HUMAN EVOLUTIONARY BIOLOGY

Edited by

MICHAEL P. MUEHLENBEIN
Indiana University, Bloomington
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INTRODUCTION

The door to the study of genetically based variation in humans cracked open at the beginning of the twentieth century with the discovery by Landsteiner (1901) of ABO blood group substances whose Mendelian mode of inheritance was later established by Bernstein (1924). Needless to say, an early trickling of discoveries has led to a flood over the past century of new and important revelations concerning the nature and significance of human genetic variation. This chapter will cover approximately three-fourths of that history as it unfolded via the discovery and elucidation of a host of markers. A helpful review of genetic markers known as of the early 1970s and their role in the study of human evolution can be found in Crawford (1973). Another recent review of classic markers and their contribution toward understanding North American Native genetic variation appeared in O’Rourke (2006). Two current textbooks that provide substantial coverage of traditional markers, along with DNA markers and other topics relevant to human biological variation, are Mielke, et al. (2006) and Molnar (2006).

Over the years markers have come to mean fairly consistently defined hereditary units. An early reference to the use of the term is found in Race and Sanger (1962) who discuss markers, in their case human blood groups, as characters that help to locate genes on chromosomes. This was, of course, early in any attempts to construct physical gene maps. Sometime later, genetic markers were associated with specific polymorphic loci that defined particular segments of chromosomes (Cavalli-Sforza et al., 1994). In essence, genetic markers today signify genes or other identifiable segments of DNA whose inheritance can be consistently documented and mapped.

For purposes of this chapter, classical markers will refer to those historically researched phenotypically observable variants that for the most part preceded DNA methodologies that presently deal with nucleotide sequences in various representations, such as mitochondrial DNA (mtDNA), variable number tandem repeats (VNTRs), single nucleotide polymorphisms (SNPs), and microsatellite polymorphisms. In short, these are DNA markers. Hence, the time frame for reviewing markers will encompass anthropological field and laboratory studies carried out beginning at turn of the twentieth century roughly through the 1980s. This end point is just prior to the widespread use of polymerase chain reaction (PCR) and the developing application of DNA markers to population studies. As a timetable guide, Roychoudhury and Nei (1988) were referred to for selecting markers from those that had been identified by the date of that publication. The major categories of human markers will include: blood group polymorphisms, serum protein and enzyme variants, and an open-ended set of markers that are of microevolutionary and anthropological interest. Polymorphism is used here to signify two or more alleles at a given locus each exceeding 1% frequency.

Tables follow that list these traditional markers. It should be noted that marker identification pertains to an earlier time period and may not conform to presently known markers and their allele or haplotype (a combination of alleles at multiple linked loci) labels. However, chromosome locations for the markers are current and were extracted from the National Center for Biotechnology Information (NCBI) website.

Some sampling of the total number of classical markers was done so as to not overwhelm the chapter. The selection process was based on how much information was available for the marker, how widely it was studied across human populations, and how informative the marker was in bringing out important points pertaining to human variation. Several classic works are available for anyone interested in a more complete and in depth compilation of markers. These include; Race and Sanger (1962), Buettner-Janusch (1966), Giblett (1969), Mourant et al., (1976), Harris (1980), Mourant (1983). Tills et al., (1983), Livingstone (1985), Roychoudhury and Nei (1988), and Cavalli-Sforza et al. (1994).
The organization for describing classical markers centers on five main topics: basics of marker identification and their expressed phenotypic physiological function, historical application of markers in classifying human populations and races, application of markers to population studies and microevolutionary processes, markers and their relationships with diseases, and contemporary use and future prospects for classic markers.

IDENTIFYING RED BLOOD CELL MARKERS

An early method for detecting variants of hereditary expression, beyond that of parental selection and crossing as devised by Mendel, were serological reactions. Discovery of blood group markers arose through unintended consequences of transfusions, involving donor–recipient mismatches that led to adverse clinical outcomes for patients. Working off these unfortunate medical mishaps, Landsteiner’s discovery in 1900 of the ABO system was based on laboratory tests of a person’s blood cells against serum from a different person. Particular combinations of cells and sera produced visible agglutination reactions between red blood cell surface antigens and corresponding bivalent antibodies found in the serum. Following extensive cross matches of this sort, Landsteiner was able to identify persons who had either A or B antigens, both A and B, or neither. The success of his work partly depended on the fact that the ABO system has naturally occurring antibodies that form shortly after birth. This then, was the launch into a succession of subsequent discoveries of both improved medical procedures requiring blood transfusion, and in identifying additional blood group marker systems.

Table 13.1 provides a listing of antigens, their chromosome location and their marker notations, and year of discovery. Some of these markers, for example in the Rh system, were originally observed in a manner similar to that noted above, that is, due to adverse transfusion reactions. Others were found through deliberate laboratory procedures of injecting human blood cells into animals (often rabbits) and extracting any produced antiserum or agglutinin, which then could be tested against humans for positive or negative reactions. An example of this is anti-N in the MN system. Interestingly, plant extracts (lectins) also were found to differentially react with receptor sites of human blood cell antigens. Also in the MN system, anti-N reagent was extracted from *Vicia graminea*, a legume. In the case of the ABO system, anti-A was made from *Dolichos biflorus*, and while *Ulex europaeus* (also a legume in the gorse bush family) differentiated A₂ from A₁ and also was used to establish that persons who lacked both A and B antigens, that is the O phenotype, did in fact possess an antigen called H. This finding provided an alternative name for the system, ABH. The H antigen is in fact present in virtually all ABO phenotypes, in decreasing amount from O type to AB. One exception to this is the Bombay phenotype O that lacks red blood cell H antigen, and carries a corresponding anti-H, as well as anti-A and anti-B in the serum.

**SOLUBLE ANTIGENS**

In addition to blood cell surfaces, antigenic markers also can appear in water soluble form through out body fluids, with particular reference here to saliva. Presence of salivary antigens in the ABH system were detected with the inhibition test, where known antisera were mixed with the tested saliva, and then checked against known red cell antigens to see which antisera had already reacted with or had been inhibited, and thus revealed the identity of the antigen.

ABH secretion (now designated as *FUT1*) of soluble antigens turned out to be just the first system that was later found to be among a linkage group on chromosome 19 that also included the Secretor locus (or *FUT2*) itself along with Lewis (or *FUT3*), Lutheran, and Auberger antigenic markers. Of historical significance, autosomal linkage of Secretor and Lutheran was the first of its kind to be shown (Mohr, 1951). Another linkage to note here is that between Kell and Sutter blood groups on chromosome 7. There is also
been evidence that Kell is linked with the PTC trait, a marker to be discussed later. Linkage detail on Kell/Sutter and the secretor loci noted above was incomplete or not known at the time of major marker compilations, such as Roychoudhury and Nei (1988). Linkage between MN and Ss was known, and allele combinations of these two systems, which undergo little recombination, should probably be treated as haplotypes. At the molecular level of the red blood cell membrane, it is now established that the M and N antigens are bound to glycophorin A (GPA) while the S and s antigens are carried by glycophorin B (GBP).

The final red blood antigen system to mention here is Xg, obviously so-named because it is located on the X chromosome. It was discovered through conventional serological methods in 1962. That year marks the end of the initial period of discovery of red blood cell polymorphisms, at least those that figured most prominently in anthropological field studies.

Modes of inheritance for red blood cell and secretor groups can be codominant (as in the MN group), dominant-recessive (as in the Rhesus group for the D antigen), a combination (as in the ABO group), or sex-linked (as in the Xg group). For some groups, detection of heterozygote phenotypes depends upon the specificity of the serological reagents. Molecular methods now make many of the earlier dominant-recessive designations obsolete or incomplete.

For a reasonably up-to-date compilation of red blood cell markers, dealing with those covered here and many more as well, the reader is referred to The Blood Group Antigen FactsBook (Reid and Lomas-Francis, 1997), wherein you will find descriptions and displays of the molecular basis of the markers along with additional categories of information befitting a complete reference source.

HEMOGLOBINS

Mode of inheritance: Two autosomal loci, segregating multiple codominant alleles, but depending upon which of the pleiotropic phenotypic expressions are considered, there can also be dominant and recessive conditions.

Table 13.1 also lists hemoglobins, which make up about 85% of the protein structure of red blood cells. Considering their early and continuing significance in microevolutionary studies, they could command a separate table. Electrophoresis was used in identifying hemoglobin variants. A primary function of hemoglobin is to bind oxygen molecules while blood has infused the lungs and transport this oxygen throughout the circulatory system where it is then released during metabolic activity. A very large number of hemoglobin variants have been found (Livingstone, 1985) but this review will focus on major variants found at the β-chain locus on chromosome 11. This locus is of particular interest here due to its maintaining elevated marker frequencies in human groups that are at increased risk for contracting endemic malaria, a topic that will be covered later on.

HLA SYSTEMS

Mode of inheritance: Multiple autosomal loci and multiple codominant linkage groups or haplotypes.

Finally, Table 13.1 contains the HLA systems. Human leukocyte antigen (HLA) haplotypes are found on white blood cells and expressed at several closely linked loci on chromosome 6. In a broader context, HLA pertains to the major histocompatibility complex (MHC) as found throughout vertebrates. The MHC is of fundamental importance in defining an individual’s immunological identity and consequently establishing a defense system against potential pathogens. The five HLA loci as listed in Table 13.1 contained markers for tracing human population relationships and for investigating associations with diseases. Three of these (HLA-A, -B, and -C) are tested through serological reactions, while the remaining two (HLA-DQ and -DR) are investigated through cytotoxic methods. Specific HLA haplotypes will be discussed later in the context of disease associations.

SERUM PROTEINS

The plasma or fluid portion of blood contains a large number of kinds of proteins, most of which were found to be polymorphic as well as variable among different human populations. Table 13.2 lists serum protein markers that will be reviewed here. The workhorse method for separating and identifying serum proteins was electrophoresis that utilized a variety of preparations, buffers, and media.

ALBUMINS

Mode of inheritance: Autosomal codominants with Al allele controlling the common albumin, and several variants, such as Al Naskapi, found in varying frequency in different populations.

Albumins make up about one-half of all serum proteins. Their genetic control is found on chromosome 4. One of their main functions is to bind and carry other serum constituents, such as fatty acids and steroids, and they also control fluid volume outside the cell. Albumin studies were regularly carried out by field researchers around the world and
several variants were discovered. One of the first of these was Al Naskapi that was found in an Indian group in Quebec (Melartin and Blumberg, 1966). Al Naskapi was subsequently observed in other Canadian and US North American Indian samples, for example among the Dogrib Indians (Szathmáry et al., 1983).

### GROUP SPECIFIC COMPONENT (GC)

**Mode of inheritance:** Two common autosomal codominant alleles.

Group specific component (Gc) is also found on chromosome 4 in close linkage with albumin. Its discovery was made by Hirschfeld (1959). Gc is well-understood to be a vitamin-D binding protein, with two common alleles, Gc$^1$ and Gc$^2$. From this function it might be expected there could be some interplay between Gc variants and the role of vitamin-D in blood cell formation, particularly in people subject to becoming anemic and in areas of reduced sunlight where human groups are at higher risk of rickets. With respect to the latter prediction, Gc$^2$ was thought to be more efficient in transporting vitamin D, and did show a higher frequency in some northern populations, but with major exceptions, notably the Saami of Norway and Sweden (Roychoudhury and Nei, 1988).

### IMMUNOGLOBULINS (GM AND INV)

**Mode of inheritance:** Multiple autosomal dominant/recessive and codominant alleles and linkage groups.

Immunoglobulins serve as the body’s defense system by forming antibodies against foreign intruders such as bacteria and viruses. By the 1960s two types of globulins were identified, namely, Gm (IgG of the heavy chain of the antibody molecule) and Inv (IgK/Km of the light chain). Gm is located on chromosome 14, while Inv is mapped to chromosome 2. Marker variants and haplotypes segregating at these two loci were observed to differ by human population and region, probably as the immune responses were adaptively tailored to specific pathogenic threats. So while there are coding genes underlying Gm and Inv, their expression is mediated by environmental circumstances. Schanfield (1980) conducted a study of the anthropological usefulness of genetic markers in differentiating regional and continental populations and concluded that Gm haplotypes, along with HLA haplotypes and the Duffy blood group, were the leaders in carrying out this task when compared against a bank of red blood cell, serum protein and enzyme markers. Two essential components of usefulness were defined in terms of uniqueness of the marker and degree of polymorphism, and on both measures, Gm scored highly.

### HAPTOGLOBINS

**Mode of inheritance:** Autosomal codominant alleles, Hp$^1$, Hp$^2$.

Haptoglobins (Hp) bind free hemoglobin (Hb) that is released from destroyed red blood cells. The Hp-Hb complex both prevents loss of hemoglobin from the body through excretion, and also apparently plays a role in reducing the risk of bacterial growth by hemoglobin (Eaton et al., 1982). Smithies (1955) was the first to demonstrate polymorphic variation in haptoglobins by using starch gel electrophoresis. Haptoglobin has since been mapped to the short arm of chromosome 16. As with other serum proteins, haptoglobin variants could be under selective forces that maintain polymorphic frequencies depending on environmental stressors. For example, Hp$^1$, which has a higher hemoglobin-binding capacity than Hp$^2$, generally reaches its highest frequency in tropically located African and Amazonian populations who face a high parasitic load and corresponding increased risk for anemia.
TRANSFERRIN

Mode of inheritance: Three autosomal codominant variants, Tf^C is common, and Tf^B and Tf^D are rare.

Transferrin, as its name implies, is iron-binding protein that carries iron from the intestine and elsewhere, and delivers it to active tissues and dividing cells. As was the case for haptoglobins, Smithies (1958) discovered the polymorphic status of the transferrin locus, now mapped to chromosome 3. Could selection be maintaining the polymorphism? Transferrin variants might be implicated in persons or groups chronically stressed by iron-deficiency anemia or who are at high risk for red blood cell destruction. Also, transferrin may be involved with removing harmful allergens present in serum. While these are bases from which selection could operate, there was no clear evidence that this has been the case.

ENZYMES

Human variation in enzymes formed a vital area of research for anthropology/human biology, leading to field studies among non-Western populations in the 1960s. The most obvious interest, and of most clinical significance, were enzyme deficiencies commonly known as inborn errors of metabolism. Several traditional biochemical markers were identified, Table 13.2 lists a selected sample. A brief introduction to these is provided here, that will be followed later by a description of how these markers varied among different populations, and some discussion of possible bases for the variation. As in the case of serum proteins, electrophoresis was the then appropriate method of investigating enzyme variants in the 1960s. When first established as hereditary markers, enzymes were promoted as prime examples of the “one gene-one protein” notion that had to be modified after subsequent discoveries, as with the G6PD locus that has numerous variants all due to mutations of one structural gene.

CARBONIC ANHYDRASE

Mode of inheritance: Two linked loci on chromosome 8, CA I and CA II, each segregating dominant alleles, along with multiple recessive variants. Current status is that there now are at least 12 carbonic anhydrase loci, some linked and others on several different chromosomes.

Second only to hemoglobin, carbonic anhydrase forms a large portion of red blood cell protein. Its major function is to release carbon dioxide in the lungs in conjunction with the respiratory cycle. Carbonic anhydrases also play a role in bone resorption and calcification, and in maintaining an acid-base balance. Early population studies did not reveal very much variation except in Australia, with regard to CA I, and Africa, in terms of CA II, which showed polymorphisms (Roychoudhury and Nei, 1988). Given the many more recent discoveries of loci controlling the carbonic anhydrases, there is the potential of finding additionally interesting population variants.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY

Mode of inheritance: Multiple codominant X-linked alleles.

Glucose-6-phosphate dehydrogenase (G6PD) is perhaps the most recognizable enzyme in anthropological study when it appears in one of several variants that result in reduced enzyme production or a deficiency. Its deficiency has received a high level of attention due to its interaction with malarial sensitivity and resistance, and hence, demonstrated increased frequencies in groups residing in endemic malarial regions. This topic will be discussed later. The normal functioning G6PD enzyme plays an important catalytic role in maintaining red blood cell membrane integrity. The enzyme is found throughout most of the body including skin and saliva. Its genetic control is located on the long arm of the X-chromosome.

HEXOSAMINIDASE A (HEXA) DEFICIENCY

Mode of inheritance: Multiple codominant autosomal alleles.

Hexosaminidase A (HEXA) is example where an enzyme deficiency can have profound effects. The mutated HEXA gene causes a lethal condition known as Tay–Sachs disease. Persons having the classical form of Tay–Sachs disease experience developmental retardation and neurological degeneration in early infancy and in most cases die before reaching their third birthday. A normal functioning HEXA gene, located on chromosome 15, produces an enzyme that catalyzes the degradation of excess ganglioside (a constituent of cell membranes), whereas the mutated variant allows for the build up of ganglioside in neurons that causes the neurodegenerative disorder. Given the dire outcome for children with Tay–Sachs, it was surprising that the condition showed such a high frequency in Ashkenazi Jews of Eastern Europe. Homozygote recessives did not survive childhood so the variant marker would be expected to exist at a very low frequency. Initial thinking proposed that random drift had by chance elevated the mutant HEXA enzyme in the comparatively small and separated Jewish communities (Fraikor, 1977). Later, heterozygote advantage
was invoked as a possible contributing explanation (Chakravarti and Chakraborty, 1978; Marks, 1995). It was argued that overcrowded urban ghettos posed severe risks for infectious diseases, for example, tuberculosis, but heterozygotes were somehow protected. A similar argument will be noted later with respect to cystic fibrosis. In a more recent report, the pendulum has swung back to explaining elevated HEXA gene frequencies as due to drift in the form of founder effect within a population experiencing rapid census growth (Frisch et al., 2004).

LACTASE DEFICIENCY

Mode of inheritance: Autosomal alleles with lactase persistence dominant over lactase deficiency.

Yet another example of an enzyme deficiency, but with comparatively low adverse consequences, involves lactase, a digestive enzyme of the milk sugar lactose. The lactase locus has been mapped to chromosome 2. Nearly all human babies produce sufficient amounts of this enzyme throughout their growing years, and then undergo a decline of enzyme output into maturity. Milk, and unfermented derived milk products, causes these adults to experience unpleasant digestive symptoms, including bloating and diarrhea. Yet adults in some parts of the world continue to produce higher amounts of lactase, and hence have none of the aforementioned symptoms. Population studies showed a strong association between cultures that had a long tradition of dairy farming and a persistence of lactase into adulthood. A genetic analysis based on family studies demonstrated that lactase deficiency was inherited as an autosomal recessive, meaning that heterozygotes and homozygotes possessing the dominant marker were lactose tolerant (Sahi, 1974). This was a classic example of a biocultural interaction. It also illustrated how environmentally dependent gene expression was, or that genetic predisposition required suitable conditions to become of significance to the organism.

PHENYLALANINE HYDROXLASE (PAH) DEFICIENCY

Mode of inheritance: Autosomal alleles, with PKU recessive.

Phenylalanine hydroxylase (PAH) is a catalytic enzyme that participates in the conversion of phenylalanine to tyrosine. A deficiency of PAH is an inborn error of metabolism that can lead to varying degrees of impaired mental functioning, and other pleiotropic effects, known as phenylketonuria (PKU). The mutated allele is located on chromosome 12. Phenylketonuria is readily diagnosable and is routinely tested for as part of newborn screening, and is preventable through careful and consistent dietary management following a phenylalanine-free regimen at least through childhood. Like lactase deficiency noted above, PKU is another case of environmentally dependent or culturally mediated expression.

OTHER MARKERS

This section concludes with a description of variable human conditions or traits that were included occasionally in population studies (see Table 13.2). Of interest, investigations of phenylthiocarbamide (PTC) polymorphism were even extended to nonhuman primates. Methods of study were quite different ranging from visual and tactile examination for cerumen (ear wax) types, initially a host of clinical diagnosis and laboratory tests for cystic fibrosis that now include genetic analysis, and serial dilution or simple test paper strips for the PTC-tasting trait. They also show a range of consequences for the individual from being rather benign for cerumen types, to profoundly affecting the well-being of cystic fibrosis patients.

CERUMEN (EAR WAX) TYPES

Mode of inheritance: Allele for wet, sticky ear wax is autosomal dominant; dry ear wax is recessive.

Cerumen markers are expressed as wet (sticky, brown) and dry types (flaky, light colored) that are controlled by a locus on chromosome 16. There is human population distribution variability in these types along with implications of selection acting on ear wax type relative to climatic variables. The dry type is most often found in northern Asian populations, while the wet type is found in tropically located Asians, as well as in Africans and Europeans. Whatever adaptive significance there is for ear wax type polymorphism is yet to be determined.

CYSTIC FIBROSIS

Mode of inheritance: Multiple autosomal alleles, with CF recessive.

Cystic fibrosis (CF) is a debilitating condition that disrupts normal pancreatic, intestinal and respiratory functioning. After some intensive genetic research, the CF gene was mapped to chromosome 7 in 1985. Since affected individuals prior to more recent therapies were at high mortality risk as children and had reduced fertility as adults, it was puzzling why the
condition had reached a high frequency in some European populations. A possible answer may be found in an association between the CF locus and risk for tuberculosis, paralleling heterozygote advantage explanations given for Tay–Sachs disease, and also for sickle cell anemia which will be discussed more fully later in the context of balancing selection and diseases.

**PTC TASTING**

Mode of inheritance: Taster allele is autosomal dominant; non-taster is recessive; variable expressivity in phenotypes.

The ability to taste the compound phenylthiocarbamide (PTC) is controlled by a major gene mapped to chromosome 7, with another locus likely involved as well. For an up-to-date confirmation of PTC chromosome mapping see Drayna et al. (2003) and for a complete historical review of this trait see Wooding (2006). Although a rigid bimodal distribution of tasters and nontasters is not observed, especially with applying the serial dilution procedure, there is a certain ease in collecting results, apparently so readily accessible that chimpanzees and rhesus monkeys became suitable subjects (Eaton and Gavan, 1965). Roychoudhury and Nei (1988) list nearly 80 human studies that had carried out PTC testing that virtually covered the world. Gene frequencies were highly variable both within and between continental samples, with no discernible patterns. There has been a suggestion of an interaction between dietary practices and thyroid function (Molnar, 2006). PTC, as a synthetic compound, serves as a proxy for a carbon-nitrogen-sulfur radical found in certain plant foods, particularly those of the cabbage family, that tasters perceive as bitter, and hence, to be avoided. This could be a protective behavior in that cabbage and its relatives may block the uptake of iodine, thereby reducing thyroid function, and resulting in depressed metabolism that in turn affected childhood growth and adult fertility. Conversely, nontasters have been shown to be more susceptible to developing nodular goiters, presumably due to a reaction of the thyroid gland to depressed amounts of iodine in the diet. Additional testing of the role selection and adaptation play in maintaining the PTC polymorphism seems warranted.

In concluding this section on basic marker identification, it should be pointed out that not all population studies utilized all of the markers described, or for that matter, had necessarily restricted their research to those that appear above. On the first point, research projects added markers as they were discovered and found to be anthropologically useful. As noted earlier, usefulness of markers was well investigated in

Schanfield and Fudenberg (1978) and Schanfield (1980), that dealt with the Gm and HLA systems and accompanying extensive tables of marker frequencies for world populations. Schanfield (1980) also notes a general problem in that certain markers could not be studied routinely because their reagents were not readily available, with particular reference to the HLA system.

Now that a set of classic markers has been introduced, the next section will offer a discussion of how these markers were applied in various contexts, the first being that of describing human biological diversity, including its most contentious application, that of classifying human races.

**BLOOD GROUP MARKERS FOR CLASSIFYING HUMAN POPULATIONS**

There is a long and tortuous history surrounding unsuccessful attempts to sort human populations into stable, mutually exclusive categories called races. Rather than extensively review that history here, the reader is referred to these works for that information (Montagu, 1964; Marks, 1995; Brace, 2005; Molnar, 2006). It is important, however, to trace the use of blood group markers as these became available to those choosing to carry out race classification. That story, as already mentioned, began with the discovery by Landsteiner in 1900 of the ABO blood group. A little more that a decade later, the ABO group was being studied by Ludwik Hirschfeld by conducting serological tests on thousands of persons, soldiers and civilians, from throughout Europe and even some from China, Japan, and Africa (Mourant, 1983). His results laid the groundwork for all subsequent studies showing serological distinctions across human populations, that is, the establishment of racial divisions. The premise applied was quite straightforward. First, accepting that the ABO blood group markers were inherited (which Hirschfeld helped to show), then frequencies of ABO blood group types (and later calculated gene frequencies) would indicate the degree of relationship between populations, the more similar they were the more closely they were related to each other, and vice versa. From there it was a matter of drawing lines between blocks of populations, a step that undoubtedly was greatly aided by geography and continental boundaries, and taken by Hirschfeld and his wife (Hirschfeld and Hirschfeld, 1919) in their defining of three ABO racial types, European, Asio-African and Intermediate (Marks, 1995). This was followed by other attempts at serological race classification (Ottenberg, 1925; Snyder, 1930; Wiener, 1948), but the effort that might have had a high potential for impacting anthropological thinking on races
was that of Boyd. In his book, *Genetics and the Races of Man: an Introduction to Modern Physical Anthropology* (1950), Boyd set forth in highly explicit terms why he considered blood group markers to be more scientifically sound for racial classification than that any of the heretofore used methods utilizing morphological characters, including anthropometry.

When Boyd’s work was published, blood group frequencies were available in large samples for the ABO, Rh, and MN systems. In addition, Boyd added PTC tasting and secretor status to his set of markers. His genetically defined races largely matched earlier classifications, particularly that of Wiener (1948). Not surprising then, Boyd’s genetic races conformed closely with geography, a point that he seems to regard as confirmation of what he expected to find regarding human population descent histories and their patterns of separation and migration. His claims for the advantages of the genetic method over earlier classifications are that it is more simply done, completely objective, and that gene frequencies do not have the genetic uncertainty that is hidden in phenotypic traits, and gene frequencies provide quantitative rather than qualitative measures of population differences along with an assessment of admixture (Boyd, 1950).

It should be noted that the erroneous claim of selective neutrality for blood group genes initially was accepted by Boyd (1950), except for maternal–fetal incompatibility in the Rh system, which then later abandoned it (Boyd, 1963a).

In this same year, Boyd (1963b) touted what he judged to be major accomplishments of the genetic method. He concluded that genetic methods had contributed to physical anthropology by: (a) confirming an Indian origin of Gypsies; (b) providing a quantitative assessment of white admixture in American Blacks; (c) establishing that Lapps were a distinctive European race; and (d) showing that Papuans of the New Guinea region were native to the South Pacific and had not migrated from Africa. With regard to one of these presumed feats there is recent caution expressed against the use of markers, sometimes single alleles, for calculating degree of admixture (O’Rourke, 2000).

By the time of Boyd’s 1950 classification of serological races, it had already been reported (Boas, 1912; Shapiro and Hulse, 1940; Lasker, 1946) that head and body measurements were subject to modification in children of migrants who accommodated to new environmental conditions. Hence, this important finding would severely question the presumed stability of those variables, such as the cephalic index, that had been so heavily relied upon by race classifiers. However, by the end of the 1960s, race classification itself was on the wane, and genetic markers were not able to sustain efforts that sought to arbitrarily apportion human variation into discrete categories.

What helped to replace racial classification were attempts to discern the nature of human population relationships in terms of cultural historical and microevolutionary processes. An even more basic task was to be able to accurately analyze whatever biological differences existed between groups without any need to classify them. A study from Boyd’s time period that illustrates this kind of endeavor was done by Sanghvi (1953). He included five endogamous Indian castes in an analysis of anthropometric versus genetic markers to discern their relationships. His list of markers, certainly short by subsequent standards, only consisted of ABO, MN and Rh blood group phenotypes, taste reactions to PTC, and red-color-blindness. He concluded that either the genetic or morphological method could be more useful in reflecting biological relationships in certain cases, but more likely they will complement each other, and hence, both should be applied using many more measurements and markers than he did. We will see in the next section that this recommendation is indeed heeded within a decade with the launching of a number of major research projects.

Physical anthropology apparently was not so convinced of Boyd’s approach not because it applied genetic markers, but because they were used to classify races. Two principal textbooks of roughly that time period perhaps best reflect the state of affairs. Montagu (1960) and Buettner-Janusch (1966) both are replete in their coverage of genetic markers, complete with tables of gene frequencies and allele distribution maps for the world. Beyond that they provided clear background information on the modes of inheritance and methods for identifying blood groups and serum proteins, and most importantly, what was then known about the selective basis of certain systems, such as the association of blood groups and diseases and the anthropological significance of hemoglobin variants at the sickle cell locus. Race classification utilizing genetic markers was seen as relatively unimportant and unproductive, in comparison with the study of selection and other microevolutionary processes that occurred within local populations. On a larger scale, research interest shifted to investigating how and when gene pools across and between continents came to differ from one another, again through microevolutionary processes. This state of affairs undoubtedly reflected the paradigm change that Washburn (1951) had proposed a decade or so earlier that the “new Physical Anthropology” should emphasize an understanding of function and process as opposed to an earlier focus on technique and description as a direct goal.

In opposition to race classification, a mid-twentieth century alternative was to view patterns of genetic variation expressed in terms of clinal distributions. Gene frequency clines joined the already recognized
gradients in human morphological variation with respect to body size and shape (Allen’s and Bergmann’s Rules), and skin pigmentation (Gloger’s Rule). These so-called “ecogeographic” rules generally explained clinal variation in morphology as due to adaptive responses of populations residing in gradients of temperature, solar radiation, and other environmental conditions that occurred in latitudinal changes. It was reasoned that gene frequency clines, or genoclines, might also be the result of natural selection gradients, such as levels of disease stress, but could as well be explained by actions of gene flow, migration, and human mobility and settlement patterns. For here, a classic example of a genocline will be presented followed by a more recent application of genocline based on Australian data that had been collected decades earlier (Birdsell, 1993).

A textbook example of a genocline is the distribution of the $B$ allele of the ABO system as its frequency was mapped from eastern Asia to the British Isles. Although it was not known why, the $B$ allele had its maximum frequency in Asia at around 25% but then declined to less than 5% in much of Western Europe (Mourant, 1954). A likely explanation for the $B$-allele cline rested in historical migrations and invasions of peoples from Asia westward over the past couple of millennia. To be sure, the $B$-allele cline was not exactly a smooth and steady transition across Eurasia. There were gaps in the big picture, and very likely if $B$-allele frequencies were filled in, a more detailed map composed of many local-level populations would show some breaks or even reversals of the general geographic trend. It is to be expected that there were historical episodes involving small founder groups that became isolated over sufficient amounts of time for genetic differentiation to have occurred. Lastly, gene flow and human population movements were not exclusively in a westerly direction.

For a more recently plotted example of a cline also from the ABO system, Figure 13.1 shows $A_1$-allele frequencies as isogenes (comparable to isotherm contour lines connecting points of equal temperature) for the Aboriginal Australian population (Birdsell, 1993). The overall range in frequency for $A_1$ is from a high of 0.53 near the center of the continent to a minimum of 0.03 at the coastal and northern island areas. As would be expected there are some steep declines along with more gradual gradients in the topographic display. Birdsell pointed out a generally recognized premise that single genes, such as the $A_1$ allele, more rapidly respond to microevolutionary processes than polygenic traits. Accordingly, in reviewing the history of human occupation of Australia, he attributed the gene frequency distribution shown in Figure 13.1 to be the

![Frequency of the $A_1$ allele showing a clinal distribution in Australian groups. From Birdsell (1993). © 1993 Oxford University Press, reprinted with permission.](image-url)
product of settlement of small founding groups (that is, founder effect), successive major migration waves, and importantly, a population structure of local bands within a larger tribal population.

Clinal distributions of classic markers, such as those for the B and A alleles just described, clearly demonstrated that race classification could not begin to capture the complexities and details of human population relationships and historical connections. In pursuit of that goal, the discussion now turns to population studies that set out to reconstruct history through an understanding of microevolution.

APPLICATION OF MARKERS TO POPULATION STUDIES AND MICROEVOLUTIONARY PROCESSES

Dynamic population study took precedence over static race labeling with the launching of a number of important human biology field research projects. One such effort was the Harvard Solomon Islands Project that was conceived by Albert Damon in the early 1960s (Friedlaender, 1987). This project served as a model of design for many more similar projects that were undertaken in roughly this time period. The Solomon Islands Project applied a multidisciplinary approach in which all four subfields of anthropology were represented, along with specialties from the biomedical sciences. It was reasoned that if population processes were to be adequately understood, it would be necessary to examine essential aspects of human behavior and decision-making. This meant that culture interacted with human biology, and in recognition of this connection a biocultural or biobehavioral approach was established. A clear illustration is to be found in population genetic measures of migration or gene flow, and even in selection and random drift. The strength of these processes very much depended upon human behavior and decision-making, such as cultural expressions in settlement patterns, mate choice and marriage customs, and culturally derived medical systems for diagnosis and treatment.

A major task within the sphere of population genetics in the Solomon Islands Project was to map biological variation among several groups on different islands with an aim to portray relationships of these groups in terms of microevolutionary processes, especially those pertaining to selection, random drift, and migration (Rhoads and Friedlaender, 1987). Among the markers included in that study were numerous blood polymorphisms, namely; eight red blood cell antigen systems, haptoglobins, transferrins, and Gm and Inv systems. Calculated allele frequencies from these markers were used in a distance analysis and other multivariate procedures that rendered comparative findings for the Solomon Islands as well as with additional samples that had been obtained earlier from Bougainville (Friedlaender, 1975), and also samples from a broader Pacific Island context. The upshot of this aspect of the study that involved markers was a complex and not easily discernible pattern of genetic variation, but it seemed to indicate at each level from local groups to that of Pacific region and even beyond, that biological heterogeneity and variability extended deep into history and could not be explained simply by random drift of small, isolated groups.

Another aspect of the Solomon Islands Project, which also was carried out in many other research efforts in that time period including the earlier Bougainville study, was to incorporate genetic markers with multiple measures of distance as a test of correspondence between these measures for potentially realizing the same or similar outcomes of population relationships. In this regard, the Solomon Islands Project combined the distance measures of geography, language, anthropometry, odontometrics, dermatoglyphics, as well as the set of genetic markers noted earlier. One of the more enlightening results showed that genetic markers, along with anthropometrics and odontometrics, less closely corresponded with language and geography than did dermatoglyphic variation (Friedlaender, 1987).

Other studies have yielded varying results in these distance correspondence analyses. A brief review of this matter can be found in Meier (1980), who noted that incongruence between distance measures could be due to such factors as sample size and composition, number and kinds of markers used, and level at which the analysis is done, from local villages to large regions. For this discussion of genetic markers, it is perhaps best summarized with the appreciation that Mendelian traits could well be subjected to short-term and relatively rapid change in frequency via random drift and founder effect (particularly in small, semi-isolated groupings), but also undergo successive generational change due to selection processes. And hence, there is a great need to understand the nature and makeup of the sample upon which the marker frequencies are based, and to fully characterize samples even though most often there was little way to control sample makeup while conducting field studies. In the end, there remains considerable theoretical uncertainty whether the degree and rate of change in frequencies of markers are expected to correspond well with the other distance measures, such as anthropometric or language change. On this matter, Lewontin remarks in his Foreword to Friedlaender (1975) that linguistic distance at that time was too simply measured. However, Lewontin praised Friedlaender's work for its strong emphasis upon the historical perspective, that is, in reconstructing the action of evolution over time.
Several population studies in the late 1960s and into the 1970s paralleled portions of the Bougainville and Solomon Islands Project design, particularly for their application of the multidisciplinary, biocultural, and historical approaches. One set of such studies can be grouped under the International Biological Programme (IBP) Human Adaptability Projects. For a brief background, the IBP was composed of seven sections that directed a global effort toward measuring and understanding ecological productivity and its interaction with human welfare. One of these sections was that of Human Adaptability (HA) which got underway in the mid 1960s. Relevant to this discussion, methods for collecting specimens, such as blood from which markers could be determined, were presented in the IBP HA Handbook that first appeared in 1965 (Weiner and Lourie, 1969). This guide did not specify which markers were to be studied but rather set forth specifics of proven field methods for securing, storing, and transporting specimens so that they could be comparably analyzed, very often in a distantly located laboratory. A common problem was hemolysis during extended periods of travel, rupturing the red blood cell membrane and spilling out constituents that would have been used for serological testing. The IBP Handbook also detailed procedures for carrying out field testing of some markers, for example screening methods for G6PD and determining PTC taster status. A major concern that needed to be addressed was that of reliability of the serological results even when the specimens reached their destinations presumably intact. This matter had received some attention at the time.

Osborne (1958) had reported some major discrepancies for blood group testing when done at three well-established laboratories. Handling problems may have been an issue in another study documenting testing discrepancies (Livingstone et al., 1960). The least stable systems involved subtyping of A in the ABO system, and in the Duffy and P markers. Thus, it was imperative that blood specimens at the very least be handled with the utmost care to avoid degradation problems. One study that did a careful analysis of such problems was Neel et al. (1964) in which they had carried out field testing on blood specimens collected from the Xavante of Brazil, and then later retested them in their laboratory in Ann Arbor, Michigan. According to their full disclosure, discrepancies seemed to relate to different testing and laboratory conditions, and it was these problem areas that the IBP Handbook hoped to rectify.

Under US IBP/HA auspices, multidisciplinary field studies that included a survey of genetic markers were carried out among human groups residing in Alaska (Inupiat Eskimos), Peru (Quechua), and Brazil (Yanomama and Makiritare), with the last cited having the greatest emphasis on applying classic markers to population genetics questions (Neel and Ward, 1972). These along with a selection of additional field studies appear in Table 13.3.

<table>
<thead>
<tr>
<th>Study area/population</th>
<th>Year begun</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wales</td>
<td>Post-WWII</td>
<td>Harper and Sunderland (1986)</td>
</tr>
<tr>
<td>Australia</td>
<td>1952</td>
<td>Birdsell (1993)</td>
</tr>
<tr>
<td>Canada/Blackfeet Indians</td>
<td>1952</td>
<td>Chown and Lewis (1953)</td>
</tr>
<tr>
<td>Brazil/Xavante</td>
<td>1962</td>
<td>Neel et al. (1964)</td>
</tr>
<tr>
<td>South Africa/San, Herero, and others</td>
<td>1963</td>
<td>Jenkins et al. (1978)</td>
</tr>
<tr>
<td>Easter Island*</td>
<td>1964</td>
<td>Etcheverry (1967); Meier (1969)</td>
</tr>
<tr>
<td>Peruvian Andes/Quechua</td>
<td>1965</td>
<td>Baker and Little (1976)</td>
</tr>
<tr>
<td>India/Gavdas</td>
<td>1966</td>
<td>Malhotra (1978)</td>
</tr>
<tr>
<td>Japan/Ainu</td>
<td>1966</td>
<td>Omoto (1978)</td>
</tr>
<tr>
<td>Bougainville</td>
<td>1966</td>
<td>Friedlaender (1975)</td>
</tr>
<tr>
<td>Solomon Islands</td>
<td>1966</td>
<td>Friedlaender (1987)</td>
</tr>
<tr>
<td>Alaska/Eskimos (Inupiat)*</td>
<td>1967</td>
<td>Jamison et al. (1978)</td>
</tr>
<tr>
<td>Southwestern United States/Papago</td>
<td>1967</td>
<td>Niswander et al. (1970)</td>
</tr>
<tr>
<td>Saharan Africa/Ideles</td>
<td>1968</td>
<td>Lefevre-Witier and Verges (1978)</td>
</tr>
<tr>
<td>Mexico/Tlaxcaltecan</td>
<td>1969</td>
<td>Crawford et al. (1974)</td>
</tr>
<tr>
<td>Central America/Black Caribs (Garifuna)</td>
<td>1975</td>
<td>Crawford (1984)</td>
</tr>
</tbody>
</table>

Note: *Due to problems, complete serological testing could not be done on the Easter Island and Inupiat blood specimens. WWII, World War II.

Particular mention should be made here of a four-volume series published under the topic of *Anthropological Genetics* (Crawford and Workman, 1973; Mielke and Crawford, 1980; Crawford and Mielke, 1982; Crawford, 1984). (A fifth volume in this series-Crawford, 2007, presented an updating of the earlier volumes by focusing upon molecular genetics.) These works in general illustrated how useful classical markers were in population study, for example of the Black Caribs of Central America (Crawford, 1984). This volume contained several differently authored chapters devoted to marker description and frequency distributions and then went on to explore critical topics that employed these data in such matters as admixture estimates, fertility differentials (in the case of the sickle cell locus), and population structure.
It was these kinds of studies done on regionally demarked human groups for which genetic, biological, and cultural information could be combined that offer sharp insight to microevolutionary processes and population dynamics.

However, there was also the big picture to deal with, that is, the relationships of neighboring populations as well as those that were distant in both geography and in their historical connections. The work that epitomizes this effort was that of Cavalli-Sforza et al. (1994). If this tome can be described briefly, it is best depicted as a worldwide geography of human genes. As customary for physical geography, there are numerous maps that depict levels of gene frequencies for the major continents and Oceania. These are referred to as synthetic maps for their handling of an array of genetic markers by a multivariate procedure, namely, principal components (PC) analysis. An example of a synthetic map is found in Figure 13.2.

These maps then are interpreted in light of historical and microevolutionary processes whereby similarities and differences in PC values (seen as peaks and valleys on the maps) can represent migrational or selection patterns, sometimes according to gradients or clines, but possibly on a more local level show sharp breaks due to population isolation and random drift. Synthetic maps of this sort also were constructed from classic markers about a decade earlier for North American Native populations (Suarez et al., 1985) that assisted in sorting out population relationships and migration patterns.

A commonly applied procedure for depicting population relationships that was based on classic markers, and continues to be used with molecular data, was that of dendrograms or phylogenetic trees. An example of a dendrogram is shown in Figure 13.3. Various statistical methods were employed to generate graphically clear representations of genetic similarities or the opposite, genetic distance among populations. There generally was no unique solution in reconstructing trees; hence, multiple trees could lead to alternative interpretations. However, dendrograms, and also synthetic maps, could be viewed essentially as methods for reducing large data sets into manageable entities that might in fact partially answer questions concerning population affinities or perhaps even more importantly, point future research toward productive, new directions.

The final work to cover in this section on population study is a review that addressed the thorny question of peopling of the New World through a congruence of variables approach (Greenberg et al., 1986). They included linguistic, dental, and genetic lines of evidence in an attempt to reconstruct the timing and number of migrations. This work is cited because it stands at the transition between the use of classical markers and the then newly developing DNA technology, at that time devoted primarily to restriction fragment length polymorphisms (RFLPs). An extensive list of references can be found in the article. These are mostly dated from the late 1970s to the mid 1980s that include original study results for Native New World populations with respect to blood
group, serum protein, and enzyme polymorphisms. The review of these markers concluded that genetics could be complementary to the other two lines of evidence but could not stand alone in supporting a tripartite migration history of New World settlement. This work also provides a critical sense of how researchers viewed the claims of Greenberg et al. (1986) through multiple authored comments that directly followed the article.

**GENETIC MARKERS AND SELECTION**

This section will focus on the role that classical markers played in assessing natural selection as a major process for understanding genetic variation within and among human populations. To some degree this development was imbedded in the history of population genetics. During the 1930s Mendelian genetics interfaced with microevolutionary theory to form population genetics that at that time had a strong mathematical and theoretical emphasis. Later, by the 1940s, the modern synthesis of evolution was established with population genetics at its foundation.

While population genetics theory certainly received application to human groups over the next several decades as noted above in population studies, it achieved a substantial boost with respect to the academic realm in 1971 with the appearance of the initial edition of *The Genetics of Human Populations* (Cavalli-Sforza and Bodmer, 1999). Three chapters in this later edition are most relevant to note here for their in-depth treatment of classical markers with regard to maintaining polymorphisms (Chapter 4), possible adaptive relationships of blood group antigens and serum proteins to selective agents, particularly disease and incompatibility (Chapter 5), and marker frequency distributions across groups (parts of Chapter 11).

In this last cited chapter the authors continued to espouse a race concept, definitely not in the previous manifestations of racial typology or strict
classification, but rather as an acknowledgement of biological differences and similarities they assumed to be based on race. It appears that their use of race is simply a matter of convenience for defining population units of study, which is to this day a vexing problem for anyone carrying out population studies. Who belongs within the sample and how will comparable study groups be defined? Island populations and semi-isolated villages offer fairly clearly demarked boundaries, and this may in fact underlie part of the attraction for the geographic selection in the field studies noted above.

In the manner in which Cavalli-Sforza and Bodmer (1999) had used race, it might have been more appropriate to have used a concept proposed by Montagu (1964), the “genogroup,” which essentially defined population differences on the basis of gene frequencies. Then again, Montagu seemed to favor “ethnic group” as a substitute for race, which has gained fairly wide application. However, “ancestry” appears to be replacing all of these terms at the present time in the context of DNA markers. Aside from constructing tree diagrams to show partial conformity with prior race classifications, Cavalli-Sforza and Bodmer (1999) more importantly demonstrated how microevolutionary processes drove the course of population change. Their text, then, established a formal educational treatment from which to pursue interests in human population genetics. Needless to say, the genes upon which most of the principles rested were classical markers. And it is a consideration of these markers with respect to selection that is covered next.

**ABO MARKERS AND DISEASE ASSOCIATIONS**

As broader world surveys of the ABO blood-group distributions became available, it also became apparent that gene frequencies varied both within highly polymorphic loci as well as considerably across populations to a degree that could not be attributed to newly arising mutation nor to random drift or to gene flow, at least above local population levels. This, of course, pointed to selection as the active force in both changing and in maintaining gene frequency levels. There was ongoing debate as to whether ABO polymorphisms were in a stable state or transient and hence subject to eventual loss of alleles. The arguments for stability rested on the presence of the ABO blood group system in nonhuman primates – antigens analogous to A and B are present in Rhesus macaques (Duggleby, 1978), and the relatively narrow range of ABO gene frequencies within certain human groups. These lines of evidence indicated that ABO polymorphisms had a long history by virtue of their existence in nonhuman primates, and then varying historical and environmental conditions for selection to operate on led to variation across different human groups. This section will cover the use of markers in trying to understand the nature of ABO polymorphisms with regard to selection.

The earliest work to implicate selection in the ABO distribution was faulty for its lack of statistical rigor. It simply amounted to collecting data showing associations between particular ABO blood types and a set of diseases, often using hospital patient records as the sampling source. However, flaws in the early research were in not having adequate control groups or unbiased samples that would allow any deviation from normal expectations to be properly ascertained. Once these study defects were corrected, many significant associations remained with respect to noninfectious diseases, but not nearly as clear as the results for infectious diseases, and these will be discussed first.

Armed with these findings, it was important to now offer possible selective mechanisms that would account for the blood group and disease associations, and that then would define directions for further investigation. Four categories of infectious disease had been identified as likely candidates for changing gene frequencies through natural selection (Vogel and Motulsky, 1997). These were: (1) acute infections, such as small pox, plague and cholera, that periodically spread as epidemics over large areas; (2) chronic infections that were highly contagious, such as tuberculosis and syphilis; (3) intestinal infections that afflicted all age groups but were likely fatal to infants and younger children; and (4) malarial infection.

These agents of selection would operate through differential mortality and fertility, although for some of them the former would be more likely in that many people would be stricken with the disease when they were past their prime reproductive years. In that situation, selection would not be acting directly on the reproductive success of the individual, but could have an impact on broader measures of fitness, e.g., inclusive fitness, due to the loss of support and resources provided by postreproductive members of the society.

Differential survival in connection with the ABO blood groups was presumed to be an increased susceptibility or resistance by virtue of certain markers a person possessed. Accordingly, it was found that individuals carrying the A antigen were more likely to contract smallpox (Vogel and Chakravararti, 1966), while persons with the O blood type were at higher risk for cholera (Glass et al., 1985). It was presumed that these blood types were in fact more susceptible because of pathogen similarity to their own genetic makeup. Hence, there was a failure of their immune system to recognize the foreign invader, likely a bacterium or virus, and consequently did not initiate an appropriate defense response that then resulted in an
increased mortality risk. Highly relevant supporting evidence for this position came out of India for its comparatively high frequency of the $B$ allele that could be explained by that country’s long history of smallpox and cholera epidemics, that would render both the A and O markers at a selective disadvantage (Buchi, 1968). Conversely, over many generations persons carrying the $B$ antigen had a proportionately higher survival rate that boosted the frequency of this marker.

Population and biomedical studies of these, and other blood group and infectious disease associations, have yielded varying and inconsistent results upholding the immunological hypothesis to explain ABO distributions. A troubling matter is that different disease associations have been found for the same locus, which raises a question of what the statistical associations actually demonstrate (Weiss, 1993). It does appear that selection acting in this manner through infectious disease probably does explain some of the worldwide marker frequency distributions. It has been reported that testing of the immunological hypothesis was discontinued sometime after the late 1970s (Vogel and Motulsky, 1997). Yet, there is at least one area of research along this line that remains very active.

During the 1950s it was shown that persons with blood type O were more susceptible to having stomach ulcers (Table 13.4). It was subsequently discovered that non-secretors of ABO substances were particularly vulnerable. This was followed by the highly significant finding that $Helicobacter pylori$ was closely associated with stomach ulcers, and that the attachment of this bacillus (bacterial infection) to the gastric epithelium was mediated by blood group antigens. In particular, it was persons who carried the Lewis $Le^b$ antigen that appeared to be most likely infected and thereby experienced an ulceration process (Boren et al., 1993). However, a later study done in an outpatient clinic was not able to confirm this result (de Mattos et al., 2002), but it did convincingly match earlier findings of $H. pylori$ infection predominantly in patients with O phenotypes. The pendulum continues to swing as a very recent report that studied monkeys concluded that the ABO blood group and secretor status of individuals may in fact be part of an evolutionarily derived innate immunity against infectious diseases (Lindén et al., 2008).

When considering noninfectious disease associations with ABO, the list is much longer and statistically stronger but an understanding of the mechanism responsible was, and still is, virtually unknown. Table 13.4 provides a selected sampling of significant associations. These represent some of the largest samples of patients and controls from worldwide series, and undoubtedly established the statistical reality of ABO markers and disease associations. The selected set focuses on the digestive system, whereas a more thorough listing would also include malignant and nonmalignant conditions of the reproductive and vascular systems. For the gastrointestinal tract, initial speculation was that there were differential immune responses by persons with different ABO antigens, especially of the soluble form. This thinking would parallel the proposal noted above for ABO associations and infectious diseases. Testing of this hypothesis, however, is complicated due to the interplay in the gut between intestinal flora, highly variable dietary practices around the world, and ABO antigen specificities. It has been concluded that the contribution of ABO polymorphisms to the etiology of digestive ailments is quite small (Vogel and Motulsky, 1997), and further that, even though these markers cannot be considered neutral, the low level and uncertain direction of selection will not explain the maintenance of variation observed in the ABO distribution (Cavalli-Sforza and Bodmer, 1999).

### INCOMPATIBILITY SELECTION AND BLOOD GROUP MARKERS

Another area of research interest that addressed the question of the persistence of blood group polymorphisms is that of incompatibility selection. Incompatibility refers to maternal/fetal situations where the mother would carry in her system antibodies against

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of series</th>
<th>No. of patients</th>
<th>No. of controls</th>
<th>Markers compared</th>
<th>Mean relative incidence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer, stomach</td>
<td>101</td>
<td>55 434</td>
<td>1 852 288</td>
<td>A:O</td>
<td>1.22</td>
</tr>
<tr>
<td>Cancer, colon</td>
<td>17</td>
<td>7 435</td>
<td>183 286</td>
<td>A:O</td>
<td>1.11</td>
</tr>
<tr>
<td>Cancer, pancreas</td>
<td>13</td>
<td>817</td>
<td>108 408</td>
<td>A:O</td>
<td>1.24</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>44</td>
<td>26 039</td>
<td>407 518</td>
<td>O:A</td>
<td>1.35</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>41</td>
<td>22 052</td>
<td>448 354</td>
<td>O:A</td>
<td>1.17</td>
</tr>
</tbody>
</table>

*Relative incidence is calculated as the ratio of, for example, A:O patients divided by A:O controls.

Source: Data taken from Vogel and Motulsky (1997, p. 221).
red blood cell antigens found in her developing fetus. For example in the ABO system, an O mother would be incompatible with a fetus having an AO or BO genotype. It is predicted under population genetics theory that ABO heterozygotes would be selected against, usually leading to a loss of genetic variability, unless there are counter selective forces that favor heterozygote survival. What these forces are for the ABO system is not yet known but probably do implicate the mother’s immune system.

In the case of the Rh system, where incompatibility is prominent in some populations, a somewhat clearer picture has emerged. This discussion will be centered on the Dd locus, since it was this marker that directly caused transfusion problems that then led to the discovery of the system, and later was shown to be a cause of death of newborns in the situation of an Rh-negative mother (dd) whose infant was Rh-positive (Dd). The child of this incompatible Rh combination was at risk for having erythroblastosis fetalis or hemolytic disease of the newborn (HDN) due to Rh positive antibodies (the small 7S gamma G type) crossing the placenta and into the fetus.

Not all Rh-positive children born to Rh-negative mothers suffered from HDN. The explanation involves several mitigating circumstances. First of all, Rh antibodies are not naturally occurring, an Rh-negative mother has to be exposed to Rh antigens in order to stimulate her system to produce antibodies. This could have come through an improper blood transfusion, or much more likely, through carrying an incompatible fetus. Some of the fetus’ red blood cells can enter the mother’s system at the time of delivery, and thereby be present to stimulate her to later form antibodies. Obviously, an initial fetus is not affected because the mother has not yet been “immunized” or sensitized prior to the baby’s birth. Subsequent incompatible conceptions increase the risk for HDN as the mother’s antibody titer is raised. There appears to be some variation in women as to how many pregnancies are required to build antibody strength to the point of causing HDN. For some time now medical intervention has protected mothers from having HDN babies through the administration of a prepared antiserum that destroys any fetal blood cells that might have entered the mother’s circulation during an incompatible pregnancy with the result that she is not sensitized. On rarer occasions, treatment has been in the form of exchange transfusions done either on the fetus or newborn. Prior to medical advancement severe HDN was often fatal, and obviously a source of much personal grief.

In the scientific realm a different kind of concern was brought out by the observation that the frequency of Rh-negative allele d was substantially higher than expected since all HDN children who were selected against were heterozygote Dd. This should mean that by losing D and d alleles equally, over time the rarer of the two eventually would be lost, and it was clear from many surveys the d allele was consistently at a lower frequency. On the other hand, surveys also showed that not only was the d allele not close to being selected out or anywhere near to a recurrent mutation rate, it seemed to be maintained at a much higher frequency than a simple selection against the heterozygote model would predict. There had to be circumstances that were counterselecting.

Two of these circumstances were noted above in that the first-born children of Rh incompatibility are not affected and that later developing fetuses may not necessarily have HDN depending upon the state of sensitivity or antibody titer of the mother. Added to these is the segregation outcome of fathers who are either DD or Dd, and of course the latter have a 50% probability have contributing a d allele and thus a compatible Rh-negative child. There is also some evidence that parents who lost a child to HDN tended to over-compensate their loss by producing more homozygote dd children, thus increasing the d allele frequency. There is a final explanation for why the d allele is elevated beyond model prediction that interestingly involved mothers who had a double incompatibility with their fetus’ for both Rh and ABO systems.

Maternal/fetal incompatibility in the ABO system does result in HDN, but this occurs in a small percentage of overall potential circumstances. In another portion of the ABO incompatible cases, say with an O mother and an A fetus, as the baby’s red blood cells enter her system they are quickly destroyed by her normally present anti-A antibody. If the blood cells also happen to carry the Rh-positive antigen and hence are Rh incompatible, then the cells are destroyed before there is time for the mother to build Rh antibody. In effect, double incompatibility serves to protect subsequent fetuses from HDN, and, of course, it would help to maintain a higher d allele frequency.

**HLA AND DISEASE ASSOCIATIONS**

From the early 1960s onward there has been increasing anthropological interest in the application of the HLA polymorphisms to investigating population relationships and to the study of microevolutionary processes, particularly selection. The HLA system has turned out to be highly useful in both regards. Certainly it is the most polymorphic system, and discovery of new alleles is still occurring. In Roychoudhury and Nei (1988) population data were compiled for 5 HLA loci and a total of 89 alleles. Updating of HLA polymorphisms can be found at an online website – IMGT/HLA Database – that currently reports nearly 2300 alleles for what is now known as Class I HLA alleles. Even going back...
to the late 1980s, allelic variation was high both within and between sampled regions. For example, the B7 allele ranged from 0.066 to 0.144 in several European countries, 0.031 to 0.060 in Asian populations, and an African sample yielded a frequency of 0.115 (Roychoudhury and Nei, 1988). Much of the variation in HLA polymorphisms can be ascribed to populations that had undergone random drift and to population movements and resultant gene flow. With regard to selection, an early and striking association was found between B27 allele and ankylosing spondylitis (AS) in a British study. It is now known that a vast majority of persons who have AS (which causes inflammation of the spine and other arthritic symptoms) possess the B27 marker. However, the marker frequency varies across ethnic groups, and indeed AS can also occur even when the B27 marker is absent. Ankylosing spondylitis is part of a group of autoimmune diseases, where the body’s immune system fails and then makes antibodies against itself.

The B27 marker, along with numerous other HLA alleles, probably predisperse their hosts to autoimmune reactions as well as to infectious diseases at times as a single allele but often in combination with other alleles in the form of haplotypes. HLA haplotypes provide a clear example of linkage disequilibrium due to selection. In one study, haplotype A1 B8 DR4 was present in nearly all hemophilic patients who showed a rapid course of developing AIDS after they were inadvertently treated with contaminated blood (Steel et al., 1988). Later research with HLA polymorphisms continued to find important marker associations with both susceptibility to and protection against disease, as will be referenced in the final section of the chapter.

**SICKLE CELL LOCUS AND MALARIAL RESISTANCE**

The marker that probably garnered the greatest amount of attention from anthropologists in the 1960s was a hemoglobin variant, Hb, the sickle cell allele. Hb is a point mutation that results from a single nucleotide substitution on the β-chain from the normal adult hemoglobin structure coded by the Hb allele. This mutation leads to one amino acid change in the hemoglobin molecule. There are a number of such variants classified as hemoglobinopathies, and two of these, Hb and Hb, will be discussed later.

Persons who are homozygote Hb/Hb episodically manifest sicklemia or sickle cell anemia due to multiple cascading effects of crescent-shaped red blood cells that are prone to hemolysis and also prevent blood from freely flowing through capillary beds. In the absence of medical attention, severe attacks are generally fatal.

Given this unfortunate outcome, it would be predicted that the Hb marker should decline in frequency ultimately to the level of a recurrent mutation rate at the locus. However, in endemic malarial regions of Africa it was proposed that there were counter-selection forces that were helping to maintain the Hb allele frequency in the population when it was combined with the normal Hb allele. Balancing selection operated against both classes of homozygotes, the Hb/Hb (from sickle cell anemia) and Hb/Hb (from malaria), and there was selection for the heterozygote Hb/Hb (protection from malarial morbidity).

The malarial parasite Plasmodium falciparum, carried by Anopheles mosquitoes, does not find a hospitable cellular environment in the heterozygote to complete its normal life cycle. This heterozygote advantage (also called overdominance) meant that both Hb and Hb alleles were being maintained at the same time that they were being selected out, a process that could lead to an equilibrium state, or balanced polymorphism. The alleles would be equal to each other in frequency only if selection against each class of homozygotes was at the same level, which it is not. Selection is much more severe against Hb/Hb than it is against Hb/Hb, so an equilibrium gene frequency would occur with the Hb allele proportionately much higher. This can be shown through the aid of some basic population genetics using the concept of relative fitness.

Relative reproductive fitness (w) is calculated with respect to specified genotypes Hb/Hb (AA), Hb/Hb (AS), and Hb/Hb (SS) and their expected frequencies, if there was Hardy–Weinberg Equilibrium at the hemoglobin locus. For a quick review, Hardy–Weinberg Equilibrium of genotypic frequencies depends on there being random mating at the locus in question and there being no evolution occurring at this locus. A further theoretical condition is that the population being studied be infinitely large. In spite of this last never-to-be-realized requirement, the Hardy–Weinberg Equilibrium was regularly found at a number of blood group loci, but not for the sickle cell locus due mainly to the action of selection.

Relative fitness for sickle cell-locus genotypes was calculated by Allison (1956) who derived his data from a number of African populations. He set the heterozygote fitness at wAS = 1.00 (which would make it relatively the most fit), and found that fitness for one homozygote was approximately wAA = 0.80 and for the other it was wSS = 0.20. These values would translate into a 20% reduction in fertility for Hb/Hb parents and an 80% drop in fertility for Hb/Hb parents. Again through an application of population genetics calculations, over time it would be expected that the frequency of the Hb allele would decrease until it reached an equilibrium of about 0.20. Since
this frequency was found in some African populations, it provided evidence for a balanced polymorphism.

However, some human population geneticists continued to question whether the sickle cell locus was in a balanced or transient state using the available evidence on differential fertility and mortality, along with computer simulation models to help predict the timing of equilibrium gene frequencies. In some African populations, \( Hb^S \) frequency appeared to be stable, as noted above, but in other regions it was subject to change (Livingstone, 1989). Complicating the picture was an interaction between \( Hb^S \) and other hemoglobin variants, especially \( Hb^C \) in West Africa, where one or the other marker was possibly being replaced. Added to this, the severity of malaria disease changed by region and altitude, and in fact very much depended upon the kind and intensity of certain horticultural practices.

Livingstone (1958) and other researchers proposed that the subsistence patterns of some African cultures shifted toward the clearing of once forested land for farming activities several thousand years ago, this established a more suitable environment for mosquito populations to flourish. As a consequence, the presence of the malarial parasite was promoted and malaria became a major disease stressor. This would set up the next step in the malaria hypothesis that involved the sickle cell locus, where probably the \( Hb^S \) allele was already present at a low frequency, and the malarial environment benefited the relatively better adapted heterozygote due to its resistance to the disease. It would be expected that this process was not strictly linear in the sense that selection for the heterozygote led to a lasting balanced polymorphism, but rather that as local populations adapted and increased in number, they also expanded in area by clearing more land and opening up broader opportunity for malarial disease, and another cycle of selection for the heterozygote. The dynamics between genetics and behavior in the sickle cell case can be considered a hallmark of biocultural interaction that is so important in understanding human population history.

The geographic distribution of the \( Hb^S \) marker, beyond Africa and into India and South Asia, has raised questions concerning the number of origins for the mutation. While Livingstone (1989) had argued for a single origin, others have proposed multiple mutations (Labie et al., 1986). Marks (1995) summarizes evidence showing that the sickle cell allele is found in five haplotypes corresponding to four African areas and one Indian source, that could be interpreted as independent origins or possibly also a single mutation that underwent successive later mutational and crossing-over events. A more complete resolution of this matter may help to answer the question as to whether or not the \( Hb^S \) marker had a single African origin and subsequently spread by gene flow beyond the continent and into Asia and Europe. On a related research front, Volkman et al. (2001) have proposed that \( P. falciparum \) has a recent origin from a single common ancestor.

**MALARIAL RESISTANCE FROM OTHER MARKERS**

By the late 1980s, \( Hb^S \) was the hemoglobin marker for which most population data were available. Limited results for \( Hb^C \) and \( Hb^E \) were beginning to show a geographic distribution coinciding with malaria that suggested that these too were adaptive in heterozygotes in providing resistance to this disease. As noted earlier, \( Hb^C \) may have been replacing \( Hb^S \) in West Africa, while \( Hb^E \) showed a high frequency in southeastern Asia, where a hilly, forested habitat fostered a different mosquito vector of malaria. Southeast Asia presented its own set of intriguing research questions with respect to genetic protection from malaria. In this region, another kind of faulty hemoglobin, referred to as thalassemias, appeared to be interacting with \( Hb^E \).

For some background, thalassemias are the result of point mutations, usually nucleotide substitutions or small deletions in regulatory genes that interfere with the normal synthesis of hemoglobin. They can occur on both the \( \alpha \) - and \( \beta \)-globin chains. Clinical significance relates to the degree of hemoglobin reduction, and ranges from mildly affected to a fatal condition called \textit{hydrops fetalis}. As in the case of hemoglobinopathies discussed above, heterozygotes for thalassemia offer protection against malaria, and once again due to balancing selection, thalassemia markers are maintained, but not necessarily balanced, polymorphisms.

Elevated frequencies for these markers were initially found in Mediterranean populations, particularly in Greece, Italy, and nearby islands where malaria is endemic. Later, polymorphic frequencies were recorded in New Guinea and Africa, as well as southeastern Asia, where the co-occurrence with \( Hb^E \) was noted above. A population genetics survey of this last region showed that after comparing gene frequencies for two populations, one had adapted via the thalassemia marker while the other gained malarial resistance from the \( Hb^E \) variant. Hence, population history would account for the different adaptive route (Vogel and Motulsky, 1997, p. 533). Additionally, the analysis went on to reveal that since the homozygote \( Hb^E/Hb^E \) was less deleterious than the homozygote thalassemia genotype, thalassemia was being replaced by \( Hb^E \) as the more effective and less costly balancing selection process.

Thalassemia was also found to co-occur geographically with an enzyme deficiency of G6PD, and it was presumed this was due to their both offering genetic resistance to malaria. The biochemical explanation for why G6PD deficiency was protective was proved by...
Friedman and Trager (1981) who showed that the malarial falciparum parasite did not survive in G6PD-deficient cells due to a lack of potassium. The island of Sardinia provided a test of the malarial selection hypothesis for both thalassemia and G6PD deficiency. Frequencies for both markers closely correlated with altitude which largely determined the malarial parasitic load. Correspondingly, the lower the altitude the greater the selection pressure at the G6PD locus.

The remaining example pertaining to the malarial selection hypothesis involves the Duffy blood group system. A laboratory was the venue for testing the susceptibility of red blood cells of the different Duffy phenotypes to infection by the malaria parasite Plasmodium vivax, a benign form and probable predecessor to the malignant derivative P. falciparum (Mourant, 1983). It was found that phenotypes carrying at least one copy of Fy0 or Fy4 were readily infected whereas the Duffy negative Fy0/Fy0 (the null Fy0 allele also has been designated as Fy", Fy, and Fy+) was highly resistant to infection. Based on these findings it was theorized that the Fy0 mutation arose from either of the common alleles, and by chance increased in frequency enough to form a few homozygotes that were then at a selective advantage when P. vivax malaria became endemic. Livingstone (1984) countered this portion of the theory by concluding that the Duffy null allele had reached a sufficiently high enough frequency to prevent vivax malaria from becoming endemic in West Africa.

The theory continues that over time the Fy0 marker progressively replaced Fy" and Fy4, and even reached fixation in some African populations, as observed today. The next phase was to theorize that tropical Africa was free of malaria until P. falciparum appeared and set off a new direction of selection operating on hemoglobin variants, including the sickle cell allele.

It is unlikely that historical details of this theory can ever be subjected to direct testing. However, considering the several examples cited above that overwhelming confirm the malarial selection hypothesis, it can be expected that portions of it will hold up to further scrutiny. Indeed, the selection hypothesis was favored more recently by Hamblin and Di Rienzo (2000) in their investigation of DNA sequence variation. In general, the early application of markers to a testing of crucial population genetic questions, especially those surrounding an understanding of how and why certain polymorphisms are maintained, must be viewed as highly successful.

ADDITIONAL AND PRACTICAL APPLICATIONS OF CLASSICAL MARKERS

Brief mention will be made here with respect to additional applications of markers. These are: parentage questions and paternity exclusion, zygosity determination in twins, and bone and mummified tissue typing.

Race and Sanger (1962) devoted chapters to “Blood Groups” and “Problems of Parentage and Identity” and to “The Blood Groups of Twins.” All of these applications relied on serological testing for red blood cell antigens from most of the common blood groups, and for identity determinations rare antigens or unusual combinations of common antigens were thought to be the most useful in assigning individuals to a race. On this last point, the examples that were provided indicated a low level of reliability for race identification. Race and Sanger (1962) did not attempt to cover forensic evidence in criminal cases, except to mention that at that time only the ABO system was applicable through specialized techniques for examining human blood and other fluid stains. Of course, present-day forensic science has an extensive array of DNA-based methods for analyzing evidence derived from criminal activities.

Little coverage of bone and tissue typing appeared in Race and Sanger (1962), which they saw as fraught with technical difficulties, some of which were inherent to the inhibition method that was being used. These problems were well documented in a study by Thieme and Otten (1957), who demonstrated that bacterial contamination could lead to false inhibition results, usually due to the transformation of A and B antigens into O. Their conclusion was that bone ABO typing was highly unreliable under certain conditions, especially if the bones had been recovered from damp soils. Later researchers made improvements in the preparation and testing procedures, and claimed to have achieved reliability from typing skeletal materials from an archaeological site in Israel (Micle et al., 1977), and mummified tissue from pre-Columbian sites in Peru (Allison, et al., 1978) and Chile (Llop and Rothhammer, 1988). Beyond the limited application and success of bone/tissue typing, the fledgling field of “paleoserology” was replaced by ancient DNA extraction and sequencing.

TAKING STOCK AND LOOKING AHEAD

Half a century after Boyd’s bold claims for accomplishments of genetic analysis it probably is pretentious to make any similar such statements, but certainly there were significant contributions between 1950 when classic markers prevailed and prior to the dominance of molecular methods that is seen today. This section will offer an overview of the various applications of traditional markers as covered earlier but now discussed in terms of how they might continue to
Contribute to our understanding of human genetic variation.

**POPULATION RELATIONSHIPS AND HISTORY**

The first point to make under this topic is that the concept of biological race possessed little practical scientific reality or application among anthropologists/human biologists investigating population affinities. To be sure, there is a research need to define units of study, but this requirement could not be met through race classification. In its place, population and variants such as deme or ancestral group or nonbiological linguistic and ethnic designations, have been used. A broad-based effort that employed language as its unit of study identifier was the Human Genome Diversity Project (HGDP). Growing out of the Human Genome Project in 1991, the HGDP had as its basic aim to map human DNA sequence diversity in order to deduce genetic history of our species. The HGDP became highly controversial due to ethical issues surrounding personal, civil, and legal rights of indigenous peoples from whom DNA would be obtained. Since its data set consists of DNA markers it is beyond the purview of this discussion, and the reader is directed to M’charek (2005) for a comprehensive review of the science behind the HGDP. Another global effort to study human genetic diversity through DNA markers is the Genographic Project. Under the direction of researchers from the National Geographic Society and IBM, the Genographic Project seeks to trace the deep migrational history of our species. Up-to-date accounts of the Project can be found at the National Geographic and IBM websites.

A recent study (Relethford, 2004) does exemplify a successful application of classical markers, along with microsatellite DNA markers and craniometrics, in a worldwide analysis of genetic variation in human populations. Relethford obtained frequencies of blood cell polymorphisms (blood groups, serum proteins and enzymes) from Roychoudhury and Nei (1988), much as he had done in a previous study (Relethford and Harpending, 1995). A major point to bring out here is that earlier databases of classical markers obviously can continue to serve contemporary research purposes. For the most part Birdsell (1993), in his study of microevolution on Australia, also followed this path in utilizing marker frequencies that he had obtained from his fieldwork of nearly a half-century earlier. These examples of data mining show that while DNA markers certainly rule the day, there still can be a place for already available classical marker frequencies to be used in the investigating human population variation and genetic history. At an even more basic level than marker frequency data sets, there are likely to be many instances of stored aliquots of human sera, saliva specimens, and hair samples that were collected during much earlier fieldwork projects that potentially could be subjected to laboratory analysis, but only after all ethical matters and human subjects concerns are satisfactorily resolved.

In sum, classical markers served their purpose well in describing human variation and in proposing plausible affinities between populations until such matters came under the finer-grained scrutiny of DNA markers. It seems fair to say that although they are no longer at the forefront of population study, classical markers will continue to contribute to these endeavors as evidenced in the examples cited above.

**MARKERS AND DISEASE ASSOCIATIONS**

It also seems appropriate to conclude that certain classical markers have been increasing over the years in their contribution to the study of selection and disease associations. For instance, the HLA system, with its numerous haplotype combinations, is highly noteworthy in this regard. Jackson’s (2000) informative discussion is replete with relatively recent examples of interaction between HLA haplotypes and several infectious diseases, notably HIV/AIDS. Along this HIV research front, a very recent study has reported a connection between HIV susceptibility and Duffy antigen status. The presence of DARC (duffy antigen receptor for chemokines) appears to increase the susceptibility to infection by HIV. But following infection, the DARC-negative phenotype leads to a slowing down of the progression of the disease (He et al., 2008).

For another marker, a major breakthrough was reported by Allen et al. (1997) in that not only did thalassemia provide malaria resistance in Papua New Guinea children it also protected against other infectious diseases, and importantly this finding may apply to other malarial resistant genes, such as HbS, as well. Finally, two very recent studies can be cited to show how active this field remains. One found that a haptoglobin phenotype was at much higher risk for cardiovascular disease in individuals with diabetes mellitus (Levy et al., 2002), while the other demonstrated a complex interaction between one of the transferrin markers and an allele at another iron metabolism locus as these posed a prominent risk factor for developing Alzheimer’s disease (Robson et al., 2004).

It probably is not surprising that these ongoing examples of classical marker associations with various diseases are still being discovered, and of course, just as in the earliest of such disease associations...
discovered decades ago, they are subject to further confirmation. There is every reason to predict that this area of research will continue to be a fruitful endeavor to pursue.

DISCUSSION POINTS

1. Which classical markers continue to be most useful in studying human variation and/or disease associations?
2. Discuss the pros and cons of Boyd’s classification of human races based on blood groups.
3. What are the ways in which variation can be maintained at a polymorphic locus?
4. On what theoretical grounds can different measures of population distance (including genetic, anthropometric, odontometric, dermatoglyphic, linguistic, geographic) be expected to correspond to or, conversely, not to agree with one another?
5. Discuss the evidence for and against the malarial hypothesis regarding the frequency distributions of hemoglobin variants, transferrins, G6PD, and Duffy markers. Include in this discussion the role of cultural activities related to subsistence patterns.
6. What are the underlying reasons that classical markers can be expected to be associated with diseases?

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