GENETIC VARIATION BETWEEN DEMES OF GEOGRAPHICALLY SEPARATED AFRICAN GREEN MONKEYS

Sandra Smith, Department of Biology
McGill University
September, 1991

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science**.

Abstract

GENETIC VARIATION BETWEEN DEMES OF GEOGRAPHICALLY SEPARATED AFRICAN GREEN MONKEYS

McGill University, 1991

This study investigates the possibility of genetic diversity between geographically separated demes of *Cercopithecus aethiops* on the islands of St. Kitts and Nevis. The island of St. Kitts was represented by two geographic demes, one from the Northern tip of the island and one from the Southern tip of the island. The island of Nevis was considered as one separate deme.

Eight plasma proteins were analyzed with polyacrylamide gel electrophoresis. A preliminary DNA investigation was also completed.

Three of the plasma proteins -- transferrin, albumin, and an unidentified protein that migrated faster than albumin -- appeared to be monomorphic. Three proteins in the alpha globulin region (between transferrin and albumin) and pseudocholinesterase were polymorphic in the total population. The DNA locus identified by the Human Molecular Fingerprinting #1 probe (locus D1Z2) was also polymorphic in all demes.

The levels of heterozygosity for North Island, South Island, and Nevis were 2.02%, 2.49%, and 2.49% respectively.

North and South Island demes were more similar to each other than either was to the Nevis deme. The South Island deme was more unlike Nevis than the North Island deme. These figures do not provide sufficient genetic evidence to evaluate divergence within the West Indian vervets according to geography.

Extrait

VARIATION GENETIQUE ENTRE DEMES DES CERCOPITHECUS AETHIOPS SEPARES GEOGRAPHIQUEMENT

Université McGill, 1991

Cette étude cherche à démontrer une diversité génétique entre les demes des Cercopithecus aethiops séparés géographiquement sur les îles de Ste-Kitts et Nevis L'île de Ste-Kitts était représentée par deux demes géographiques, le premier à la pointe nord de l'île et le deuxième à la pointe sud de l'île. L'île de Nevis était considerée comme un seul deme

Huit protéines sérales ont été analysées par électrophorèse sur gel polyacrylamide. Une investigation préliminaire de l'ADN a aussi été entreprise.

Les trois protéines sérales -- transférrine, albumine et une protéine inconnue (dont la migration était plus rapide que celle d'albumine) -- ont paru comme étant monomorphiques Trois protéines dans la région alpha-globuline (entre transférrine et albumine) et pseudocholinesterase étaient polymorphiques à travers la population entière. De plus, le locus d'ADN, identifié avec la sonde "Human Molecular Fingerprinting #1" (locus D1Z2), était polymorphique pour tous les demes.

Les niveaux d'hétérozygosité pour les groupes Nord et Sud de l'île de Ste-Kitts ainsi que de l'île de Nevis étaient respectivement 2.02%, 2 49%, et 2.49%.

Une comparaison pairée des demes Nord et Sud de l'île de Ste-Kitts a démontré moins de variation qu'avec le groupe de Nevis. Le groupe de l'île Sud a démontré moins de ressemblance au groupe de Nevis que le groupe de l'île Nord. Ces données ne fournissent pas une preuve génétique suffisante d'evaluer la divergence parmi des vervets Ouest Indiens selon un critère géographique.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I think that this section of my thesis could be as long as all the other sections put together The number of people that contributed to the completion of this work is infinite as all the people who have contributed to my life have allowed me the courage and strength to be here and achieve my goals

First, I would like to thank the Natural Sciences and Engineering Research Council for support during my graduate work McGill University and the faculty and staff of the Department of Biology have been kind supporters Special thanks to the members of my supervisory committee: Rima Rosen, Kurt Sittman, and especially Ken Morgan for the "always-open" door. Also thanks to Robert Lamarche for endless computer and photography consultation and help

Gratitude is extended to all the folks in St. Kitts who helped me to obtain the necessary samples Appreciation is felt for all the third and fourth floor people of the Allan Memorial Institute R & T Building as well as the administration More pointedly, I'll never forget the encouragement and support received from my direct cohorts: Anna Marie, Andrew, Jillian, Tim, and Leigh Thanks to Colette for the endless help and advice.

I must not, and certainly will NOT, forget the gracious hospitality I received from David Rosenblatt's lab during my DNA adventure. Never-ending thanks to Estelle for all her god-like information and the girls: Susan, Adele, and Sue for the open ears and uplifting conversation.

Although not all scientists, my friends from Swim Club and my other endeavors helped me remain sane during frustrating times. I thank you all. But my biggest Montréal supporters can never be thanked enough - what you put up with!! I love you guys - Katherine and Jessica!!!

As a foreigner to Montréal, the home-town connection was very important. The loving support of my parents and grandparents will always be remembered and cherished. The Manitoban friends made me realize that friends triumph and Bell Canada wins!!!!!! Special thanks to Phil. Finally, (truly!!) my heartfelt thanks and love to Frank and Roberta for their personal and scientific allegiance. Thanks RP for helping me get through - "just RELAX"!!!!!!

With my deepest love

Mom and Dad
and
Gram and Gramp

GENETIC VARIATION BETWEEN DEMES OF GEOGRAPHICALLY SEPARATED AFRICAN GREEN MONKEYS

> The sufficient set of state variables for describing an evolutionary process within a population must include some information about the statistical distribution of genotypic frequencies. It is for this reason that the empirical study of population genetics has always begun with and centered around the characterization of the genetic variation in populations.
>
> Richard Lewontin, 1974.

In the last twenty years there have been many studies looking at genetic variation within defined species (Prakash *et al*, 1969; Selander *et al*, 1969, Nozawa *et al*, 1975; Lucotte *et al*, 1982). In addition, much work has been carried out comparing different species to provide insight into evolutionary paths followed over time. These interspecific studies include comparisons at the levels of morphology (Colyn *et al*, 1987; Ashton and Zuckerman, 1950-51, 1960), cytogenetics (de Grouchy *et al*, 1978; Finaz *et al*, 1977), biochemical genetics (Kawamoto *et al*, 1986), and most recently, molecular genetics (Diamond, 1988; Maeda *et al*, 1988).

Intraspecies Population Studies

A more microscopic view of evolution concerns the development of genetically diverse subgroups within a single species. An example of this intraspecific research is the 1969 study of Selander and Yang on a wild population of house mouse (*Mus musculus*). Their aims included increasing the number of loci available for studies of wild populations and assessing the extent of genic polymorphism in wild populations of this species to use for comparison with estimates available for other organisms. Like Selander and Yang, Prakash *et al*, also in 1969, described the extent of genetic variation in described populations of *Drosophila pseudoobscura*

using percent polymorphic loci and percent heterozygosity as measures Similarly, Nozawa *et al*, in 1975, quantified genetic variation within and between troops of *Macaca fuscata fuscata* They also characterized troops as genetically open or genetically closed Whether a troop is closed or open determines whether gene exchange exists between groups of the species Theoretically, a completely closed troop could be regarded in the same manner as a geographically isolated population of the species Nozawa's team concluded that individual migration occurred between adjacent groups and thus the troops studied did not act as genetic isolates

When the migration rate and effective gene dispersion from a troop were studied, the migration rate was found to be quite low, approximately 5% in a generation (Nozawa *et al*, 1975) More important was the actual distance of gene dispersion from a troop Because troop size and migration rate between troops were not constant across the population, the exchange of genes was not necessarily restricted to adjoining troops Troops were compared pairwise by looking at genetic distance between troops, both as an independent measure and in relation to geographic distance between troops Nozawa *et al* concluded that troops on separate islands or separated by more than 100km behave independently, and can thus be regarded as isolated from one another

Nozawa and his colleagues inferred general characteristics of the population such as the tendency to form many local demes and the susceptibility of the population to genetic drift . Small demes and minimal migration between demes make the population susceptible to random genetic drift and may be responsible for the low genetic variability within troops and for the marked genetic differentiation between troops

In 1975, Anderson and Giblett studied intraspecific variation of red cell enzymes in the pigtailed Macaque (*Macaca nemestrina*) They found several loci to be monomorphic; 27% of the analyzed enzymes were found to be variable in more than 1% of the individuals Phosphoglucose isomerase (PGI) polymorphisms revealed two cases of mistaken paternity in the mating records of a large primate center. This group of researchers also studied the population variation between demes of monkeys separated by a body of water. These two sets of animals were found to be less similar to each other than any other pairs of demes. It should be noted that caution

was taken to state that this phenomenon may have been due to a smaller sample size in one of the monkey demes

D J Melnick has been studying Rhesus monkeys (*Macaca mulatta*) in Asia for several years In 1988, he described work that included 25 genetic loci. His group found that 59.1% of the genetic variation found among wild troops of Rhesus was due to variation within a single troop; 8 4% was due to gene differences between members of different troops in the same local population; 23 8%, to variation between monkeys in different local populations within the same geographic region; and 8.7%, to gene differences between members in different geographic regions. To put this into easier categories: 59.1% of natural genetic variation was found between members of the same troop, 32 2% of gene differences were between troops in the same geographic area, and only 8 7% of variation was accounted for by differences between individuals found in different geographic areas. As suggested by Melnick in the above paper, and earlier by Slatkin (1987), small differences between recent geographically fragmented species may copy the genetic structure of a "species with moderate gene flow between its populations" This is simply due to inadequate passage of time to allow for the level of genetic differentiation to reach a level at which it can be detected to be true genetic subspecies differentiation

In 1987, Meera Kahn stated that "biochemical genetic markers and the expected future wealth of DNA markers will help in tracing the paths of evolutionary divergence during the most recent geological past with a reasonable level of accuracy among isolated populations". Thus it would seem that studying troops of animals that are geographically separated by distances which mimic actual geographic isolation would be a good tool for studying evolutionary divergence as it occurs.

Studies on *Cercopithecus aethiops*

Some groups of investigators have completed similar studies of intraspecific genetic variation in *Cercopithecus aethiops*. In 1982, Lucotte *et al* described electrophoretic polymorphism in different subspecies of this species The animals were taken from three morphologically and geographically different populations (Senegal, Ethiopia, and South Africa), and the amount of polymorphism and heterozygosity was calculated to

provide clues of possible evolutionary pathways Genetic distances between the three subspecies were also computed, the distances were significant In 1983, Dracopoli *et al* did a large study in which vervets from four localities in central and southern Kenya were described in terms of genetic variation Their results lead to conclusions on population structure and also hypotheses on the forces behind gene fixation in some populations of the monkeys. The study of Kawamoto *et al* in 1986, using monkey samples obtained from Ethiopia, was an extensive project that looked at polymorphism and heterozygosity within species, and genetic distances between three genera of the family of Cercopithecidae Their aim was to elaborate on the possible paths of diversification in this family Most recently, Meera Kahn, 1987, has used electrophoretic analysis of blood proteins to study the evolution of physiological traits and their biological significance during speciation in various monkey species

All of the groups which have published data comparing troops of *Cercopithecus* have obtained their samples from Africa Ashton and Zuckerman in 1950-51 compared morphological data from the Kittitian monkeys (Eastern Caribbean, West Indies) and the West African Green monkeys They found that the skulls and teeth of the Kittitian monkey were larger and less variable although in the normal range of other Cercopithecidae In 1960, Ashton concluded that while variations existed, they were insufficient for taxonomic divisions higher than the subspecific level. Yet Ashton also strongly stated that the teeth of the St Kitts monkey would double in size in approximately 5000 years. This work provides evidence that the monkeys have diverged somewhat from their West African ancestors It may be possible that subspeciation resulted from a founder effect combined with three centuries of genetic drift or gradualistic selection (W.W. Denham, 1987). But Denham does purport that differences may only be due to a complex history of migration that has yielded monkeys of mixed parentage

Beyond the Ashton and Zuckerman studies, Palmour *et al*, 1980, reported protein variation in monkey samples from St. Kitts and Nevis, but no emphasis was placed on population structure and the origin of the samples Furthermore, no research teams have used DNA markers within their population studies. Therefore the study described here is the first attempt to

detail geographically separated demes of *C. aethiops* on these islands via protein and DNA analysis. The main emphasis is to study genetic variation and to begin an investigation into the evolutionary patterns of these West Indian vervets

History of the Vervet on St. Kitts and Nevis

St. Christopher, better known as St. Kitts, was first settled by the British in 1623. Later that same year, a French group took a foothold. The island was inhabited by both French and English without strife until the mid 1600's. Control of the island then switched hands several times until the early eighteenth century. At this time the English built Brimstone Hill, a huge battery atop an advantageous hill looking over the gateway between the Atlantic Ocean and the Caribbean Sea. The country is now a self-governing state within the British Commonwealth. Its sister nation, Nevis, was settled in 1628 and is also a member of the British Commonwealth.

St. Kitts is an island of 65 square miles whereas Nevis comprises 36 square miles St. Kitts has a human population of 36,000; Nevis has 9,500. Both countries attract some tourism but remain primarily agricultural islands.

It has been estimated that the African Green monkey first arrived on St. Kitts around the 1650's. The exact date of the first appearance of the vervet is not known but it was declared an agricultural vermin in Barbados in 1682, indicating that the monkeys were present among trade ship goods in the West Indies and that they had reached a significant population size. Furthermore, their presence was acknowledged in the personal journals of a French Catholic priest, Father Labat, in 1700 (McGuire, 1974). It would appear that the monkeys were originally brought as pets and either escaped or were released when they reached an age and size that made them undesirable to have in the home.

Trading of goods with St. Kitts originated from the West coast of Africa (McGuire, 1974) The immigration of monkeys continued for several decades after which their rapidly growing presence and agricultural menace lead the human inhabitants to inhibit their influx into the country. The initial founding population has been estimated to be 1700 individuals which were direct descendents of the West African Green monkey.

The continued presence of the St. Kitts vervet throughout the following

years has been confirmed in many other historical documents (cited in McGuire, 1974) To this date, *Cercopithecus aethiops* is still considered an agricultural pest. The government pays bounty for proof of death/capture and some residents hunt them for food. Although population estimates for the vervet are subject to numerous errors, the most recent and accepted figure for the St. Kitts population is 40,000 individuals (F.R. Ervin, personal communication). This population represents the descendents of approximately sixty vervet generations.

Approach in this study

Island populations have been popular study groups for evolutionary biologists since Charles Darwin's work began to be appreciated. Darwin recognized the quick adaptation and divergence of the finches in accordance to natural selection placed on the birds by environmental forces. Quick divergence of island species from their ancestral parent is largely due to an isolated gene pool Absence of gene flow, new genes entering or present genes leaving the population, allows favored genes to increase in frequency without competition or dilution from new, outside genes The St Kitts and Nevis monkey populations represent similar isolated populations The present investigation revolves around genetic variation obtained and maintained between the two islands and possibly between two geographically separated demes on St. Kitts.

Observed polymorphisms have been assumed to be controlled by autosomal alleles showing codominant features (Kawamoto *et al*, 1986; Dracopoli *et al*, 1983; Nozawa *et al*, 1975). Thus, a simple gene counting method can be implemented to determine gene frequencies at loci studied Using this method, both protein and DNA analysis have been used in an attempt to look for genetic variation between three groups of *Cercopithecus aethiops* located on St. Kitts and Nevis.

Nei and Roychoudhury (1974) state that to estimate heterozygosity and genetic distance one should study many loci; the more loci, the more reliable the estimates Polyacrylamide gel electrophoresis with a general protein stain was used to this end. The sample size is not as important as the number of loci studied but it does affect the probity of the allele frequency estimates. So, in this type of study it is a

compromise between the number of loci and the number of individuals when time, money, and manpower are limited. The use of the polyacrylamide gel and the general protein stain was to optimize both aspects of data collection. The primary consideration for the choice of loci studied was methods and equipment available for use in data collection.

Choice of Loci

A large bank of plasma samples was available for study of this population. Furthermore, red cell proteins had been well studied for this particular group; little information on plasma proteins had been collected. Also, plasma proteins are good candidates for informative polymorphisms in intraspecific studies (Palmour *et al*, 1980). A DNA analysis was added to the the study when the opportunity presented itself during the course of the work.

Albumin has the highest concentration of all the serum proteins in plasma (Giblett, 1969). When analyzing plasma, albumin is easily identified as it shows up as a large wide band in small dilutions of plasma.

Transferrin was a protein of particular interest. First, transferrin is known to be polymorphic in other monkey species such as the macaques (Shotake *et al*, 1977; Goodman *et al*, 1965) and the baboons (Buettner-Janusch, 1963; Barnicot *et al*, 1965) Furthermore, transferrin shows some variation in vervets in Ethiopia (Turner, 1981; Kawamoto *et al*, 1986; Lucotte *et al*, 1982) as well as in Senegal and South Africa (Lucotte *et al*, 1982). The vervets studied in Lucotte's study are of three recognized subspecies of *Cercopithecus aethiops*, one of which (the Senegalese vervet) presumably shares ancestors with the Carribean vervet . Thus it makes for an interesting study into the evolutionary background and progress of the island monkey to study a protein that has been studied in its African "cousins". There was also some indication that there might be some low level of variation at this locus in these island populations (Palmour *et al*,1980). To investigate the level of this variation further, the transferrin locus was studied.

Meera Kahn (1987) observes that "allelic distribution patterns among populations have indicated trends of subspeciation" in groups of monkeys that he studied which were geographically isolated. In conclusion he stipulates that "relative activity profiles of certain enzymes may be helpful in studies of the evolution of physiological traits and their biological significance during speciation".

Serum pseudocholinesterase (EC 3.1.1.8) is a liver-manufactured plasma

enzyme that came to genetic attention because persons with rare genetic variants were found to have reduced activity. Pseudocholinesterase inactivates anaesthetics such as suxamethonium (a complex of two acetylcholine molecules also known as succinyl dicholine) (Giblett, 1969); when this anaesthetic is administered to these people they may experience such symptoms as muscle paralysis, apnea, and possible coma. Pseudocholinesterase has a molecular weight of approximately 348,000 daltons. Its activity is measurable with the technology present within the lab and is also of personal interest to the author due to variants in her family.

Studies from Africa show polymorphism for the pseudocholinesterase protein in Ethiopian vervets (Turner, 1981) but not in Kenyan vervets (Dracopoli *et al*, 1983) However, the Ethiopian vervets studied by Kawamoto *et al* (1986) are observed to be monomorphic at this locus. All three groups utilized horizontal starch gel electrophoresis. Assuming the electrophoretic bands to be direct products of the genes for pseudocholinesterase, allele frequencies were calculated. A study of the St. Kitts/Nevis monkey populations might lend evidence to their historical origins as well as offer some comparison between African and Carribean vervets. Thus, a study of this locus ensued.

Alpha-1-antitrypsin is medically a very interesting protein. Deficiencies of this enzyme have been linked to several severe medical problems including disabling lung disease and liver disorder (Kueppers, 1973; Talamo *et al*, 1968; Cox, 1981) Alpha-1-antitrypsin variants are also known to be differentially sensitive to environmental irritants, such as smoke and asbestos (Kueppers, 1973). If there were variants in the monkey population, this would offer an excellent test system for gene-environment interaction studies. King and Wilson (1975) indicate the position of several serum proteins, observed using immunodetection, on starch gels. Alpha-1-antitrypsin was found to migrate between transferrin and albumin. Variation in this protein could be detected by the general protein polyacrylamide gel and further investigated by isoelectric focussing if medical complications existed. Using the knowledge of previous studies and extrapolating possible electrophoretic migrations patterns, a preliminary scan was made of this protein in this sample of monkeys.

Rationale and Objectives

This project attempts to continue an extensive investigation into the genetic

variation of this island monkey population as well as an initial investigation into evolutionary patterns. A few protein loci were studied as well as a DNA polymorphism. More work should follow to round out this "sketch" of the population.

The project includes estimates of the percent of polymorphic loci and the average heterozygosity for each subpopulation separately. The percent polymorphic loci would simply be the percent of loci that are polymorphic (Selander and Yang, 1969). A polymorphic locus is defined as one for which the frequency of the most common allele does not exceed 0.99 (Kawamoto *et al*,1986; Nozawa *et al*, 1975). Heterozygosity is theoretically one minus the Hardy-Weinberg homozygosity. To measure the heterozygosity taking into account varying sample sizes at each locus, a weighting system was used to obtain an average heterozygosity across the loci.

As well, because differences in allele frequency estimates were observed between the isolated demes, a pairwise comparison of the populations in terms of the genetic distance ensued. These measurements were first analyzed using Nei's (1972) measure of genetic distance with a correction for small sample size (Nei, 1978). However, due to the sparseness of the data, the data were re-analyzed using a heterogeneity χ^2 test of the groups (three pairwise tests) using the most reliable and informative data.

In terms of application, studies describing genetic variation of particular non-human primate populations are important because they provide genetically defined populations that may serve as laboratory models of relevance to studies of man (Anderson and Giblett, 1975; Palmour *et al*, 1980). The usefulness of animal models can be placed in three categories, according to Siciliano (1974):

1. to suggest mechanisms which operate in man.
2. to suggest experiments which may be carried out in man.
3. to provide systems in which to study mechanisms which almost certainly operate in man.

Furthermore, as stated by Prakash *et al* (1969), " in order to understand evolutionary phenomena in natural populations, one has to start by determining the pattern of genetic variation in populations which come from different ecogeographic regions of the species range and represent different degrees of isolation from the other populations of the species ".

These studies provide clues to evolutionary stability or change and enable researchers to focus on areas that would most probably give useful data in more comprehensive structural analysis (Anderson and Giblett, 1975)

There are several reasons behind conducting a study such as the one entailed: 1) breeding groups have been set up with maternal lineages recorded, and genetic description aids in paternal inference. This type of information can be used for genetic management in breeding groups; 2) since this is a young isolate in terms of evolution, this population provides an excellent tool with which researchers can study rapidly evolving DNA with firm comparisons with the direct descendents in Africa Knowledge of the genetic background would allow one to control for the heterogeneity within each of the different populations so a picture of any significant differences could be interpreted ; 3) population genetic information can be used to study phenomena such as inbreeding, migration, mating behavior, and behavior, in general; 4) genetic information can also lend information to susceptibility to genetic diseases; and, finally, 5) genetic description and distance calculations of this particular group of monkeys could provide insight into the rate at which speciation and divergence occurs between recently separated groups of a species.

As stated by Lewontin, in the study of speciation, it is important to follow the divergence of related populations at the beginning and as it proceeds. To understand the mechanisms and forces behind speciation, one must observe the phenomenon as it occurs.

Material and Methods

SAMPLES

Plasma samples from Nevis and St. Kitts vervets were provided by the Behavioral Sciences Foundation, St. Kitts. Plasma samples were collected into 10 ml EDTA-coated tubes and then refrigerated. Sample centrifugation permitted separation of plasma from rest of blood (IEC clinical centrifuge - 12 minutes at 3000rpm). Plasma was removed into transparent polypropylene screw cap tubes and stored at -20 °C. If DNA was to be extracted from the sample the plasma was removed as above and the procedure for extraction was followed as described below without any modification. All samples for the population study were, to the best of knowledge, from individuals who were not closely related to one another.

MATERIALS:

Tubes for plasma collection were purchased from Corning. Acrylamide was purchased from Kodak Eastman Company. Glycine was obtained from Biorad. Polypropylene screw-cap tubes and transfer pipettes for DNA isolation and screw-cap eppendorf tubes were purchased from Sarstedt. Clinical assay kits for serum pseudocholinesterase were generously provided by Sclavo Diagnostics. Sodium monobasic and dibasic phosphate salts were purchased from BDH ACS. NYTRAN nylon membrane (0.45µm) was purchased from Xymotech Biosystems Inc. Affinity purified rabbit anti-goat IgG was purchased from Biorad. Phenol for DNA isolation was purchased from IBI Laboratories. Dextran sulfate was obtained from Pharmacia. Sodium dodecyl sulphate and formamide were purchased from BDH biochemicals Sealer and sealer bags for DNA studies were obtained from Kapak. The DNA probe, hMF #1, was made available commercially through VenProbe of Canada. Restriction enzyme, Multiprime DNA labelling and Nick Translation kits were purchased from Amersham Co. Unless otherwise stated other chemicals were purchased from Sigma or Fisher Scientific.

PROTEIN STUDIES:

Acrylamide Gel Electrophoresis.

Plasma electrophoresis with general protein staining provided insight into seven proteins. Plasma used for electrophoretic analysis was prepared as a 1:4 dilution using a diluant of 20% sucrose in running buffer. Also added to the sample was bromophenol blue to act as a tracking dye, at approximately 0.5% w/v.

The running buffer was a 0.0575M Glycine-TRIS solution made at a concentration of 20x strength, pH 8.3. When needed this stock was diluted to proper running strength with doubly deionized water (see Appendix A, #5).

The gel buffer for the separating gel was 0.375M TRIS-HCl, pH 8.9 solution (see Appendix A, #1).

The gel buffer for the stacking gel was 0 0658M TRIS-H_3PO_4, pH 6.9 solution (see Appendix A, #3).

Separation gels for general protein staining were 15% acrylamide gels (see Appendix A, #6) Ammonium persulfate and TEMED were used to aid in polymerization A concentration of 8 5% polyacrylamide was used to analyze serum pseudocholinesterase qualitatively (see Appendix A, #8).

The amount of buffer, catalyst, and TEMED did not vary, volumes were made up with water when necessary.

Stacking gels were identical for all acrylamide gel electrophoresis experiments (see Appendix A, #7).

Gels were all loaded at room temperature and then run at 4 °C, using a Searle Buchler Instruments power supply (#3-1155):

general	7-7 1/2 hours at 600V
pseudocholinesterase	6-6 1/2 hours at 600V

Gel Staining and Destaining/Fixing:

General protein: 0.04% Coomassie Blue in 10% perchloric acid overnight (see Appendix A, #9).

Destain in 10% acetic acid and then store in deionized water

Pseudocholinesterase: A positive chemical assay in which the substrate was alpha-napthyl acetate and the dye was Fast Red TR (see Appendix A,#10). Scoring was by positive chemical detection (Harris and Hopkinson, 1977).

Specific enzyme assay for pseudocholinesterase:

To determine exact enzymatic activity in the plasma a clinical enzyme assay kit was obtained. The optimum assay for monkey plasma used butyrylthiocholine as a substrate. Pseudocholinesterase hydrolyzes C-S and C-O bonds in the butyrylthiocholine and thus liberates thiocholine (Koelle, 1949). This enzyme-released thiocholine is detected by 5-5'-dithio-bis-2-nitrobenzoic acid (DTNB). The photometric assay was carried out at a wavelength of 405nm and 37 °C as suggested by the manufacturer. Samples were read at 10 seconds and then 30 seconds later. Activity was determined by the change per 30 seconds and converted into accepted international units.

Scoring of protein gels:

Given the fact that this study of plasma proteins used polyacrylamide gel electrophoresis there are few reports on which to model phenotype classifications Much of the study of plasma proteins has involved starch or cellulose acetate electrophoresis. Thus, even though there exist phenotype classifications for some of the proteins such as pseudocholinesterase and alpha-1-antitrypsin the phenotypic band scoring cannot simply be transferred to a new medium of study. To be able to use the same classifications as past reports one would have to identify the known phenotypes with the phenotypes identified using the same method which, in this case, is polyacrylamide gel electrophoresis.

Since such a comparison was not conducted a very simple method of band assignment was utilized. The most common band was given the number "1" class. As new variants within a protein class were discovered they were assigned the next number class (ie. "2" bands would be the next band type observed). To enable the reader to have some idea as to migratory behavior, the faster band is always given priority when denoting the phenotype (ie. if the "2" band is faster than the "1" band then the phenotype is denoted 2-1).

Western Blotting:

Transfer of proteins from acrylamide gel to NYTRAN (0.45µm) was accomplished by electrical transfer utilizing the concepts of protein transfer detailed by Gershoni (1988). The apparatus was constructed within the Biology Department of McGill University according to previous units (Mike DeVouge and Tony Brisa).

Once electrophoretic migration had occurred, the gel was removed from the glass plates and placed in transfer buffer. This transfer buffer was the same Tris-Gly buffer as used in the electrophoresis; methanol was not needed, as in SDS electrophoresis, because the proteins were in native form during migration. A "blotting sandwich" was then constructed. A saturated piece of NYTRAN was placed next to the gel and this complex was then surrounded by two saturated pieces of Whatman 3MM filter paper on each side. Bubbles were removed and the complex was placed in the transfer cassette. The transfer ran the proteins from cathode (to which the gel was closest) to anode (to which the Nytran was closest) Most plasma proteins are negatively charged at this pH (8.3) and therefore they will move toward the positive pole when in an electrical field.

Immunodetection of proteins:

Determination of certain bands on the general protein gel was accomplished by transferring the proteins onto NYTRAN nylon membrane as discussed above. Following successful transfer the membrane was quenched with skim milk proteins to prevent non-specific binding of the specific serum antibodies to the membrane. A blocking solution of 3% skim milk powder (SMP) in Tris Buffered Saline (TBS, see Appendix A, Western blotting #1) was used at room temperature for 1 hour with agitation. [Bovine serum albumin was tried as a blocking protein but was found to give a very high background; the skim milk powder totally eliminated this problem]. The membrane was then bathed in a solution containing the specific serum antibody in 1.0%SMP in TBS at room temperature for 1.5 to 2 hours with slow agitation. The serum antibodies used were directed toward the particular human serum proteins and were raised in a goat host.

Excess antibody and nonspecifically bound antibody were removed by vigorous washing. Washing consisted of three rinses of the membrane for 10 minutes each,

in TBS with 0.1% Triton X-100, at room temperature with vigorous agitation

The second antibody was then introduced to the membrane. The second antibody is an enzyme-conjugated antibody that allows chromogenic detection of the particular protein being sought In this case, an affinity-purified rabbit(host) anti-goat IgG (heavy and light chain) horse radish peroxidase conjugate was used. The second antibody was in a solution of 1%SMP in TBS and the reaction was at room temperature with slight agitation for even mixing for 1 to 1.5 hours. This was followed by washing as before.

To detect the protein-antibody-HRP antibody complex, the substrate for the enzyme is introduced to the membrane. Peroxide was present at a concentration of 0.1% in TBS as well as 0.04% 4-chloro-1-naphthol dissolved first in absolute methanol (see Appendix A, Western blotting #2). This treatment was carried out at room temperature with slight agitation for an appropriate amount of time in which signal development was significant. Development on the membrane was halted by rinsing the membrane in an excess of distilled water for 10 minutes at room temperature with agitation.

DNA STUDIES:

DNA isolation:

DNA was isolated from fresh, whole African green monkey blood collected in 10ml EDTA-coated tubes. These tubes were spun at 1900 rpm for 10 to 12 minutes in a table top centrifuge following which the plasma was removed. The remaining contents of the tube, the red cells and the lymphocytes, were transferred to 15ml capped polypropylene conical tubes. The volume of this conical tube was brought up to approximately 11ml with RSB buffer (see Appendix A, DNA #2) and the contents mixed well (without vortexing). 60µl of nonidet P-40 , a strong non-ionic detergent, was then added to the tube to lyse cell membranes. Therefore, following nonidet P-40 treatment, there were broken cell membranes, organelles and whole nuclei in the reaction tube.

The intact nuclei were spun to the bottom of the tube by centrifugation at 1900rpm for 10 minutes. The supernatant, which contained the lysed red cells, was poured off and the pellet was resuspended in approximately 0.5ml of RSB buffer. Following resuspension, via gentle shaking, 3ml of SDS solution was added and

mixed in SDS solution contained RSB, NaCl, SDS, and EDTA (Appendix A, DNA #4). The SDS solution contains an ionic detergent which lyses the nuclear membrane The lysis of this membrane releases the DNA and other contents of the white blood cell. To enhance DNA isolation, 3ml of UNC-phenol solution was then added and mixed in The UNC-phenol solution is a mixture of molecular grade phenol, TRIS-base, 8- hydroxyquinoline, m-cresol, B-mercaptoethanol, and water (Appendix A, DNA #1). The m-cresol is an antibacterial agent, 8-hydroxyquinoline is an antioxidant and partial RNase inhibitor as well as a weak chelator of metal ions, B-mercaptoethanol reduces phenol's DNA destructive properties, and phenol mainly acts to denature proteins Because phenol can destroy DNA, this stage was carried out as quickly as possible

Proteins and any remaining cellular debris were pelleted by centrifugation at 3000rpm for 10 minutes, following which, the DNA remained in the aqueous phase This phase was removed by wide-mouthed, sterile, plastic transfer pipette into a fresh conical tube These pipettes were used to prevent mechanical shearing of the DNA. An equal volume of chloroform (3ml) was added and mixed in to denature any further proteins (mainly the histone proteins that are physically close to the DNA) These proteins were pelleted by centrifugation at 1900rpm for 5 minutes

The DNA remained in the aqueous phase. This phase was removed, via transfer pipette, into a sterile glass scintillation vial to which two volumes (approximately 6ml) of cold 90% ethanol were added The contents of the vial were shaken roughly (no vortex) after which the ethanol caused precipitation of the DNA out of aqueous phase The visible DNA was then spooled with a sterile capillary tube or a sterile eppendorf tip. The DNA was placed in a sterile eppendorf tube, which was labelled with the proper monkey I.D. number and left to air dry (to evaporate any remaining ethanol). Following drying, the DNA was resuspended in TE^{-4} (Appendix A, DNA #3) and stored at 4 °C for further dissolution before storage below freezing point (-20 °C).

Determination of DNA purity and concentration:

A 1:40 dilution of DNA sample was made with sterile water. The dilutions was vortexed to break up the large DNA complex. This dilution was analyzed spectrophotometrically (Maniatis *et al*, 1982) using a Beckman DU - 64 spectrophotometer (all spectrophotometric work was carried out with this machine)

All samples were exposed to light at 260nm and then 280nm The ratio of the absorbance at 260nm to the absorbance at 280nm was used to determine purity The absorbance at 260nm was used to calculate the concentration of DNA in the original sample

Restriction Enzyme Digest

Four units of restriction enzyme were used per µg of DNA. The restriction enzymes were accompanied by the buffer in which the enzyme worked optimally. Generally, EcoR1 digests were done at room temperature overnight but occasionally they were done at 37 °C. When the latter temperature was used, half of the restriction enzyme was added and the digest was incubated for 30 minutes in a 37 °C waterbath, then the remainder of the enzyme was added and the digest left to incubate for a further hour.

The digest was checked by running 4µl of the digest with 1µl of bpb (Appendix A, DNA #7) on a small 1% agarose gel (55V for about 1 to 1.5 hours, using H4BRL1025 horizontal submerged gel electrophoresis system and a BIORAD 200/2 0 power supply) using TPE as the running buffer (Appendix A, DNA #5). U.V. light was used to visually check for DNA digestion. Then a large gel was set up. When the thirty well comb was used, only 10µl could be loaded on the gel, therefore precipitation and resuspension of the digest in a smaller volume was necessary. This was accomplished by adding NaCl to bring the salt concentration of the sample up to 0.5M and followed by two volumes of cold ethanol, shaking, and allowing precipitation at -80 °C for about 1 hour. The ethanol was then poured off, the sample dried and resuspended in 9µl water and 1µl bpb. These samples were loaded on the large gel.

All samples were run on 1% agarose gels for between 1300 and 1360V-h (using the H5BRL1087 submerged horizontal electrophoresis unit along with the BIORAD power supply) with buffer cooling to reduce overheating of the gel. The sample of DNA loaded was 4 µg, determined by optical density assay of the samples at 260nm. Samples were checked for protein contamination and accepted if the abs_{260}/abs_{280} ratio was between 1.7 and 2.0. Otherwise they were reisolated with a chloroform rinse followed by ethanol precipitation.

Following electrophoresis, the gels were washed with water then with alkali buffer (0.4M NaOH / 0.6M NaCl; Appendix A, DNA #8). This is to denature the gel

somewhat so transfer of the DNA out of the gel is facilitated DNA was transferred to NYTRAN nylon membranes (0 45 µm) by capillary action as first described by Southern (1975) His original protocol was modified somewhat using the Rapid Alkali Transfer Method This technique depends on the binding capacity of the nylon matrix when exposed to an alkaline buffer and eliminates the extra step of neutralizing the gel prior to standard capillary transfer

After at least 2 hours of transfer action, the membrane was washed with 5XSSC (Appendix A, DNA #10) to remove the alkali buffer and the membrane was baked for at least 1 hour, but not more than 3 hours, in an 80 °C oven, to fix the DNA to the nylon membrane.

Pre-prehybridization:

The dry Nytran membrane was incubated at 65 °C in 0.1XSSC / 0.5%SDS for 30 minutes, then placed in a sealer bag to allow smaller volumes of working reagents to be used.

Prehybridization:

5ml of solution was used per 100cm^2 of membrane. Blots were generally 20cm by 20cm which is 400cm^2, therefore 20ml of solution was prepared for each membrane:

6XSSC
10XDenhardt's
1%SDS
100 µg/ml of boiled Herring Sperm DNA
distilled water to bring up volume to 20ml

Herring sperm was boiled for 5 minutes to denature it and rapidly cooled on ice for 5 minutes to prevent reannealing. All ingredients of the prehybridization solution can be found in Appendix A in the DNA section (#9, #12, #11, #15).

Following the addition of the herring sperm DNA, the blot was incubated for at least 2 hours immersed in a 42 °C waterbath. This was used as a stopping point when needed The prehybridization bag was stored at 4 °C until further work could be done.

Probe Description

The probe used in this study was the hMF#1 probe developed in David Hoar's lab at the University of Calgary. It is the human molecular fingerprinting probe which maps to a region on the human chromosome 1 (D1Z2; 1p36.1) and has homology to chromosome 1 of *C. aethiops*. It has been used to look at hypervariable regions in humans such that each individual can be distinguished from another. It recognizes a 40 base pair tandem repeat unit (Tynan and Hoar, 1989) with a highly variable copy number, between 6.25×10^3 and 1.25×10^4 copies in humans (Nakamura *et al*, 1987). This probe was of interest because of its ability to detect such subtle differences between individuals and also because a study had been done showing cross reactivity with a single *Cercopithecus aethiops* DNA sample (Tynan and Hoar, 1989).

The probe was bought in dessicated form and resuspended in sterile water (50µg in 5ml).

Probe Labelling:

The hMF #1 probe was labelled by random multiprime labelling. The Lambda probe for detecting the lambda molecular weight markers was labelled by nick translation. The radioactive label was in the form of ^{32}P-dCTP. Specific activity of the probes was in the range of 5×10^8 - 8×10^9 and 1.5×10^6 cpm/µg for hMF #1 and Lambda respectively.

Unincorporated label was removed using Sephadex G-50 Fine columns. The probe mixture was applied to the column; the column was then spun for 20 minutes at 2000rpm. Unincorporated label stuck to the column and probe solution was collected in a small screw-cap eppendorf tube.

Hybridization:

One uses 7.5ml per 100cm^2 of membrane, therefore a total of 30ml is normally required for a regular large gel blot:

6XSSC

50% formamide at pH 7.4

5% dextran sulfate
1%SDS
100 µg/ml of boiled Herring Sperm DNA
distilled water to bring up volume to 30ml
30,000,000cpm of hMF#1 probe
250,000cpm of lambda probe

The hybridization solution was added after the removal of the pre-hybridization solution. Bubbles were removed, the bag sealed, and incubated at 46/47 °C for at least 18 hours but no more than 24 hours. For the recipes of the individual ingredients of the hybridization solution, see Appendix A (DNA).

Removal of Non-specifically Bound Probe from the Nytran:

Before testing the Nytran for the location of the bound radioactive probe, one wants to remove any probe that is not bound to the complementary sequence on the membrane. In other words, one removes any non-specifically bound probe so that it doesn't cloud the regions that have specifically bound probe. This is achieved by washing the membrane in soapy solutions, for several rinses, and at high temperatures.

two washes	2XSSC / 0.1% SDS :	5 minutes room temp.
	then	15 " " "

followed by:

three washes	0.1XSSC / 0.1% SDS:	15 minutes room temp.
	then	2X 30 minutes at 65 °C

finally:

	0.1XSSC:	5 minutes room temp.

The Nytran was then blotted with good white paper towelling (White Swan 321-001) and scanned with a geiger counter to ensure that the majority of the radioactivity (which would be nonspecifically bound probe) had been washed away.

Autoradiography:

Once the nonspecifically bound probe had been sufficiently removed from the Nytran, the blot was wrapped in Saran Wrap and placed next to a sheet of Kodak XAR-5 film. The blot and the film were sandwiched with two intensifying screens (Dupont Lightning-plus Cronex Intensifying screens), to increase the sensitivity of the film. Using the intensifying screen sandwich increases the sensitivity of the film to ^{32}P by 8 to 10 fold (Maniatis *et al*, 1982). The entire cassette was placed in the -80 °C freezer and left to expose for 10 to 12 days. Exposure of the film at very low temperatures is to prolong the period of fluorescence during which the majority of the events are recorded by the film. The response of the film to low intensities of light is very non-linear therefore increasing the period in which this response occurs reduces this non-linear effect (Maniatis *et al*, 1982).

Following exposure, the X-ray film was removed under safety light and developed using an automatic X-ray film processor.

DATA MANIPULATION AND STATISTICS:

Data files were stored in Excel files (Microsoft Corporation; version 1.5); calculations involving the gene frequencies and phenotype frequencies were performed within the Excel worksheets. Graphs were created using MacIntosh Cricket Graph (Cricket Software; version 1.3).

Gene frequency estimation, as stated in the introduction, was generally achieved by simple gene counting under the assumption of autosomal codominance.

Heterozygosity, h, is basically $1-\Sigma p_i 2$, where p_i is the point estimate for an allele frequency, or $2p_ip_j$, from the Hardy-Weinberg equilibrium statement where p_i and p_j represent allele frequency estimates. When taken over several loci a weighting system must be used if sample sizes for loci vary. A weighting system suggested by Hedrick (1985) was implemented with a slight modification:

$$H = \frac{\Sigma(h \cdot N_i)}{\Sigma N_i \cdot m}$$

h = heterozygosity at a single locus
N_i = monkeys sampled at a single locus
m = number of loci

When Mendelian inheritance was established or inferred by analogy to a related species, the locus was tested for fit to Hardy-Weinberg equilibrium. The degrees of freedom on each test statistic was determined by the following formula:

$$d.f. = [\ \{\ s(s+1)/2\ \}\ -\ 1\ ]\ -\ \{\ s\ -\ 1\ \}$$

s = number of alleles observed at the locus

Nei's measure of standard genetic distance was calculated as follows:

$$D = -\ln I$$

$$I = Jxy/(JxJy)^{1/2}$$

$$Jxy = \Sigma\, j_{xy}/\ r$$

$$J_x, J_y = \Sigma\, j_{x,y}/r$$

$j_{xy} = \Sigma(x_i \cdot y_i)$ for loci of pairs of groups studied

$j_{x,y} = \Sigma\, x_i^2, \Sigma\, y_i^2$ for locus from population X or Y

r = number of loci used in comparison

x_i, y_i = frequency estimate of *i*th allele

The statistic, I, is the normalized identity of the genes between population,X, and population, Y. It indicates the percentage of genes that are common to both populations. The genetic distance between the groups, D, indicates the percentage of accumulated allelic differences per locus (Nei, 1972).

In 1978, Nei published a statistical method that attempted to account for the use of small numbers of individuals when estimating genetic distance. It takes the following corrective format and was used in the calculations conducted in this study

$$j_x' = (2n_x j_x - 1)/(2n_x - 1)$$

where j_x is as above and n_x is the number of individuals used to study a particular locus. This adjustment affected the remainder of the calculations in the following way:

$$J_x' = \Sigma j_x'/n_x$$

$$I' = Jxy/(Jx'Jy')^{1/2}$$

$$D' = -\ln I'$$

The minimum genetic distance was also originally determined; also according to Nei (1987):

$$D_m = \Sigma\, d_k / r$$

$$d_k = (j_x' + j_y')/2\ -\ j_{xy}$$

k = for the *k*th locus

j_x', j_y' as stated above for adjusted j's

r = number of loci

To analyze the error in the genetic distances, the variance was originally determined Nei (1978) states that it is simpler to work with the minimum genetic distance than the standard genetic distance. Variance for the standard genetic distance can be calculated but it is very complicated and a computer program is now available from Nei's lab if one needs to calculate this statistic. Although a very good estimator of the variance of D over all loci would be the sum of the intralocus variance and the variance of the population heterozygosity, the variance of the population heterozygosity is known to increase with the increase of the mean genetic distance (Li and Nei, 1975). Because the genetic distances were small, this factor was not calculated as it was likely to be negligible. The variance of D(m), when r loci are studied is:

$$V(D_m) = V(d)/r$$

$$V(d) = \Sigma (d_k - D_m)^2 / (r-1)$$

Following analysis of the data with the above statistical methods, the data was reanalyzed for genetic distance due to sparseness of data that had been demonstrated to show Mendelian inheritance and that were polymorphic. The only data that could be included in the above distance calculations were the hMF #1 data and the data from the two monomorphic proteins: transferrin and albumin. Thus genetic distance analysis was simplified by conducting pairwise heterogeneity χ^2 tests on the hMF #1 data alone. (This data was the only data that could be used when assuming Mendelian segregation as a base assumption in conducting the test. Remember, monomorphic data such as that data from transferrin and albumin simply show zero distance between the groups as all groups share the one allele present in each system.) The χ^2 statistic was determined using the following formula:

$$\chi^2_{xy} = 2n_x n_y \cdot \Sigma \left\{ \frac{(x_i - y_i)^2}{(x_i n_x + y_i n_y)} \right\}$$

where x_i and y_i represent sample allele frequency estimates

The degrees of freedom on each statistic would be the number of alleles minus one as the test is using the allele frequency estimates.

Results

The number of individuals representing each region is given in Appendix B Not all loci were analyzed for each sample; therefore the number of samples for each locus is given by deme in Table 1.

Table 1 Number of individuals investigated

Protein or DNA locus	North	South	Nevis
pseudocholinesterase	72	62	20
transferrin	107	94	74
globulin 4	80	73	39
globulin 3	76	62	39
globulin 2	99	83	73
globulin 1	96	77	72
albumin	107	94	74
prealbumin	107	90	74
hMF #1 (D1Z2)	19	20	20

General Protein gels:

Figure 1 shows a typical 15% polyacrylamide gel treated with a general protein stain. Figure 2 gives a schematic representation of the different bands of the several proteins scored on the 15% general protein polyacrylamide gel.

Albumin was readily identified because of its high concentration(Giblett, 1969). Its migration corresponded well with the fact that it is known to have a smaller molecular weight than many other plasma proteins (Vander, Sherman, &

Luciano,1980) and with the isoelectric point of human and other non-human primate albumins. Albumin variants in man have typically been revealed by electrophoresis of dilute samples on starch,cellulose acetate, or agarose (Arai *et al*, 1989; Huss *et al*, 1988, Brennan *et al*, 1988). We did not observe any albumin variants either in plasma diluted 1:4 (standard) or 1:150. Vervets from Ethiopia as well as those from Senegal and South Africa were also monomorphic at this locus (Turner, 1981; Lucotte *et al*, 1982; starch gel electrophoresis). Samples from this population were not tested in borate or acetate or on starch or agarose. However, a previous study (Palmour *et al*,1980) using cellulose acetate as supporting medium also revealed no albumin variants.

Transferrin was identified electrophoretically by comparison to a similar study on chimpanzee (King and Wilson, 1975) and previous studies on transferrins identified by ^{59}Fe binding from various vertebrate species (Palmour and Sutton, 1971; Palmour *et al*,1980). The thin, sharp band migrated toward the anode about 1/3 of the gel length, which is in good accord with its molecular weight of 73,000-76,000 daltons (Giblett, 1969). No variants were found in this study. Even though there has been some indication of transferrin variation in this population (Palmour *et al*, 1980), it does not reach polymorphic levels. The transferrin locus was found to be monomorphic (or perhaps better stated to be non-polymorphic) in all sampled demes of the West Indian vervet.

Four groups of bands were found migrating between transferrin and albumin; these were termed the globulins according to classic nomenclature(Putnam, 1960). These groups were labelled by number, one through four, according to mobility, with one being fastest and four being the slowest. As seen in Figures 1 and 2, Globulin 4 (closest to transferrin) was found to be variant in all demes with three bands identified. The number of individuals sampled is shown in Table 1: North, 80; South,73; Nevis, 39.

Globulin 3, migrating slightly faster than Globulin 4, was also found to show electrophoretic variation in all three demes. Three different electrophoretic mobilities were observed. An individual could exhibit zero, one, or two bands at this region. All three demes showed some variation, but the presence of all five phenotypic categories (non-detectable, 1, 1-2, 2, and 3-1) only occurred in the North Island monkey deme. The number of individuals sampled is shown in Table 1: North, 76; South, 62; Nevis, 39.

Globulins 2 and 1 were scored in a similar fashion. Globulin 2 showed no

variation and globulin 1 showed variation (see Figure 3)

One of these latter two globulin proteins was thought to be alpha-1-antitrypsin because of the chimpanzee study previously mentioned (King and Wilson, 1975) When western blotting and immunofixation were carried out using anti-human alpha-1-antitrypsin, it was determined that both the globulin 1 and globulin 2 areas of the gel were associated with the presence of this enzyme. Figure 3 shows the blots from this experiment. The blots from these experiments are light sensitive and prone to fading and diffusion. The one shown has diffused somewhat, but does show that there are two areas in which the presence of alpha-1-antitrypsin is indicated. The first positive area is the region that is slightly slower than the large albumin band. This first region falls in the same region as that which was assigned to Globulin 1 and Globulin 2. However, band matching between the positive area and the Globulin 1 and 2 regions was not possible. The second positive area is a large clump of darkly-staining bands with low mobility (much slower than transferrin). The second stained clump may represent cross-reactivity of the antibody with some homologous protein, such as alpha-1-antichymotrypsin or reactivity of the antibody with a polymerized version of the alpha-1-antitrypsin protein.

Even though the first positive alpha-1-antitrypsin coincides well with the region which had been scored as Globulin 1 and 2, it is not clear that the bands recognized under general protein staining are due to this enzyme activity. Therefore to err on the side of caution this region will be designated Globulin 1 and Globulin 2.

Alpha-1-antitrypsin deficiency in humans results in severe lung diseases and liver disorders (Kueppers, 1973; Talamo *et al*, 1968; Cox, 1981). Furthermore the frequency of the null allele at the human locus is quite low (Pi $^{-}$), less than 0.01 (Fagerhol, 1967; Kueppers, 1971). If one were to theorize that the globulin 1 and 2 regions did represent alpha-1-antitrypsin protein, the frequency of individuals lacking this protein (in particular, referring to Globulin 1 locus) would be higher than expected. Figure 4 describes the variation observed at the Globulin 1 region. However, given the general good health of the sampled monkey populations and the lack of good family data with this population using this system, a great deal of caution should be taken in interpreting this data.

The absence of the bands in this mobility region may be due to a second locus preventing the production of the protein or a mutation resulting in a drastic change

in electrophoretic mobility or nongenetic reasons such as sample sensitivity. It is quite clear that a thorough family study should be conducted to analyze the genetic transmission of this group of electrophoretic bands. Certainly if family data does substantiate the high frequency of a null variant and that additional information indicates that this locus is indeed alpha-1-antitrypsin protein these monkeys could be a useful model in biomedical research.

The pre-albumin band is a diffuse band with a fast mobility on the 15% polyacrylamide gel; it was named "pre-albumin" because it ran faster than or before albumin. It was thought that this band might represent alpha-1-acid-glycoprotein because of its location (King & Wilson, 1975). Western blotting and immunodetection were performed but no positive results were obtained. There could be difficulties with cross reaction of an antibody for human alpha-1-acid-glycoprotein with the monkey version of this particular protein. Regardless of identity, this area was found to be invariant in all three demes of monkeys.

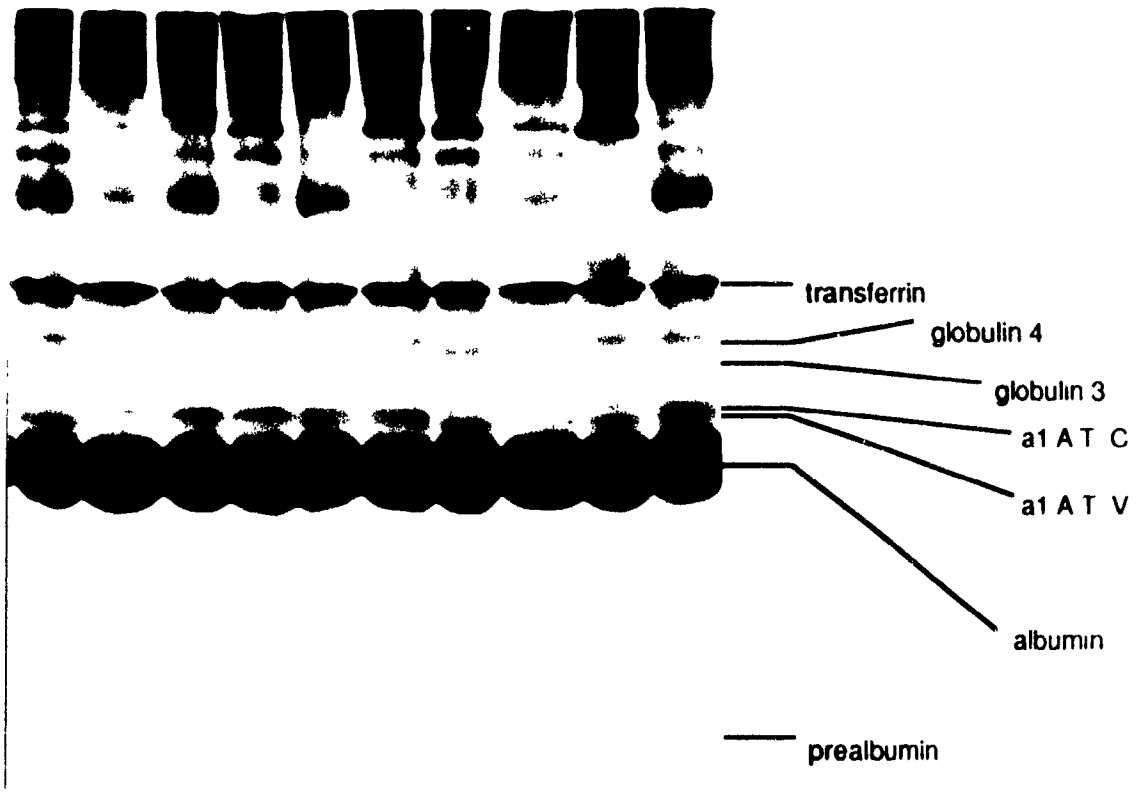

Figure 1 **Photograph of typical 15% polyacrylamide gel run with monkey plasma samples for 7-7 1/2 hours at 600 volts. Staining was by Coomassie blue protein stain. Six regions indicating the presence of protein were scored from this type of gel.**

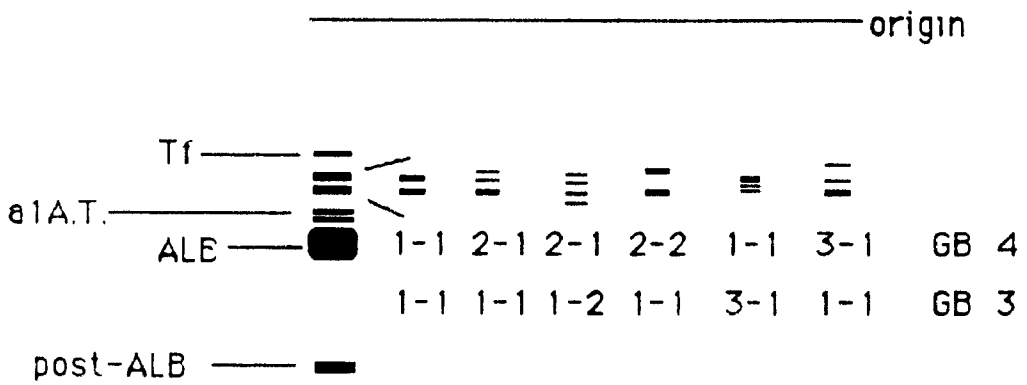

Figure 2 **Schematic representation of the plasma protein bands scored for each monkey deme using the 15% polyacrylamide gel.**

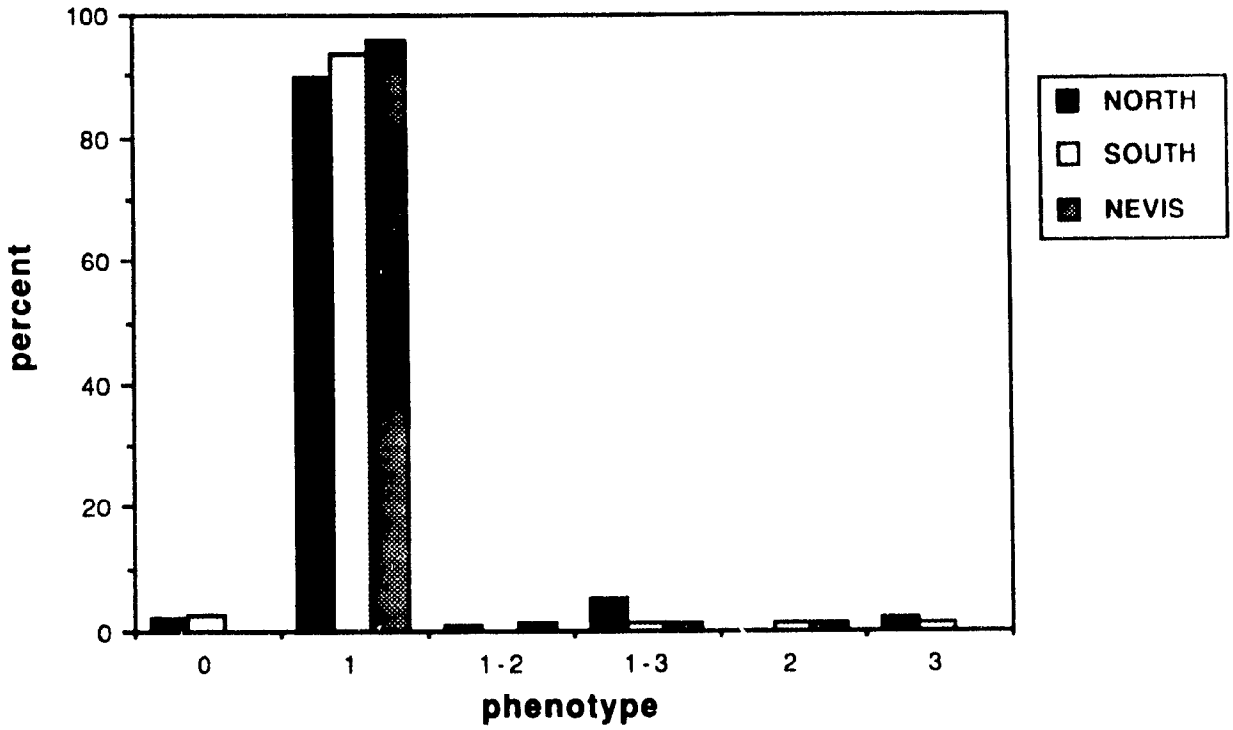

Figure 3 Phenotypic distribution for the Globulin 1 region of the 15% PAGE gel. The sample sizes ranged were 96, 77, and 72 for North, South, and Nevis groups respectively.

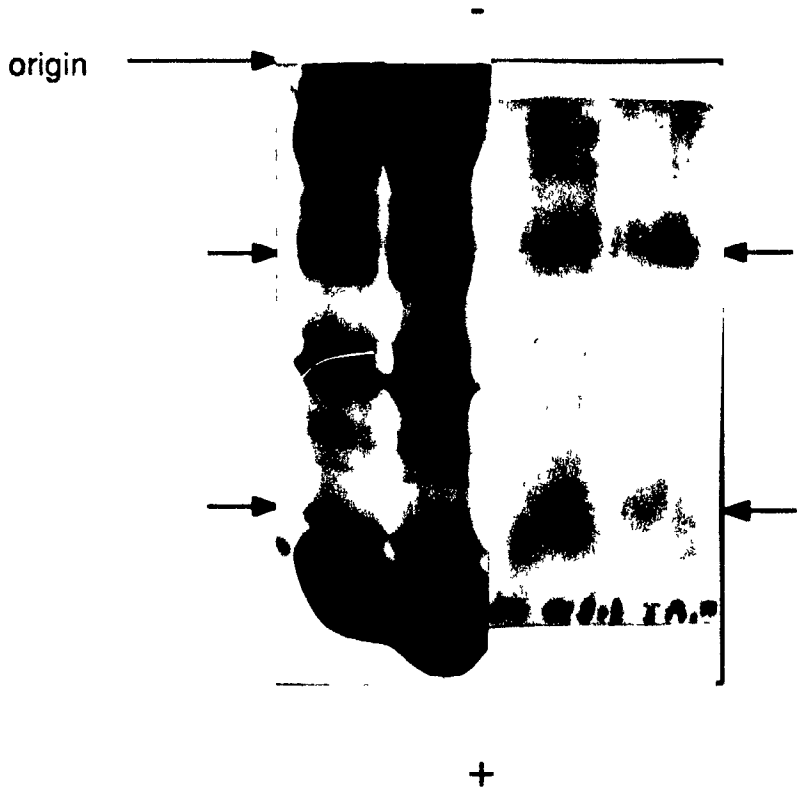

Figure 4 **Nytran membrane blots were prepared after electrophoresis of monkey plasma proteins. The blot was tested for the presence of alpha-1-antitrypsin; two areas tested positive. As can be seen from the control protein gel, one positive area was that which was previously labelled globulin 2 and 1. The slower migrating area was not scored and may represent the formation of complexes of the alpha-1-antitrypsin molecule or cross reactivity with a similar enzyme inhibitor, alpha-1-antichymotrypsin.**

Pseudocholinesterase:

Two types of analyses were used at this locus, qualitative and quantitative Qualitative analysis was achieved with the 8 5% acrylamide gel and Tris-Gly (pH8.3) to display electrophoretic mobility variants. Quantitative analysis of enzyme activity utilized a clinical kit from Sclavo Diagnostics Butyrylthiocholine was found to be the best substrate for pseudocholinesterase testing in this monkey Acetylthiocholine and succinyldithiocholine iodide were also tested and it was found that butyrylthiocholine gave 10 fold higher activities

Appendix C details the raw data of the phenotypes scored for all three demes, Appendix D provides the phenotypic counts There were 72 samples from the North deme, 62 samples from the South deme, and 20 samples from the Nevis deme (Table 1). Figure 5 is a photograph of a polyacrylamide gel exemplifying the type of visual results that were obtained and used for scoring at the pseudocholinesterase protein, there is variation in both mobility and in intensity of staining. Figure 6 shows a schematic diagram of the different mobility phenotypes, with priority given to the faster band in the phenotypic nomenclature within this study.

Figure 7 illustrates a comparison between the electrophoretic data and the enzyme activity data. The mean for each phenotype subgroup was calculated 237.08 (non-detectable), 525.55 (1-1), 151.13 (1-4; only one individual), and 317.30 (3-1; only one individual) (see table 2). A complete comparison could not be made as data for many individuals in the study population was available for only one of the two systems.

There is clearly a great deal of variation in individual pseudocholinesterase activity. But a definite correlation between the electrophoretic and the in vitro data cannot be drawn; more data must be collected if this goal is to be achieved.

First, informative family studies must be conducted in both systems. The electrophoretic bands from the gel data must be determined to be derived from straightforward Mendelian inheritance. Nongenetic and other genetic factors such as sample degradation or second locus interfaction must be ruled out. Although samples were freshly collected and promptly analyzed, no controlled degradation studies were done. Second, if the gel data are to be correlated with the clinical kit activity data, then the kit data need also be shown to follow some genetic pattern whether it be Mendelian or quantitative. With these pieces of data perhaps the

systems can be used adjacently to clarify genotypes which are difficult to assign.

Some problems that may be predicted involve those individuals that appear to lack pseudocholinesterase activity on the gel. If these observations have genetic etiology the system may contain a silent allele or a variant with very low activity In such a situation a homozygote of a "visible allele" may not be distinguished from a heterozygote for the silent allele or very low activity allele. This could result in an underestimation of the frequency for the silent or very low activity allele and an overestimation for the frequency of the other alleles involved. However, there are mathematical estimation procedures that do account for these type of genetic systems (Hedrick, 1985).

Figure 8 illustrates the phenotypic distribution of individuals across the three demes of monkeys.

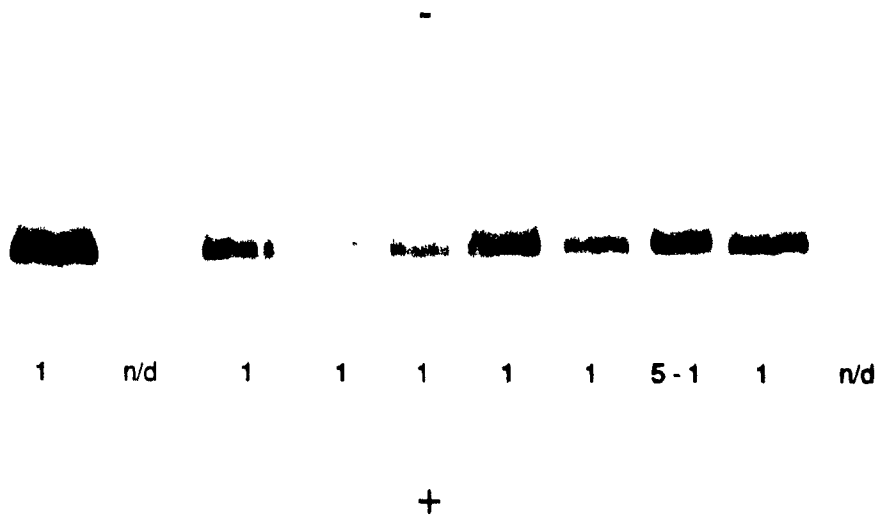

Figure 5 **Photograph of 8.5% polyacrylamide gel used to determine phenotypes of monkeys at the pseudocholinesterase locus. Gel was run for 6 - 6 1/2 hours at 600 volts and then stained using alpha-napthyl acetate as a substrate and Fast Red TR as a dye. Notice the definite variation in intensity of staining particularly at the region of the electrophoretic band 1. Comparable amounts of protein were loaded into each well of the gel. The abbreviation "n/d" denotes non-detectable.**

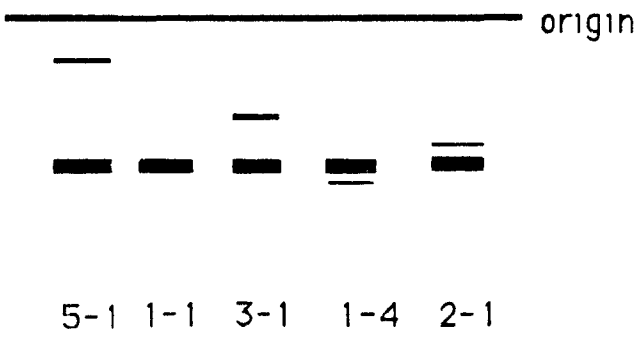

Figure 6 **Schematic diagram of mobility variants identified for the pseudocholinesterase locus.**

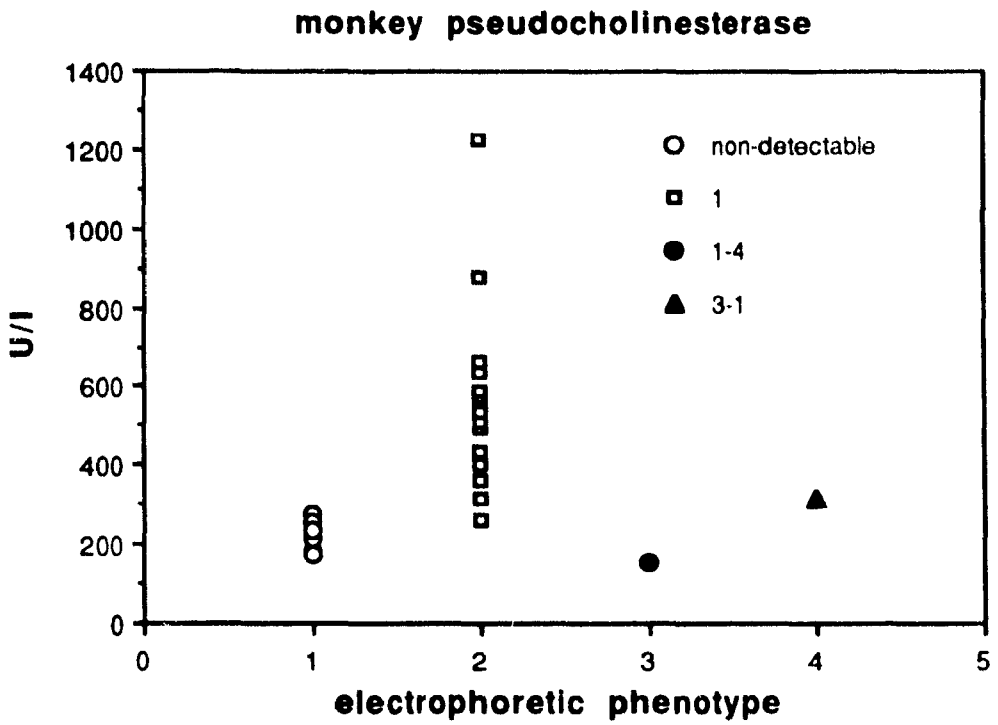

Figure 7 **Histogram of compiled qualitative and quantitative data at the pseudocholinesterase locus. Each symbol represents one monkey sampled using both electrophoretic and spectrophotometric methods. The number system on the horizontal axis is simply to separate four electrophoretic phenotypes determined from the gel. The numbers one to four represent the phenotypes: non-detectable, 1, 1-4, and 3-1 respectively.**

Table 2 Compilation of enzyme activities and gel phenotypes of monkeys for serum pseudocholinesterase.

The activity is expressed as micromoles of 5-thio-2-nitrobenzoic acid liberated per minute per liter of serum at 37 degrees Celsius. This unit is based on the international enzyme unit (SCLAVO Diagnostics).

Phenotype is defined, for this purpose, as visual banding displayed by a sample when separated on 8.5% acrylamide gel and chemically assayed with alpha-napthyl acetate as a substrate and Fast Red TR as an indicator of activity.

Phenotype	sample size	mean activity	standard deviation
non-detect	8	237.1	34.0
1	19	525.6	222.89
1-4	1	151.13	-
3-1	1	317.36	-

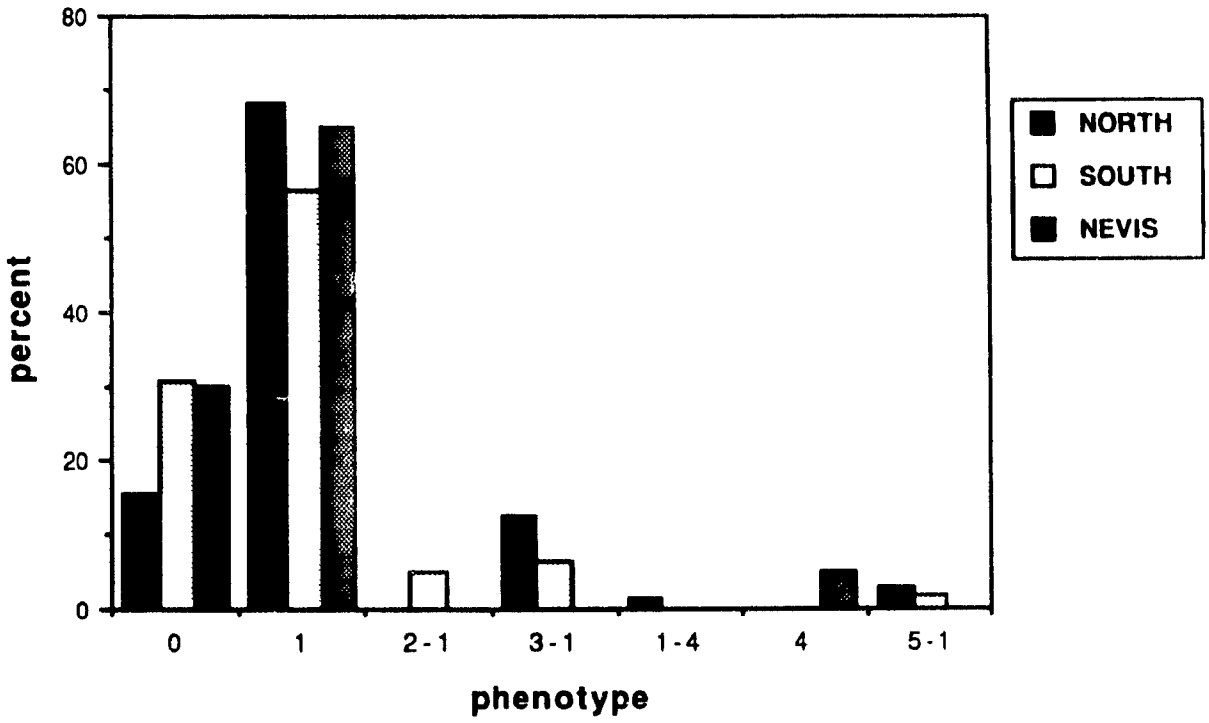

Figure 8 **Electrophoretic mobility phenotype distribution among the three demes of monkeys for the pseudocholinesterase protein. The sample sizes were 72, 62, and 20 for North, South, and Nevis groups respectively.**

DNA polymorphism:

EcoR 1-digested DNA defined by the Human Molecular Fingerprinting probe #1 (hMF #1 recognizes sequences located at the D1Z2 locus; human 1p36.1) and is polymorphic in all three demes. Family studies were completed, showing that alleles are codominantly inherited (see figure 9). This autoradiogram shows a family group which includes one male with a baby from each of three different females. A second family group (a male, a female, and their four offspring), and other male-female-single offspring groups also expressed hybridization patterns which were consistent with Mendelian inheritance (not shown). Appendix E gives the complete collection of data supporting this conclusion.

Further study of the monkey population seems to suggest that there are at least six alleles defined by hMF#1 probe. These can be seen in figure 9. Although standard molecular weight markers were used, it was difficult to assign alleles by the inferred molecular weights of the bands. This type of procedure can detect differences anywhere from 150 to 400 base pairs; the differences between bands from different monkeys were seemingly as small as 40 base pairs. Thus, measurement imprecision due to technical limitations confused the assignment of alleles. With this in mind, scoring was achieved with reference to family data, the reference gel that delineates the six alleles in figure 9, and the few individuals that were repeated on different gels.

The alleles were assigned by electrophoretic mobility; 0 for the slowest and 4 for the fastest. The allele 3b was discovered following the assignment of allele 4. It was, therefore, assigned 3b to permit the scheme indicating electrophoretic mobility to remain intact.

The allelic frequencies can be found in Table 3 and the number of sampled individuals in Table 1: North, 19; South, 20; Nevis, 20. A comparative graph of the allelic frequencies for EcoR 1-digested monkey DNA at the hMF#1 locus is located in figure 10 . The monkey DNA was also cut with the restriction endonucleases Hinf 1 and Taq 1. The Hinf 1 digests consistently failed. Several lots of the enzyme and two different buffers were tried to no avail. One reason for the failure of Hinf 1 to digest the vervet DNA could be methylation of nucleotides within the Hinf 1 recognition site, as Hinf 1 is known to be sensitive to nucleotide methylation (Pech *et al*, 1979; Huang *et al*, 1982; Nelson and McClelland, 1987; Washio *et al*, 1990).

Taq 1 digests resulted in a complex set of bands when hybridized with the hMF #1 probe (up to 12 or more bands). Several regions that appeared as thick bands may actually have been doublets that would be detected if technical sensitivity was improved (reduce exposure time to film). One family group was analyzed along with four randomly chosen unrelated individuals. The family group had been previously studied using EcoR 1 and consisted of two potential fathers, a mother, and a child. The Taq 1 results agreed with the EcoR 1 results, in that Mendelian inheritance of the Taq 1 fragments is suggested. More family data would be required for acceptance of this conclusion. In addition, further study is necessary to determine whether a second DNA polymorphism defined by Taq 1 digestion and hMF #1 probe exists.

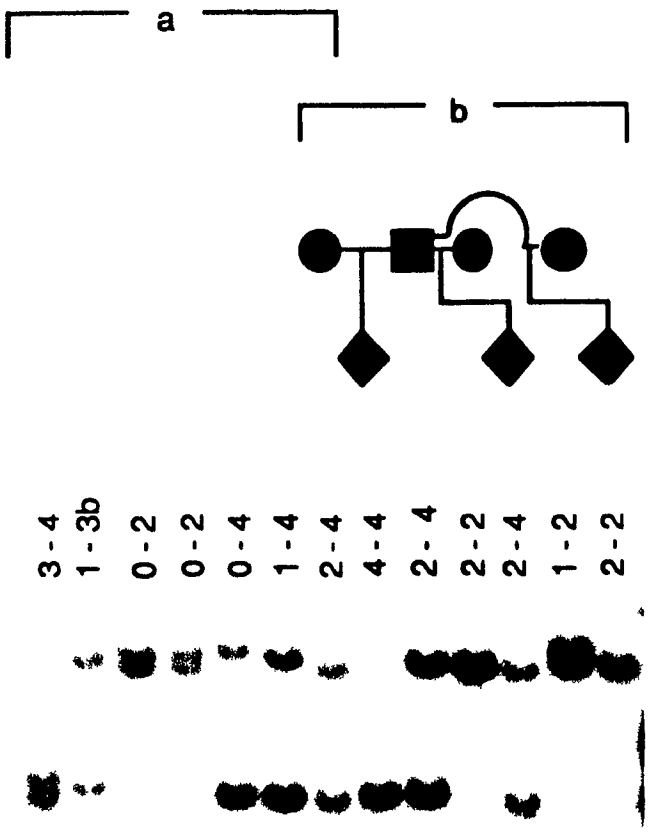

Figure 9 **Human Molecular Fingerprinting #1 probe (D1Z2;1p36.1) defined these alleles in the West Indian vervet. Section (a), exhibits reference standards that delineate all six alleles. Section (b) shows a subset of the family data.**

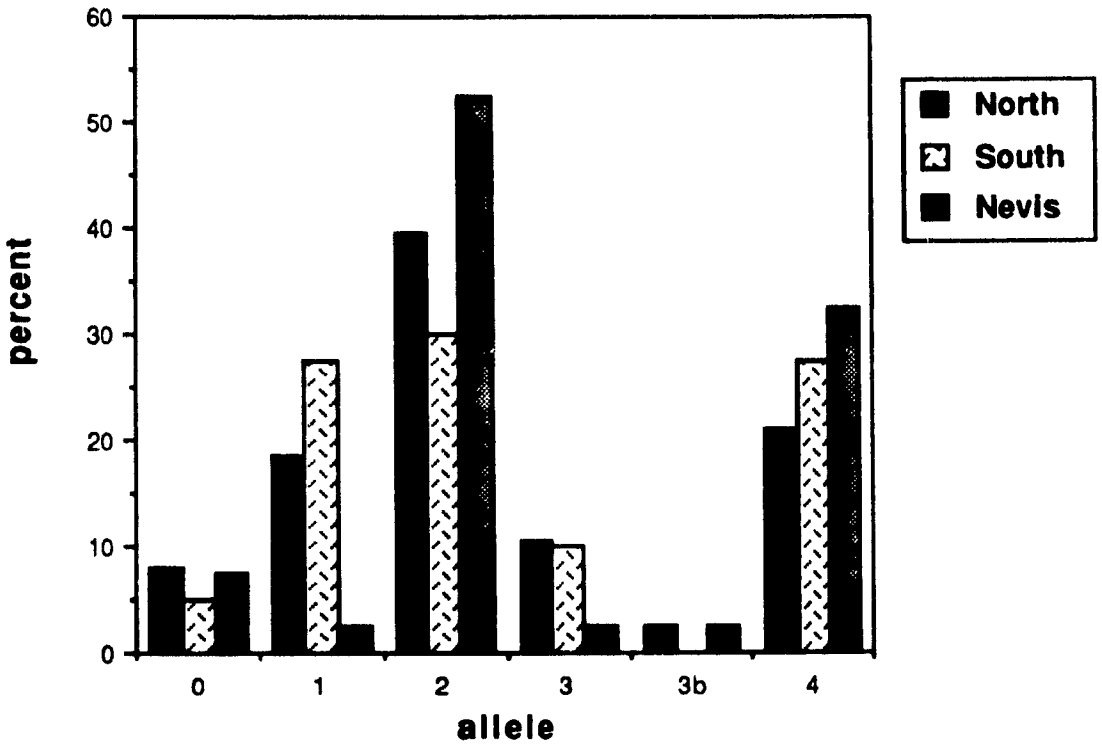

Figure 10 **A comparison between geographically separated demes of monkeys at the locus identified by the hMF #1 probe (D1Z2; 1p36.1) using EcoR 1-digested DNA. The sample sizes within the demes were 19, 20 and 20 for North, South, and Nevis respectively.**

Table 3 Estimated allelic frequencies for some geographically separated demes of monkeys in the West Indies

locus	North	South	Nevis
hMF #1			
0	0.079	0.050	0.075
1	0.158	0.250	0.025
2	0.447	0.325	0.525
3	0.105	0.100	0.025
3b	0.026	0.000	0.025
4	0.184	0.275	0.325

χ^2 Analysis for Goodness of Fit:

The purpose of the χ^2 test is to test the fit of observed data to a particular model To test for fit, an hypothesis about the inheritance of the involved locus must be constructed. An assumed model can only be constructed when some amount of data suggests a certain mode of inheritance. For this reason some data cannot proceed past the stage of simple observation and presentation. These data include all information on the globulin 3 and 4 regions of the polyacrylamide gel

Furthermore, when a locus appears monomorphic in a population, comparing observations and expected values yields no information on the population's genetic structure. Data which fall into this category are: transferrin, albumin, and prealbumin.

When a locus has two alleles that are quite common and several rare alleles there exist several phenotypic classes that have few observations. As a general rule for the χ^2 analysis, the expected number in each class should be greater than five (Hedrick, 1985, p.64). If the observations are lower in certain classes, then appropriate combinations should be made so that the results of the test are not simply functions of the small number of observations in these classes. Loci that would fall into this class include pseudocholinesterase, alpha-1-antitrypsin, and hMF #1.

However, both the pseudocholinesterase and Globulin 1 (alpha-1-antitrypsin?) loci were insufficiently tested with regard to Mendelian inheritance to allow further analysis. Literature suggests inheritance in a Mendelian fashion for electrophoretic phenotypes at the alpha-1-antitrypsin locus (Fagerhol and Gedde-Dahl, 1969, Kueppers, 1973; Garver *et al*, 1986) and for enzyme activity phenotypes at the pseudocholinesterase locus (Oki *et al*, 1964; Simpson and Kalow, 1964). Also, for the Globulin 1 (alpha-1-antitrypsin?) locus, one small family group was found to be consistent with Mendelian inheritance:

	father	**child**	**mother**
monkey	O1118	O704-3	O704
phenotype	1-2	1	1-3

Nonetheless this information is not sufficient for reliable assignment of genetic

transmission. Thus, a χ-square test was not applied to the data for pseudocholinesterase and alpha-1-antitrypsin obtained in this study.

However, the DNA locus was studied with regard to its mode of inheritance, which proved to be autosomal codominant. Due to many possible phenotypes and small sample sizes, many phenotype classes for the hMF #1 locus were observed to have fewer than five individuals. Therefore, the phenotype classes were combined into three classes: individuals with only the slow-moving bands (U), individuals with only the fast-moving bands (L), and individuals that had a slow and a fast-moving band (U-L). The raw data can be found in appendix F. When these phenotype classes were collapsed, each of the three demes was found to be in Hardy-Weinberg equilibrium for the hMF#1 locus. The statistics are displayed in table 4 below (also see Appendix F).

Table 4 **χ^2 values for test of fit to Hardy-Weinberg equilibrium for sampled monkey demes.**

LOCUS	d.f.	χ^2 Values			
		NORTH	SOUTH	NEVIS	OVERALL
hMF #1	1	0.0125	0.0863	0.6851	0.0726

Population Measures:

Prior to calculating population measures which characterize these monkey demes, the data should be analyzed in terms of its suitability. There are several criteria under which loci should be chosen, some of which include:

1. random selection
2. reliable detection of variation with methods available
3. pattern of inheritance known or demonstrable

With these simple criteria a few of the loci previously detailed must be omitted from the measures of genetic variation within and between the demes of monkeys on St. Kitts and Nevis.

Albumin was found, using 15% PAGE, TRIS-Gly (pH 8.3), to be monomorphic in all three demes. However, if variants do exist and have not been recognized either due to technical insensitivity, erroneous conclusions could be drawn if the albumin data were included in the measure calculations, particularly for heterozygosity measures. Albumin data was thus excluded from the population measures calculations.

Globulin 3 and 4 are unknown proteins. Although both "loci" are polymorphic, neither should be used in determining the level of polymorphism because the inheritance of these visual electrophoretic bands is not known. These data must be excluded.

As stated previously no extensive family data were collected at the alpha-1-antitrypsin and the pseudocholinesterase loci. Thus, these data must be excluded from the population measures calculations.

Although the DNA data itself is of interest and indicates possible deme differences, it most definitely was not chosen without some indication of its potential level of variation within the study population. By definition, the hMF #1 probe is a HVR probe (hypervariable region probe) - it recognizes highly variable regions of DNA; it showcases very polymorphic regions of DNA.

Although published works using the hMF #1 probe were largely on humans, a cross-species study was carried out (Tynan and Hoar, 1989). But, it should be noted that the number of individuals representing each species was very small (1 or 2 individuals). The human studies indicated high levels of polymorphism. It was likely that this probe would identify polymorphic sequences in the present study group (Weiss, 1989). The DNA probe was chosen for its potential ability to detect

genetic variation if it existed and thus population measures based on DNA variation will be biased toward showing differentiation. This bias will be integral to the interpretation of the results obtained

Hedrick (1985) states that the most widely used measure of genetic variation is heterozygosity. A version of his formula was used to estimate the average heterozygosity at each locus for each deme. The average heterozygosity was 2.02%, 2.49%, and 2.49% for North Island, South Island, and Nevis respectively. The heterozygosity calculations included data from the following loci: transferrin, prealbumin, and hMF #1 (see Appendix G).

The percentage of polymorphic loci for each group was 33.3%. This measure included the same data as was included in the heterozygosity calculations.

The original intent of this project was to study genetic variability between demes of monkeys. To determine whether further pursuit of this goal would be fruitful, the most polymorphic data from this study was used to conduct a pairwise heterogeneity χ^2 test of the groups. This trial analysis used the χ^2 test for small numbers designed by C.A.B. Smith (1986), a conservative method for determining whether genetic differences between groups are significant. The following results were obtained

North-South	3.55
South-Nevis	12.41
North-Nevis	7.53

Testing whether a significant difference exists between two groups requires looking at the distribution of the χ^2 statistic about its mean. Because this statistic can only be positive the distribution is obviously skewed; thus it is more helpful to look at its square root and it variance (Smith, 1986). The standardized normal variate, z_2, an indication of the level of difference between the two demes being tested was calculated for each pair:

North-South	-0.46
South-Nevis	2.26
North-Nevis	0.92

At a confidence level of 95%, a significant difference is noted when z_2 is greater

than 1.65. This would suggest that the allele frequencies at this locus differ between the South and the Nevis demes (z_2 =2.26, p=0.02). No significant differences can be suggested between North and South demes and North and Nevis demes in light of the above data

Discussion

The purpose of this study was to determine whether genetic diversity could be measured between three geographically separated demes of *Cercopithecus aethiops* on the islands of St. Kitts and Nevis. The main question was whether monkeys from distant groups could be genetically distinguished from one another, prior to determining gene flow between adjacent regions. The population measures that were generated from this study and which are discussed below are, unless otherwise stated, based on the information provided from the sampling of the following loci: transferrin, prealbumin, and hMF #1 (D1Z2).

In all three demes, one out of the three reliable loci was polymorphic.

A test for Hardy-Weinberg equilibrium on each deme separately indicated that all three populations were in equilibrium. When the data from the three demes were aggregated and treated as one large population, this large group was also found to be in Hardy-Weinberg equilibrium.

When average heterozygosity was determined for each deme the following results were obtained:

North	2.02%
South	2.49%
Nevis	2.49%

A comparison of these results indicates that no significant genetic differences exist between the demes with respect to the loci studied.

Using the most reliable data available from this study, the DNA data, a pairwise heterogeneity χ^2 test of groups was performed. The χ^2 values obtained from that test were as follows:

North-South	3.55
South-Nevis	12.41
North-Nevis	7.53

As stated by Smith (1986), a more reliable measure of the significance of a difference between two groups of observations is the square root of the χ^2 statistic and its standardized normal variate. The measure of the significance is found in a statistic termed z_2. The z_2 values for the pairwise comparisons were found to be

North-South	-0.46
South-Nevis	2.26
North-Nevis	0.92

At a confidence level of 95%, the only pair that displays genetic difference is the South versus Nevis pair (z_2=2.26, p=0.02). In other words, a genetic "distance" was observed.

When searching for characteristics that may be used to distinguish closely related groups it is useful to look at DNA markers as they, in general, exhibit more polymorphism than protein markers. Within protein markers it is wise to canvass plasma proteins as they generally display more polymorphism than enzymes.

One particular area of protein variation that is often problematic, but was studied in this and many other investigations, is quantitative variation. Many of the problems encountered by other investigators were also experienced in this study of pseudocholinesterase.

A major problem arises when trying to assign allelic designations on the basis of activity levels (Harris *et al*, 1963). The main reason this task is near-impossible is the overlapping distributions of enzyme activities in each phenotypic class. In reference to the present study, the measured activity of a plasma sample in the most common electrophoretic phenotype (1) could range from 264.47 to 1224.11 micromoles 5-thio-2 nitrobenzoic acid liberated per liter serum at 37 degrees Celsius. The "non-detectable" phenotype had an enzymatic activity range of 173.80 to 272.03. The other two rarer phenotypes that were measured fell into one of these ranges; 1-4 into the range for non-detectable and 3-1 into the range for the 1 phenotype. More importantly, the ranges of enzyme activities for the two most frequently observed phenotypes overlap. Although the overlap is quite small, there is not a decisive distinction between the two phenotypes in terms of enzyme activities such that phenotype could be assigned by activity alone. Trying to decipher the actual genotypes involved would be incredibly difficult.

The troubles confronted in this study of pseudocholinesterase are not unlike those faced by other researchers investigating the quantitative variation of proteins

such as red cell acid phosphatase (Hopkinson *et al*,1964).

Another vexatious area in the study of protein variation and one that arose during this project is that of the null allele (Breed and Jolly, 1988). The question is always the same, "Does the null exist or is it an anomaly?". Some of the possible explanations for the loss of a band on a protein gel include:

1. technical insensitivity - the "missing" band may be present but is being masked by another band or group of bands; this situation would indicate the presence of another allele or protein modification not previously observed.
2. storage problems - the "missing" band may simply be further down the gel, broken down by degradation related to poor storage conditions or age (Juul, 1968).
3. second locus interference - the "missing" band may actually be missing due to interference in protein production by the gene action of a second locus, rather than the presence of a null allele.

Even with the limitations of the present study, there are clear differences between the West Indies populations and the African populations. A detailed comparison of West African monkeys with the Caribbean monkeys would truly be of interest for evolutionary study. An investigation of genetic variation is the study of the evolutionary potential of a population. Therefore, a comparative study of the genetic variability in the old world and new world populations would inherently provide insight into the mechanisms and pathways of evolution.

Average heterozygosity has been reported for a few populations of African vervets. In Ethiopian animals, Turner (1981) reported an average heterozygosity of 5.6%. Dracopoli and colleagues (1983) reported an average heterozygosity of 5.0% for vervet troops in central and southern Kenya. Lucotte and coworkers (1982), who studied different subspecies of *C. aethiops* from Senegal, Ethiopia, and South Africa, reported an average heterozygosity of 2.1% for *C. aethiops sabaeus* (which is, taxonomically, the same species as the monkeys located in St. Kitts and Nevis). The average heterozygosities observed in St. Kitts and Nevis demes were 2.02%, 2.49%, and 2.49% for North, South, and Nevis respectively.

Compared to the studies done by Dracopoli *et al* (1983) and Turner (1981), the West Indian populations show a low level of variability. However, it should be noted

that these differences may well be due to the involvement of different subspecies or simply different methodologies. As noted by Lucotte *et al*(1982), different subspecies of *C. aethiops* exist across Africa. Ethiopia and South Africa have different subspecies than Senegal. When Senegalese monkeys - *C. aethiops sabaeus* - are compared to the West Indian monkeys the levels of average heterozygosity coincide to a greater degree (Senegal - 2.1% and St. Kitts/Nevis - 2.02% - 2.49%). But, it should also be noted that Lucotte's group observed a lower level of variability across all three subspecies - lower than those levels observed by the other two studies of the African vervet.

Of further interest in the comparison of heterozygosity levels between the African studies and the Carribean study, some of the loci studied were common to both sets yet their treatment regarding their use in heterozygosity calculations was different Both Dracopoli *et al* (1983) and Turner (1981) seem to have used data from their analysis of pseudocholinesterase in their heterozygosity calculations even though the genealogical information was either not available or not presented. Lucotte *et al* (1982) also used information from loci that had questionable genetic foundation such as the prealbumin 1 protein. Recall that any questionable loci in this study were omitted from the heterozygosity calculations, for example, the pseudocholinesterase data Therefore the direct comparisons of these population variation measures with those presented here are not possible. The levels of variation within the troops on the islands of St. Kitts and Nevis are the most reliable estimates given the information obtained thus far.

What reasons could be behind the low variability found in the West Indian populations? There are a few reasons that could be hypothesized. First, and probably most obvious, is a founder effect. If these island populations stemmed from a small population then the variation present in the founding group would be the main source of variability. A founding group with little variability would result in a present-day population with low level variation. However, it has generally been accepted that the St. Kitts/Nevis populations were repeatedly introduced from West Africa in small groups over a period of about 100 years (McGuire, 1974) This mode of introduction would provide a broader sample of the genetic variation in the original West African populations Furthermore, if a restricted founding group was responsible for the present population the level of variation should be even lower because the Senegalese vervet has a level of variation that is very similar to that of the St. Kitts/Nevis populations (Lucotte, 1982). It appears unlikely that the low

present-day variability is due solely to founder effect.

Stringent selection could also result in the low level of variation that was observed in the West Indies populations but this seems unlikely because these monkeys have no natural predators or any great problem with locating subsistence

This particular study is useful or could lead, by example, to useful information for the researchers that use this primate group as a tool in scientific research. Genetic typing of the monkeys found in the compounds of the Behavioral Science Foundation on St. Kitts could be used to document paternity, as well as genetic and physiological integrity of the breeding and experimental groups. In fact, when studying the hMF #1 locus, paternity was determined in a case where there were two possible fathers.

Furthermore, because extensive behavioral data is collected, genetic and behavioral data may be compared and analyzed to investigate the roles of nature and nurture in individual development.

No inference about the process of evolution can be made by examining data from one point in time. However, if one looks at a population at different time points one can make some inferences about the various groups of the same species and whether they are diverging or converging. Thus, this study stands as a frame in the moving picture of the West Indian monkeys on the islands of St. Kitts and Nevis.

Appendix A - Recipes for Solutions

General Electrophoresis:

1. HCl-Tris:

3M Tris, 36.3g (Sigma no T-1503)
0.48M, 48ml 1N HCl (concentrated HCl is 12M=12N)
Make up to 100ml with doubly deionized water. Adjust to pH 8.9 with solid TRIS or 1N HCl.

2. 30% Acrylamide:

300g acrylamide made up to 1L with doubly deionized water.
Mix thoroughly and then add: 5g charcoal, activated.
Stir for about 1 hour in a glass container.
Filter slowly by gravity on a buchner or regular funnel. May have to be refiltered if some charcoal leaks through. Can also centrifuge for 10 minutes at 2000rpm
Then add 8.0g of N-N'-methylene-bis-acrylamide
Mix thoroughly.
Store in glass and at 4 °C.

3. H_3PO_4 - TRIS:

0.47M Tris, 5.7g
25.6 ml 1M H_3PO_4 (concentrated H_3PO_4 is 15M)
Make up to 100ml with doubly deionized water.
Adjust to pH 6.9 with solid Tris or 1M H_3PO_4.

4. 2X Ammonium Persulfate - Catalyst:

0.28g ammonium persulfate
make up to 100ml with doubly deionized water.
Should not be used if more than a week old.

5. Tris-GLY Running Buffer 20X stock:

0.15M Tris, 18g
1.15M GLY, 86.4
Make up to 1 liter with doubly deionized water.
Adjust to pH 8.3 with solid Tris or 1N HCl.
Used as a running buffer, dilute 1:20 with doubly deionized water.

6. 15% acrylamide gel: 32ml/gel

4ml of Tris-HCl
16ml of 30% acrylamide
8ml 2X catalyst
4ml doubly deionized water
15µl of N,N,N'-Tetramethylethylenediamine (TEMED). Used to quickly initiate polymerization of the gel.

7. Stacking Gel (approximately 5%):

1.4 ml of Tris - H_3PO_4
1.8 ml of 30% acrylamide
3.5 ml 2X catalyst
3.3 ml doubly deionized water
10µl TEMED

8. 8.5% acrylamide gel for pseudocholinesterase typing:

4 ml of Tris - HCl
9.1 ml of 30% acrylamide
8 ml of 2X catalyst
10.9 ml doubly deionized water
15 µl TEMED

9. 0.04% Coomassie Blue stain used for general protein staining:

0.2g Brilliant Blue R

500 ml 10% perchloric acid (perchloric acid generally comes as a 60% solution therefore use 83ml concentrated perchloric acid and make up the volume to 500ml with doubly deionized water).

10. Fast Red TR stain for qualitative gel pseudocholinesterase typing (for 1 gel):

245ml 0.2M phosphate buffer, pH 7.1

5 ml 1% alpha-naphthyl acetate dissolved in 50% aqueous acetone (lab grade)

98mg Fast Red TR Salt

11. Phosphate buffer:

125 ml 0.2M Na Phosphate monobasic (27.6g/L)

375 ml 0.2M Na Phosphate dibasic(28.4g/L)

Mix thoroughly, pH to 7.1 by adding the needed solution, monobasic or dibasic

Western Blotting:

1. 20X Tris Buffered Saline(TBS):

87.6g NaCl

12.11g Tris base

adjust to pH 7.4 with 1N HCl; use at 1X strength, diluting with deionized water.

2. Signal Detection solution:

0.04g 4-chloro-1-napthol in 10ml absolute methanol

then add 90ml 1XTBS

just before use, add 100µl hydrogen peroxide

This solution is light sensitive (as is hydrogen peroxide itself), so make just before using.

DNA Work:

1. UNC - Phenol:

500g of molecular grade phenol (distilled)
150ml of 2.0M Tris, pH 7.9 (121.1g Tris/500ml)
150ml doubly deionized water
0.75g 8-hydroxyquinoline
37.5ml m-cresol
1.5ml ß-mercaptoethanol

Dissolve the phenol slightly and divide into two brown bottles (reducing agents are light sensitive, can just wrap the bottle in aluminum foil). Add half of each of remaining ingredients to each bottle and let equilibrate overnight or for a couple days (if not in a hurry!!!). Remove unused buffer which will be on the top of the solution. Store in small "light-protected" bottles in the freezer. These aliquots can be refrozen, if necessary, but generally one can leave the "active" bottle at room temperature under a fumagation hood, and thaw aliquots as needed. When using the phenol mixture, take from the bottom of the bottle as it is difficult to remove all of the unused equilibrating buffer.

2. RSB Buffer:

10mM Tris, 1.211g
10mM KCl, 0.746g
10mM $MgCl_2$, 2.033g
Make up to 1L with Millipore water.
Adjust with solid Tris or 1N HCl, to pH 7.6.

3. TE^{-4} Buffer to reconstitute dried DNA:

10mM Tris, 0.606g
0.1mM Disodium Ethylenediaminetetraacetate (EDTA)
Make up to 500ml with Millipore water.
Adjust the pH to 8.0.

4. SDS Solution for DNA isolation:

0.5M NaCl, 2.92g
0.5% Sodium dodecyl sulphate, 0.5g
2mM EDTA, 0.074g
Make up to 500ml with 1X RSB buffer.

5. 10X TPE Buffer:

0.89M TRIS, 108g
0.23M H_3PO_4, 15.1ml conc. H_3PO_4
5mM EDTA, 7.45g
Make up to 1 liter with Millipore water.

6. Gel Running Buffer:

100ml 10X TPE
900ml Millipore water
100µl Ethidium Bromide solution (10mg/ml water)

7. Gel loading/tracking dye solution:

0.1% bromophenol blue,0.01g
0.1M EDTA, 0.372g
0.1% SDS, 0.01g
50% glycerol, 5.0ml
Make up volume to 10ml with Millipore water.

8. Alkaline Blotting Buffer (2 liters/gel):

100ml 4M NaOH (160.04g/L in Millipore water)
120ml 5M NaCl (292.2g/L in Millipore water)
780ml Millipore water

9. 20XSSC:

3M NaCl, 175.3g
0.3M Na Citrate, 88.2g
make up to 1L with Millipore water.
adjust to pH 7.4 with 1N HCl

10. 5XSSC for washing Nytran to remove alkali following transfer:

125ml 20XSSC
375ml Millipore water

11. 20% SDS for use in washing nonspecifically bound probe from the blot:

20g SDS per 100ml of autoclaved water

12. 100X Denhardt's Solution:

2% polyvinylpyrrolidone, 1g (PVP)
2% Ficoll 400, 1g
2% BSA, 1g
Dissolve in Millipore water and make up volume to 50ml.
Filter to sterilize.
Make aliquots and freeze.

13. 50% Dextran Sulphate:

50% dextran sulphate, 25g
Dissolve in 50ml of autoclaved, Millipore water.
Separately autoclave the beaker and stir bar prior to use.
Store in fridge, DO NOT FREEZE.

14. Deionized formamide.

6g mixed bed resin
100ml of formamide (BDH no. ACS 360-76)
Stir for 15-30 minutes until pH is 7.4.
Filter, by gravity, and store in aliquots in the freezer. Use from the freezer each time.

15 10mg/ ml Herring Sperm DNA:

Put 1g of Herring Sperm DNA (Boehringer/Manneheim no. 223646) in 100ml autoclaved, Millipore water. Use flamed tweezers for the transfer. Cover the erlenmeyer with parafilm and let dissolve overnight in the fridge.

Sonicate the DNA in small aliquots so that the size of the fragments is about 500 base pairs. Store in small aliquots in the freezer for future use, keep small aliquot in fridge for daily use (say around 5 ml). It can be boiled repeatedly. Checking for good shearing is important to good blocking. Put 1µg on a minigel, it should run with the bromophenol blue, which is runs around 500 base pairs.

16. Prehybridization Solution:

6 ml 20XSSC
4 ml 50XDenhardt's solution
1 ml 20%SDS
0 2 ml of H S DNA (10µg/µl)
distilled water to bring up volume to 20ml

Herring sperm was boiled for five minutes to denature it, rapidly cooled on ice for five minutes to prevent reannealing, and then added to rest of ingredients

17. Hybridization Solution

9 ml 20XSSC
15 ml 100% formamide at pH 7.4
3 ml 50% dextran sulphate

1 5 ml 20%SDS

0 3 ml of H S DNA (10μg/μl), boiled

distilled water to bring up volume to 30ml

30,000,000cpm of hMF#1 probe

250,000cpm of lambda probe

APPENDIX B. Locations found in St Kitts geographic study areas

Summary of monkeys and their locations

location	number	deme	total
St. Pauls	54	North	127
Parsons	8		
Saddlers	32		
Newton's Ground	28		
(Mason)	1		
North	4		
Salt Pond	79	South	102
Frigate Bay	16		
Bird Rock	1		
Banana Bay	3		
South	3		
St. George	1	Nevis	94
St. Thomas	2		
St. James	7		
Butlers	2		
Nevis	82		

APPENDIX C Raw data

NOTE: Within the following tables of raw data are contained descriptions of observed band patterns On occasion there existed banding patterns that appeared unusual, the following are some abbreviations used to describe/classify data obtained on these occasions·

X could not be scored

abs absence of band

phenotype? certainty of phenotype was not absolute

two phenotypes given for the same individual indicates that the individual had been scored twice for that particular system

Data from North Island region

	A	B	C	D	E	F	G	H
1	monkey	location	Tf	GB 4	GB 3	GB 2	GB 1	ALB
2	O568	ST. PAULS	+	1 - - 1	2 - - 2	1 - - 1	1 - - 3	+
3	O578	PARSONS	+	X	X	1 - - 1	1 - - 1	+
4	O591	ST. PAULS	+			X	1 - - 3	+
5	O594	ST. PAULS	+			1 - - 1	1 - - 2	+
6	OL606	ST. PAULS	+			1 - - 1	1 - - 1	+
7	O659	ST. PAULS	+			1 - - 1	1 - - 1	+
8	O685	ST. PAULS	+			1 - - 1	1 - - 1	+
9	O694	(Mason)	+			1 - - 1	1 - - 3	+
10	O774	ST. PAULS						
11	O1077	PARSONS						
12	O1227	ST. PAULS	+	2 - - 1	1 - - 2 ?	1 - - 1	1 - - 1	+
13	O1228	ST PAULS	+	1 - - 1	1 - - 1 ?	1 - - 1	1 - - 1	+
14	O1232	ST. PAULS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 3	+
15	O1238	ST. PAULS	+			1 - - 1	1 - - 3	+
16	O1248	ST. PAULS	+			1 - - 1	1 - - 1	+
17	O1257	ST PAULS	+			1 - - 1	3 - - 3	+
18	O1271	ST. PAULS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
19	O1272	ST. PAULS	+			1 - - 1	1 - - 1	+
20	O1273	PARSONS	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+

	I	J	K	L
1	pre-ALB	pseudo. gel	pseudo. assay	hMF #1
2	+			
3	+			
4	+			
5	+			
6	+	1--1 s.	664 95	2--4
7	+			
8	+			
9	+			
10		1--1 w		2--2
11				2--2
12	+			
13	+	1--1 v.w	317.36	4--4
14	+			
15	+		544 05	2--2
16	+			
17	+			
18	+			
19	+			
20	+			

	A	B	C	D	E	F	G	H
21	O1274	PARSONS	+	1--1	1--1	1--1	1--1	+
22	O1278	ST PAULS	+	1--1	3--1	1--1	1--1	+
23	O1309	PARSONS	+	2--1	1--2	1--1	1--1	+
24	O1315	SADDLERS	+	2--1	1--1	1--1	1--1	+
25	O1320	ST. PAULS	+	2--1	1--2?	X	1--1	+
26	O1321	ST. PAULS	+	2--1	1--1	1--1	1--1	+
27	O1322	PARSONS	+	1--1	1--1	1--1	1--1	+
28	O1328	ST. PAULS	+	2--1	3--1	1--1	1--1	+
29	O1341	PARSONS	+	2--1	1--2	1--1	X	+
30	O1343	ST. PAULS	+	2--2	1--2	1--1	1--1	+
31	O1344	ST PAULS	+	X	X	X	X	+
32	O1346	PARSONS	+	2--1	1--1	1--1	1--1	+
33	O1349	ST. PAULS	+	2--2	1--1	X	X	+
34	O1357	ST. PAULS	+	X	X	X	X	+
35	O1358	ST. PAULS	+	2--1	1--2	X	X	+
36	O1359	ST. PAULS	+	2--1	1--1	1--1	1--1	+
37	O1360	NEWTON	+	X	1--1	1--1	1--1	+
38	O.361	NEWTON	+	2--1	1--2	1--1	1--1	+
39	O1362	ST. PAULS	+	2--2	1--2	1--1	1--1	+
40	O1363	ST. PAULS	+	X	X	X	X	+
41	O1365	NEWTON	+	2--1	1--1	1--1	1--1	+
42	O1366	NEWTON	+	2--1	1--1	1--1	1--1	+
43	O1367	SADDDLERS	+	2--2	1--2	1--1	abs.	+
44	O1368	SADDDLERS	+	2--1	1--1	1--1	1--1	+
45	O1369	ST PAULS	+	2--2	X /abs.	1--1	X	+
46	O1371	ST. PAULS	+	2--1	1--1	1--1	1--1	+
47	O1373	NEWTON	+	2--1	1--1	1--1	1--1	+
48	O1375	NEWTON	+	2--1	1--1	1--1	1--1	+
49	O1376	ST. PAULS	+	2--1	1--1 v.s.	X	X	+
50	O1377	ST. PAULS	+	2--1	1--1	1--1	1--1	+
51	O1378	ST. PAULS	+	2--2	X	1--1	1--1	+
52	O1379	ST. PAULS	+	2--1	1--2	1--1	1--1	+
53	O1380	SADDDLERS	+	X	X	1--1	1--1	+
54	O1381	ST. PAULS	+	2--2	1--1	1--1	1--1	+
55	O1385	NEWTON	+	2--2	1--1	1--1	1--1	+
56	O1386	ST. PAULS	+	2--2	1--2	1--1	1--1	+
57	O1387	ST. PAULS	+	2--2	3--1	1--1	1--1	+
58	O1388	SADDLERS	+	2--2	1--1	1--1	1--1	+
59	O1389	SADDLERS	+	2--2	1--2	1--1	1--1	+
60	O1390	SADDLERS	+	2--1	1--2	1--1	1--1	+
61	O1391	SADDLERS	+	3--2	1--2	1--1	1--1	+
62	O1392	ST. PAULS	+	2--1	1--2	1--1	1--1	+
63	O1395	SADDLERS	+	2--2	1--2	1--1	1--1	+
64	O1396	ST. PAULS	+	2--1	1--2	1--1	1--1	+

	I	J	K	L
21	+			
22	+	1--1 s		
23	+	3--1 s.		
24	+	3--1 s.		
25	+	1--1 w		
26	+	3--1 s		
27	+	3--1 s.?		
28	+	abs/abs	226.69/241.80	1--3
29	+	1--1 w.		
30	+	1--1 s.	513 83/634 73	0--2
31	+	1--1s/1--1	408 04	2--3
32	+	1--4 s.	151.13	1--4
33	+	2--1 s.?		
34	+	1--1 s		
35	+	1--1 w		
36	+	1--1 w.		
37	+	4--4 v.s ?		
38	+	1--1 v s		
39	+	1--1 w		
40	+	1--1 w.		
41	+	1--1 w		
42	+	1--1 w		
43	+	1--1 s		
44	+	1--1?		
45	+	1--1?		
46	+	1--1 s		
47	+	1--1 s.		
48	+	1--1 s.		
49	+	1--1s/1--1vs	1224 11	2 -2
50	+	1--1 v.s.		
51	+	abs		
52	+	abs/abs		1--3
53	+	1--1 s		
54	+	X		
55	+	X		
56	+	X		
57	+	abs		
58	+	5--1 w.		
59	+	3--1 s.		
60	+	3--1 s.		
61	+	1--1 w.		
62	+	3--1 v.s.		
63	+	3--1 s.		
64	+	1--1 v.s.		

	A	B	C	D	E	F	G	H
65	O1397	NEWTON	+	2--2	1--1	1--1	1--1	+
66	O1398	ST PAULS	+	2--1?	1--2	1--1	1--1	+
67	O1399	ST. PAULS						
68	O1400	NEWTON	+	2--1	1--2	1--1	1--1	+
69	O1401	SADDLERS	+	2--2	1--2	1--1	1--1	+
70	O1404	ST. PAULS	+	2--1	2--2	1--1	1--1	+
71	O1405	NEWTON	+	1--1	1--2	1--1	1--1	+
72	O1406	SADDLERS	+	2--1	1--2	1--1	1--1	+
73	O1407	SADDLERS	+	2--2	1--2	1--1	1--1	+
74	O141,	NEWTON	+	2--1	abs.	1--1	1--1	+
75	O1412	NEWTON	+	2--2	1--2	1--1	1--1	+
76	O1419	SADDLERS	+	2- 2	1--2	1--1	1--1	+
77	O1420	NEWTON	+	1--1	1--2	1--1	1--1	+
78	O1438	SADDLERS	+	2--1	X	1--1	1--1	+
79	O1439	NEWTON	+	1--1	X	1--1	1--1	+
80	O1449	NORTH						
81	O1482	SADDLERS						
82	O1545	ST. PAULS						
83	O1547	ST. PAULS						
84	O1551	NEWTON						
85	O1554	ST. PAULS						
86	O1556	NORTH						
87	O1557	NEWTON						
88	O1558	NORTH						
89	O1571	NEWTON						
90	O1576	SADDLERS						
91	O1582	ST. PAULS						
92	O1590	ST. PAULS						
93	O1593	NEWTON						
94	OL1709	ST. PAULS						
95	OL1720	SADDLERS						
96	OL1984	ST. PAULS	+			1--1	3--3	+
97	L3000	SADDLERS						
98	OL4020	ST. PAULS	+	1--1	1--2	1--1	abs.	+
99	OL5001	SADDLERS	+	X	1--2vs	1--1w.	1--1w.	+
100	OL5011	ST. PAULS	+	X	X	1--1	1--1	+
101	OL5026	SADDLERS	+	2--1	X	1--1	1--1	+
102	OL5027	SADDLERS	+	2--1?	3--1	1--1	1--1	+
103	OL5028	SADDLERS	+	2--1?	3--1	1--1	1--1	+
104	OL5030	ST. PAULS	+	X	3--1	1--1	1--1	+
105	OL5032	SADDLERS	+	2--1	1--2 vs	1--1	1--1 w.	+
106	OL5040	NEWTON	+	2--1	3--1?	1--1 s.	1--1 s.	+
107	OL5041	SADDLERS	+	2--2	3--1?	1--1	1--1	+
108	OL5042	SADDLERS	+	2--1	1--2	1--1	1--1 w.	+

	I	J	K	L
65	+	1--1 v.s.		
66	+	1--1 w ?		
67				
68	+	abs		
69	+	2--1 w.?		
70	+	3--1 v.s		
71	+	1--1 v.s		
72	+	1--1 w.		
73	+	abs		
74	+	1--1 s		
75	+	abs		
76	+	1--1 s		
77	+	X		
78	+	1--1 w.		
79	+	3--1 s.		
80				2--2
81				
82		1--1s		1--3
83				
84				
85		1--1s	408 04/438 26	1--3b
86			528.94	0--2
87		1--1s		
88			302.25	0- 2
89				
90				
91				2--2
92		1--1s		1--4
93				
94			725 4	4--4
95				
96	+			
97				
98	+	1--1 w.		
99	+	1--1 v.s		
100	+	1--1 w.		
101	+	X		
102	+	X		
103	+	X		
104	+	1--1 w.		
105	+	abs		
106	+	1--1 w.		
107	+	1--1 v.s.		
108	+	1--1 s		

	A	B	C	D	E	F	G	H
109	OL5044	ST PAULS	+	2--1	1--1	1--1	1--1	+
110	OL5045	SADDLERS	+	2--1	2--2 s	1--1	1--1 w.	+
111	OL5048	SADDLERS	+	2--2	3--1	1--1 s.	1--1 s.	+
112	OL5049	SADDLERS	+	2--2	1--2	1--1	1--1	+
113	OL5052	NEWTON	+	2--2	1--2	1--1	1--1	+
114	OL5059	ST. PAULS	+	2--2	1--2	1--1	1--1 w.	+
115	OL5067	SADDDLERS	+	1--1	1--1	1--1	1--1	+
116	OL5068	ST. PAULS	+	2--2	1--2	1--1	1--1 w.	+
117	OL5075	NEWTON	+	2--2	1--2	1--1	1--1 w.	+
118	OL5076	NEWTON	+	2--2	1--2	1--1	1--1 w.	+
119	OL5083	NEWTON	+	2--2	3-1/1-2?	1--1	1--1 s.	+
120	OL5085	ST. PAULS	+	X	X	1--1	abs.?	+
121	OL5090	SADDLERS	+	2--2	2--2	1--1	1--1 w.	+
122	OL5098	NEWTON	+	2--2	abs.	1--1	1--1	+
123	OL5099	NEWTON	+	2--1	1--2	1--1	1--1	+
124	OL6009	SADDLERS	+	1--1	1--2	1--1	1--1	+
125	OL6012	NEWTON	+	2--1	1--2	1--1	1--1	+
126	OL6017	NEWTON	+	X	X	1--1	1--1 w.	+
127	OL6026	SADDLERS	+	X	X	1--1	X	+
128	OL6028	NEWTON	+	X	X	1--1	X	+

	I	J	K	L
109	+	5--1 v.s./s.		
110	+	1--1 s.		
111	+	abs		
112	+	1--1 s		
113	+	1--1 s.		
114	+	X		
115	+	1--1 w.		
116	+	X		
117	+	X		
118	+	3--1 v.s.?		
119	+	3--1 v.s.?		
120	+	abs		
121	+	3--1 v.s.?		
122	+	1--1 w.		
123	+	1--1 s.		
124	+	abs		
125	+	1--1 w.		
126	+	1--1 w.		
127	+	1--1 v.s.?		
128	+	1--1 v.s.?		

Data from South Island region

	A	B	C	D	E	F	G	H
1	monkey	location	Tf	GB 4	GB 3	GB 2	GB 1	ALB
2	O322	SALT POND	X	X	X	X	X	X
3	O323	SALT POND	+	1 - - 1	X	X	X	+
4	O355	SALT POND	X			X	X	X
5	O356	SALT POND	+			X	X	+
6	O357	SALT POND	+			X	X	+
7	O419	SALT POND	+			X	X	+
8	O612	SALT POND	+	1 - - 1	1 - - 2	1 - - 1	1 - - 2 / 1 - - 3	+
9	O613	SALT POND	+	2 - - 2	1 - - 1	1 - - 2	1 - - 1	+
10	O626	SALT POND	+	1 - - 1	1 - - 1	1 - - 1	3 - - 3	+
11	O678	SALT POND	+	2 - - 1	1 - - 1	1--1 S.	3 - - 3 ?	+
12	O841	SALT POND	+			1 - - 1	1 - - 1	+
13	O893	SALT POND	+			1 - - 1	1 - - 1	+
14	O894	SALT POND	+			1 - - 1	1 - - 1	+
15	O895	SALT POND	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
16	O902	SALT POND	+	X	1 - - 1	1 - - 1	1 - - 1	+
17	O903	SALT POND	+	X	1 - - 1	1 - - 1	1 - - 1	+
18	O904	SALT POND	+			1 - - 1	1 - - 1	+
19	O905	SALT POND	+			1 - - 1	1 - - 1	+
20	O907	SALT POND	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
21	O908	SALT POND	+			1 - - 1	1 - - 1	+
22	O912	SALT POND	+	2 - - 1	1 - - 1	1 - - 1 S	X /1--1?	+
23	O935	SALT POND	+			1 - - 1	1 - - 1	+
24	O936	SALT POND	+	2 - - 2	1 - - 1	1 - - 1	1 - - 1	+
25	O937	SALT POND	+	1 - - 1	1 - - 1	1 - - 1	1 - - 1	+
26	O940	SALT POND	+	1 - - 1		1 - - 1	1 - - 1	+
27	L949	SALT POND	+	X	X	1 - - 1	1 - - 1	+
28	L953	SALT POND	+	X	X	1 - - 1	1 - - 1	+
29	L961	SALT POND	+	X	X	1 - - 1	1 - - 1	+
30	L962	SALT POND	+	2 - - 1	1 - - 1	X	X	+
31	L970	SALT POND	+	2 - - 2	3 - - 1	1 - - 1	1 - - 1	+
32	L971	SALT POND	+	2 - - 2	1 - - 1	1 - - 1	1 - - 1	+
33	L972	SALT POND	+	2 - - 2	1 - - 1	1 - - 1	1 - - 1	+
34	L987	SALT POND	+	1 - - 1	1 - - 1	1 - - 1	1 - - 1	+
35	L1016	FRIGATE BAY	+	2 - - 1	3 - - 1	1 - - 1	1 - - 1	+
36	O1020	FRIGATE BAY	+	2 - - 1	2 - - 2	1 - - 1	1 - - 1	+
37	O1021	FRIGATE BAY	+	1 - - 1	1 - - 2	1 - - 1	1 - - 1	+
38	L1025	SALT POND	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
39	O1028	BIRD ROCK	+			1 - - 1	1 - - 3	+
40	O1032	SALT POND	+	2 - - 1	2 - - 2	1 - - 1	1 - - 1	+
41	O1033	SOUTH						
42	O1040	SALT POND	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
43	O1041	SALT POND	+	1 - - 1	1 - - 2	1 - - 1	1 - - 1	+
44	O1042	SALT POND	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+

	I	J	K	L
1	pre-ALB	pseudo. gel	pseudo. assay	hMF #1
2		1--1 v.s.		
3		abs/abs/abs		
4		abs/4s--3		
5		1--1 w.		
6		abs		
7				
8	+			
9	+			
10	+			
11	+			
12	+			
13	+			
14	+			
15	+			
16	+			
17	+			
18	+			
19	+			
20	+			
21	+			
22	+			
23	+			
24	+			
25	+			
26	+			
27	+	abs		
28	+	1--1 s.		
29	+	abs		
30	+	abs		
31	+	1--1 w.		
32	+	1--1 w.		
33	+	1--1 s.		
34	+	1--1 s.		
35	+	1--1 w.		
36	+	1--1 s.		
37	+	1--1 s.		
38	+	abs		
39	+	1--1 w.		2--2
40	+	3--1 w.	317.36	2--2
41		1--1 w.	362.7	4--4
42	+	X		
43	+	1--1 v.s.?		
44	+	X		

	A	B	C	D	E	F	G	H
45	O1046	SALT POND	+	2--1	1--2	1--1	1--1	+
46	O1050	SALT POND	+	2--1	1--2	1--1	1--1	+
47	O1051	SALT POND	+	2--1	1--2	1--1	1--1	+
48	O1061	SALT POND	+	2--2	2--2	1--1	1--1	+
49	O1062	SALT POND	+	2--1	2--2	1--1	1--1	+
50	O1091	BANANA BAY	+	2--1	1--2	1--1	1--1	+
51	O1105	BANANA BAY						
52	O1118	FRIGATE BAY	+	2--1	2--2	1--1	1-2/1-1?	+
53	O1127	FRIGATE BAY	+	2--1	2--2	1--1	1--1	+
54	O1137	SALT POND	+	2--1	1--2	1--1	1--1	+
55	O1144	SALT POND	+	2--1	1--2	1--1	1--1	+
56	O1171	SALT POND	+	2--2	1--2	1--1	1--1	+
57	O1172	SALT POND	+	2--1	1--2	1--1	1--1	+
58	O1173	SALT POND	+	2--1	2--2	1--1	1--1	+
59	O1174	SALT POND	+	2--2	2--2	1--1	1-3/1-1?	+
60	O1175	SALT POND						
61	O1176	SALT POND	+	1--1	1--2	1--1	1--1	+
62	O1177	SALT POND	+	2--1	2--2	1--1	abs.	+
63	O1178	SALT POND	+	2--2	2--2	1--1	1--1	+
64	O1281	SALT POND						
65	O1281	SALT POND	+	X	X	1--1 s.	1--1 s.	+
66	O1285	SALT POND	+	2--2	1--2 s.	1--1	1--1	+
67	O1292	SALT POND	+	2--1	1--1	1--1	1--1	+
68	O1293	SALT POND	+	2--1	1--1	1--1	1--1	+
69	O1294	SALT POND	+			1--1	1--1	+
70	O1297	South						
71	O1298	SALT POND	+	2--2	3--1?	1--1 w.	1--1 w	+
72	O1299	SALT POND	+	2--2	3--1	1--1	abs.	+
73	O1300	SALT POND	+	2--1	1--1	1--1	1--1	+
74	O1303	SALT POND	+	2--2	3--1?	1--1	1--1	+
75	O1304	SALT POND	+	2--1	1--1	1--1 w.	1--1	+
76	O1305	SALT POND	+	2--2	3--1?	1--1	1--1	+
77	O1323	FRIGATE BAY	+	2--2	1--1	1--1	1--1	+
78	O1326	SALT POND	+	2--1	3--1	1--1 w	1--1 w.	+
79	O1332	SALT POND	+	2--2	1--2 s.	1--1 w.	1--1 w	+
80	O1333	SALT POND	+	1--1	1--2	X	X	+
81	O1546	FRIGATE BAY	+	2--1	X	1--1 w.	1--1 w	+
82	O1561	FRIGATE BAY	+	X	X	1--1 s.	abs ?	+
83	O1568	South						
84	O1573	SALT POND	+	2--1	1--2	1--1 s.	2--2	+
85	O1575	FRIGATE BAY	+	2--1	1--2	1--1	1--1	+
86	O1578	SOUTH						
87	OL1583	SALT POND	+	2--1	1--1	1--1	1--1	+
88	OL1584	SALT POND	+	2--1	1--1	1--1	1--1	+

	I	J	K	L
45	+	X		
46	+	X		
47	+	X		
48	+	1--1 w.?		
49	+	1--1 s.?		
50	+	1--1 w		
51		1--1 s.	876.53	2--4
52	+	1--1 v.s.	544.05	2--4
53	+	abs	181.35	2--2
54	+	1--1 s.		
55	+	1--1 w.	408.04	1--4
56	+	abs		
57	+	abs		
58	+	abs		
59	+	1--1 s.		
60		abs		
61	+	1--1 w.		
62	+	3--1 v.s.		
63	+	abs ?		
64				0--2
65	+	null/1-1 w.	166.24	3--3
66	+	abs		
67	+	abs		
68	+	abs		
69	+			
70				2--2
71	+	abs		
72	+	abs		
73	+	abs/1-1	528.94	1--1
74	+	abs/abs	272.03	1--4
75	+	abs		
76	+	5--1 s.		
77	+	abs		
78	+	1-1 s.	423.15/574.28	1--3
79	+	3--4 w.?		
80	+	1-1s/1-1s	634.73	1--1
81	+	3-1w/1-1s	377.81	1--3
82	+	1--1 w.		
83		1--1s		0--4
84	+	1--1 w.		
85	+	1--1 s.		
86		1--1s		2--4
87	+	1--1 w.		
88	+	1--1 w.?		

	A	B	C	D	E	F	G	H
89	O1586	FRIGATE BAY	+	2--1	X	1--1	1--1	+
90	OL1849	SALT POND	+	2--1	X	X	X	+
91	OL1908	FRIGATE BAY	+	X	X	1--1	1--1	+
92	L2029	SALT POND	+	2--2	1--1	1--1	1--1	+
93	L2035	SALT POND	+	X	X	1--1	1--1	+
94	L2038	SALT POND	+	2--2	1--1	3--1?	1--1	+
95	L2039	FRIGATE BAY	+	2--2	X	1--1	1 -1	+
96	OL2061	SALT POND	+	2--1	1--1	X	X	+
97	OL2062	SALT POND	+	2--2	1--1	X	X	+
98	OL2112	SALT POND						
99	OL3008	SALT POND	+	2--2	1--2	1--1	X	+
100	L3009	SALT POND	+	2--2	X	1--1	1--1	+
101	OL5070	FRIGATE BAY	+	2--2	X	X	X	+
102	OL8016	FRIGATE BAY	+	3--2	X	1--1	1--1	+
103	OL8079	SALT POND	+	3--2	X	1--1	1--1	+
104	OL8087	FRIGATE BAY	+	2--1	2--2	1--1	1--1	+
105	OL8089	FRIGATE BAY	+	2--1	2--2	1--1	1--1	+

	I	J	K	L
89	+	3--1 v.s./abs		2--4
90	+	3--1 v.s.		
91	+	3--1 v.s.		
92	+	2--1 v.s.		
93	+	abs		4--4
94	+	2--1 v.s.		
95	+	2--1 v.s.		
96	+	1--1 w.		
97	+	1--1 s.		
98				1--1
99	+	1--1?		
100	+	1--1 v.s.		
101	+	1--1 w.		
102	+	1--1 w.		
103	+	1--1 w.		
104	+	1--1 v.s.		
105	+	1--1 v.s.		

Data from Nevis group

	A	B	C	D	E	F	G	H
1	monkey	location	Tf	GB 4	GB 3	GB 2	GB 1	ALB
2	N46	NEVIS	+	2 - - 2	1 - - 1	1 - - 1	1 - - 1	+
3	N66	NEVIS	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
4	N82	NEVIS	+			1 - - 1	1 - - 3	+
5	N245	NEVIS	+			X	X	+
6	N337	NEVIS	+			1 - - 1	1 - - 2	+
7	N419	NEVIS	+			1 - - 1	1 - - 1	+
8	N445	NEVIS	+			1 - - 1	1 - - 1	+
9	N448	NEVIS	+			1 - - 1	1 - - 1	+
10	N454	NEVIS	+			1 - - 1	1 - - 1	+
11	N459	NEVIS	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
12	N476	NEVIS						
13	N489	NEVIS						
14	N498	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
15	N515	NEVIS	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
16	N621	NEVIS	+	X	X	1 - - 1	1 - - 1	+
17	N622	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
18	N667	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
19	N670	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
20	N671	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1--1 ?	+
21	N673	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
22	N674	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
23	N676	NEVIS	+	X	1 - - 2	1 - - 1	1 - - 1	+
24	N677	NEVIS	+	1 - - 1	1 - - 2	1 - - 1	1 - - 1	+
25	N678	NEVIS	+	2 - 1	1 - - 1	1 - - 1	1 - - 1	+
26	N679	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
27	N680	NEVIS	+	2 - - 1	1 - - 2	1 - - 1	1 - - 1	+
28	N683	NEVIS	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
29	N716	NEVIS	+			1 - - 1	1 - - 1	+
30	N825	NEVIS	+			1 - - 1	1 - - 1	+
31	N826	NEVIS	+			1 - - 1	1 - - 1	+
32	N831	St James	+	2 - - 1		1 - - 1	1 - - !	+
33	N843	NEVIS	+	2 - - 1		1 - - 1	1 - - 1	+
34	N852	NEVIS	+	X		1 - - 1	1 - - 1	+
35	N853	NEVIS	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
36	N859	St Thomas	+	2 - - 1	1 - - 1	1 - - 1	1 - - 1	+
37	N860	St Thomas	+	2 - - 1		1 - - 1	1 - - 1	+
38	N861	NEVIS	+	X	X	1 - - 1	1 - - 1	+
39	N870	NEVIS	+		1 - - 2	1 - - 1	2 - - 2	+
40	N871	NEVIS	+		1 - - 2	1 - - 1	1 - - 1	+
41	N872	NEVIS	+			1 - - 1	1 - - 1	+
42	N873	NEVIS	+			1 - - 1	1 - - 1	+
43	N874	NEVIS	+			1 - - 1	1 - - 1	+
44	N876	NEVIS	+		1 - - 1	1 - - 1	1 - - 1	+

	I	J	K	L
1	pre-ALB	pseudo. gel	pseudo. assay	hMF #1
2	+			
3	+			
4	+			
5	+			
6	+			
7	+			
8	+			
9	+			
10	+			
11	+			2- 4
12		abs	272 01	2- 2
13		1--1	438 26	2--3
14	+			
15	+			
16	+			
17	+			
18	+			
19	+			
20	+			
21	+			
22	+			
23	+			
24	+			
25	+			
26	+			
27	+			
28	+			
29	+			
30	+			
31	+			
32	+			
33	+			
34	+			
35	+			
36	+			
37	+			
38	+	abs		
39	+			
40	+			
41	+			
42	+			
43	+			
44	+			

	A	B	C	D	E	F	G	H
45	N877	NEVIS	+	1--1	1--1	1--1	1--1	+
46	N878	NEVIS	+		1--1	1--1	1--1	+
47	N879	NEVIS	+	1--1		1--1	1 -1	+
48	N880	NEVIS	+	1--1	1--1	1--1	1--1	+
49	N881	NEVIS	+		3--1?	1--1	1--1	+
50	N882	NEVIS	+			1--1	1--1	+
51	N883	NEVIS	+			1--1	1 -1	+
52	N884	NEVIS	+			1--1	1--1	+
53	N885	NEVIS	+			1--1	1--1	+
54	N886	NEVIS	+			1--1	1--1	+
55	N887	NEVIS	+			1--1	1--1	+
56	N889	NEVIS	+	2--1	1--2	1--1	1--1	+
57	N890	NEVIS	+	2--1	1--2	1--1	1--1	+
58	N891	NEVIS	+	2--1	1--2	1--1	1--1	+
59	N892	NEVIS	+	2--1	1--2	1--1	1--1	+
60	N893	NEVIS	+	2--1		1--1	1--1	+
61	N894	NEVIS	+			1--1	1--1	+
62	N896	St.James	+			1--1	1--1	+
63	N897	St.James	+			1--1	1--1	+
64	N898	NEVIS	+			1--1	1--1	+
65	N899	NEVIS	+			1--1	1--1	+
66	N900	St James	+			1--1	1--1	+
67	N901	St James	+			1--1	1--1	+
68	N905	St James	+	1--1	1--1	1--1	1--1	+
69	N907	Butlers	+	2--1	1--1	1--1	1--1	+
70	N908	NEVIS	+	2--1	1--1	1--1	1--1	+
71	N910	St James	+	1--1	1--1	1--1	1--1	+
72	N911	Butlers	+	1--1	1--1	1--1	1--1	+
73	N915	NEVIS						
74	N929	NEVIS						
75	N933	NEVIS						
76	N936	NEVIS						
77	N996	NEVIS						
78	N998	NEVIS						
79	N1005	NEVIS						
80	N1006	NEVIS						
81	N1030	NEVIS						
82	N1031	NEVIS						
83	N1038	NEVIS						
84	N1039	NEVIS						
85	N1040	NEVIS						
86	N1041	NEVIS						
87	N1044	NEVIS						
88	N1045	NEVIS						

	I	J	K	L
45	+			
46	+			
47	+			
48	+			
49	+			
50	+	4- 4?s		
51	+	abs	256 91	2--4
52	+			
53	+			
54	+			
55	+			
56	+			
57	+	1--1	544 05/528 94	2--4
58	+			
59	+			
60	+			
61	+			
62	+			
63	+			
64	+	abs	196 46/151 13	2--4
65	+			
66	+			
67	+			
68	+			
69	+			
70	+			
71	+			
72	+			
73		1--1	438 26/574 28	2--2
74		abs	256 91/166 24	2--2
75		1--1w	302 25/332 48	2--2
76				
77				
78				
79				
80		1--1s	619 61/559 16	2--4
81			544.05/377.81	1--2
82		abs	226 69/241.80	0--4
83		1--1vs		0--4
84		1--1		0--2
85		1--1		4--4
86		1--1w.		4--4
87		1--1s.		2--3b
88		1--1w		

	A	B	C	D	E	F	G	H
89	NL1276	NEVIS						
90	NL1525	NEVIS	+	2--1	1--1	1--1	1--1	+
91	NL1526	NEVIS	+	2--1	X	1--1	1--1	+
92	NL1981	NEVIS	+	2--1	1--1	1--1	1--1	+
93	NL1982	NEVIS	+	2--1	1--1	1 -1	1--1	+
94	NL1996	St.Georges	+	2--2	1--1	1--1	1--1	+
95	L8007	NEVIS						

	I	J	K	L
89			770.74/785.85	
90	+	1--1	317.36/211.58	2--4
91	+	1--1	362 70/438.26	2--2
92	+			
93	+			
94	+			
95				2--4

Appendix D Counts of phenotypes for each loci studied

	A	B	C	D	E
1	**locus**	**phenotype**	**North**	**South**	**Nevis**
2	GB 4	1	11	10	7
3		2 - - 1	39	38	30
4		2	29	23	2
5		3 - - 2	1	2	0
6		**SUM**	80	73	39
7					
8	GB 3	non-detectable	3	0	0
9		1	23	26	21
10		1 - - 2	39	20	17
11		2	4	12	0
12		3 - - 1	7	4	1
13		**SUM**	76	62	39
14					
15	hMF #1	0 - - 2	3	1	1
16		0 - - 4	0	1	2
17		1	0	3	0
18		1 - - 2	0	0	1
19		1 - - 3	3	2	0
20		1 - - 3b	1	0	0
21		1 - - 4	2	2	0
22		2	6	4	5
23		2 - - 3	1	0	1
24		2 - - 3b	0	0	1
25		2 - - 4	1	4	7
26		3	0	1	0
27		4	2	2	2
28		**SUM**	19	20	20
29					
30	pseudocholin	non-detectable	11	19	6
31		1	50	35	13
32		2 - - 1	0	3	0
33		3 - - 1	9	4	0
34		1 - - 4	1	0	0
35		4	0	0	1
36		5 - - 1	2	1	0
37		**SUM**	73	62	20
38					
39	GB 1 (a1 A.T ?)	non-detectable	2	2	0
40		1	86	72	69
41		1 - - 2	1	0	1
42		1 - - 3	5	1	1
43		2	0	1	1
44		3	2	1	0
45		**SUM**	96	77	72

Appendix E: Familial data for hMF #1 probe during DNA analysis

mother		child		father	
N890	2-4	N890-1	4	O1256	2-4
N933	2	N933-1	2-4	O1256	2-4
O1198	0-2	O1198-2	2	O1256	2-4
O1228	4	O1228-1	2-4	O1256	2-4
O1281	4	O1281-1	2-4	O1118	2-4
N476	2	N476-4	2	01111	2
N459	2-4	N459-5	4	O1118	2-4
		N459-6	4		
O704	3-4	O704-3	2-3	O1118	2-4
		O704-4	2-3		
		O704-5	2-4		
		O704-6	2-3		
N489	2-3	N489-4	2-3	O1111	2

Appendix F: χ^2 test for Hardy-Weinberg equilibrium - hMF #1 (D1Z2 locus)

North data (U allele group includes alleles 0, 1, and 2, L allele group includes alleles 3, 3b, and 4)

	A	B	C	D	E	F
1	allele	frequency	phenotype	observed	expected	(O-E)**2/E
2	Upper	0.6842105	U--U	9	8.89473616	0.00124573
3	Lower	0.3157895	U--L	8	8.21052668	0.00539813
4			L--L	2	1.89473716	0.00584792
5	sum freq.	1				
6				19	sum chi	0.01249178

South data

	A	B	C	D	E	F
1	allele	frequency	phenotype	observed	expected	(O-E)**2/E
2	Upper	0.625	U--U	8	7.421875	0.04503289
3	Lower	0.375	U--L	9	8.90625	0.00098684
4			L--L	3	2.671875	0.04029605
5	sum freq.	1				
6				20	sum chi	0.08631578

Nevis data

	A	B	C	D	E	F
1	allele	frequency	phenotype	observed	expected	(O-E)**2/E
2	Upper	0.625	U--U	7	7.8125	0.0845
3	Lower	0.375	U--L	11	9.375	0.28166667
4			L--L	2	2.8125	0.23472222
5	sum freq.	1				
6				20	sum chi	0.60088889

Overall: all data combined.

	A	B	C	D	E	F
1	allele	frequency	phenotype	observed	expected	(O-E)**2/E
2	Upper	0.6440678	U--U	24	24.4745765	0.00920232
3	Lower	0.3559322	U--L	28	27.0508473	0.03330361
4			L--L	7	7.47457613	0.03013181
5	sum freq.	1				
6				59	sum chi	0.07263774

Appendix G: Calculations for Heterozygosity

	A	B	C	D	E	F	G	H
1	locus	allele	north(X)	south(Y)	nevis(Z)	X*X	Y*Y	Z*Z
2								
3	transferrin	1	1	1	1	1	1	1
4	sum (Xi)*(Xi)					1	1	1
5	N for locus					107	94	74
6	heterozygosity					0	0	0
7	h*N					0	0	0
8								
9	pre-albumin	1	1	1	1	1	1	1
10	sum (Xi)*(Xi)					1	1	1
11	N for locus					107	90	74
12	heterozygosity					0	0	0
13	h*N					0	0	0
14								
15	hMF#1	0	0 079	0 05	0 075	0.0062	0.0025	0 0056
16		1	0 158	0 25	0 025	0 025	0.0625	0 0006
17		2	0.447	0.325	0.525	0 1998	0.10563	0.2756
18		3	0 105	0 1	0.025	0.011	0 01	0.0006
19		3b	0 026	0	0.025	0 0007	0	0 0006
20		4	0 184	0 275	0.325	0.0339	0.07563	0 1056
21	sum (Xi)*(Xi)					0.2766	0 25625	0 3888
22	N for locus					19	20	20
23	heterozygosity					0 743	0.76282	0.6269
24	h*N					14.117	15 2564	12.538
25								
26	sum (h*N)					14 117	15 2564	12.538
27	sum (Ni)					233	204	168
28	sum (Ni)*m					699	612	504
29	est H over loci					0.0202	0.02493	0 0249

Appendix H: Heterogeneity χ^2 Test of Groups (pairwise)

	A	B	C	D	E	F	G	H
1	locus	allele	north(X)	south(Y)	nevis(Z)	sq. (X-Y)	sq. (Y-Z)	sq. (X-Z)
2								
3	hMF#1	0	0.079	0.05	0.075	0.00084	0.00063	1.6E-05
4		1	0.158	0.25	0.025	0.00846	0.05063	0.01769
5		2	0.447	0.325	0.525	0.01488	0.04	0.00608
6		3	0.105	0.1	0.025	2.5E-05	0.00563	0.0064
7		3b	0.026	0	0.025	0.00068	0.00063	1E-06
8		4	0.184	0.275	0.325	0.00828	0.0025	0.01988
9	n		19	20	20			
10								
11								
12		upper (U)	0.684	0.625	0.625	0.00348	0	0.00348
13		lower (L)	0.316	0.375	0.375	0.00348	0	0.00348
14								
15								

note: the U allele group includes alleles 0, 1, and 2, the L allele group includes alleles 3, 3b, and 4

	I	J	K	L
1		chi frac.X/Y	chi frac.Y/Z	chi frac.X/Z
2				
3		0.00033627	0.00025	5.3316E-06
4		0.00105774	0.00920455	0.00505111
5		0.00039273	0.00235294	0
6		6.2578E-06	0.00225	0.00256513
7		0.00136842	0.00125	1.006E-06
8		0.00138687	0.00029674	0.0019889
9	sum---->	0.00514828	0.01560422	0.00961148
10	chi stat. ->	3.91269261	12.483378	7.30472257
11				
12		0.00013653	0	0.00027848
13		0.00046413	0	0.00046413
14	sum---->	0.00060066	0	0.00074261
15	chi stat. ->	0.45650506	0	0.56438613

Bibliography

J E Anderson and E R Giblett, Intraspecific red cell enzyme variation in pigtailed macaque (*Macaca nemestrin*) , Biochem Genet , 13, 189 (1975)

K Arai, K Huss, J Madison, F Putnam, F M Salzano, M H L P Franco, S E B Santo, M J M Freitas, Amino acid substitutions in albumin variants found in Brazil , P N A S , 86, 1821 (1989)

E H Ashton, The influence of geographic isolation on the skull of the green monkey (*Cercopithecus aethiops sabaeus*) 5 The degree and pattern differentiation in the cranial dimensions of the St Kitts green monkey , Proc roy Soc , Lond , 151, 563 (1960)

E H Ashton and S Zuckerman, The influence of geographic isolation on the skull of the green monkey (*Cercopithecus aethiops sabaeus*) 1 A comparison between the teeth of the St Kitts and African green monkey , Proc roy Soc , Lond , 137, 212 (1950)

E H Ashton and S Zuckerman, The influence of geographic isolation on the skull of the green monkey (*Cercopithecus aethiops sabaeus*) 2 The cranial dimensions of the St Kitts and African green monkey , Proc roy Soc , Lond , 138, 204 (1951a)

E H Ashton and S Zuckerman, The influence of geographic isolation on the skull of the green monkey (*Cercopithecus aethiops sabaeus*) 3 The developmental stability of the skulls and teeth of the St Kitts and African green monkey , Proc roy Soc , Lond , 138, (1951b)

E H Ashton and S Zuckerman, The influence of geographic isolation on the skull of the green monkey (*Cercopithecus aethiops sabaeus*) 4 The degree and speed of dental differentiation in the St Kitts green monkey., Proc roy Soc , Lond , 138, 354 (1951c).

D A Barnicot, C J Jolly and P.T Wade, Protein variation and primatology , Am J. Phys Anthrop , 27, 343 (1965)

R T Breed and C J Jolly, Variation in rates of activity and inhibition of serum cholinesterase among anthropoid primates , Int J of Primatol , 9(2), 97 (1988).

S O Brennan, P M George, R J Peach, Charactensation of a slow component of normal serum albumin , Clin Chim Acta, 176, 179 (1988)

J Buettner-Janusch, Hemoglobins and transferrins of baboons , Folia primat , 1, 73 (1963)

M M Colyn and W N Verheyen, Considerations sur la validite de l'holotype de *Cercopithecus mitis maesi* Lonneberg,1919 et description d'une nouvelle sous-espece *Cercopithecus mitis heymansi* , Mammalia, 51, 271 (1987)

D W Cox, New variants of alpha-1-antitrypsin comparison of Pi typing techniques , Am J Hum Genet , 33, 354 (1981)

J deGrouchy, C Turleau and C Finaz, Chromosomal phylogeny of the primates, Ann Rev Genet ,12, 289 (1978)

W W Denham, West Indian green monkeys problems in historical biogeography., (Karger Publishing, New York,1987)

J M Diamond, DNA based phylogenies of the three chimps , Nature, 332, 685 (1988)

N C. Dracopoli, F L Brett, T R Turner and J C Jolly, Patterns of genetic variability in the serum proteins of the Kenyan vervet , Am J Phys Anthropol , 61, 39 (1983)

M K Fagerhol, Serum Pi types in Norwegians , Acta Pathol Micobiol Scand , 70, 421 (1967)

M K Fagerhol and T Gedde-Dahl Jr , Genetics of the Pi serum types , Human Hered , 19, 354 (1969)

C Finaz, N. VanCong, C Cochet, J Frezal and J deGrouchy, Fifty-million-year evolution of chromosome 1 in the primates , Cytogenet Cell Genet , 18, 160 (1977)

R I Garver Jr , J-F Mornex, T Nukiwa, M Brantly, M Courtney, J-P Le Cocq, R G Crystal, Alpha-1-antitrypsin deficiency and emphysema caused by homozygous inheritance of non-expressing alpha-1-antitrypsin genes , N E J M , 314 (12), 762, (1986)

J M Gershoni, Protein blotting a manual, Methods of Biochemical Analysis, 33, 1 (1988)

E R Giblett, Genetic markers in human blood, (Blackwell Scientific Publications, Great Britain, 1969)

M Goodman, A Kulkal, E Poulik and E Rekly, Species and geographic differences in transferrin polymorphism of the macaques , Science, 147, 884 (1965)

H Harris and D A Hopkinson, Handbook of enzyme electrophoresis in human genetics, (North-Holland Publishing Company, Amsterdam, The Netherlands, 1977)

H Harris, D A Hopkinson, E B Robson and M Whittaker, Genetical studies on a new variant of serum cholinesterase detected by electrophoresis , Ann Hum Genet , 26, 359 (1963)

P.W Hedrick, Genetics of populations, (Jones and Bartlett Publishers Inc ,Boston,Mass , 1985).

D A Hopkinson, N Spencer and H Harris, Genetical studies on human red cell acid phosphatase , Hum Genet , 16(1), 141 (1964)

L -H Huang, C M Farnet, K C Ehrlich, and M Ehrlich, Digestion of highly modified bacteriophage DNA by restriction endonucleases , Nuc Acids Res., 10, 1579 (1982)

K Huss, F W Putnam, N Takahashi, Y Takahashi, G A. Weaver, T Peters,jr , Albumin Cooperstown a serum albumin variant with the same (313 Lys to Asn) mutation found in albumins in Italy and New Zealand , Clin Chem , 34(1), 183 (1988)

P Juul, Human plasma cholinesterase isoenzymes , Clin Chim Acta, 19, 205 (1968)

Y Kawamoto, T Shotake and K Nozawa, Genetic differentiation among three genera of Cercopithecidae , Primates, 223, 272 (1986)

M -C King and A C Wilson, Evolution at two levels in humans and chimpanzees , Science, 188, 107 (1975)

G B Koelle, Proc Soc Exptl Biol Med , 70, 617 (1949)

F Kueppers, Alpha-1-antitrypsin physiology, genetics, and pathology , Humangenetik, 11, 177 (1971)

F. Kueppers, Alpha-1-antitrypsin , Am J Hum Genet , 25, 677 (1973)

R.C. Lewontin, The genetic basis of evolutionary change , (Columbia University Press, Columbia, 1974).

W H Li and M Nei, Drift variances of heterozygosity and genetic distance in states, Genet Res , 25, 229 (1975)

G Lucotte, C Gautreau, G Galat and A Galat-Luong, Polymorphisme electrophoretique des differentes sous-especes de Cercopithecus aethiops , Folia primatol , 38, 183 (1982)

N Maeda, C Wu, J Bliska and J Reneke, Molecular evolution of intergenic DNA in higher primates pattern of DNA changes, molecular clock, and evolution of repetitive sequences , Mol Biol Evol , 5, 1 (1988)

T Maniatis, E F Fritsch and J Sambrook, Molecular cloning a laboratory manual , (Cold Spring Harbor Laboratory , Cold Spring Harbor, N Y , 1982)

M. T. McGuire, The St Kitts Vervet , Contr Primatol , 1, 1 (1974)

P Meera Kahn, Isozymes as bioprobes for genetic analysis of nonhuman primates , Genetica, 73, 25 (1987)

D Melnick, The genetic structure of a primate species rhesus macaques and other Cercopithecine monkeys , Intl J Primatol , 9(3), 195, (1988)

Y Nakamura, C Julier, R Wolff, T Holm, P O'Connell and M Leppert et al , Characterization of a human "midisatellite" sequence , Nucleic Acids Res , 15(6), 2537, (1987)

M Nei, Estimation of average heterozygosity and genetic distance from a small number of individuals , Genetics, 89, 583 (1978)

M Nei, Genetic distance between populations , Amer Naturalist, 106, 283 (1972)

M Nei, Molecular Evolutionary Genetics , (Columbia Publishers Inc , New York, New York, 1987)

M Nei and A K Roychoudhury, Sampling variances of heterozygosity and genetic distance , Genetics, 76, 379 (1974)

M Nelson and M McClelland, The effect of site-specific methylation on restriction modification enzymes , Nuc Acids Res , 15 (suppl), r219 (1987).

K Nozawa, T Shotake, Y. Ohkura, M Kitajima and Y Tanabe, Genetic variations within and between troops of *Macaca fuscata fuscata*, Contemporary Primatology Fifth International Congress Primatology, Karger and Basel., (1975), p 75.

Y Oki, W T Oliver and H S Funnell, Multiple forms of cholinesterase in horse plasma, Nature, 203, 605 (1964)

R. M. Palmour, J E Cronin, A Childs and B W. Grunbaum, Studies of primate protein variation and evolution microelectrophoretic detection , Biochem Genet , 18, 793 (1980)

R.M Palmour and H.E. Sutton, Vertebrate transferrins molecular weights, chemical compositions and iron-binding studies Biochem , 10, 4026 (1971)

M Pech, R E. Streeck, and H G Zachau, Patchwork structure of a bovine satellite DNA, Cell, 18, 883 (1979)

S. Prakash, R C. Lewontin and J L Hubby, A molecular approach to the study of genic heterozygosity in natural populations 4. Patterns of genic variation in central, marginal, and isolated populations in *Drosophila p* , Genetics, 61, 841 (1969)

F. Putnam, The plasma proteins , (Academic Press, New York, 1960)

R. K. Selander and S. Y. Yang, Protein polymorphism and genic heterozygosity in a wild population of the house mouse (*Mus musculus*)., Genetics, 63, 653 (1969).

T. Shotake, Y. Ohkura and K Nozawa, Genetic polymorphisms of blood proteins in the troops of Japanese macaques, *Macaca fuscata* 6 Serum transferrin polymorphism., Primates, 18, 291 (1977)

M J Siciliano, D A Wright and C R. Shaw, Biochemical polymorphisms in animals as models for man , Progress in Medical Genetics, 10, 17 (1974).

N E Simpson and W Kalow, The "silent" gene for serum cholinesterase , Am J. Hum. Genet , 16(2), 180 (1964)

M Slatkin, Gene flow and the geographic structure of natural populations., Science 236, 787 (1987).

C. A B Smith, Chi-squared tests with small numbers, Ann. Hum Genet., 50, 163 (1986).

E.M Southern, Detection of specific sequences among DNA fragments separated by gel electrophoresis , J Mol Biol , 98, 503 (1975)

R C. Talamo, J D Allen, K F Austen, Hereditary alpha-1-antitrypsin deficiency., NEJM 278(7), 345, (1968)

T.R Turner, Blood protein variation in a population of Ethiopian vervet monkeys (*Cercopithecus aethiops aethiops*) , Am J. Phys. Anthro , 55, 225 (1981).

K.M Tynan and D I Hoar, Primate evolution of a human chromosome 1 hypervariable repetitive element , J Mol. Evol.28(3), 212 (1989)

A J. Vander, J H. Sherman and D.S. Luciano, Human physiology: the mechanisms of body function., (McGraw-Hill Book Company, United States of America, 1980).

K Washio, S Ueda, and S. Misawa, Effects of cytosine methylation at restriction sites on DNA typing, J For Sci , 35(6), 1277 (1990).

M L. Weiss, DNA fingerprints in physical anthropology., Am. J. Hum. Bio , 1, 567 (1989).