of these methods for studies of WHV-free and WHV-infected woodchuck populations appears promising.

The data for Pep-GL-4 and Pgld in this study indicate significant genetic differences between the New York and Delaware samples. A similar amount of heterogeneity and comparable values for Nei genetic identity have been reported for black-tailed prairie dog populations from northern to southern New Mexico (Chesser, 1983). There are no comparable data for closely related species over the woodchuck’s natural range in eastern North America, but Wilson (1982) found two heterogeneous enzyme loci between central New York and southern Pennsylvania populations of the pine vole (*Microtus pennsylvanicus*), a rodent species widely distributed, as is the woodchuck, near areas of agricultural cultivation. Thus, woodchucks are similar to the pine vole in that populations separated by >300 km are genetically differentiated, but we do not yet know whether differentiation is present on a finer scale as is observed in colonies of prairie dogs separated by much shorter distances (Chesser, 1983).

Observed geographic differences in WHV infection led Tyler et al. (1981) to suggest that WHV resistance might differ characteristically between the northern subspecies *M. monax rufescens* and the southern subspecies *M. monax monax*. New York woodchucks would classically be considered *M. monax rufescens* and the Delaware animals *M. monax monax* (Hall, 1981), but recent studies in genetic variation of mammals have tended to erode the subspecies concept rather than to confirm it (Futuyama, 1979). Regardless of subspecies designation, the northern and southern populations that we sampled were genetically distinct.

WHV is a member of the hepatadnavirus group which includes human hepatitis B virus (HBV). The two viruses produce similar responses in their respective hosts (Summers et al., 1978). Genetically mediated variations in host response to HBV have been suggested, including differential rates of chronic HBV infection in individuals with certain allelic variants of the major histocompatibility complex (Hillis et al., 1977). Geographically and genetically distinct human populations differ significantly in the incidence of HBV infection (Black et al., 1986) and by determining the probability that a host would become a chronic carrier of HBV rather than recovering, such genetic differences could dramatically influence the distribution of HBV in a population. Our data suggest that woodchuck populations in the region of WHV hyperendemism are genetically distinct from WHV-free populations, but whether genetically determined differences between these populations influence the response to WHV infection remains to be determined. Specific data on the major histocompatibility antigens in these populations would be of particular interest, since these have been implicated in some studies of HBV infection. We are also sampling additional populations between central New York and Delaware to assess more fully the relationship between genetic differentiation and the prevalence of WHV infection.

**ACKNOWLEDGMENTS**

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LITERATURE CITED


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