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# Y-specific DNA and population structure in natural populations of the house mouse, Mus domesticus.

Kathleen A. Hill
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# Y-SPECIFIC DNA AND POPULATION STRUCTURE IN NATURAL POPULATIONS OF THE HOUSE MOUSE, *MUS DOMESTICUS*

by

KATHLEEN A. HILL

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## ABSTRACT

The absence of random mating subdivides a species into populations and populations into smaller subgroups. Restricted gene flow in such a structured population enhances the effects of genetic drift, resulting in genetic divergence when natural selection is not considered. Analysis of genetic variation and population structure is essential in understanding evolutionary processes.

Information concerning population structure has generally been obtained through the analysis of autosomally inherited variants and less often through X-linked variants. Recently, mitochondrial DNA (mtDNA) which is maternally inherited and clonally transmitted and DNA sequences unique to the Y chromosome (Y-specific) which do not recombine with other sequences and are transmitted from father to son have also begun to provide unique insight into population structure. Specifically, these provide information on matrilineal and patrilineal gene flow, respectively.

In this investigation, natural house mouse (Mus domesticus) populations were screened for Y-specific DNA restriction fragment length polymorphisms. Animals were collected during a 7 year sampling period from 25 corn cribs distributed within 9 sampling regions in southwestern Ontario. A battery of 21 Type II restriction endonucleases allowed the direct analysis of portions of the DNA sequence. Y-specific DNA restriction fragments were detected using a biotinylated YDNA probe (the Y-chromosomal clone 145SC5) and a nonradioactive detection system. At least 8 and as many as 38 wild caught mice from different sampling sites were screened for Y-specific DNA variability with each restriction endonuclease.

Hybridization of the YDNA probe to female inbred mouse DNA was not detected. Y-specific DNA sequence variation was not detected in natural

house mouse populations of southwestern Ontario. The Y-specific DNA sequences detected were identical to those observed in the inbred mouse strains studied.

Either of two situations may explain the lack of detection of Y-specific DNA variability in these mouse populations. First, considerable Y-specific DNA sequence variation may exist but was not detected. Second, Y-specific DNA variability may be infrequent in the mouse populations analyzed. It seems unlikely, considering the number and type of restriction endonucleases, the choice of probe and the animals screened that the present investigation was not capable of detecting common Y-specific DNA variants.

Low frequency of variation in Y-specific DNA sequences may result either from the loss of new neutral mutations in large populations or in small populations having high levels of gene flow or from strong selective pressures. Considering the small effective population size for Y-linked alleles and the population structuring of these mouse populations, strong selective pressures appear to be the most likely explanation for the present findings.

Recently, Y-specific DNA sequence variation in wild-caught mice of Montreal, Quebec has been detected. The apparent contradiction between this finding and both the effects of selection upon Y-linked alleles and the results of the present study is yet to be resolved.

# DEDICATION

I wish to dedicate this thesis to my family, David and Sheila Hill and Sharon, Maegan and Kelly. I am so fortunate to have their love and encouragement. Such support makes all things possible.

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# I INTRODUCTION

## A Population Structure

Population structure is the product of the absence of random mating throughout a species. Ecological and/or behavioral factors are at the basis of population structuring. A species may become subdivided into smaller subgroups if, for example, a suitable habitat is not continuous or the organisms exhibit territorial behavior. Population structuring may occur at several levels in a species, subdividing the species into a number of populations and frequently further subdividing each population into smaller subgroups.

Population subdivision affects allelic frequencies in the subgroups of a population and the populations of a species. Subdivision increases the magnitude of genetic drift (the random fluctuations in allelic frequencies) and thus enhances the potential for genetic divergence among the different subdivisions of a species. Population subdivision also restricts gene flow and thus enhances the potential for increased genetic divergence between subgroups of a population over time (Wright, 1931, 1940, 1943, 1965; Slatkin, 1985). Thus, genetic drift and gene flow are two opposing forces affecting genetic differentiation in a structured population in the absence of selective pressures. Analysis of population structure and specifically, gene flow, through examination of gene frequency data and direct observations of dispersal provides insight into genetic divergence and is essential in gaining knowledge of evolutionary processes (Mayr, 1963; Ehrlick and Raven, 1969; Jackson and Pounds, 1979).

## B Analysis of Allelic Frequencies and Population Structure

The Hardy-Weinberg principle states that single-locus genotypic frequencies after one generation of random mating can be represented by a binomial (with two alleles) and a multinomial (with multiple alleles) function

of the allelic frequencies (Hedrick, 1985). Hardy-Weinberg genotypic proportions exist in randomly-mating populations in the absence of factors that change allelic frequency (selection, mutation, gene flow and genetic drift) and do not change over time if such conditions persist. Any deviation of genotypic proportions from those predicted by the Hardy-Weinberg principle is indicative that one of these factors is affecting allelic frequency. The absence of random mating results in population division with limited gene flow between each subdivision and consequently the possibility for genetic drift. Therefore, deviations from Hardy-Weinberg genotypic proportions can serve as an indication of the possible existence of population structuring.

If random-mating subgroups exist, then each subgroup can establish a Hardy-Weinberg equilibrium often at a different allelic frequency than that of other subgroups. If the allelic frequencies from all the subgroups are pooled and are treated as if they represented a single unstructured population, then an excess of homozygotes and a lower than expected number of heterozygotes is observed as compared with Hardy-Weinberg proportions. This effect is called Wahlund's principle (for a review, see Hedrick, 1985).

Fixation or F coefficients (Wright, 1951 and 1965) have been used to describe the reduction in heterozygosity in subdivided populations. The F statistic measures departure from the expected random-mating proportions in the whole population and within the subgroups. These coefficients describe the degree to which a population is subdivided. A subdivided population may have three or more levels of complexity. For instance, if there are three levels, these would be the individuals (I), the subgroups (S) and the total population (T). The three F coefficients are F(ST), F(IT) and F(IS). Allelic frequencies are required to calculate values of F. If the proportion of

heterozygosity of an individual in a subgroup is $\underline{H}(\underline{I})$, the expected heterozygosity of an individual in an equivalent random-mating subgroup is $\underline{H}(\underline{S})$, with the mean value of all subgroups being $\overline{\underline{H}}(\underline{S})$ and the expected heterozygosity of an individual in an equivalent random mating total population is $\underline{H}(\underline{T})$, then $\underline{F}(\underline{IS})$, $\underline{F}(\underline{ST})$ and $\underline{F}(\underline{IT})$ can be calculated.

$$F(IS) = (\overline{H}(S) - H(I))/\overline{H}(S)$$

$$F(ST) = (H(T) - \overline{H}(S))/H(T)$$

$$F(IT) = (H(T) - H(I))/H(T)$$

Unless other forces, such as selection favouring the heterozygote, are operating in a population, $\underline{F}(\underline{ST})$ is always greater than or equal to zero. $\underline{F}(\underline{ST})$ is a measure of the reduction in heterozygosity of a subgroup due to population subdivision and random genetic drift. $\underline{F}(\underline{IS})$ and $\underline{F}(\underline{IT})$ are measures of deviation from Hardy-Weinberg proportions within the subgroups and the total population, respectively. Positive values of $\underline{F}(\underline{IS})$ and $\underline{F}(\underline{IT})$ indicate deficiency of heterozygotes. Negative values indicate an excess of heterozygotes. The three coefficients are interrelated as can be seen from the following equation (Wright, 1951 and 1965):

$$F(ST) = (F(IT) - F(IS)) / (1 - F(IS))$$

Allelic frequencies are required to make such calculations. Nei (1977) has demonstrated how the $\underline{F}$ coefficients can be expressed in terms of observed and expected genotypic and allelic frequencies with two alleles and one locus and in cases of multiple alleles and multiple loci.

## C Models of Population Structure

Many models of population structure have been developed (for a review, see Felsenstein, 1976 and Slatkin, 1985). The models permit description of

changes in allelic frequencies due to population structuring. Populations may be described as either discrete (containing isolated subgroups of individuals) or continuous (composed of continuously distributed individuals). For simplicity, nonoverlapping generations are considered in most of the models. Individual models may vary in the physical arrangement of populations (one, two and three dimensional models), in different patterns of gene flow and in the incorporation of different distances between individuals and/or subgroups. The parameters of the models have also been expanded to assess the effects of infinite or finite population size and the absence or presence of selective pressures on the maintenance of genetic variation.

Of the many populations models developed, there are four classic examples. The continent-island model (Wright, 1940) describes unidirectional gene flow from a large population to a small subgroup. In the island model (Wright, 1951), a population is subdivided into many subgroups of equal size. Gene flow is assumed to occur equally between all subgroups. In the isolation by distance model (Wright, 1943), a continuous population is described. The population has uniform density and random mating is limited by the average distance of dispersal. The stepping-stone models (Kimura and Weiss, 1964; Weiss and Kimura, 1964) describe limitations to gene flow among subgroups of a population due to the geographic distances between the different subgroups. In the one-dimensional model, the subgroups are linearly oriented with gene flow occurring only between adjacent subgroups. The two- and three-dimensional stepping-stone models describe gene flow from one subgroup to adjacent subgroups, spatially distributed in two and three dimensions, respectively.

D Analysis of Natural Populations

Important information pertaining to population structure can be derived from the analysis of genetic variation. Genetic variability can be defined as the existence of two or more genetically different phenotypes in the same population. Included among the factors that affect genetic variation are the breeding system, natural selection, genetic drift, gene flow and mutation. Information on genetic variability, initially, yields insight into the breeding system, gene flow and genetic drift within a population and ultimately, into population structure.

The development of new techniques applicable to the analysis of inherited variants has permitted examination of different gene products and even the direct analysis of the DNA sequence itself. Morphological and cytological variants were easily identified and were the focus of early studies. Electrophoretic protein variants have been intensively studied since the 1960's. Recent DNA sequencing and restriction fragment length analyses have focused upon variation in the DNA sequence itself. Generally, autosomally-inherited variants and less often X-linked traits have provided information on population structure. To date, the analysis of genetic variation has involved hundreds of thousands of individuals from thousands of populations and many species.

Buri (1956) described how finite population size affected allelic frequency using a morphological variant. The two alleles at the brown locus which determine eye colour in Drosophila melanogaster were selectively nearly neutral. These alleles permitted the analysis of genetic drift independent of any effects of natural selection. Buri (1956) showed that finite population size did not change the mean allelic frequency but increased the variance in allelic frequencies over replicate populations and over time. Also, the

heterozygosity declined with time.

Morphological variation involving two alleles at the agouti coat colour locus of the house mouse (Mus domesticus) was analyzed by Petras (1967c). Observation of the different phenotypes in natural populations revealed a deficiency of heterozygotes at this locus. One explanation for this deficiency was the existence of inbreeding and thus population subdivision in natural house mouse populations.

Widespread morphological polymorphism at the T locus in natural house mouse populations has been reported (Anderson, 1964 and Petras, 1967b). The alleles at this locus affect the development of axial structures in the caudal region of the mouse. Some of the t alleles when present in the homozygous condition are lethal and others affect male fertility. The major force, keeping the alleles in the population is an abnormal transmission ratio which favors t bearing sperm of heterozygous males. In one analysis of this morphological variant (Petras, 1967b), the allelic frequency of a t allele in natural house mouse populations was considerably lower than the allelic frequency predicted from models incorporating the transmission advantage. It was concluded that some other factor affected the frequency of the t allele. Inbreeding (population subdivision) in natural house mouse populations was suspected and incorporation of an inbreeding coefficient into a deterministic model resulted in predicted t allele frequences that were consistent with observed frequencies (Petras, 1967b).

Wild oats (Avena fatua) have several polymorphic morphological traits observed throughout central California (Jain and Marshall, 1967). Populations of this plant consist of small subgroups clustered at the base of trees in orchards. Jain and Rai (1974) estimated the gene flow among subgoups to

be quite low (1 percent or less). The subgroups were small (17-191 plants) and the effective subgroup size was estimated to be between 14.6 and 40.3 individuals. Genetic variation at the lemma color locus was examined and a considerable amount was found between subgroups but not over time in the same subgroup. The information available concerning the effective population size, migration rate and self-fertilization, together with the observed pattern of genetic variation, was consistent with that of a large population subdivided into numerous subgroups. However, the possiblity of selection affecting the genetic variation among subgroups could not be discounted.

Cytological variants have included such classical examples as the inversion sequences in Drosophila chromosomes (Dobzhansky, 1943; Anderson et al., 1975), the translocations of chromosomes of the plant genus, Clarkia (Bloom and Lewis, 1972) and the variation in the length of the Y chromosome in humans (Bender and Gooch, 1961; Bishop et al., 1962 and De la Chapelle et al., 1963). Although these cytological variants, discussed in detail below, have been detected and described, their analysis has not been extended to an examination of population structure.

In Drosophila, the chromosomes of the salivary gland are large and diplotene which permits recognition of specific chromosome banding patterns. The third chromosome has polymorphisms for inversions of one or more chromosomal segments. The different morphs can be recognized by the loop patterns of homologous chromosomes in heterozygotes. Many of the inversions are highly polymorphic in natural populations of Drosophila pseudoobscura (Anderson et al., 1975). The frequencies of the inversion types were characterized by striking genetic variation between sites, distinct temporal

patterns at single sample sites and major seasonal fluctuations.

In the plant genus Clarkia, chromosomal translocations are common and rings of chromosomes of different sizes form in hybrid populations. Bloom and Lewis (1971) examined the transition zone between two Clarkia speciosa races. Crosses between individuals located at the extremes of the transition zone were infertile because of the production of chromosomally unbalanced gametes. Between the two races there exists a gradual stepwise replacement of one chromosomal race by the other over a distance of only 20 miles.

Variation in the length of the long arm of the Y chromosome is frequent in human populations (Bender and Gooch, 1961; Bishop et al., 1962; De la Chapelle et al., 1963). Between 2 and 3 per cent of males have a Y chromosome of variant length, in most cases unusually long. Wennstrom and De la Chapelle (1963) suggested that the cause of the varying chromosome lengths was due to inherited differences in the coiling of the heterochromatin of the long arm of the Y chromosome.

By far the most extensive analysis of population structure has employed electrophoretic protein variants. Gel electrophoresis permits the rapid analysis of many loci of any organism and distinguishes between homozygotes and heterozygotes. Extensive genetic variation has been detected using electrophoretic techniques (for reviews, see, Selander, 1976; Powell, 1975; Hamrick, 1979 and Nevo, 1978). The estimates of average heterozygosity obtained by the analysis of electromorphs from at least 14 loci for three major taxa, plants, invertebrates and vertebrates were 0.104, 0.112 and 0.049, respectively. (Nevo, 1978 and Hamrick, 1979). In the analysis of 42, 41 and 71 loci, the number of polymorphic loci determined for Drosophila melanogaster, Mus domesticus and Homo sapiens was 19, 20 and 28, respectively (Lewontin,

1973). Thus extensive inherited protein polymorphism analyzed with the use of gel electrophoresis was found in natural populations for structural genes coding for enzymes (Lewontin, 1973).

Levin et al. (1979) examined electrophoretic variants of the malic dehydrogenase locus in the plant Oenothera organensis. A heterozygote deficiency, compared with Hardy-Weinberg proportions was discovered, suggesting the occurrence of population subdivision.

Singh and Rhomberg (1987) obtained estimates of gene flow from rare alleles in Drosophila melanogaster in an extensive electrophoretic study of 117 loci for genetic variation in 15 populations throughout the world. Estimates of the number of migrants exchanged per generation among populations were 1.09 in East Asian populations and 2.66 in west coast North America. These estimates of gene flow suggest that even with great geographic distance, gene flow between neighboring populations was extensive. If the alleles examined were selectively neutral, little differentiation among neighboring populations is expected. Yet, despite extensive gene flow, genetic differentiation exists. In this instance gene flow may play a minor role in the geographic genetic differentiation and natural selection may play a more important role.

Petras (1967a) examined 2 loci controlling biochemical variants (esterase-2 and hemoglobin) in mouse populations. A numerical deficiency of heterozygotes was found at both loci. Population structuring was a possible explanation for this. Direct observation of mouse populations indicated the existence of restricted movement of mice in stable environments (for review, see Petras, 1967a and Reimer and Petras, 1968). The observation of social behaviour of mice in population cages revealed the existence of territoriality

and the formation of small random-mating units or demes (Reimer and Petras, 1968; Topping, 1975; Philpott, 1982). An inbreeding coefficient ($\underline{F}$ = 0.06 to 0.30) and the effective size of the demes or breeding units (between 6 and 80 individuals) were estimated (Petras, 1967a). Hoeg (1978) analyzed 7 loci controlling biochemical variants and again found a numerical deficiency of heterozygotes existing at 5 of the 7 loci studied in mice. A discontinuous, two-dimensional stepping-stone model was used to describe mouse population structure (Hoeg, 1978). This model was used because of the existence of demes and the discontinuous distribution of suitable habitats (corn cribs) for mouse populations in southwestern Ontario. Hoeg (1978) found that a migration rate of 0.2981, with the deme size of 10 individuals accounted for the variance in allelic frequencies at the 7 loci studied when natural selection was not considered.

Recently, DNA sequence variation has been the focus of the analysis of genetic variation (for review, see Clegg and Epperson, 1985). This eliminates the complications of differential gene expression and post-translational changes in proteins and also permits analysis of DNA sequences not coding for proteins. Few studies of DNA sequence variation have been applied to the study of population structure (Clegg and Epperson, 1985). Analysis of DNA sequence variability is complex and time consuming and thus not readily suitable for studies of large numbers of individuals in a population.

The use of Type II restriction endonucleases (RE) has permitted the direct analysis of a portion of a DNA sequence for variation (for review, see, Wilson, 1988; Ewens et al., 1981). Each Type II RE cleaves double stranded DNA at sites which have a specific sequence of nucleotides (recognition sequences). The recognition sequences typically consist of 4, 5 or 6 nucleotide

base pairs. The RE cleave the DNA into fragments that can be isolated by size using gel electrophoresis. A DNA sequence, as a result, has a specific electrophoretic pattern of restriction fragment sizes. Mutations in the recognition sequence may cause gains or losses in the number of cut sites, resulting in restriction fragments of different sizes and subsequently different electrophoretic patterns or restriction fragment length polymorphisms (RFLPs). Deletions and/or insertions in the DNA sequence may also result in restriction fragments of different size. RFLPs are differences in restriction fragment size, when comparing homologous DNA sequences digested with a particular RE.

Although, at present the analysis of genomic DNA RFLP has not been used in studies of population structure, two DNA sequences appear very promising given the present technology. These are mitochondrial DNA (mtDNA) and Y chromosome-specific DNA sequences.

Analysis of mtDNA RFLPs has been extensive (for review, see, Brown, 1983), but application of mtDNA variability in studies of gene flow within populations and population structure has been limited (DeSalle et al., 1986). Avise et al. (1979) first described intraspecific mtDNA heterogeneity within populations of Peromyscus. Individuals from the same location showed less than 0.5% sequence divergence and individuals from conspecific populations separated by 50 to 500 miles differed by 1.5%. In Drosophila silvestris and Drosophila heteroneura, measures of population subdivision indicated 50-60 per cent of the observed mtDNA variability was due to interdemic subdivision (DeSalle et al., 1986).

Great potential exists for use of mtDNA sequence variability in studies of gene flow and population structure since mtDNA is rapidly evolving (Brown

et al., 1979). Hence much interspecific variation has been detected in a variety of organisms, including primates, mice and plants (Brown et al, 1982; Ferris et al., 1983b; Brown and Vinograd, 1974; Palmer, 1985). Intraspecific mtDNA variation has been detected in the genus Peromyscus (Avise et al., 1979) and in Drosophila silvestris and Drosophila heteroneura (DeSalle et al, 1986). MtDNA does not recombine with other genetic elements and is clonally transmitted to offspring. Most importantly to studies of population structure, mtDNA is maternally inherited and as such can be used to study matrilineal or female-mediated gene flow. Information obtained concerning population structure using mtDNA variation should complement previous studies of autosomal variants (for review, see, Moritz et al., 1987 and Lansman et al., 1981).

Complementary to the mtDNA is the DNA of the Y chromosome. The transmission of the Y chromosome is patrilineal and the major portion of the Y chromosome contains sequences that are unique to the Y chromosome (termed, Y-specific) and hence are clonally replicated. This component exists in a constant haploid or hemizygous state in males. A small portion of the Y chromosome contains sequences homologous to those of the X chromosome and undergoes meiotic recombination with it. Y-specific DNA represents the genomic analogue of mtDNA. Analysis of Y-specific DNA sequence variability should provide information pertaining to population structure through analysis of male-mediated gene flow.

Until recently, suitable Y-chromosome genetic markers were not available for the study of natural populations. The use of somatic cell hybridization (Bishop et al., 1983; Pritchard et al., 1987), flow cytometry (Baron et al., 1984; Bishop et al., 1985) and recombinant DNA techniques (Lamar

and Palmer, 1984) have permitted the isolation of Y-specific DNA and the development of appropriate probes. Such probes are being used in the analysis of Y-specific DNA RFLP.

Erickson (1987) has identified a human Y-specific sequence that was male-specific in humans and great apes. Another human Y-specific sequence was found to be present on the X chromosome and autosomes of the great apes and gibbon. A DNA sequence on human X and Y chromosomes was conserved in other primates, as were some RE sites (Erickson, 1987). Thus, analysis of primate DNA using human Y-specific probes can permit analysis of the evolutionary relationships among primates.

Casanova et al. (1985) detected Y-specific RFLPs in human populations and described the frequency of the Y-specific variants in Algerian, Sardinian and Northern European human males. Lucotte et al. (1989), using a highly polymorphic Y-specific probe, reported the existence of Y-specific DNA polymorphism in the human African populations of Bantus and Pygmies. This particular probe has identified 16 Caucasian Y-haplotypes (Ngo et al., 1986) and 2 Pygmy-specific Y-haplotypes (Lucotte et al., 1989). Thus, in human populations variation in Y-specific DNA sequences has been detected and analysis of human population structure and gene flow has begun.

Bishop et al. (1985) identified two Y chromosome haplotypes in inbred mouse strains. The two Y chromosome types represented interspecific variability in Y-specific DNA between the species Mus domesticus and Mus musculus. This finding was supported by the similar analyses of Lamar and Palmer (1984) and Nishioka and Lamothe (1986). In analysis of 6 species of Mus, Nishioka and Lamothe (1986 and 1987) identified further interspecific variation in Y-specific DNA. These mouse Y-specific sequences were also

detected in both male and female rats, indicating a lack of Y-specificity of these particular fragments in rats. These researchers failed to detect the mouse Y-specific sequences in hamster and guinea pig DNA, indicating a lack of conservation of these sequences in other rodents. Platt and Dewey (1987) also reported detection of interspecific variability in mouse Y-specific DNA sequences.

Singh et al. (1988) have identified a DNA sequence derived from the mouse Y chromosome which has detected hypervariability in the sex-determining region of the mouse Y chromosome. The DNA sequence of the probe consisted largely of repeats of the tetranucleotide GATA. Variability in Y-specific DNA was detected between 4 mouse species (Mus musculus, Mus domesticus, Mus poschiavinus and Mus spretus). In this investigation, individual mice of natural populations of the species Mus domesticus each had a distinct Y-specific DNA restriction fragment pattern.

Vanlerberghe et al. (1986) have demonstrated the combined use of autosomally-inherited electrophoretic variation, mtDNA RFLP and Y-specific DNA RFLP to describe gene flow. In their study, the gene flow described, occurred between two European mouse species in a zone of hybridization. Their analysis permitted description of exchange of autosomal traits between the two species and determination of the different contributions of female- and male-mediated migration to the total analysis of gene flow. Such a study is, in principle, applicable to the analysis of gene flow between the subgroups of a population in order to obtain information concerning population structure. However, mtDNA and Y-specific DNA variants within such a population must first be identified.

## E The Present Investigation

The present study was the second part of a two part analysis of genetic variation in natural house mouse populations. DNA samples were first screened for mtDNA RFLPs and then for Y-specific RFLPs since the time, effort and cost of the combined analysis was much less than that of two independent studies. The purpose of the present investigation was to gain information on population structure, through the identification of Y-specific DNA variation with a subsequent description of male-mediated gene flow. Specifically, natural house mouse (*Mus domesticus*) populations of southwestern Ontario were screened with a battery of 21 Type II restriction endonucleases and a mouse Y-specific DNA probe for Y-specific DNA RFLPs.

Evidence of Y-specific DNA variation was not detected. The implications of this finding will be discussed in light of some of the models that have been proposed to explain genetic variability involving Y-specific DNA. Finally, the insight that Y-specific DNA contributes to an understanding of structure in house mouse populations of southwestern Ontario, will be considered.

# II GENERAL METHODS

## A Source of Mice and Sampling Procedures

The mice (Mus domesticus) used in this study were collected from nine sampling regions located in three counties of southwestern Ontario: Essex, Kent and Elgin. These sampling sites (see Table 1 and Figure 1) are located east of the Detroit River between Lakes St. Clair and Erie. The samples were collected from 1983 to 1989. A total of 25 corn cribs located in the nine sampling regions were used in the present investigation.

Mice were collected during spring and summer months when corn cribs were being emptied. A hardware cloth barrier was set up about a metre from the concrete base of the corn crib and Sherman live-traps were placed within the enclosed area. This trapping procedure permitted the capture of over 75 percent of mice in a corn crib. Corn crib sites and sampling procedures have previously been described in detail by Hawkeswood (1975) and Topping (1975).

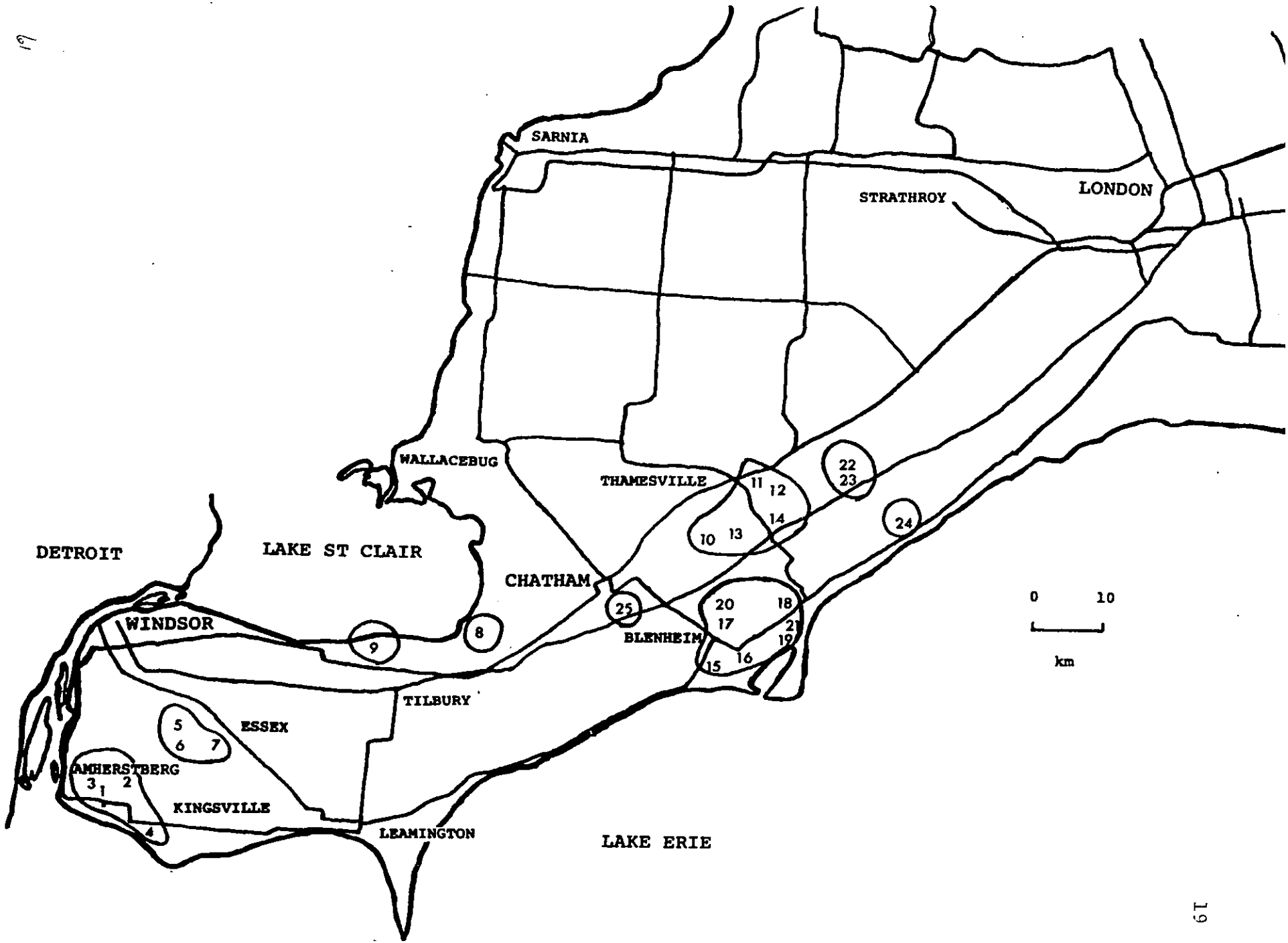
Following capture, all mice were brought to the laboratory, numbered, and a record of sex, age and collection site was made. Matings between males and females captured from the same crib were established to obtain offspring for use in the present investigation. Captured animals are referred to as "wild" mice and their offspring as "wild offspring". Four to seven male wild offspring from each wild male and female pair were required to obtain a sufficient quantity of DNA for the analysis of Y and mtDNA RFLPs with 21 restriction endonucleases.

Male and female mice of two inbred strains were also included in this investigation. The inbred mouse strains used, were C57Bl/6J and C3HeB/Fej (Bar Harbour, Maine).

Table 1. Regions and corn cribs sampled in southwestern Ontario

| Region | Corn Crib | No. |
|---|---|---|
| 1. Malden | Bondy | 1 |
| | Goolin | 2 |
| | Renaud | 3 |
| | Wright | 4 |
| 2. Colchester North | Farrough | 5 |
| | Rochleau | 6 |
| | McKim | 7 |
| 3. Tilbury | Baute | 8 |
| 4. Tilbury North | Houle | 9 |
| 5. McKay's Corners | Brown | 10 |
| | Cudmore | 11 |
| | Kegel | 12 |
| | Parsons | 13 |
| | Smoulder | 14 |
| 6. South of Ridgetown | Bokor | 15 |
| | Clendenning | 16 |
| | Demaitre | 17 |
| | McKinley | 18 |
| | Myslick | 19 |
| | Newcomb | 20 |
| | Rose | 21 |
| 7. Orford | Carniero | 22 |
| | Lucio | 23 |
| 8. Aldborough | Oscar | 24 |
| 9. Chatham East | Charron | 25 |

Figure 1. A map of southwestern Ontario showing the locations of the sampling sites. The numbers indicate the approximate locations of the corn cribs listed in Table 1. The circled areas indicate the nine regions listed in Table 1.


SARNIA
LONDON
STRATHROY
WALLACEBUG
THAMESVILLE
DETROIT
LAKE ST CLAIR
CHATHAM
WINDSOR
BLENHEIM
TILBURY
ESSEX
AMHERSTBERG
KINGSVILLE
LEAMINGTON
LAKE ERIE
1
2
3
4
5
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B Isolation of Genomic and Mitochondrial DNA

Food was removed from the mice at least 20 hrs prior to sacrifice. Mice were sacrificed by cervical spinal dislocation. The livers were removed, minced and homogenized at 4 C. For a sample representing each wild mouse, livers were removed from enough adult male wild offspring of that mouse to obtain about 4 g of tissue.

The liver samples were minced with scissors and a scalpel into small cube-shaped pieces. The tissue was added to cold homogenization buffer (10 ml/g tissue) in a 55 ml glass homogenizer. The homogenization buffer consisted of 1 part 1.5 M sucrose in TE (10 mM Tris, 1mM EDTA, pH 7.5) and 5 parts 10 mM NaCl in TE, pH 7.5 (Brown, personal communication). The sample was then briefly homogenized (4 passes with the homogenizer) using a power drill (1 000 rpm) and a teflon pestle (Wheton, Fisher). The homogenate was filtered through 2 layers of bleached cheesecloth into a 30 ml centrifuge tube (Nalgene).

The homogenate was centrifuged for 5 min at 1 200 x g and at 4 C (Sorvall RC2B JA 20, rotor SS 34). This centrifugation was performed four times. The pellets of the second and third centrifugation containing both nuclei and mitochondria were resuspended in digestion buffer and combined in a sterile Falcon tube (15 ml, Fisher). Following the fourth run, the supernatant was centrifuged once more at 23 000 x g for 20 min at 4 C. The pellet of the final high speed centrifugation was also resuspended in digestion buffer and transferred to a sterile Falcon tube (15 ml). Thus two types of samples were obtained, one from low speed centrifugation containing mitochondria and nuclei, the other from high speed centrifugation enriched for mitochondria but still contaminated with nuclei.

The digestion buffer (after Ausubel et al., 1989) used to degrade cellular proteins was prepared in two parts. Part A (100 mM NaCl, 10 mM Tris-HCl, pH 8 and 25 mM EDTA, pH 8) was used to resuspend and transfer pellets to Falcon tubes. Part B (6% sodium dodecyl sulfate and 1.2 mg/ml proteinase K, Bethesda Research Laboratories, in part A digestion buffer) was subsequently added to the resuspension. Pellets were resuspended with 1 ml of part A digestion buffer per 0.75 g of tissue extracted and 1 ml of part B digestion buffer was subsequently added per 0.25 g of tissue extracted. The samples were incubated in a 50 C waterbath for 28 hr with periodic agitation. Following digestion, the samples were stored overnight at 4 C.

Samples were placed in 1.5 ml microcentrifuge tubes in 700 ul aliquots prior to phenol extraction (Maniatus et al., 1982). Samples were extracted first with phenol (Terochem Laboratories, (TL)), then with a 1:1 mixture of phenol and chloroform (TL), and finally with chloroform. With each extraction samples were mixed until an emulsion formed (15 min) and then centrifuged for 10 min in an Eppendorf centrifuge at room temperature.

Following extraction with chloroform, 300 ul aliquots of the aqueous phase were placed in 1.5 ml microcentrifuge tubes and DNA was precipitated using a 0.5 volume of cold 7.5 M ammonium acetate (TL) and 2 volumes of ice cold 95% ethanol. Samples were stored in 95% ethanol at -20 C until needed.

Samples were centrifuged in an Eppendorf centrifuge for 15 min at 4 C to sediment DNA. The supernatant was decanted and the pellet was washed with 800 ul of 70% ice cold ethanol. After centrifugation for another 5 min at 4 C, the supernatant was again decanted and any traces of ethanol evaporated from the DNA pellet.

The DNA pellet was resuspended in 20 ul of TE containing 10 mg/ml bovine pancreas Ribonuclease A (Sigma). RNase digestion was carried out in a 37 C waterbath for at least 7 hr.

C Restriction Endonuclease Digestion of Genomic and Mitochondrial DNA

The Type II Restriction Endonucleases (RE) used in the present investigation and their recognition sequences are listed in Table 2. Hae II and Ban I were purchased from Terochem Laboratories. All other restriction endonucleases were purchased from Bethesda Research Laboratories (BRL). Immediately following RNase treatment, RE digestions were carried out employing temperature and buffer conditions recommended by the supplier. Overnight digests contained 0.5 ul of RE and 2 hr digests contained 1 ul of RE. Digestion was stopped with addition of Endo R Stop (ERS) Solution (BRL, 0.3 ul ERS/ul RE digestion mixture) and incubation at 65 C for 10 min. ERS contained 50% glycerol, 0.1 M Na2EDTA, 1% sodium dodecyl sulfate and 0.1% bromophenol blue. After cooling to 23 C, the samples were electrophoresed.

D Agarose Gel Electrophoresis

Electrophoresis of DNA fragments was performed at room temperature in horizontal 1.0 % agarose (BRL) gels (15 x 15 x 0.5 cm) in 1 X TBE buffer (89 mM Tris; 89 mM boric acid; 2 mM EDTA, pH 8.0). The gel solution contained ethidium bromide (Sigma) at a concentration of 1 mg/ml TE (pH 8.0). The electrophoresis was carried out at 20 V for 2 hr and subsequently at 60 V for 3 to 3.5 hr.

Table 2. Restriction endonucleases used in the present study and their recognition sequences

| Restriction Endonuclease | Recognition Sequence (5′-3′) | Base Cutter |
|---|---|---|
| Acc I | GT/(AC)(TG)AC | 6d |
| Alu I | AG/CT | 4 |
| Ava I | C/PyCGPuG | 6d |
| BamH I | G/GATCC | 6 |
| Ban I | G/GPyPuCC | 6d |
| Dra I | TTT/AAA | 6 |
| EcoR I | G/AATTC | 6 |
| EcoR V | GAT/ATC | 6 |
| Hae II | PuGCGC/Py | 6d |
| Hae III | GG/CC | 4 |
| Hinc II | GTPy/PuAC | 6d |
| Hind III | A/AGCTT | 6 |
| Hinf I | G/ANTC | 5d |
| Hpa II | C/CGG | 4 |
| Mbo I | /GATC | 4 |
| Msp I | C/CGG | 4 |
| Nsi I | ATGCA/T | 6 |
| Sca I | AGT/ACT | 6 |
| Taq I | T/CGA | 4 |
| Tha I | CG/CG | 4 |
| Xba I | T/CTAGA | 6 |

/ cut site
() either of 2 nucleotides
d degenerate
Py Pyrimidine
Pu Purine
N any nucleotide

E Detection of mtDNA Restriction Fragments

Following electrophoresis, the gel was placed on a UV transilluminator (Spectroline) and mtDNA fragments were photographed. A Polaroid MP-4 Land Camera, Wratten gelatin filter (No. 22, Kodak) and film Type 667 coaterless black and white Land film (Polaroid) were used to photograph the ethidium bromide stained mtDNA fragments.

F Southern Blotting

Immediately following the photography of the gel, the DNA fragments within the gel were Southern blotted (Southern, 1975; Ausubel et al., 1989) to a 0.4 u charge modified nylon membrane (Sigma). Depurination was carried out in 0.25 M HCl and denaturation was carried out in 0.5 M NaOH. The transfer solution was 20 X SSC (3 M NaCl and 0.3 M sodium citrate, pH 7.0) and the transfer proceeded overnight. The nylon membrane was wetted in distilled water and soaked in transfer solution just prior to use. Following Southern blotting and while still damp, the nylon membrane was placed over several layers of Whatman No. 1 filter paper saturated with distilled water and the DNA-containing side was UV-irradiated, for 2 min (UV) with a germicidal (General Electric) 30 W tube at a distance of 24 cm (Khandjian, 1987). The nylon membrane was then thoroughly rinsed in 2 X SSC. The membrane at this stage was either used immediately or dried between sheets of Whatman No. 1 filter paper and stored.

G YDNA Probe

A cloned mouse Y chromosome-derived sequence was used as a probe to detect Y-specific restriction fragments. The probe, 145SC5 (Nishioka, 1988), is a Y chromosomal repetitive sequence. At least 200 copies of 145SC5-related

sequences are contained on the mouse Y chromosome. The isolation of the mouse Y chromosomal sequence 145SC5 has previously been described in detail (Nishioka and Lamothe, 1986 and 1987; Nishioka, 1988). The 145SC5 DNA was received, precipitated in ethanol (Kindly provided by Dr. Y. Nishioka, McGill University).

The 145SC5 1.5 Kb EcoR I fragment ligated to the plasmid pUC 8 (Viera and Messing, 1982) was introduced into the JM107 strain of Escherichia coli (Kindly provided by Mr. A. Chatterjee, University of Windsor) by a calcium chloride transformation procedure (after Maniatus et al., 1982). Transformed colonies were selected for Ampicillin (50 ug/ml, Sigma) resistance. Plasmid DNA was isolated from cultures of a transformed colony (Maniatus et al., 1982). The 1.5 Kb EcoR I fragment was identified following electrophoresis of digested plasmid DNA in a 1.0 % agarose gel with ethidium bromide staining. Cultures of transformed colonies were stored in glycerol stocks (15 and 50 % glycerol) at -20 C and -70 C with and without dimethylsulphoxide (16 % DMSO). The insert was not isolated from the vector prior to probing genomic DNA for Y-specific restriction fragments.

H Preparation of Biotinylated YDNA Probes

Rapid, small scale isolation of plasmid DNA (2 ug) was carried out using a boiling method (after Maniatus et al., 1982). The alcohol precipitated DNA was dried, resuspended in TE containing RNase (10 mg/ml) and incubated at 37 C for 1.5 hr. After digestion with EcoR I, the insert and vector DNA fragments were identified following electrophoresis in a 1.0 % agarose gel. Probe DNA was phenol extracted and ethanol precipitated as described above for mouse DNA.

Probe DNA was resuspended in 37.5 ul TE (pH 8.0) in preparation for nick translation. The probe DNA was labelled with biotin-7-dATP (BRL) using a nick translation kit (BRL). Labelled probe DNA was stored in 95% ethanol at -20 C. The labelled probe DNA was ethanol precipitated to remove unincorporated nucleotides. After ethanol precipitation, the dry DNA pellet was resuspended in 100 ul hybridization solution and stored at 4 C in preparation for hybridization.

I Hybridization

Blots were prehybridized and hybridized using the conditions specified by the BluGENE Nonradioactive Nucleic Acid Detection System (BRL). Several modifications were necessary. Blots were prehybridized in 5 X SSC, 50 % formamide (BRL), 25 mM sodium phosphate (pH 6.5), 0.5 mg/ml freshly denatured sheared herring sperm DNA (Sigma) and 9.0 % powdered skim milk (Carnation). The prehybridization solution was prepared immediately prior to use, heated for 10 min at 95 C and immediately chilled on ice. The cold solution was added to the hybridization bag (BRL) containing the blot. Prehybridization was carried out for 6 hr at 42 C with agitation.

Hybridization was carried out overnight at 42 C with agitation. The hybridization solution contained 5 X SSC, 45 % formamide, 20 mM sodium phosphate (pH 6.5), 0.2 mg/ml freshly denatured sheared herring sperm DNA, 3 % powdered skim milk and 0.5 ug/ml freshly denatured probe DNA. The probe DNA was denatured by heating at 95 C for 10 min followed by cooling on ice. The hybridization solution was heated at 95 C for 10 min and then immediately placed on ice to denature the herring sperm DNA.

The hybridization solution was stored at 4 C and reused. The solution was discarded only when high background was detected on nylon membranes.

To minimize background on nylon membranes, formamide, 5 X SSC and the sodium phosphate solution were filter sterilized using a sterile 0.2 u cellulose acetate membrane and syringe filter (Nalgene) prior to inclusion in prehybridization and hybridization solutions.

Post hybridization washes of the blots were carried out as outlined in the BluGENE Kit. The final washes were performed at 65 C as suggested for nylon membranes (BRL).

J Detection of YDNA Restriction Fragments

The protocol for the detection of biotinylated probes was followed as outlined in the BluGENE Kit. Several modifications were necessary to minimize background on nylon membranes. All solutions in contact with blots were filtered using a 0.45 u cellulose acetate membrane (Nalgene). BluGENE Buffer 1 contained 0.25 M instead of the recommended 0.15 M NaCl.

The Streptavidin-Alkaline Phosphatase (SA-AP) conjugate was pretreated to remove material which bound nonspecifically to nylon membranes. A 5 x 15 cm strip of untreated nylon membrane was incubated with agitation in 10 ml prehybridization solution at 42 C for 6 hr. This membrane was then washed briefly in 5 X SSC. Diluted SA-AP solution (140 ml) was prepared as outlined in the BluGENE Kit and incubated with the prehybridized membrane for 4 hr at 42 C with agitation. The nylon membrane was discarded. The treated SA-AP solution was recovered and stored at 4 C.

Blots were incubated in the treated SA-AP solution for no more than 10 min. It was essential to wash the SA-AP solution from the blots using a 40-fold greater volume of Buffer 1 than the volume of the SA-AP solution suggested in the BluGENE protocol. This step was repeated once. Finally,

all blots were incubated in the NBT and BCIP dye solution for only 3 hr, otherwise the background became too dark.

## III GENERAL SURVEY FOR Y-SPECIFIC DNA SEQUENCE VARIATION

### A Combined Analysis of Mitochondrial and Y-Specific DNA

A collection of 21 Type II restriction endonucleases (RE) was used to screen each of the sampling regions for both mitochondrial and Y-specific DNA sequence variation. The mitochondrial DNA (mtDNA) restriction fragments were separated on the basis of size, using agarose gel electrophoresis and were observed using ethidium bromide staining. Y-specific DNA restriction fragments, in the same DNA sample, were then analyzed. DNA was Southern blotted to nylon membranes and biotinylated 145SC5 DNA was used to identify Y-specific DNA restriction fragments. The simultaneous screening for mt and Y-specific DNA restriction fragment length polymorphisms is novel. The combined analysis does not duplicate sample collection or DNA restriction fragment preparation. Thus, the time, effort and cost of the combined analysis is much less than that of two independent studies.

Examination of Y-specific DNA sequence variation complements that of mtDNA variation. Mouse mtDNA is clonally transmitted and maternally inherited. The inheritance of Y-specific DNA is holandric, that is, from father to son. The inheritence of the two DNAs provides two different perspectives of the analysis of genetic variability. Namely, mtDNA variability permits description of female-mediated gene flow and Y-specific DNA variability provides information concerning male-mediated gene flow. Simultaneous description of both Y-specific and mtDNA variation therefore, permits a comprehensive analysis of gene flow and complements the conclusions reached using autosomally inherited variation.

B Restriction Endonucleases

From previous surveys of mtDNA diversity, in which 6, 9, 11 and 18 RE (Avise et al., 1979; Yonekawa et al., 1980; Ferris et al., 1983b and Brown, 1980, respectively) were used, it seemed reasonable to employ a maximum of 21 RE in the present survey. The selection of the 21 different RE (see Table 2) was made on the basis of a number of criteria: (a) the availability and the low cost of each enzyme; (b) the ability of each RE to recognize a unique DNA sequence; (c) the representation in the battery of 4, 5 and 6 base recognition sequences as well as degenerate RE; (d) the previous detection of mouse mtDNA or Y-specific DNA sequence variation with particular enzymes; (e) the previous use of the RE with the YDNA probe 145SC5; (f) the ability of the RE to permit rapid analysis of mtDNA size variation and (g) the high REDI value of the RE in analysis of mouse mtDNA RFLP.

The enzymes, Alu I, BamH I, EcoR I, Hae III, Hind III and Msp I had detected interspecific sequence variation in Y-specific DNA of Mus domesticus and Mus musculus (Singh et al., 1988; Lamar and Palmer, 1984; Nishioka and Lamothe, 1986 and 1987; Vanlerberghe et al., 1986 and Bishop et al., 1985, respectively). Furthermore, Alu I has detected intraspecific variability in European wild caught mice of the species Mus domesticus (Singh et al., 1988). The enzymes, BamH I, EcoR I and Hind III have detected interspecific differences in mouse mtDNA sequence (Yonekawa et al., 1980). Ferris et al. (1983a) also reported detection of interspecific mouse mtDNA variation with the RE, Hinf I, Mbo I and Xba I. An extensive analysis of mtDNA evolution in eight species of Mus identified the RE, Hae III, Hinc II, Hinf I, Hpa II, Mbo I, Taq I and Xba I as capable of detecting interspecific variability

(Ferris et al., 1983b). EcoR I was the only enzyme for which Y-specific DNA sequences had been detected using the Y probe 145SC5 (Nishioka, 1988). This enzyme permitted the testing of southern blotting, hybridization and detection protocols. Ava I, EcoR V, Hae II, Hind III, Nsi I and Sca I were enzymes that were specifically chosen to permit rapid analysis of mtDNA size variation. Such enzymes produced mtDNA fragments (888 to 16,295 bp in size), all of which were detected with the resolution attained using 1% agarose gels.

Since the sequence of mouse mtDNA has been determined (Bibb et al., 1981), it is possible to predict the number of recognition sequences and the size of the DNA restriction fragments obtained with a particular RE (Nei & Li, 1979). Such a prediction is based upon the length of the DNA sequence, the guanine and cytosine content of the DNA sequence and the recognition sequence of the RE. Degeneracy is defined as the ability of a RE to recognize more than one recognition sequence (Smith, 1979). The Restriction Endonuclease Digestion Index (REDI, Sands and Petras, 1988) incorporates the degeneracy of a RE into a model similar to that of Nei and Li (1979). The REDI model predicts the ability of a RE to detect mutations. Two assumptions are made in the REDI model: (a) there is a random distribution of all nucleotides in the DNA sequence and (b) there is an equal probability of single transition or transversion events occurring within a recognition site. REDI values range from 0 to 1. The higher the REDI value, the more effective the RE is at detecting mutations. The REDI test was used to select restriction endonucleases having high REDI values (see Table 3) for the analysis of mtDNA sequence variation. The REDI test was not used to select RE appropriate for the analysis of Y-specific DNA variability. Too little is known of the sequence of mouse YDNA to permit use of the REDI model.

Table 3. The REDI values calculated for the 21 restriction endonucleases used in the present study.

| Enzyme | Recognition Sequence (5′-3′) | Base Cutter | REDI value |
|---|---|---|---|
| Acc I | GT/(AC)(TG)AC | 6d | 0.2187 |
| Alu I | AGCT | 4 | 0.2440 |
| Ava I | C/PyCGPuG | 6d | 0.2070 |
| BamH I | GGATCC | 6 | 0.1870 |
| Ban I | G/GPyPuCC | 6d | 0.2070 |
| Dra I | TTT/AAA | 6 | 0.2089 |
| EcoR I | G/AATTC | 6 | 0.1977 |
| EcoR V | GAT/ATC | 6 | 0.1977 |
| Hae II | PuGCGC/Py | 6d | 0.2070 |
| Hae III | GG/CC | 4 | 0.2317 |
| Hinc II | GTPy/PuAC | 6d | 0.2187 |
| Hind III | A/AGCTT | 6 | 0.1977 |
| Hinf I | G/ANTC | 5d | 0.2442 |
| Hpa II | C/CGG | 4 | 0.2317 |
| Mbo I | /GATC | 4 | 0.2440 |
| Msp I | C/CGG | 4 | 0.2317 |
| Nsi I | ATGCA/T | 6 | 0.1977 |
| Sca I | AGT/ACT | 6 | 0.1977 |
| Taq I | T/CGA | 4 | 0.2440 |
| Tha I | CG/CG | 4 | 0.2317 |
| Xba I | T/CTAGA | 6 | 0.1977 |

/ cut site
() either of 2 nucleotides
d degenerate
Py pyrimidine
Pu purine
N any nucleotide

Methylation of mouse YDNA has been indicated by the different cleavage patterns obtained using the RE, Hpa II and Msp I (Nishioka and Lamothe, 1986). The usual target dinucleotide of restriction modification methylation is CpG. Hpa II and Msp I are isoschizomers, that is, they have the same recognition sequence (5′ CCGG 3′). Hpa II does not cleave if the 3′ cytosine of its recognition sequence is 5-methylcytosine or either cytosine is 4-methylcytosine. Msp I has the same recognition sequence as Hpa II, but cleaves when the 3′ cytosine is 5-methylcytosine but not when the 5′cytosine is 5-methylcytosine. These two RE were chosen to permit analysis of methylation of YDNA near sequences homologous to 145SC5 DNA.

The ability of the RE to identify their recognition sequences is affected by methylation of bases within their recognition sequences. The overall effect of this is a reduction in the total amount of DNA sequence analyzed. The effects of methylation on the ability of the individual RE to detect their recognition sequence(s) are summarized in Table 4 (adapted from the BRL catalog).

C Animals

Female inbred (C57BL/6J and C3HeB/FeJ) mice were included in this analysis to verify the Y-specificity of the probe. Male inbred (C3HeB/FeJ) mice were included in the survey to permit a comparison between inbred and wild mouse Y-specific DNA restriction fragments.

A description of the wild-caught animals used in this survey is presented in Table 5. Animals, captured over a 7 year sampling period, were included in this analysis. The wild mouse samples were chosen to represent as many different corn crib populations and sampling regions as possible. Animals captured from nonrelease sites were preferred in this analysis. Release sites

Table 4. The methylation specificity of the 21 restriction endonucleases used in the present analysis.

| Enzyme | Recognition Sequence (5′-3′) | Methylation Specificity (N6-methyladenine and 5-methylcytosine) |
|---|---|---|
| Acc I | GT/(AC)(TG)AC | 3′ A |
| Alu I | AGCT | A or C |
| Ava I | C/PyCGPuG | 3′ C |
| BamH I | GGATCC | 5′ C |
| Ban I | G/GPyPuCC | not known |
| Dra I | TTT/AAA | not known |
| EcoR I | G/AATTC | 3′ A or C |
| EcoR V | GAT/ATC | 5′ A |
| Hae II | PuGCGC/Py | 5′ C |
| Hae III | GG/CC | 5′ C |
| Hinc II | GTPy/PuAC | 3′ A |
| Hind III | A/AGCTT | 5′ A or C |
| Hinf I | G/ANTC | 5′ A |
| Hpa II | C/CGG | 3′ C 5mC or 4mC |
| Mbo I | /GATC | A |
| Msp I | C/CGG | 5′ C |
| Nsi I | ATGCA/T | not known |
| Sca I | AGT/ACT | not known |
| Taq I | T/CGA | A |
| Tha I | CG/CG | C |
| Xba I | T/CTAGA | 3′ A or C |

/ cut site
() either of 2 nucleotides
Py pyrimidine
Pu purine
N any nucleotide
5mC 5-methylcytosine
4mC 5-methylcytosine

Table 5. Wild caught male mice screened for Y-specific DNA sequence variation

| Animal No. | Sampling Year | Crib | Release Site | Sampling Region | No. of Enzymes Used |
|---|---|---|---|---|---|
| 713 | 83 | Houle | N | 4 | 11 |
| 287/286 | 84 | McKim | N | 2 | 7 |
| 65/67,68 | 85 | Rochleau | N | 2 | 14 |
| 161/173 | 85 | Goolin | N | 1 | 14 |
| 248 | 85 | Renaud | N | 1 | 2 |
| 275 | 85 | Oscar | N | 8 | 4 |
| 312/318/325 | 85 | McKinley | N | 6 | 5 |
| 360 | 85 | Clendenning | N | 6 | 13 |
| 72 | 86 | Rochleau | Y | 2 | 3 |
| 174 | 86 | Rose | N | 6 | 7 |
| 373 | 86 | Bokor | N | 6 | 9 |
| 451/487/489/498/433 | 86 | Farrough | N | 2 | 17 |
| 501/502/519 | 86 | Baute | N | 3 | 11 |
| 714 | 86 | Rose | N | 6 | 1 |
| 24/29 | 87 | McKim | Y | 2 | 4 |
| 144/156 | 87 | Parsons | N | 5 | 6 |
| 177/181 | 87 | Brown | N | 5 | 9 |
| 183 | 87 | Lucio | N | 7 | 11 |
| 341/360/365/370 | 87 | Demaitre | N | 6 | 9 |
| 421/430 | 87 | Newcomb | N | 6 | 12 |
| 490/668 | 87 | Smoulder | N | 5 | 8 |
| UK | 88 | Myslick | N | 6 | 2 |
| UK | 88 | McKim | Y | 2 | 2 |
| 9/21 | 88 | Rochleau | Y | 2 | 4 |
| 49/52/59/61/65 | 88 | McKim | Y | 2 | 8 |
| 86/104 | 88 | Wright | N | 1 | 10 |
| 108/113 | 88 | Bondy | N | 1 | 16 |
| 156/280/307 | 88 | Parsons | N | 5 | 3 |
| 172 | 88 | Lucio | Y | 7 | 3 |
| 194 | 88 | Wright | N | 1 | 2 |
| 217 | 88 | Carniero | Y | 7 | 2 |
| 329/335/343/353 | 88 | Myslick | Y | 6 | 5 |
| 553 | 88 | Newcomb | Y | 6 | 1 |
| 397 | 88 | Charron | N | 9 | 2 |
| 421/426 | 88 | Kegel | N | 5 | 11 |
| 507 | 88 | Cudmore | N | 5 | 2 |
| 659 | 88 | McKim | Y | 2 | 3 |
| 668/677/702 | 88 | Rose | Y | 6 | 4 |
| 484 | 88 | McKinley | N | 6 | 1 |
| UK | 89 | Charron | N | 9 | 1 |
| 91 | 89 | Parsons | N | 5 | 1 |

/ more than one sample
, either of 2 males in a single sample
UK wildoffspring of captured pregnant female

(see Table 6) were those areas into which wild male mice captured from other regions and inbred female mice had been introduced in previous years for other mouse population studies (Sands, in preparation). Such sites were avoided if possible in the present survey to prevent screening of inbred mtDNA unnecessarily and to prevent confusion as to the source of Y-specific DNA variation if it was detected.

The majority of the screening (with at least 10 RE) involved animals from 11 different cribs. Individual mice were generally not screened with the entire battery of 21 RE. Often, enough wildoffspring were not available to extract DNA in sufficient quantity for the screening of 21 RE. Finally, the animals used in this investigation were selected to eventually permit the identification of Y-specific DNA variation within corn crib populations, between cribs of the same region, between sampling regions and over different sampling years.

D Y-Specific DNA Probe

Table 7 lists DNA sequences derived from the Y chromosome of the mouse and available for use as probes in studies of YDNA variation. Several criteria were established prior to the selection of a suitable Y-specific DNA probe. The most important characteristic of the probe was its ability to bind to male-specific DNA sequences, i.e., sequences unique to the Y chromosome. Secondly, a Y-specific DNA sequence was sought that was highly repetitive, in order to permit the detection of many Y-specific DNA restriction fragments. Finally, a probe was sought that would bind to several regions of the Y chromosome. It was hoped that the analysis of several regions of the Y chromosome would reduce the probability of analyzing a single conserved region of the Y chromosome.

Table 6. Corn cribs and fields into which foreign mice had been released in previous studies.

| Site Name | Region | Year of Release (month) | |
|---|---|---|---|
| | | Corn Crib | Field |
| Baute | 3 | 86(5), 86(11) | 87(9), 88(9) |
| Carniero | 7 | 87(10) | 88(7) |
| Clendenning | 6 | 85(11) | 86(7) |
| Goolin | 1 | 85(11) | 86(11), 87(9), 88(9) |
| Lucio | 7 | 87(10) | 88(7) |
| McKim | 2 | 86(10) | 87(9), 88(9) |
| Newcomb | 6 | | 87(6) |
| Renaud | 1 | 85(11) | 86(6), 86(7) |
| Rochleau | 2 | 85(11) | |
| Rose | 6 | 86(11), 87(6) | 87(9), 88(9) |
| Smoulder | 5 | | 87(7) |

Table 7. Mouse YDNA sequences available for use as probes.

| YDNA clone | Reference |
|---|---|
| AC11 (ACC1, ACC2, ACC3) | Nishioka and Lamothe (1986 & 1987) |
| 145SC5 | Nishioka (1988) |
| pDP1171 | Page et al. (1987) |
| pY353/B | Bishop et al. (1985)<br>Vanlerberghe et al. (1986) |
| pY1, pY2, pY3 | Lamar and Palmer (1984) |
| M720, pBM19, pErs5A | Eicher et al. (1983) |

Ultimately, the availability of a Y-specific DNA probe was critical and limited the choice of probes to three YDNA sequences. pDP1171 was kindly provided by Dr. D. Page, Whitehead Institute for Biomedical Research, Cambridge. This probe is a Balb/C mouse genomic 5 Kb EcoR I fragment cloned into the EcoRI site of the vector, pBR322 (Page et al., 1987). It was derived from the mouse Y chromosome. pDP1171 detects 2 distinct Y-linked loci, Zfy-1 and Zfy-2 and an X-linked locus, Zfx. Thus pDP1171 cannot be used as a Y-specific probe. The sequences to which it hybridizes are also not highly repetitive. In fact, the 2 copies of the sequence are both located in the testis determining region of the mouse Y chromosome. It was felt that this probe was not suitable for an efficient analysis of Y-specific DNA polymorphism.

The Y chromosomal clone AC11 was kindly provided by Dr. Nishioka, McGill University, Montreal. AC11 is a mouse Y chromosomal clone that detects Y-specific sequences which are highly repetitive (300 copies on the mouse Y chromosome; Nishioka and Lamothe, 1986). AC11 also contains a banded krait minor satellite (Bkm) DNA sequence (for a description see, Singh et al., 1984) that will bind to both male and female DNAs. AC11 must be restriction endonuclease digested with both BamH I and Taq I and the fragment ACC1 isolated to remove the Bkm sequence and obtain a Y-specific probe (Nishioka and Lamothe, 1987).

The Y chromosomal clone 145SC5 (Nishioka, 1988) was chosen for use in this analysis because it had two key characteristics. The probe 145SC5 has been shown to detect Y-specific sequences and it hybridizes to a repetitive YDNA sequence present in 200 copies on the long arm of the mouse Y chromosome (Nishioka, 1988). The DNA sequence of the clone 145SC5 and the

function of the homologous repetitive YDNA sequences is not known. No YDNA RFLP analysis had previously been performed using this mouse YDNA sequence, so no information was available on the detection of variation in the recognition sequences involved in the present study. Finally, the clone 145SC5 could be used as a probe without prior isolation of the mouse YDNA sequence from the vector pUC 8.

E Nonradioactive Detection System

The potential health hazards, disposal problems and instability associated with radioisotopes were reasons for the selection of a nonradioactive detection system in the present study. The BluGENE detection system detects biotin-labeled probes using a streptavidin-alkaline phosphatase conjugate (for review, see Gebeyehu et al., 1987).

The enzyme, alkaline phosphatase is covalently linked to streptavidin. Streptavidin is a tetrameric protein that binds noncovalently to the vitamin biotin with high affinity and near irreversibility. The high affinity of the binding is the result of multiple hydrogen bonds and van der Waals interactions with surface polypeptide loops that bury the biotin molecule in the protein interior (Weber et al., 1989). Biotin-7-dATP is a nucleotide analog, synthesized by attachment to the amino-nitrogen at the hydrogen bonding position of the base (i.e., 6-position) of an aliphatic linker, specifically, (CH2)6, to which the biotin is bound (Gebeyehu et al., 1987). Biotin can be conjugated to amino groups by reaction with the biotin ester, Biotinyl-N-hydroxysuccinimide (Gebeyehu et al., 1987). Nick translation of double stranded DNA (greater than 1000 bp) includes incubation with DNase I, E. coli DNA polymerase I and a deoxynucleotide mixture containing one labeled nucleotide (Biotin-7-dATP). Probe-target hybrids are visualized due to the formation of a coloured

precipitate resulting from the reaction of alkaline phosphatase and the substrate, 5-bromo-4-chloro-3-indoylphosphate (BCIP) and the dye, nitroblue tetrazolium (NBT).

Biotin-labelled probes are stable at -20 C for at least 1 year. For Southern blotting, 0.25 pg of DNA can be detected in less than one hour with maximum signal development occurring in only 3 h. The sensitivity of this detection system is sufficient for detection of single copy genes in a Southern blot of mammalian DNA (Gebeyehu et al., 1987). The entire detection procedure is rapid, with results available in two days.

F Results of the General Screening for Y-Specific DNA Sequence Variation

Hybridization of 145SC5 DNA to female inbred mouse DNA was not detected. The probe was found to hybridize to restriction fragments of male mice only. Thus, only Y-specific restriction fragments were detected.

For 8 RE (Acc I, Alu I, EcoR I, Hae III, Hind III, Mbo I, Nsi I and Tha I) no differences in cleavage patterns were detected between wild caught and inbred males. Screening of inbred male mice with the remaining 13 RE was not performed.

Table 8 summarizes the results of the general survey for YDNA variability in wild mice of southwestern Ontario using the probe 145SC5 and 21 different restriction endonucleases. The 21 RE failed to detect Y-specific DNA variability in comparisons of at least 8 and as many as 38 different wild caught males. Not all animals, cribs or regions were screened with the entire battery of 21 RE. The screening performed in each of the nine sampling regions is summarized in Table 9 and the number of cribs screened within the 9 sampling regions is also indicated.

Table 8. The results of the general screening for Y-specific DNA variability in wild mice of southwestern Ontario using the probe 1455C5 and 21 restriction endonucleases.

| Restriction Endonuclease | No. of Wild Males | Inbred Animals | No. of Males from Nonrelease Cribs | No. of Males from Release Cribs | No. of Cribs | No. of Regions | No. of Sampling Years | No. of Y-Specific DNA Fragments Observed | Variation Observed |
|---|---|---|---|---|---|---|---|---|---|
| Acc I | 9 | M/F | 9 | 0 | 8 | 5 | 5 | 8 | None |
| Alu I | 9 | M/F | 9 | 0 | 6 | 5 | 5 | 1 | None |
| Ava I | 11 | F | 3 | 8 | 6 | 3 | 4 | 1 | None |
| BamH I | 11 | F | 9 | 2 | 9 | 5 | 4 | 4 | None |
| Ban I | 13 | F | 13 | 0 | 8 | 4 | 4 | 8 | None |
| Dra I | 19 | 2F | 19 | 0 | 14 | 6 | 4 | 1 | None |
| EcoR I | 38 | 2F/M | 24 | 14 | 19 | 7 | 6 | 5 | None |
| EcoR V | 17 | F | 16 | 1 | 14 | 7 | 5 | 7 | None |
| Hae II | 20 | 2F | 20 | 0 | 16 | 8 | 6 | 1 | None |
| Hae III | 20 | 2F/1M | 20 | 0 | 16 | 8 | 6 | 1 | None |
| Hinc II | 15 | 2F | 13 | 2 | 11 | 5 | 5 | 3 | None |
| Hind III | 8 | 2F/2M | 7 | 1 | 7 | 6 | 4 | 1 | None |
| Hinf I | 9 | F | 9 | 0 | 7 | 4 | 4 | 2 | None |
| Hpa II | 10 | F | 10 | 0 | 9 | 5 | 4 | 1 | None |
| Mbo I | 9 | M | 9 | 0 | 7 | 5 | 5 | 2 | None |
| Msp I | 11 | F | 9 | 2 | 10 | 5 | 4 | 2 | None |
| Nsi I | 11 | F/M | 11 | 0 | 11 | 6 | 5 | 6 | None |
| Sca I | 18 | 2F | 18 | 0 | 13 | 5 | 4 | 2 | None |
| Taq I | 36 | F | 28 | 8 | 17 | 6 | 6 | 5 | None |
| Tha I | 15 | 2F/M | 9 | 6 | 7 | 6 | 4 | 1 | None |
| Xba I | 20 | 2F | 19 | 1 | 15 | 7 | 5 | 2 | None |

F female
M male

Table 9. The number of males and corn cribs from 9 different sampling regions screened with 21 restriction endonucleases.

| Enzyme | Sampling Region No. (No. of Cribs) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1*(4)** | 2(3) | 3 | 4 | 5(5) | 6(7) | 7(2) | 8 | 9 |
| Acc I | 3(2)*** | 2(2) | 1 | 1 | | 2(2) | | | |
| Alu I | 3(2) | 2 | 1 | 1 | | 2 | | | |
| Ava I | 1 | 6(2) | | | | 4(3) | | | |
| BamH I | 4(3) | 3(2) | | 1 | 1 | 2(2) | | | |
| Ban I | 2(2) | 1 | | | 4(2) | 6(3) | | | |
| Dra I | 3(2) | 4(2) | 1 | | 3(3) | 7(5) | 1 | | |
| EcoR I | 4(3) | 14(3) | 3 | 1 | 4(4) | 9(5) | 3(2) | | |
| EcoR V | 2(2) | 3(2) | 1 | 1 | 3(3) | 5(4) | 2 | | |
| Hae II | 3(3) | 4(3) | 1 | 1 | 3(3) | 6(3) | 1 | 1 | |
| Hae III | 3(3) | 6(3) | 1 | 1 | 4(3) | 3(3) | 1 | | 1 |
| Hinc II | 4(3) | 6(3) | | 1 | 1 | 3(3) | | | |
| Hind III | 1 | 1 | 2 | | 1 | 2(2) | 1 | | |
| Hinf I | | | | 1 | 3(3) | 3(2) | 2 | | |
| Hpa II | 3(2) | 1 | | | 1 | 4(4) | 1 | | |
| Mbo I | 3(2) | 2 | 1 | 1 | | 2(2) | | | |
| Msp I | 1 | 4(3) | | | 1 | 4(4) | 1 | | |
| Nsi I | 3(3) | 3(3) | 1 | | 1 | 2(2) | | 1 | |
| Sca I | 4(3) | 3(2) | | | 2(2) | 8(5) | 1 | | |
| Taq I | 3(3) | 8(3) | | | 8(4) | 14(5) | 1 | | 2 |
| Tha I | 2 | 6(2) | 3 | | 1 | 2(1) | 1 | | |
| Xba I | 3(2) | 4(3) | | 1 | 4(4) | 6(3) | 1 | 1 | |

* Names of Sampling Regions.

| | | |
|---|---|---|
| 1 Malden | 4 Tilbury North | 7 Orford |
| 2 Colchester North | 5 McKay's Corners | 8 Aldborough |
| 3 Tilbury | 6 South of Ridgetown | 9 Chatham East |

** Total number of cribs in the region. Where no number is given only one crib was sampled.

*** Number of cribs screened in the region. Where no number is given only one crib was sampled.

The Y-specific restriction fragments observed following digestion of genomic DNA with each of the 21 RE are listed in Table 10 and illustrated in Figure 2. A total of 64, Y-specific DNA restriction fragments were observed. Thus, a maximum of 128 recognition sequences were analyzed with the battery of RE, assuming that each restriction fragment was the product of two recognition sites of the RE and the fragments themselves, were distantly arranged from one another on the Y chromosome and therefore did not share common recognition sequences. The RE, Acc I and Ban I have degenerate 6 base recognition sequences and 8 fragments, the highest number of Y-specific sequences detected in this study.

The different cleavage patterns observed with the two RE, Hpa II and Msp I, indicate the presence of methylation of YDNA in the vicinity of 145SC5-related sequences on the mouse Y chromosome. Hpa II digested genomic DNA produced a smear of Y-specific DNA fragments that were 9 to 23 Kb in size. This pattern was similar to that of undigested genomic DNA probed with 145SC5 DNA. Two distinct bands were detected with the RE, Msp I.

G Technical Difficulties

The 0.45 u charge modified nylon membranes, in one particular lot supplied by Sigma Chemical Co., were unable to allow efficient binding of bovine serum albumin to block protein binding sites. This defect in the membrane caused nonspecific binding of the streptavidin-alkaline phosphatase conjugate to the nylon membrane and darkening of the entire membrane occurred.

The BluGENE Detection System Instruction Manual (Bethesda Research Laboratories) contains a typing error. The 10 min incubation of the nylon membrane with the streptavidin-alkaline phosphatase conjugate is not repeated

Table 10. The number of Y-specific DNA restriction fragments detected using the Y-specific probe 145SC5 and 21 restriction endonucleases.

| Enzyme | Recognition Sequence (5′-3′) | Base Cutter | No. of Y-specific DNA fragments observed |
|---|---|---|---|
| Acc I | GT/(AC)(TG)AC | 6d | 8 |
| Alu I | AGCT | 4 | 1 |
| Ava I | C/PyCGPuG | 6d | 1 |
| BamH I | GGATCC | 6 | 4 |
| Ban I | G/GPyPuCC | 6d | 8 |
| Dra I | TTT/AAA | 6 | 1 |
| EcoR I | G/AATTC | 6 | 5 |
| EcoR V | GAT/ATC | 6 | 7 |
| Hae II | PuGCGC/Py | 6d | 1 |
| Hae III | GG/CC | 4 | 1 |
| Hinc II | GTPy/PuAC | 6d | 3 |
| Hind III | A/AGCTT | 6 | 1 |
| Hinf I | G/ANTC | 5d | 2 |
| Hpa II | C/CGG | 4 | 1 |
| Mbo I | /GATC | 4 | 2 |
| Msp I | C/CGG | 4 | 2 |
| Nsi I | ATGCA/T | 6 | 6 |
| Sca I | AGT/ACT | 6 | 2 |
| Taq I | T/CGA | 4 | 5 |
| Tha I | CG/CG | 4 | 1 |
| Xba I | T/CTAGA | 6 | 2 |

/ cut site
() either of 2 nucleotides
d degenerate
Py pyrimidine
Pu purine
N any nucleotide

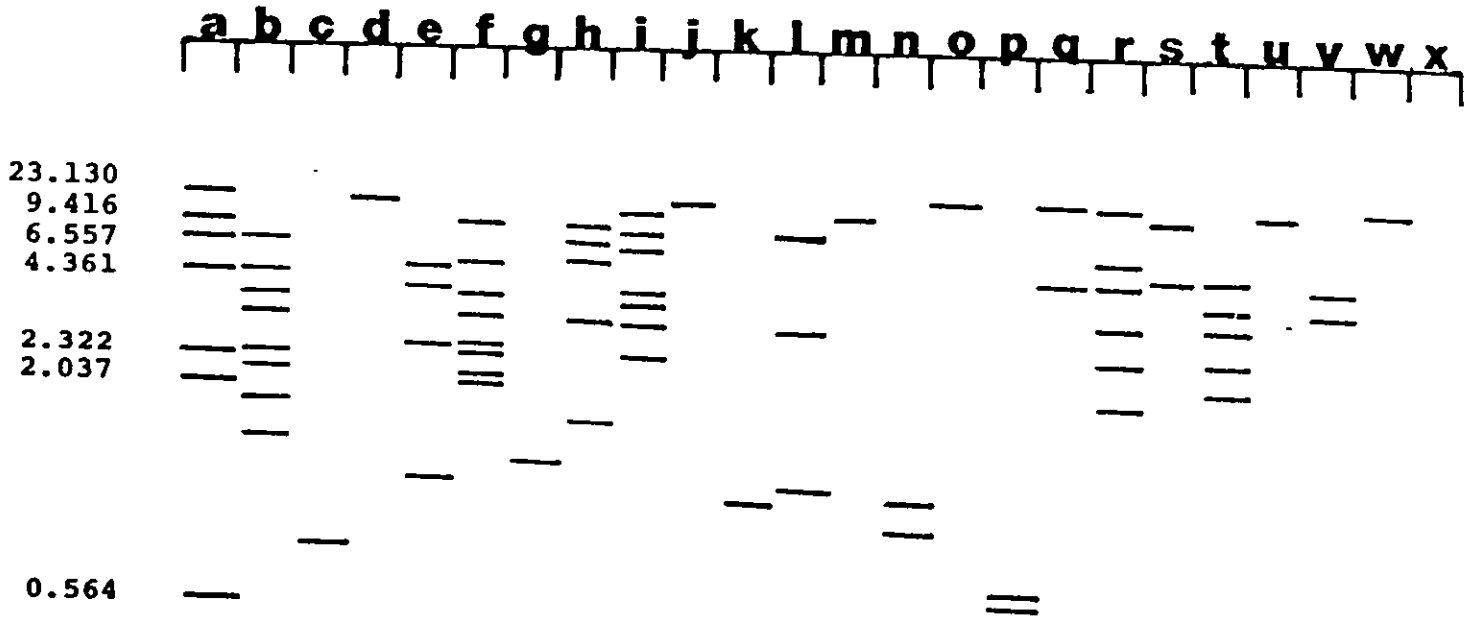


Figure 2. Diagrammatic representation of the 21 restriction endonuclease digestion phenotypes of wild mouse genomic DNA probed with 1458C5 DNA. The lanes are marked with the letters a through x. Lane a represents the lambda Hind III size standards (Kb). Lanes b through v represent genomic DNA of male mice digested with the 21 restriction endonucleases (arranged in alphabetical order from left to right). Refer to Table 10 for a listing of the restriction endonucleases. Lane w represents undigested genomic DNA of a male mouse. Lane x represents female mouse genomic DNA.

for an additional 10 min with fresh conjugate solution. The result of this error is the same as that obtained with the use of the defective nylon membranes.

Finally, the streptavidin-alkaline phosphatase conjugate solution cannot be used as received but instead must be treated prior to use to remove contaminants which bind nonspecifically to the entire nylon membrane. Again, the results of the use of untreated conjugate were darkening of the entire membrane.

The defective nylon membranes, the BluGENE Detection System, the Nick Translation System, Biotin-7-dATP and hybridization bags involved in these difficulties were all replaced by their suppliers at no cost.

# IV DISCUSSION AND CONCLUSIONS

## A Y-Specific DNA Variability and Natural Populations

The present investigation was in fact the second part of a two part analysis of genetic variation in natural house mouse populations. DNA samples were first screened for mtDNA RFLPs and then for Y-specific RFLPs, since the time, effort and cost of the combined analysis was much less than that of two independent studies. Natural house mouse (Mus domesticus) populations of southwestern Ontario were screened with a battery of 21 Type II restriction endonucleases (RE) and a mouse Y-specific probe for Y-specific DNA restriction fragment length polymorphism (RFLP). The purpose of the present investigation was to obtain additional information about the structure of natural house mouse populations.

Y-specific DNA sequence variation was not detected in natural house mouse populations of southwestern Ontario. Either of two conditions may explain this finding. First, considerable variability in the Y-specific DNA sequences of the mouse populations may exist but was not detected due to insufficient screening. Second, Y-specific DNA variability may be rare in the mouse populations analyzed.

It is unlikely that the battery of RE used in this study would not facilitate the detection of DNA sequence variability for three reasons. First, RE that had detected Y-specific DNA sequence variation in previous studies were included in the battery. Second, the number of RE chosen was consistent with previous RFLP analyses in which interspecific and intraspecific DNA sequence variation was detected (Avise et al., 1979; Yonekawa et al., 1980; Ferris et al., 1983b and Brown, 1980). Third, unique recognition sequences were identified by each of the RE chosen.

The enzymes, Alu I, BamH I, EcoR I, Hae III, Hind III and Msp I detected interspecific sequence variation in Y-specific DNA of mice of the species Mus domesticus and Mus musculus (Singh et al., 1988; Lamar and Palmer, 1984; Nishioka and Lamothe, 1986 and 1987; Vanlerberghe et al., 1987 and Bishop et al., 1985, respectively). This variation was detected in many inbred mouse strains (Lamar and Palmer, 1984; Nishioka and Lamothe, 1986 and 1987; Bishop et al., 1985) and in wild-caught mice of northern Italy (Singh et al., 1988), Denmark and Bulgaria (Vanlerberghe et al., 1987). Intraspecific variability in Y-specific DNA of wild caught mice (Mus domesticus) of northern Italy has been identified using Alu I (Singh et al., 1988). Recently, Taq I and Msp I have detected Y-specific DNA sequence variation in wild caught mice (Mus domesticus) of the Montreal, Quebec region (Nishioka, personal communication, 1989).

The number of recognition sequences analyzed in this study was consistent with previous RFLP analyses in which inter- and intraspecific mtDNA sequence variation was detected. In the present study, a total of 64 Y-specific DNA restriction fragments were observed and hence a maximum of 128 recognition sequences were analyzed. This assumes that each restriction fragment was the product of two recognition sequences at either end of the fragment, that the fragments themselves were distantly arranged from one another on the Y chromosome and thus did not share common recognition sequences. Gyllensten and Wilson (1987) analyzed 162 cleavage sites using 11 RE. Brown (1980) analyzed 217 cleavage sites, using 7 RE having 4 base recognition sequences. Ferris et al. (1983b) screened 300 cleavage sites with 11 RE and Ferris et al. (1983a) analyzed 76 cleavage sites with 3 RE. These studies detected interspecific mtDNA variability in humans and mice. Avise

et al. (1979) screened 36 cleavage sites with 6 RE and detected intraspecific mtDNA variability in populations of the genus Peromyscus.

The mouse Y chromosomal clone 145SC5 (Nishioka, 1988) is appropriate for the identification of intraspecific variation in Y-specific DNA sequences. It detects Y-specific DNA sequences, present in 200 copies located along the length of the long arm of the mouse Y chromosome. Recently, using this same probe and the restriction endonucleases Taq I and Msp I, in an analysis of 6 mice captured from a barn in Montreal, Quebec, Nishioka (personnal communication, 1989) has identified polymorphisms in the Y-specific DNA sequences. Thus, 145SC5 DNA has detected intraspecific DNA sequence variation in natural house mouse populations.

The screening approach employed in the present project should have been sufficient for the identification of common Y-specific DNA variants. At least 8 individuals from different sampling regions were analyzed with each of the 21 RE. This screening generally involved 11 different crib populations, from 7 different sampling regions (sampling regions 1-7, see Table 1 and Figure 1). The 11 crib populations were screened with at least 10 RE. The greatest geographic distance between cribs that were screened in the present study was 110 km. It is possible to calculate the probability of missing a variant at a particular recognition sequence. For 10 RE used in this study, with a maximum of 56 recognition sequences, if the frequency of a variant is as low as 0.30 and if 15 mice were tested, the probability of missing that variant is on the order of 0.005. So the absence of a common Y-specific DNA sequence variant does not appear to be due to sampling problems.

A second explanation for the lack of detection of Y-specific DNA sequence variation is the low frequency of such variability. Two unique characteristics of Y-specificity may be involved. 1) The constant haploid state and associated lack of recombination may be important. Intragenic recombination results in new combinations of DNA sequences not found in parental genomes and is a source of new alleles. Therefore, reduced variability of Y-specific DNA sequences may in part be due to the lack of recombination between Y-specific and other DNA sequences. 2) The haploid state of Y-specific DNA may be important because of the effects of natural selection on Y-linked variability. Loss of new neutral mutations in a large population and similarly in many small populations having high levels of gene flow is also a possible situation resulting in the low frequency of Y-specific DNA sequence variation.

B Y-linked Polymorphism and Natural Selection

Population models involving haploid systems and selection have been well developed (for review, see, Hedrick, 1985). Since there are many similarities between strictly Y-linked alleles and the alleles of haploids the dynamics of strictly Y-linked allelic frequencies can best be described in terms of haploid selection models. The major difference between the two is that Y-linked loci are found only in males and haploid loci are found in all individuals of a population. The frequency of a Y-linked allele in a population is therefore identical to the proportion of male genotypes in the population containing the allele.

Population models, in which it is assumed that the Y chromosome does not recombine with other DNA sequences and two alleles coexist at one locus in a panmictic population, permit analysis of the conditions required to

maintain Y-linked polymorphism in the presence of selective forces. The Y chromosome is hemizygous and a lethal allele would be expressed phenotypically and eliminated from the population in one generation. An allele with reduced fitness (but not lethal) would also be rapidly eliminated from the population over time. The rate of loss of the allele is a function of its initial frequency and the selection coefficient (a measure of the selective pressure). In these simple, constant fitness models (Clark, 1987), strictly Y-linked polymorphisms cannot be maintained.

Clark (1987) described four more complex population models involving selection that focused particularly on conditions required to maintain Y-linked polymorphism. The first model considered Y-specific traits in which many types of selection including viability, segregation distortion, fecundity and sexual selection were all determined by a single Y-linked locus. In this model, the Y chromosome did not recombine with any other genetic element. Stable Y-linked polymorphism could not be maintained under the conditions of this model.

The second model described interactions between homologous segments of the X and Y chromosomes. Different combinations of the two X chromosomes and the two Y chromosomes were considered. Each resulting genotype had a specified viability. However, again, no conditions could be described that would permit the existence of a stable Y-linked polymorphism under the conditions of this model.

In the third model, X-and Y-linked loci influenced meiotic drive. In this model the only component of selection was the differential production of gametes by heterozygous females or males. Again, stable Y-linked polymorphism could not be achieved.

In the fourth selection model described by Clark (1987), both viability and meiotic drive differed among genotypes. This model allowed both viability and meiotic drive to be determined by a pair of alleles at a homologous locus on the X and Y chromosomes. Only when viability and meiotic drive were jointly affected by both X- and Y-linked genes could a Y-linked polymorphism be maintained.

Clark (1987) performed numerical simulations, including 100,000 trials on models 2 through 4. The numerical simulations were a measure of the plausibility of the models relevance. For the fourth model, 100,000 sets of viability and meiotic drive parameters were tested and populations were simulated until 20,000 generations or a stable equilibrium was attained. Stable Y-linked polymorphism occurred in less than 2% of the trials. It is apparent that the conditions necessary for maintenance of Y-linked polymorphism by natural selection as expressed in Clark's models (1987) are stringent and unlikely to be satisfied by many random combinations of parameters. Therefore combinations of different viabilities and meiotic drives rarely result in a stable Y-linked polymorphism. If Y-linked polymorphism is maintained by natural selection then it cannot be explained with these constant fitness models.

Interaction between a Y-linked locus and a locus that confers differences in viabilities permits the existence of stable Y-linked polymorphism (Clark, unpublished). A possible example has been reported by Singh et al. (1988). Hypervariability in DNA sequences of the sex-determining region of the mouse Y chromosome was detected using a probe that consisted of repeats of the tetranucleotide GATA (Singh and Jones, 1982; Singh et al., 1988). The dominant autosomal sex-determining locus called T-associated sex reversal (Tas) on

chromosome 17 has also been shown to contain GATA repeats (Keil-Metzger and Erickson, 1984). The Tas locus is closely linked to the T complex (Washburn and Eicher, 1983) which is associated with differences in viability.

Haldane and Jayakar (1964) described a model in which selective pressures varied over time. In diploids a situation can exist where there is a tendancy to maintain polymorphisms, that is a protected polymorphism. In this situation both alleles will increase in frequency when rare and will not be lost as a result of random fluctuations in fitnesses. Protected polymorphism occurs when there is heterozygote advantage. Such a situation is not possible in haploids or Y-linked alleles and thus the maintenance of polymorphism in haploids or Y-linked alleles is not possible with temporal variation in fitness.

Maintenance of Y-linked polymorphisms by natural selection could involve frequency-dependent fitness (Hedrick, 1985). Frequency-dependent selection describes a situation in which genotypic frequencies dictate the magnitude of selective pressures. In models of frequency-dependent selection that lead to a balanced polymorphism, the relative fitnesses of the genotypes are usually some inverse function of their frequencies in the population. That is, if an allele is rare the genotype containing that allele would have a higher fitness than genotypes involving more common alleles (for review, see, Ayala and Campbell, 1974).

Geographic structuring of fitness, that is, spatial variation in selective pressures, may also maintain polymorphism in a haploid population. Gliddon and Strobeck (1975) used Levene's model (Levene, 1953) of population structure to describe the conditions required for the maintenance of a stable polymorphism in a hypothetical haploid population. Levene's model describes

a discontinuous but random-mating population, the subgroups of which are located in different niches and are subjected to different selective pressures within each niche. Individuals of such a population mate randomly then distribute to separate niches and are affected by the selective pressures of that niche. Such spatial variation in selective pressures can hence maintain Y-specific DNA polymorphisms.

Reports of Y-specific variation in mouse populations are rare. Y-unique sequences were not analyzed in the study of Singh et al., (1988). So the variability in that study may involve homologous loci on the Y chromosome and chromosome 17. Geographic variation in fitness values may be operating in some European populations and may also explain the results obtained by Singh et al. (1988). Nishioka (personal communication, 1989) has identified Y-specific variability in natural mouse populations. This variability is being studied to see how it relates to the above-mentioned models.

In the present investigation, Y-specific variation was not detected in natural house mouse populations. This finding is consistant with temporal variation in fitnesses and with the various constant fitness models which indicate that stable Y-linked polymorphism cannot be maintained. This lack of detected variation is expected because of the hemizygosity of Y-linked alleles. The findings would be unexpected if frequency-dependent selection or geographic variation in fitness exist. Such conditions should be able to maintain Y-specific DNA variation. There is no evidence, however, for such conditions operating on Y-specific traits in natural mouse populations of southwestern Ontario.

C Autosomal Variation and Population Structure

Another possible explanation for the present findings is the loss of new neutral mutations in large populations or small populations with high levels of gene flow. The plausibility of these two situations can be assessed from current knowledge of population structuring obtained from previous analyses of natural house mouse populations.

Natural populations of Mus domesticus which inhabit corn cribs offer many advantages for the study of population structure (for a review, see Reimer, 1965; Hawkeswood, 1975) but it has been difficult to assess if mice inhabiting a crib represent a single, random-mating population or whether population subdivision exists. The cribs represent discrete areas of optimal habitat that have capacity for large population sizes and can be carefully monitored. The cribs are filled with dried corn (Zea mays) and thus provide food, cover and nesting material for mice. The corn crib mouse populations are discontinously distributed and are replicated several hundred times across southwestern Ontario. However, in corn crib mouse populations, it is difficult to directly obtain information regarding the size and composition of the founder population and the formation, size and number of subgroups within a single crib population. Thus direct assessment of the population strucutre is impeded.

The possible dynamics of corn crib mouse populations have been described (Hawkeswood, 1975). Corn is placed in the cribs in late September and early October. Mice from fields enter the crib, since food and cover are lacking in the fields. These immigrants form founder populations. Breeding resumes in the spring and the population sizes increase. Corn is removed from cribs between May and August. Mice leave the cribs at this time and enter the fields where survivorship is low, probably due to the lack of adequate

cover. This series of events is repeated annually.

Evidence suggesting population structuring exists in natural house mouse populations has been obtained using a variety of indirect techniques (Crowcroft, 1955; Anderson, 1964; Reimer, 1965; Lidicker, 1976). Monitoring of animal position in population cages suggests the formation of small breeding units or demes (Reimer, 1965). A deme appeared to be composed of a single dominant male, several females and several subordinate males. Young males remained as subordinates or were forced to leave a territory. Dominance shifts were also observed. Males fiercely defended territories if space was a limiting resource as in a population cage (Reimer, 1965) but not so fiercely in the larger barrel system (Hawkeswood, 1975; Philpott, 1982). Some female territory defence was observed in the population cage studies (Reimer, 1965). Physical migration between established demes was observed in the population cage studies. Demes were monitored weekly and 4 of the 24 females in the population cage system moved to new territories within 136 days of initiating the study (Reimer, 1965). Introduction of mice into established demes resulted in 91% of foreign males and 72% of foreign females being killed (Reimer, 1965). Thus, established demes have been described as stable and generally genetically isolated with possible genetic exchange occurring via limited female-mediated migration.

Hawkeswood (1975) also observed deme formation in corn-filled interconnected barrels, a system which simulated the corn crib environment. Steel drums (200 L) interconnected with poly-vinyl chloride tubing were one-third filled with corn. The barrrels were kept in a barn and mice were introduced. Strange males introduced into established demes were killed by deme members. Demes formed in the barrel system were highly isolated,

forming non-overlapping territories. Evidence indicating the participation of females in the formation and protection of territories was not obtained (Hawkeswood, 1975). Therefore, under somewhat artificial conditions, there exists evidence of a behavioral basis for population structuring in natural house mouse populations.

Physical migration of mice inhabiting barns and cribs has been described in mouse populations with high population densities. Trapping studies carried out in fields around corn cribs filled with corn resulted in the capture of significantly more males than females. Trapping carried out in barns resulted in the capture of significantly more females than males. Also, fewer males than females have been captured upon emptying cribs with high population densities (Hawkeswood, 1975). These findings are consistent with the exodus of males from defended territories within barns and corn cribs (Reimer, 1965).

Trap-and-release studies (Reimer, 1965) permitted estimation of physical migration of mice on a farm site. An average of 2.9 feet was travelled between recaptures in a corn crib. An average of 18.6 feet and a maximum of 400 feet was travelled between recaptures within a barn. Limited movement (7% of released mice recaptured) between buildings and fields was observed. Other estimates of physical migration rates have been obtained: 5%, Petrusezicz and Andrzejewski, (1962); 2%, Evans, (1949); 17%, Petras (1965). Brown (1953) has reported a lack of detected movement of mice between buildings and the surrounding fields. These data indicate limited dispersal of house mice and thus restricted gene flow resulting in population subdivision. All of these studies have been done in an undisturbed situation. This may be drastically changed when the habitat is disturbed, as in the case of corn crib emptying.

The analysis of electrophoretic biochemical variants produced indirect evidence for population subdivision within natural mouse populations. Reimer (1965), in southwestern Ontario, found a deficiency of heterozygotes at both the hemoglobin and esterase-2 loci. The inbreeding coefficient estimated for these two loci ranged from 0.12 to 0.20. An effective deme size of 9 to 23 mice was estimated using Wright's population models, the estimated inbreeding coefficient and an estimated migration rate of 0.07. These findings were best explained through population structuring, when considering territoriality and limited dispersal of mice.

Petras (1967a) analyzed allelic frequencies at both the hemoglobin and esterase-2 loci in natural mouse populations in Michigan. A numerical deficiency of heterozygotes was discovered at both loci. The inbreeding coefficients obtained from analysis of these two loci were in the range of 0.06 to 0.30. Wright's models of population structure were used to estimate the effective size of the demes and a range of 6 to 80 individuals was obtained. The deficiency was best explained by population structuring. Petras (1967a) described the structuring of mouse populations on three levels, specifically, within buildings, between buildings of the same farm and between farm complexes.

Hoeg (1978) analyzed 7 polymorphic biochemical loci controlling renal enzymes (isocitrate dehydrogenase, malic enzyme, glutamate oxaloacetate transaminase, dipeptidase-1, glucose-6-phosphate dehydrogenase and two loci controlling phosphoglucomutase). The study involved corn crib mouse populations. At 5 of the 7 polymorphic loci, numerical deficiencies of heterozygotes were detected. Heterogeneity in allelic frequencies between crib populations was also evident. These findings were consistent with the

existence of population subdivision. However, Hoeg (1978) found a slight correlation (at the 5 percent level of significance) between geographic distance and genetic divergence. This suggested that random genetic drift in the discontinuous populations was being counteracted by other factors such as selection and/or migration. Hoeg (1978) obtained an estimate of the migration rate of 0.2981 based upon a deme size of 10, to account for the variability in allelic frequencies observed at the 7 loci under consideration. This estimate of the migration rate is an overestimate if selective pressures are also counteracting the effects of genetic drift.

Hawkeswood (1975) analyzed allelic frequencies for the hemoglobin and glucose phosphate isomerase loci in corn crib mouse populations and found no significant deviation from Hardy-Weinberg expectations. Pooled allelic frequencies involving isolated corn crib populations were also in agreement with Hardy-Weinberg expectations (Hawkeswood, 1975). This suggested either random mating in the absence of selection or the existence of population subdivision with heterosis.

Baker (1981) introduced individual mice (Mus domesticus) carrying an unusual hemoglobin allele into a natural mouse population to obtain a direct estimate of gene flow between mouse populations inhabiting chicken coops. Females carrying the variant were introduced at an initial frequency of 5% of a total chicken coop population. The variant was found to spread to neighboring populations within two years. The rapid spread of the introduced allele through the entire farm population indicated that gene flow rather than genetic drift was a major factor affecting gene frequency.

D General Population Models

The pioneering work of Wright (1951) used the F statistic or inbreeding coefficient to measure the increase in homozygosity as a result of a departure from random mating. It is thus a measure of subdivision in a population. If F is greater than 0, subgroups exist within a population, as opposed to one large panmictic population. Wright also described F in terms of the effective population size of a panmictic unit (Ne) and the degree of gene flow (m).

Wright's island model describes a discontinuous population subdivided into many small, randomly breeding units isolated from one another by distinct barriers. When m is small:

$$F \tilde{=} 1/(4Nem + 1)$$

If m = 0, in the case of complete isolation of subgroups, then F approaches one. F is equal to 0 if the population is not subdivided but is panmictic.

In the isolation by distance model (Wright, 1951), a continuous population is described. No distinct barriers exist between subgroups. The population has uniform density and random mating is limited by the average distance of dispersal (determined by density and availability of suitable habitat). In this model:

$$F \approx 1 - [1 - (1 - m)^2]1/Ne$$

The inbreeding coefficient can be calculated directly from allelic frequencies:

$$F = (He - Ho)/He$$

Where He is the number of heterozygotes expected in a panmictic population and Ho is the number of heterozygotes observed. The F value is a quantitative

measure of population structuring calculated from allelic frequencies. If the F value is calculated from allelic frequencies and the value of m is available from estimates of dispersal, Ne may be calculated. In both models of population structure the degree of inbreeding (F) and the effective subgroup size (Ne) are inversely related.

Values of F, m and Ne have been obtained for natural house mouse populations using both of Wright's models. Petras (1965) determined the effective deme size to be 6 to 80 individuals using the island model and 8 to 20 individuals, using the isolation by distance model. A range of values was obtained in each case because a range of values for physical migration rate (0.02 to 0.20) has been reported in previous studies. Petras (1967a) also determined values for the inbreeding coefficient in his study to range between 0.06 and 0.30. F estimates can however be distorted by selection operating on the genotype involved. As a result Ne and/or m can be biased. Such values describe small breeding units with limited gene flow between units.

Estimates of the effective population size for corn crib populations have been obtained by several methods. Corn cribs, emptied after only 90 days of being filled with corn, permit capture of the founder population and description of its size and composition (age and sex of mice). The effective population size is determined largely by the size of the founder population and less so by the size of the population in each subsequent generation. Philpott (1982) described the founder population of 6 corn cribs. A lower estimate of the size of the founder population (5) was the mean number of mice estimated to be ninety days of age or more based upon analysis of eye lens weight. An upper estimate of 10 was based upon the mean number

of all the mice caught from the 6 cribs emptied in February. The estimate of the effective population size in corn cribs has also been obtained by analyzing the total number of mice captured from cribs emptied in the summer months (Petras and Topping, 1983). For instance, an average of 60 mice was captured from such cribs and if two generations are assumed to have occurred since the crib was initially populated in the previous fall and assuming an average litter size of four and maturation age of 8 weeks, the upper estimate of the effective population size in a crib is on the order of 14 individuals. Such estimates are upper limits since all mice may not contribute equally to the gene pool, founders may be related, and more than two generations may have occurred. If there is more than one breeding unit or deme in a crib, the Ne of that unit may be considerably smaller. The estimates of Ne derived from census of animals in cribs are similar to values of Ne calculated from estimates of inbreeding and migration rates.

Hoeg (1978) suggested the best model of population structure for of corn crib mouse populations is a discontinuous two-dimensional stepping-stone model (Kimura and Weiss, 1964). This model permits incorporation of different geographic distances between cribs, making it more realistic than Wright's island model. The stepping-stone model suggests that geographic variation in gene frequency should increase with distance. Hoeg (1978) however, did not observe this in the analyses of several loci. The migration rate estimates were not lower for regions separated by greater distance as expected from the model (Hoeg, 1978). The effects of genetic drift may be counteracted by the forces of selection and/or gene flow.

## E X-Linked loci and Population Structure

Generally, description of natural house mouse populations has involved analysis of autosomally inherited variants (Reimer, 1965; Petras, 1967; Hawkeswood, 1975 and Hoeg, 1978) but information obtained from the analysis of X-linked variants complements that provided by autosomal loci. Screening for electrophoretic X-linked variants has been performed in 1408 mice from 46 locations in southwestern Ontario, using 4 loci (phosphoglycerate kinase, glucose-6-phosphate dehydrogenase, alpha-galactosidase and ornithine transcarbamylase loci) but failed to identify genetic variability (Sands and Petras, 1986). Similar findings in other mouse populations have been obtained for the X-linked hypoxanthine phosphoribosyl transferase locus (Chapman, personal communication). Similar studies of 4, 126 and 1124 X-linked loci in kangaroo, Drosophila and human populations, respectively, have revealed levels of genetic variation (18.2%, 23% and 8.1% of X-linked loci were polymorphic, respectively) comparable to that reported for autosomal loci (Cooper et al., 1979). The X-linked variants described in human populations have, however, generally been associated with disease states (McKusick, 1975) but this does not appear to be the case in kangaroo and Drosophila populations. A lack of X-linked variability appears to be unique to mouse populations.

In mouse populations inhabiting corn cribs, population subdivision and numerical bottlenecks enhance genetic drift. It has been shown that the effective population size for X-linked alleles is smaller than that for autosomal alleles (Charlesworth et al., 1987) because females contain two-thirds and males one-third of X-linked alleles. In contrast, Ne for autosomal alleles is equal to the total population size N and Ne for X-linked alleles is equal to 2/3 N (assuming the sex ratio is 1:1). This suggests that variability should

be greater at X-linked loci than at autosomal loci.

It has been demonstrated with simple deterministic models that maintenance of polymorphism is more difficult for alleles controlled by X-linked loci than for autosomally inherited alleles (Pamilo, 1979). The hemizygosity of males permits the direct effect of selection to operate upon X-linked alleles. Selection has been suggested as the prime agent responsible for the lack of detectable X-linked enzymatic loci variability (Sands and Petras, 1986).

F Mitochondrial DNA and Population Structure

Analysis of mtDNA variability in natural house mouse populations also complements information obtained from autosomal variants. As well, the maternal inheritence of mtDNA permits the description of matrilineal affiliations of mice within and between populations (Avise et al., 1979; Vanlerberge et al, 1987). Analysis of mtDNA sequence variability is currently in progress for house mouse corn crib populations of southwestern Ontario (Sands, in preparation). That investigation has screened the mice used in the present study with the same 21 RE and detected mtDNA polymorphism with only one RE (Hinf I).

G Y-Specific DNA and Population Structure

The maintenance of Y-specific DNA sequence variation can also be analyzed using population models. If a sex ratio of 1:1 is assumed then *Ne* is equal to (1/2)*N*. Thus, for Y-linked alleles, the effective population size is smaller than that for X-linked and autosomal alleles and the potential for genetic divergence among populations is greater for Y-linked alleles. However this is not consistant with the absence of Y-specific DNA variability

detected in natural house mouse populations.

The loss of new neutral mutations from a population could explain a lack of detected variability (for review, see, Hedrick, 1985). Population size is important in determining whether a new single muatation will be lost from a population. There is a greater probability that a single new mutation will be lost from a larger population than from a smaller one. An absence of Y-specific variability is consistant with the loss of new neutral mutations from a population of large size. Also, new neutral mutations may be lost from many small populations having high migration rates. However, considering the structuring of house mouse populations and the small effective population size of Y-linked alleles, one would expect that occasionally a new mutant would become established in such a population due to genetic drift. Such variability was not detected in the present investigation.

Singh et al. (1988) have identified Y-specific DNA sequence variation in wild caught mice (Mus domesticus). The Y-specific DNA sequences of mice trapped in Germany, Switzerland and Italy were each distinct. Reduced gene flow due to great geographic separation and discontinuity in habitats between mouse populations may account for the Y-specific DNA variation detected. The mouse populations analyzed in the present investigation were not separated by such great geographic distances. Singh et al. (1988) also detected distinct Y-specific DNA sequences in five mice trapped in 5 different locations in northern Italy. The Y-specific DNA variability in the Italian mouse populations may be maintained by the presence of environmental barriers to gene flow. The 5 sampling sites of Singh et al. (1988) were situated in the plains and alps of northern Italy. Differences in the habitats of plains and alps of northern Italy may bring in differences in selection. Southwestern

Ontario, unlike northern Italy is geographically homogeneous. As well, the habitats of the present study were very similar across the sampling region. Gene flow between mouse populations of southwestern Ontario is thus not restricted by the major environmental barriers proposed in maintaining the Y-specific DNA variation identified by Singh et al. (1988).

Nishioka (personal communication, 1989) has identified Y-specific DNA sequence variation in 6 mice trapped in a single barn in Montreal, Quebec. It is not known if this polymorphism is common or rare in Quebec mouse populations. One possible source of Y-specific DNA variation, more likely to occur in the Montreal region as opposed to that of southwestern Ontario, is the frequent introduction of foreign mice from the extensive shipping industry in the Montreal region. Further study of the mouse populations of Quebec and Ontario are necessary to fully understand the apparent differences in Mus domesticus Y-specific DNA sequence variation between these two geographic locations.

In summary, previous studies of natural house mouse populations have provided evidence supporting a behavioural basis for population structuring in artificial populations and indirect evidence supporting population structuring in natural house mouse populations. In the present study, the lack of Y-specific DNA variability detected could be explained by the loss of new neutral mutations in large populations or in many small populations having high levels of gene flow. However, considering the small effective population size for Y-linked alleles and the structuring of house mouse populations it seems likely that occasional variants would become established because of genetic drift. The lack of detection of Y-specific DNA variability is perhaps best explained by the direct effects of natural selection upon the hemizygous Y-

linked alleles.

In apparent contradiction with the present findings and the effects of natural selection, Y-specific DNA variability has been identified in mice of the Montreal, Quebec region. This variability could be due to a recent mutation and may not have reached an equilibrium situation. Further analysis of this variability is essential to fully understand, the differences between Quebec and southwestern Ontario house mouse populations and the role of natural selection in the maintenance of Y-specific DNA polymorphism.

# V SUMMARY

1. The present investigation was the second part of a two part analysis of genetic variation in natural house mouse populations. DNA samples were first screened for mtDNA RFLPs and then for Y-specific RFLPs.

2. Mice (Mus domesticus) were collected during a 7 year sampling period from 25 corn cribs located throughout southwestern Ontario.

3. Natural house mouse populations were screened for Y-specific restriction fragment length polymorphisms using 21 Type II restriction endonucleases, a biotinylated Y-chromosomal clone (145SC5) and a nonradioactive detection system.

4. Hybridization of the Y chromosomal clone to female inbred mouse DNA was not detected.

5. The Y-specific restriction fragment patterns for 8 different restriction endonucleases were identical in inbred and wild caught mice.

6. The different cleavage patterns observed with the two restriction endonucleases, Hpa II and Msp I, indicate the presence of methylation of YDNA in the vicinity of 145SC5-related sequences on the mouse Y chromosome.

7. Y-specific DNA sequence variation was not detected in natural house mouse populations of southwestern Ontario.

8. It was unlikely, considering the number and type of restriction endonucleases, the choice of probe and the animals screened, that the present investigation was not capable of detecting common Y-specific DNA sequence variation.

9. Low frequency of variation in Y-specific DNA sequences may result from the loss of new neutral mutations in large populations or in small

populations having high levels of gene flow. However, considering the population structuring of these house mouse populations and the small effective population size for Y-linked alleles, it would seem likely that occasionally, new neutral mutations would become established in the populations due to genetic drift. Yet, Y-specific DNA sequence variation was not detected in these populations.

10. Y-specific DNA sequence variation may be in low frequency in natural populations due to the enhanced susceptibility of hemizygous DNA sequences to the effects of natural selection. This hypothesis is supported by several constant fitness models of natural selection for Y-linked alleles.

11. Recently, Y-specific DNA variability was identified in mice of the Montreal, Quebec region. This finding is in apparent contradiction with, the results of the present study and the predictions of constant fitness models of natural selection for Y-linked alleles. This variability could be due to a recent mutation and may not have reached an equilibrium state.

# APPENDIX I

## ANIMALS SCREENED FOR Y-SPECIFIC DNA VARIATION

ANIMALS SCREENED FOR YDNA RFLP'S USING THE Y PROBE 145SC5 AND 21 DIFFERENT RESTRICTION ENDONUCLEASES

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| ACC I | 83 | 713 | HOULE | N | 4 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 85 | 65 | ROCHLEAU | N | 2 |
| | 86 | 433 | FARROUGH | N | 2 |
| | 86 | 501 | BAUTE | N | 3 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 88 | 113 | BONDY | N | 1 |
| | INBRED M | | | | |
| | INBRED F | | | | |
| ALU I | 83 | 713 | HOULE | N | 4 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 86 | 501 | BAUTE | N | 3 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 86 | 433 | FARROUGH | N | 2 |
| | 87 | 421 | NEWCOMB | N | 6 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 88 | 113 | BONDY | N | 1 |
| | INBRED M | | | | |
| | INBRED F | | | | |
| AVA I | 84 | 287 | MCKIM | N | 2 |
| | 86 | 872 | ROCHLEAU | Y | 2 |
| | 87 | 421 | NEWCOMB | N | 6 |
| | 88 | 659 | MCKIM | Y | 2 |
| | 88 | 329 | MYSLICK | Y | 6 |
| | 88 | 677 | ROSE | Y | 6 |
| | 88 | 65 | MCKIM | Y | 2 |
| | 88 | UK | MCKIM | Y | 2 |
| | 88 | 668 | ROSE | Y | 6 |
| | 88 | 108 | BONDY G D | N | 1 |
| | 88 | 21 | ROCHLEAU | Y | 2 |
| | INBRED F | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| BAMH I | 83 | 713 | HOULE | N | 4 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 86 | 373 | BOKOR | N | 6 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 88 | 108 | BONDY G D | N | 1 |
| | 88 | 86 | WRIGHT | N | 1 |
| | 88 | 421 | KEGEL | N | 5 |
| | 88 | 113 | BONDY | N | 1 |
| | 88 | 49 | MCKIM | Y | 2 |
| | 88 | 65 | MCKIM | Y | 2 |
| | INBRED F | | | | |
| BAN I | 85 | 312 | MCKINLEY | N | 6 |
| | 85 | 248 | RENAUD | N | 1 |
| | 85 | 325 | MCKINLEY | N | 6 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 87 | 365,370 | DEMAITRE | N | 6 |
| | 87 | 490 | SMOULDER | N | 5 |
| | 87 | 144 | PARSONS | N | 5 |
| | 88 | 194 | WRIGHT | N | 1 |
| | 88 | 280 | PARSONS | N | 5 |
| | 88 | 329 | MYSLICK | N | 6 |
| | 88 | 343 | MYSLICK | N | 6 |
| | 88 | UK | MYSLICK | N | 6 |
| | 88 | 307 | PARSONS | N | 5 |
| | INBRED F | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| DRA I | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 67,68 | ROCHLEAU | N | 2 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 85 | 65 | ROCHLEAU | N | 2 |
| | 86 | 501 | BAUTE | N | 3 |
| | 86 | 373 | BOKOR | N | 6 |
| | 86 | 174 | ROSE | N | 6 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 87 | 370 | DEMAITRE | N | 6 |
| | 87 | 156 | PARSONS | N | 5 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 177 | BROWN | N | 5 |
| | 87 | 360 | DEMAITRE | N | 6 |
| | 87 | 341 | DEMAITRE | N | 6 |
| | 88 | 421 | KEGEL | N | 5 |
| | 88 | 113 | BONDY | N | 1 |
| | 2 INBRED F | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| ECOR I | | | | | |
| | 83 | 713 | HOULE | N | 4 |
| | 84 | 287 | MCKIM | N | 2 |
| | 85 | 67,68 | ROCHLEAU | N | 2 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 86 | 072 | ROCHLEAU | Y | 2 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 86 | 489 | FARROUGH | N | 2 |
| | 86 | 501 | BAUTE | N | 3 |
| | 86 | 502 | BAUTE | N | 3 |
| | 86 | 519 | BAUTE | N | 3 |
| | 87 | 668 | SMOULDER | N | 5 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 87 | 421 | NEWCOMB | N | 6 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 156 | PARSONS | N | 5 |
| | 87 | 360 | DEMAITRE | N | 6 |
| | 87 | 365,370 | DEMAITRE | N | 6 |
| | 87 | 29 | MCKIM | Y | 2 |
| | 87 | 341 | DEMAITRE | N | 6 |
| | 87 | 181 | BROWN | N | 5 |
| | 88 | 217 | CARNIERO | Y | 7 |
| | 88 | 61 | MCKIM | Y | 2 |
| | 88 | 59 | MCKIM | Y | 2 |
| | 88 | 52 | MCKIM | Y | 2 |
| | 88 | 9 | ROCHLEAU | Y | 2 |
| | 88 | 104 | WRIGHT | N | 1 |
| | 88 | 65 | MCKIM | Y | 2 |
| | 88 | 172 | LUCIO | Y | 7 |
| | 88 | 329 | MYSLICK | N | 6 |
| | 88 | 659 | MCKIM | Y | 2 |
| | 88 | 677 | ROSE | Y | 6 |
| | 88 | 421 | KEGEL | N | 5 |
| | 88 | 668 | ROSE | Y | 6 |
| | 88 | 108 | BONDY G D | N | 1 |
| | 88 | 21 | ROCHLEAU | Y | 2 |
| | 88 | UK | MCKIM | Y | 2 |
| | INBRED M | | | | |
| | 2 INBRED F | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| ECOR V | 83 | 713 | HOULE | N | 4 |
| | 85 | 65 | ROCHLEAU | N | 2 |
| | 85 | 67,68 | ROCHLEAU | N | 2 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 86 | 373 | BOKOR | N | 6 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 86 | 174 | ROSE | N | 6 |
| | 86 | 501 | BAUTE | N | 3 |
| | 87 | 668 | SMOULDER | N | 5 |
| | 87 | 421 | NEWCOMB | N | 6 |
| | 87 | 177 | BROWN | N | 5 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 88 | 108 | BONDY G D | N | 1 |
| | 88 | 172 | LUCIO | Y | 7 |
| | 88 | 421 | KEGEL | N | 5 |
| | 88 | 86 | WRIGHT | N | 1 |
| | INBRED F | | | | |
| HAE II | 83 | 713 | HOULE | N | 4 |
| | 84 | 286 | MCKIM | N | 2 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 275 | OSCAR | N | 8 |
| | 85 | 65 | ROCHLEAU | N | 2 |
| | 86 | 501 | BAUTE | N | 3 |
| | 86 | 714 | ROSE | N | 6 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 86 | 373 | BOKOR | N | 6 |
| | 87 | 376 | DEMAITRE | N | 6 |
| | 87 | 341 | DEMAITRE | N | 6 |
| | 87 | 367,370 | DEMAITRE | N | 6 |
| | 87 | 360 | DEMIATRE | N | 6 |
| | 87 | 177 | BROWN | N | 5 |
| | 87 | 183 | LUCIO | N | 7 |
| | 88 | 86 | WRIGHT | N | 1 |
| | 88 | 156 | PARSONS | N | 5 |
| | 88 | 113 | BONDY | N | 1 |
| | 88 | 421 | KEGEL | N | 5 |
| | 2 INBRED FEMALE | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| HAE III | 83 | 713 | HOULE | N | 4 |
| | 84 | 287 | MCKIM | N | 2 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 67,68 | ROCHLEAU | N | 2 |
| | 85 | 65 | ROCHLEAU | N | 2 |
| | 86 | 373 | BOKOR | N | 6 |
| | 86 | 433 | FARROUGH | N | 2 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 86 | 174 | ROSE | N | 6 |
| | 86 | 501 | BAUTE | N | 3 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 156 | PARSONS | N | 5 |
| | 88 | 113 | BONDY | N | 1 |
| | 88 | 421 | KEGEL | N | 5 |
| | 88 | 86 | WRIGHT | N | 1 |
| | 88 | 397 | CHARRON | N | 9 |
| | 88 | 353 | MYSLICK | N | 6 |
| | 88 | 507 | CUDMORE | N | 5 |
| | 88 | 426 | KEGEL | N | 5 |
| | 2 INBRED F | | | | |
| | 1 INBRED M | | | | |
| HINC II | 83 | 713 | HOULE | N | 4 |
| | 84 | 286 | MCKIM | N | 2 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 318 | MCKINLEY | N | 6 |
| | 85 | 368 | CLENDENNING | N | 6 |
| | 85 | 65 | ROCHLEAU | N | 2 |
| | 86 | 433 | FARROUGH | N | 2 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 86 | 373 | BOKOR | N | 6 |
| | 88 | 113 | BONDY | N | 1 |
| | 88 | 65 | MCKIM | Y | 2 |
| | 88 | 49 | MCKIM | Y | 2 |
| | 88 | 421 | KEGEL | N | 5 |
| | 88 | 86 | WRIGHT | N | 1 |
| | 88 | 103 | BONDY G D | N | 1 |
| | 2 INBRED F | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| HIND III | | | | | |
| | 85 | 275 | OSCAR | N | 8 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 86 | 519 | BAUTE | N | 3 |
| | 86 | 581 | BAUTE | N | 3 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 87 | 365,370 | DEMAITRE | N | 6 |
| | 87 | 177 | BROWN | N | 5 |
| | 88 | 702 | ROSE | Y | 6 |
| | 2 INBRED F | | | | |
| | 2 INBRED M | | | | |
| HINF I | 83 | 713 | HOULE | N | 4 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 87 | 177 | BROWN | N | 5 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 421 | NEWCOMB | N | 6 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 87 | 668 | SMOULDER | N | 5 |
| | 88 | 172 | LUCIO | N | 7 |
| | 88 | 421 | KEGEL | N | 5 |
| | INBRED F | | | | |
| HPA II | 85 | 360 | CLENDENNING | N | 6 |
| | 85 | 65 | ROCHLEAU | N | 2 |
| | 86 | 174 | ROSE | N | 6 |
| | 86 | 373 | BOKOR | N | 6 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 87 | 668 | SMOULDER | N | 5 |
| | 88 | 113 | BONDY | N | 1 |
| | 88 | 86 | WRIGHT | N | 1 |
| | 88 | 108 | BONDY G D | N | 1 |
| | INBRED F | | | | |
| MBO I | 83 | 713 | HOULE | N | 4 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 86 | 433 | FARROUGH | N | 2 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 86 | 581 | BAUTE | N | 3 |
| | 87 | 421 | NEWCOMB | N | 6 |
| | 88 | 113 | BONDY | N | 1 |
| | INBRED M | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| MSP I | 85 | 65 | ROCHLEAU | N | 2 |
| | 85 | 260 | CLENDENNING | N | 6 |
| | 86 | 373 | BOKOR | N | 6 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 86 | 174 | ROSE | N | 6 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 87 | 24 | MCKIM | Y | 2 |
| | 88 | 61 | MCKIM | Y | 2 |
| | 88 | 113 | BONDY | N | 1 |
| | 88 | 421 | KEGEL | N | 5 |
| | INBRED F | | | | |
| NSI I | 84 | 286 | MCKIM | N | 2 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 67,68 | ROCHLEAU | N | 2 |
| | 85 | 275 | OSCAR | N | 8 |
| | 85 | 318 | MCKINLEY | N | 6 |
| | 86 | 498 | FARROUGH | N | 2 |
| | 86 | 501 | BAUTE | N | 3 |
| | 87 | 181 | BROWN | N | 5 |
| | 87 | 341 | DEMAITRE | N | 6 |
| | 88 | 104 | WRIGHT | N | 1 |
| | 88 | 108 | BONDY | N | 1 |
| | INBRED M | | | | |
| | INBRED F | | | | |
| SCA I | 85 | 360 | CLENDENNING | N | 6 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 65 | ROCHLEAU | N | 2 |
| | 86 | 373 | BOKOR | N | 6 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 86 | 174 | ROSE | N | 6 |
| | 87 | 370 | DEMAITRE | N | 6 |
| | 87 | 177 | BROWN | N | 5 |
| | 87 | 341 | DEMAITRE | N | 6 |
| | 87 | 365,370 | DEMAITRE | N | 6 |
| | 87 | 360 | DEMAITRE | N | 6 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 430 | NEWCOMB | N | 6 |
| | 87 | 668 | SMOULDER | N | 5 |
| | 88 | 108 | BONDY G D | N | 1 |
| | 88 | 113 | BONDY | N | 1 |
| | 88 | 86 | WRIGHT | N | 1 |
| | 2 INBRED F | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| TAQ I | 84 | 287 | MCKIM | N | 2 |
| | 85 | 67,68 | ROCHLEAU | N | 2 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 85 | 325 | MCKINLEY | N | 6 |
| | 85 | 248 | RENAUD | N | 1 |
| | 85 | 312 | MCKINLEY | N | 6 |
| | 86 | 72 | ROCHLEAU | Y | 2 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 86 | 174 | ROSE | N | 6 |
| | 87 | 144 | PARSONS | N | 5 |
| | 87 | 156 | PARSONS | N | 5 |
| | 87 | 24 | MCKIM | Y | 2 |
| | 87 | 421 | NEWCOMB | N | 6 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 490 | SMOULDER | N | 5 |
| | 88 | 194 | WRIGHT | N | 1 |
| | 88 | 21 | ROCHLEAU | Y | 2 |
| | 88 | 668 | ROSE | Y | 6 |
| | 88 | 65 | MCKIM | Y | 2 |
| | 88 | 659 | MCKIM | Y | 2 |
| | 88 | 677 | ROSE | Y | 6 |
| | 88 | 108 | BONDY G D | N | 1 |
| | 88 | 426 | KEGEL | N | 5 |
| | 88 | 507 | CUDMORE | N | 5 |
| | 88 | 335 | MYSLICK | N | 6 |
| | 88 | 353 | MYSLICK | N | 6 |
| | 88 | 397 | CHARRON | N | 9 |
| | 88 | 553 | NEWCOMB | Y | 6 |
| | 88 | 307 | PARSONS | N | 5 |
| | 88 | 343 | MYSLICK | N | 6 |
| | 88 | 329 | MYSLICK | N | 6 |
| | 88 | 280 | PARSONS | N | 5 |
| | 88 | UK | MYSLICK | N | 6 |
| | 88 | 484 | MCKINLEY | N | 6 |
| | 89 | UK | CHARRON | N | 9 |
| | 89 | 91 | PARSONS | N | 5 |
| | INBRED F | | | | |

| ENZYME USED | YEAR OF CAPTURE | ANIMAL NO. | CRIB | RELEASE | REGION |
|---|---|---|---|---|---|
| THA I | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 67,68 | ROCHLEAU | N | 2 |
| | 86 | 501 | BAUTE | N | 3 |
| | 86 | 502 | BAUTE | N | 3 |
| | 86 | 519 | BAUTE | N | 3 |
| | 87 | 29 | MCKIM | Y | 2 |
| | 87 | 365,370 | DEMAITRE | N | 6 |
| | 87 | 177 | BROWN | N | 5 |
| | 87 | 370 | DEMAITRE | N | 6 |
| | 88 | 217 | CARNIERO | Y | 7 |
| | 88 | 61 | MCKIM | Y | 2 |
| | 88 | 59 | MCKIM | Y | 2 |
| | 88 | 9 | ROCHLEAU | Y | 2 |
| | 88 | 49 | MCKIM | Y | 2 |
| | 2 INBRED F<br>INBRED M | | | | |
| XBA 1 | 83 | 713 | HOULE | N | 4 |
| | 85 | 67,68 | ROCHLEAU | N | 2 |
| | 85 | 319 | MCKINLEY | N | 6 |
| | 85 | 173 | GOOLIN | N | 1 |
| | 85 | 161 | GOOLIN | N | 1 |
| | 85 | 275 | OSCAR | N | 8 |
| | 85 | 360 | CLENDENNING | N | 6 |
| | 86 | 451 | FARROUGH | N | 2 |
| | 86 | 487 | FARROUGH | N | 2 |
| | 87 | 341 | DEMAITRE | N | 6 |
| | 87 | 365,370 | DEMAITRE | N | 6 |
| | 87 | 370 | DEMAITRE | N | 6 |
| | 87 | 360 | DEMAITRE | N | 6 |
| | 87 | 177 | BROWN | N | 5 |
| | 87 | 183 | LUCIO | N | 7 |
| | 87 | 668 | SMOULDER | N | 5 |
| | 87 | 156 | PARSONS | N | 5 |
| | 88 | 61 | MCKIM | Y | 2 |
| | 88 | 184 | WRIGHT | N | 1 |
| | 88 | 421 | KEGEL | N | 5 |
| | 2 INBRED F | | | | |

# APPENDIX II

A SOUTHERN BLOT OF MALE AND FEMALE MOUSE GENOMIC DNA DIGESTED WITH SEVERAL RESTRICTION ENDONUCLEASES AND HYBRIDIZED WITH BIOTIN-LABELLED 145SC5 DNA

Male and female mouse genomic DNA restriction endonuclease digested, fractionated, transferred and hybridized with biotin-7-dATP labelled 145SC5 DNA. Lanes 1 through 4 contain male DNA digested with Nsi I. Lane 5 contains female DNA digested with Nsi I. Biotinylated lambda Hind III DNA fragments are shown in lane 6. Lanes 7 through 9 contain male DNA digested with EcoR V. In lane 10 female DNA was EcoR V digested. In lanes 11 through 16 male DNA was digested with the enzymes Xba I, Nsi I, Hind III, EcoR V, Xba I and Hind III, respectively.

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