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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 glycyl 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. Heparinized blood samples were obtained from the femoral 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 immediately in crushed dry ice, then stored at −70 C for 2 to 7 days. Horizontal starch gel electrophoretic 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 enzymes or tissue proteins under each of four standard buffer systems (references in Table 1). The survey determined which proteins, tissue fractions, and buffer systems would produce scorable 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 unrelated woodchucks maintained in a WHV negative breeding colony at Cornell University that had been trapped in Tompkins County, New York. The Delaware sample included 18 woodchucks trapped during the summer of 1983 in northern Delaware and purchased from a commercial source (Hazleton-Dutchland Laboratories, 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 fraction that had been resolved in the initial survey. For each specific protein stain, bands of differing 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 protein-coding locus. Expected heterozygosity ($\bar{H} = \Sigma [1 - p_i^2]/[\text{number of loci}]$, where p_i is frequency of allele i) at each locus, and a chi-square statistic for conformance of genotype frequencies to Hardy-Weinberg expectation, were calculated for each population. Overall genetic similarity between the two populations was calculated as Nei's (1972) genetic identity using all loci. For each polymorphic locus, we tested statistically significant differentiation between the New York and Delaware samples measured by chi-square test of heterogeneity of allele frequencies (Workman and Niswander, 1970). For all statistical tests, $P < 0.05$ was accepted as indicating significance.

To establish that the observed enzyme variants were genetically determined, we performed electrophoresis on blood taken in October–November 1985 from 52 laboratory-born descendants of 19 of the New York and Delaware woodchucks. For each of six established polymorphic loci, the phenotype of each descendant was compared with the phenotypes of its parents and examined for congruence to a Mendelian model of inheritance. Where possible, we compared the genotypes of offspring to expected Mendelian ratios by chi-square analysis.

RESULTS

Table 1 summarizes the specific plasma protein and erythrocyte enzymes examined 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) appeared 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 Delaware, 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; Pgk; 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 genetic 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 1. Enzymes screened for genetic polymorphism in woodchucks.

Enzyme name	Abbreviation	Optimal (and alternate) buffer system*
Acid phosphatase	ACP	–
Aconitase	AC	– –
Adenosine deaminase	ADA	–
Adenylate kinase	AK	–
Alcohol dehydrogenase	ADH	– –
Aldolase	ALD	– –
Alkaline phosphatase	AKP	–
Alphaglycerophosphate dehydrogenase	AGP	– –
Aspartate aminotransferase	AAT	CT
Catalase	CAT	–
Creatine kinase	CK	–
Diaphorase	DIA	CT (4)
Esterase	EST	–
Fructose diphosphatase	FDP	– –
Fumarase	FUM	– –
Galactosaminidase	GAM	– –
Glucokinase	GK	– –
Glucose-6-phosphate dehydrogenase	G6PDH	–
Glucose-1-phosphate transferase	G1PT	– –
Glucosephosphate isomerase	GPI	RSL (CT, 4)
β-Glucosidase	BGLU	– –
Glutamic dehydrogenase	GDH	– –
Glutamic pyruvic transaminase	GPT	– –
Glutathione reductase	GR	–
Glyceradehyde-3-phosphate dehydrogenase	GAPDH	CT
Guanine deaminase	GDA	– –
Isocitrate dehydrogenase	IDH	CT (4)
Lactate dehydrogenase	LDH	CT (4)
Leucine aminopeptidase	LAP	MF (CT, 4)
Malate dehydrogenase	MDH	CT
Malic enzyme	ME	CT
Mannosephosphate isomerase	MPI	RSL (MF, 4)
Menadione reductase	MR	– –
Methylumbellifery phosphatase	MUP	CT (4)
Nucleoside phosphorylase	NP	RSL (CT)
Octanol dehydrogenase	ODH	– –
Peptidase with glycyl-leucine	PEP-GL	4 (CT)
Peptidase with leucyl-alanine	PEP-LA	RSL
Peptidase with leucyl-leucyl-leucine	PEP-LLL	4
Peptidase with phenyl-alanyl-proline	PEP-PAP	4
Phosphoglucomutase	PGM	–
Phosphogluconate dehydrogenase	PGD	4
Phosphoglycerate kinase	PGK	CT (4)
Pyruvic kinase	PK	– –
Sorbitol dehydrogenase	SDH	– –
Superoxide dismutase	SOD	MF
Triosephosphate isomerase	TPI	–
Xanthine dehydrogenase	XDH	– –
Protein	PRO	RSL

* 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 $n = 19$.

Locus	Allele	Frequency	
		New York	Delaware
Dia-1	1	0.789	0.917
	2	0.211	0.083
Ldh-2	1	0.909	0.944
	2	0.091	0.056
Mpi	1	0.068	0.028
	2	0.886	0.944
	3	0.045	0.028
Pep-GL-4[a]	1	0.432	0.111
	2	0.568	0.889
Pgd[a]	1	0.795	1.000
	2	0.205	0.000
Pro-4	1	0.947	0.778
	2	0.053	0.111
	3	0.000	0.111
Heterozygosity ($\bar{H}$) over 24 loci		0.066	0.039

[a] 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* ($\bar{H}$ = 0.107) (Hanken and Sherman, 1981), black-tailed prairie dog, *Cynomys ludovicianus* ($\bar{H}$ = 0.066) (Foltz and Hoogland, 1983), and yellow-bellied marmot, *Marmota flaviventris* ($\bar{H}$ = 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 Pgd in this study indicate signficant 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 pinetorum*), 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 (Futuyma, 1979). Regardless of subspecies designation, the northern and southern populations that we sampled were genetically distinct.

WHV is a member of the hepadna virus 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.

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