Genetic Variation in Arthropod Vectors of Disease-Causing Organisms: Obstacles and Opportunities

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INTRODUCTION

Rationale for Studying Vector Genetics

Some groups of people have long believed that certain blood-sucking organisms were the cause of particular (mainly febrile) diseases that afflicted them and their livestock. However, it was not until about a century ago that scientific evidence for involvement of arthropods in these diseases began to accumulate. Today, many viruses, bacteria, protozoa, and helminths are known to be transmitted to humans and their domestic animals by hematophagous arthropods.

In industrialized countries, our main concerns with arthropod-borne pathogens are with those that affect our livestock. However, in tropical countries, these pathogens are major causes of disease among both humans and domestic animals. It is difficult to obtain reliable estimates of the number of cases of human diseases or of losses to the livestock industry as a result of arthropod-borne pathogens in the third world. One reason for this is the lack of an adequate data-gathering infrastructure throughout much of the tropics. Another problem is that the data available are biased by the fact that many studies are restricted to areas where a particular disease is already known to be present at a significant level and extrapolations from these data are tenuous at best.

Control of the diseases caused by arthropod-borne pathogens has usually been carried out by vector control, most commonly by reduction of the vector population either by "environmental sanitation" or by killing the vectors with various biocides. The latter approach was deceptively simple and encouraged many to believe that it was possible to achieve worldwide eradication of malaria. The rise of insecticide resistance soon dashed such hopes. Although insecticides and acaricides have short-term usefulness in protecting against arthropod-borne pathogens, they cannot, on their own, provide a method for disease control or eradication. This realization has sparked renewed interest in alternatives, including genetic methods, for arthropod control.

Development of genetic methods to control transmission of arthropod-borne pathogens is strong justification for studies of vector genetics. There are, however, other compelling reasons for such studies. During transmission of pathogens, arthropods interact with at least two very diverse groups of organisms:
their vertebrate hosts and the pathogenic organisms being transmitted. Genetic studies of vectors should provide insights into the fundamental nature of these interactions, and understanding these interactions will provide opportunities for novel control strategies. Furthermore, vectors provide opportunities for the study of microevolution; people transport them to new localities where the vectors may be subjected to evolutionary forces that they have not experienced previously, and we carry out a variety of control measures that provide novel selective pressures to which the vectors respond.

The discovery that certain hematophagous arthropods may transmit pathogens soon led to the realization that each pathogen is transmitted by a limited number of arthropods and that each arthropod has the ability to transmit only certain pathogens. This provided the first indication that arthropod genetics was involved in determining vector competence. (Throughout this review, the term "vector competence" refers to the innate ability of a vector to acquire pathogens and to pass enough of them to a susceptible host to infect that host. The term "pathogen" is used in reference to an arthropod-borne organism which is, or may be, pathogenic in a vertebrate.) The first experimental demonstration of genetic control of the susceptibility of an arthropod to a pathogen was obtained by Huff (65, 66) with Culex pipiens and a bird malaria parasite, Plasmodium cathemerium. Since then, there have been many studies on the genetics of vectors, and much of this information has been reviewed from several perspectives. Several reviews, published during the past decade, have focused on the genetics of various taxa such as mosquitoes (8, 134, 153), tsetse flies (38, 42), and blackflies (131) or on the genetics of vector susceptibility to pathogens or vector competence (24, 25, 39, 54, 57, 68, 99, 157, 165). Two reviews (54, 157) speculate on the potential use of vector genetics for control of arthropod-borne diseases. Readers interested in molecular genetics should consult references 8 and 157.

Scope of the Review

The objective of this review is to provide an overview of the genetic variation in arthropods that transmit pathogens and to speculate on the significance of this variation. To accomplish this, I review briefly the evidence of vector-pathogen interactions that could provide the selective pressure for evolution of genetic control of vector competence. I then summarize the genetic basis of vector susceptibility to various pathogens, the first critical step in establishing vector competence. The amount of biochemical genetic variation in vectors is summarized, and I discuss the ways in which genetic studies have been used to address questions about vector biology that are particularly pertinent to pathogen transmission. Finally, I discuss the implications that genetic variation in vectors has for control of pathogens.

In preparing this review, I concentrated upon, but did not restrict myself to, publications appearing during the last decade. I did not attempt to cover the vast amount of literature on insecticide resistance or to review environmental aspects of vector competence (see reference 25 for the latter). With respect to surveys of genetic variation within vector populations, I restricted the review to biochemical genetics. This was done because of the uneven application of cytogenetic and molecular techniques to vector species and because of space constraints.

General Pattern of Pathogen Development in Vectors

Arthropods may transmit pathogens mechanically, with the pathogens on the external surface of the vector and with the vector providing them with nothing more than transportation to a new vertebrate host. Alternatively, the vector and the pathogen may have one of several biological associations in which the pathogen develops and/or multiplies within the vector and thus the vector provides the means of both dispersal and nutrition. Although vectors may acquire pathogens from their mothers (vertical transmission) (13, 127, 137) or as a result of mating with infected conspecifics (sexual transmission) (137), the most common association of the pathogen with the vector begins when the pathogen arrives with the blood meal in some part of the digestive tract. From here, the parasite moves to a site where it either develops or multiplies. Multiplication may or may not be accompanied by morphological changes in the vector. Some pathogens may migrate to several sites before reaching the site, usually the salivary glands, from which they leave the vector. For some pathogens, the migrations alternate with multiplication phases. In all cases except mechanical transmission, it is theoretically possible that during any migration, multiplication, or developmental phase there is an opportunity for a vector-pathogen interaction to cause failure of the pathogen to complete its development or to reach the appropriate point of exit from the vector.

Vector-Pathogen Models

For a variety of reasons, researchers often use vector-pathogen models that do not occur in nature. In some cases the deviation from a natural association is at the species level, and in others it is at the strain level, with for example, the vector and pathogen coming from different areas. The usefulness of the results obtained from studies of these artificial associations depends upon the objectives of the research. An example of the limitations of such studies is given below.

Although not natural vectors of the monkey malaria parasite Plasmodium cynomolgi, Anopheles gambiae strains that are highly susceptible or highly refractory by virtue of an ability to encapsulate oocysts have been selected (18). These strains show the same properties with three other species of simian malaria parasites (Plasmodium gonderi, Plasmodium inui, and Plasmodium knowlesi), a rodent malaria parasite (Plasmodium berghei), and an avian malaria parasite (Plasmodium gallinaceum). The refractory strain encapsulates more than 90% of the oocysts of Plasmodium vivax (of Asian origin), 66% of the oocysts of Plasmodium ovale (of African origin), and approximately 90% of the oocysts of Plasmodium falciparum (of Asian or New World origin). However, the P. cynomolgi-refractory strain encapsulates less than 14% of the oocysts of three strains of Plasmodium falciparum of African origin and none of the Plasmodium malariae oocysts (African origin) (18). This particular example demonstrates three limitations to laboratory studies of vector susceptibility or vector competence. (i) Within any species of parasite, there may be considerable variation in the ability to overcome defense mechanisms of the vector. (ii) A parasite from the same geographical region as the vector may possess mechanisms for overcoming the defense mechanisms of the vector. This point serves to emphasize the necessity of using strains of vector and parasite from the same geographic area if the work has applied rather than basic goals. (iii) Refractoriness or susceptibility in one vector-pathogen model does not ensure the same degree of refractoriness or susceptibility to closely related parasites. The same point can be demonstrated with Anopheles gambiae and Plasmodium yoelii nigeriensis and Plasmodium falciparum (48) and with Aedes aegypti and members of the filarioid genus Dirofilaria (103). However, experiments with Aedes aegypti and seven members of the viral genus Flavivirus (108) showed similar responses.
with different viruses, as did experiments with *Aedes albopictus* and two members of the viral genus *Alphavirus* (167). Similarly, the *Aedes aegypti* genes for susceptibility and refractoriness to subperiodic *Brugia malayi* controlled the development of other filarioid worms (periodic *Brugia malayi*, *Brugia pahangi*, and *Wuchereria bancrofti*) that develop in the thoracic muscles (90), and heritable factors controlling the susceptibility of *Glossina morsitans morsitans* to midgut infections with *Trypanosoma congolense* control susceptibility to midgut infections with *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense* (101). One reason for these diverse results is that these studies were conducted in the absence of an understanding of the underlying mechanisms in the interactions between the parasites and the vector. Without such an understanding, it is not possible to predict accurately how a vector will respond to a pathogen.

**Vector-Pathogen Interactions**

Although there have been many reports that pathogens do not affect their normal arthropod vectors (see citations in references 141 and 142), it is possible that many of these reports are due to the choice of the parameter studied or to the precision with which measurements were made. However, there are now several demonstrations that pathogens, including at least five viruses (31, 52, 164, 170, 186, 187), three protozoans (143, 144, 188), and two filarioid worms (6, 7) can affect several aspects of vector biology including development rate (164), egg production (170), feeding (52, 170, 188), midgut morphology (186, 187), midgut proteinase activity (144), mortality rate (31, 144), and spontaneous flight activity (6, 7). Two unanswered questions are whether (i) the damage is sufficient to reduce the fitness of the vector and (ii) enough young vectors are subjected to selective pressure to change the vector competence of a population. The first question could be addressed experimentally by exposing a genetically heterogeneous vector population to pathogens for several generations. If pathogens adversely affect the fitness of susceptible members of the population, we would expect to see a decrease in the level of susceptibility and/or vector competence of the population. An answer to the second question will require considerable field work, probably supplemented by computer simulations.

There are two distinctly different reasons why development of a pathogen in a vector may terminate prematurely: (i) the presence of specific agents that adversely affect the pathogen (i.e., antiblastic agents) and (ii) the lack of specific materials required by the pathogen (i.e., atreptic effects). It is likely that both are under genetic control. If pathogens reduce the fitness of infected vectors, antiblastic agents and atreptic effects could be subject to genetic selection under appropriate conditions. There is substantial evidence of antiblastic agents that affect the development of pathogens, but the evidence for atreptic effects is equivocal.

Antiblastic, or potentially antiblastic, agents vary from enzymes (12) and lectins (150, 191) produced in response to a blood meal to melanin-like materials that encapsulate parasites (18, 119, 126). However, the cause-and-effect relationship between the presence of the lectins and trypanosome refractoriness in tsetse flies has been questioned (87, 123).

After a blood meal, females of *Aedes aegypti* strains that are refractory to *Brugia malayi* produce seven peptides that are not found in a susceptible strain (184, 185). Unfortunately, it has not been established that these peptides are controlled by the gene for refractoriness (184, 185), nor has it been established that the peptides interact with the parasite as it develops in the thorax (184). However, the discovery of specific peptides in filaria-resistant strains of blood-fed *Aedes aegypti* opens the possibility of using molecular approaches to study the mode of action of these peptides (if, in fact, they have a role in resistance to filariae). Furthermore, these peptides may provide a lead to transgenic approaches for filaria control in mosquitoes such as Asian populations of *Culex pipiens quinquefasciatus*, important vectors of human filariasis, in which selection for refractoriness has been unsuccessful (125, 148, 196).

A treptec effects are inherently difficult to demonstrate, because one is essentially required to prove that the lack of one thing is the cause of another, when one has little indication of the nature of the missing substance. Until the vector-pathogen interactions have been elucidated, it is difficult to ascertain whether vector refractoriness is due to antiblastic or to atreptic effects. For example, a vector strain may be refractory to low pathogen titers but susceptible to high titers. This could be explained equally well by high titers overwhelming the vector defenses (antiblastic agents) or by high titers overcoming an atreptic effect such as a low concentration of receptor sites.

Even though it is difficult to establish that atreptic effects limit pathogen development, these may be the simplest explanation for some vector-pathogen interactions. Barriers that prevent viruses from escaping from the mosquito midgut (western equine encephalomyelitis virus in *Culex tarsalis* [82] and La Crosse virus in *Aedes triseriatus* [128]), from penetrating the salivary glands (western equine encephalomyelitis virus in *Culex tarsalis* [82]), or from escaping from the salivary glands (La Crosse virus in *Aedes hensoni* [127, 128]) may be due to the absence of specific molecules. Lectins produced by *Rhodnius prolixus* stimulate the maturation of *Trypanosoma cruzi* (129), and others produced by *Glossina morsitans morsitans* are apparently required for the maturation of *Trypanosoma congolense* and *Trypanosoma brucei rhodesiensis* (102, 189, 190). Low concentrations of these lectins may produce atreptic effects. The work on lectins in *Rhodnius prolixus* and *Glossina* spp. may provide models for further work with other vector-pathogen models. For example, given that lectins appear to provide cues for maturation of trypanosomes, a systematic search could be undertaken to see if lectins have similar effects in other vector-pathogen models and, in particular, to relate these to intraspecific variation in vector competence. Similarly, studies are needed to see whether lectins can stimulate the maturation of parasites in various in vitro cultivation systems (99).

**GENETICS OF SUSCEPTIBILITY AND VECTOR COMPETENCE**

Above, I argued that pathogen development in the vector could be terminated at any of several steps and that this could result from either antiblastic factors or atreptic effects. Below, I summarize evidence showing that genetic control of resistance to pathogens may be relatively simple in vectors (involving maternally inherited factors or only one gene) or a complex polygenic system (involving genes for both susceptibility and resistance).

**Methodology**

Most studies of the genetics of vector competence begin by demonstrating intra- or interpopulation variation in some vector-pathogen interaction such as threshold level for infection, number of parasites established or maturing in the vector, duration of extrinsic incubation period, and oral transmission ability. This is followed by a selection program to obtain “high-
ly susceptible” and “highly refractory” strains. Unfortunately, because of the multitude of interactions between the pathogen and its vector, there is little assurance that selection is acting upon the same loci in the two lines (194), and usually the strains obtained are neither completely susceptible nor completely refractory. For traits controlled by a few loci or by maternally inherited factors, the selection program requires only a few generations; for polygenic traits, it may be a very prolonged process. However, prolonged selection has been required for some models which, upon analysis, appeared to have few loci involved in susceptibility or refractoriness (181). Once suitable lines have been established, reciprocal crosses, backcrosses, etc., are carried out and the parameter in question is assessed in each of these to test various genetic models. For an excellent discussion of appropriate methodologies, including the use of isofemale lines, see reference 158; for methods to infer the genotype of nonhematophagous males, see reference 89; and for the application of molecular techniques, see references 8, 64, and 157.

**Association with Visible and Biochemical Marker Genes**

There have been several attempts to associate susceptibility to pathogens with visible phenotypes of the vectors (see reference 39 for a summary and references 16 and 119 for recent studies). Few conclusions can be drawn from this work, because causal relationships between the visible phenotypes and susceptibility have not been established. Somewhat more interesting are attempts, summarized below, to relate susceptibility to biochemical features of the vector.

Midgut trypsin activity is slightly lower in a strain of *Anopheles stephensi* that is susceptible to *Plasmodium gallinaceum* than it is in a highly refractory strain (35). However, with this species, trypsin activity is the same in strains that differ in susceptibility to *Plasmodium falciparum*, but the refractory strain takes larger blood meals, begins degradation of the hemoglobin sooner, and has higher levels of midgut aminopeptidase than does the susceptible strain (33). In *Anopheles gambiense*, refractoriness and susceptibility to *Plasmodium cynomolgi* are associated with esterase banding patterns that are observed by gel electrophoresis of mosquito tissue. Refractoriness is associated with the Est A phenotype, whereas susceptibility is associated with the Est C phenotype; this association persists in a mass-reared colony and in lines selected for Est A or Est C (174). It was subsequently established that there are two esterase loci (Est1, a cholinesterase locus with four alleles, and Est2, a carboxyesterase locus with two alleles) and that different alleles at these loci and alleles for refractoriness or susceptibility to *Plasmodium cynomolgi* are inextricably linked by an inversion in the left arm of chromosome 2 (23). Although a causal relationship between the esterase(s) and refractoriness has yet to be established, these discoveries will no doubt greatly aid in the identification and location of the locus responsible for refractoriness to *Plasmodium cynomolgi*. Salmon-eyed *Glossina morsitans morsitans*, mutants which have high levels of tryptophan owing to an inability to metabolize this amino acid, have greater than normal susceptibility to *Trypanosoma congolense* (26) and to *Trypanosoma brucei brucei* (92), and the elevated levels of tryptophan in the mutants may benefit the trypanosomes (42).

**Genetic Control of Vector Susceptibility and Competence**

The first aspect of the genetics of vector competence to attract the attention of medical entomologists was that of vector susceptibility to vertebrate pathogens. During more than 60 years that this subject has been studied, at least eight genetic mechanisms have been elucidated in laboratory models (Table 1). Below, I discuss several aspects of this information.

Most vectors appear to have one major gene controlling susceptibility or refractoriness (Table 1), but this generalization must be viewed cautiously for several reasons. (i) Some data indicate that there may be minor genes affecting susceptibility (56, 107, 154). (ii) Most studies have used long-standing laboratory colonies, and it is possible that selection or drift within these colonies has eliminated alleles at some “minor” loci. (iii) For most models, expression of the genes for susceptibility or refractoriness has been demonstrated in only one genetic background. (iv) Usually, the influence of environmental factors has been minimized during genetic studies. The expression of genes for refractoriness or susceptibility has been assessed under relatively constant environmental conditions that are near optimal for the adult vectors, and the experimental animals have been obtained by rearing well-nourished individuals under uncrowded conditions. (v) It has yet to be established, with any vector-pathogen model, that the genetic mechanism identified in the laboratory operates in natural populations.

Most genetic mechanisms that influence vector competence appear to do so at an early stage of development of the pathogen, often when it is in or near the midgut. Exceptions include the mechanisms that limit the development of filarioid worms in mosquitoes. These usually affect worms as they develop in the thoracic muscles, fat body, or Malpighian tubules, but in some strains of *Aedes aegypti*, development of *Dirofilaria immitis* is arrested at the microfilarial stage (103). If the damage done by the pathogen reduces the fitness of the vector, it would be advantageous for the vector to develop a mechanism that kills pathogens early in their development. Mechanisms that destroy pathogens late in their development may risk being circumvented, especially by pathogens that have a sexual cycle and extensive multiplication in the vector.

Although maternal inheritance is a major mechanism controlling susceptibility among members of only one group of vectors (tsetse flies), there are several vector-pathogen models in which nuclear genes control susceptibility but in which there are indications of maternal effects (53, 194). There are examples also in which the paternal genes for susceptibility or refractoriness appear to be overexpressed. Such results are observed among F1 females in reciprocal crosses of *Anopheles gambiense* lines that are refractory and susceptible to *Plasmodium berghei* (2) or to *Plasmodium yoelii nigeriensis* (48). The reason for the disproportionately greater influence of the paternal gene is not known.

The genetic linkages of loci for susceptibility have been established in only a few vectors, even though such information is critical in defining the genetic basis of vector competence, and may have practical value. If a colony of vectors carrying genes for refractoriness is used to replace a natural population of vectors, it is essential that the genetics of susceptibility and refractoriness as well as the chromosomal or linkage map location(s) for the gene(s) be known. This is particularly true for vectors, such as mosquitoes, in which males are not hematophagous, are the heterogametic sex, and have intrachromosomal recombination. If a susceptibility gene is closely linked to the sex-determining gene (as it is for susceptibility to filarioid worms in *Aedes aegypti* [151, 163]), the putative refractory colony could have males carrying susceptibility alleles at loci that are closely linked to the allele for maleness. This may escape detection in the colony, because only females are readily monitored for refractoriness, but intrachromosomal recombination will eventually result in linkage of the allele for...
susceptibility and the allele for femaleness and susceptible females would eventually occur in the population.

In most vector-pathogen models, there appears to be a relatively simple genetic control of vector susceptibility to pathogens. An exception is control of bluetongue virus susceptibility in Culicoides variipennis (154). Although only one locus has been described, the phenotype of an individual is determined by its mother’s genotype, with the dominant allele in the mother being the allele inherited from her father (154). It is possible that other loci are involved or that there are environmental effects, since susceptibility to bluetongue virus is often higher in laboratory-reared midges than in field-collected ones (154).

**Interpopulation Variation in Vector-Pathogen Relationships**

In laboratory colonies, susceptibility and resistance to pathogens are usually determined by relatively simple genetic mechanisms (Table 1). The frequencies of such genes within a population will be influenced by the usual evolutionary forces of mutation, selection, founder effects, genetic drift, etc. It is therefore not surprising to find interpopulation differences in levels of susceptibility to pathogens. This has been demonstrated with ticks (84), phlebotomine sand flies (193), mosquitoes (3, 27, 34, 51, 103, 167), and tsetse flies (109). Interpopulation variation in susceptibility to pathogens is not always the case (3, 168). In most studies, the comparisons have used too few vector populations to permit patterns to be discerned. Furthermore, most interpopulation comparisons of vectors have used only one strain of pathogen. A recent study of the susceptibility of Culicoides variipennis sonorensis to bluetongue virus showed the importance of using several strains of carefully characterized pathogens (106). Midges from colonies AA and AK are equally susceptible to three serotypes but differ with respect to two serotypes; colony AA midges are highly susceptible to serotype 13 and have low susceptibility to serotype 11, but the reverse is true for colony AK midges.

| Pathogen | Vector | Genetic mechanism<sup>a</sup> | Reference |
|----------|--------|-----------------------------|-----------|
| Viruses  |        |                             |           |
| Bluetongue | C. variipennis | Paternal gene dominant (\(b^u/b^u\)) | 154 |
| Western equine encephalomyelitis | C. tarsalis | Susceptibility dominant | 56 |
| Dengue | A. albopictus | Susceptibility incomplete dominant | 53 |
| Yellow fever | A. aegypti/formosus hybrids | Codominance | 108 |
| Protozoa |        |                             |           |
| L. major | P. papatasi | >1 gene, codominance | 194 |
| T. congolense | G. m. morsitans | Maternally inherited factors | 100 |
| P. gallinaceum | A. aegypti | Susceptibility incomplete dominant | 181 |
| P. gallinaceum | A. aegypti | Susceptibility dominant (p/s/+) | 78 |
| P. berghei | A. gambiae | Susceptibility incomplete dominant | 2 |
| P. yoelii nigeriensis | A. gambiae | Refractoriness incomplete dominant | 48 |
| P. cynomolgi | A. gambiae | Susceptibility gene (\(Pf^f/Pf^f\)) | 175 |
| Nematoda |        |                             |           |
| W. flexicauda | A. aegypti | Susceptibility sex-linked recessive (\(f^m-f\)) | 163 |
| D. repens | A. aegypti | Susceptibility sex-linked recessive | 19 |
| D. immitis | A. aegypti | Susceptibility sex-linked recessive | 195 |
| B. malayi | A. aegypti | Susceptibility sex-linked recessive (\(f^m-F\)) | 89 |
| B. malayi | A. scutellaris | Complexity | 166 |
| B. pahangi | A. scutellaris | More than one chromosomal gene | 107 |
| W. bancrofti | Complex | Refractoriness incomplete dominant | 121 |
| B. malayi | C. pipiens | Susceptibility sex-linked recessive (\(s^b\)) | 121 |

<sup>a</sup> The genetic mechanism refers to establishment of the pathogen in the vector. Unless indicated otherwise, only one gene is involved. Loci and alleles, if designated in the reference cited, are given in parentheses. Incomplete dominant refers to the situation in which the \(F_1\) progeny have a phenotype that is intermediate between those of the parental lines.

<sup>b</sup> \(f^m-f\), the allele causing susceptibility to Waltonella flexicauda, which develops in the fat body, is allelic to \(f^m\), the allele causing susceptibility to Brugia malayi, which develops in the thoracic muscles.

<sup>c</sup> Because of a lack of intraspecific polymorphism for susceptibility to Brugia spp., interspecies crosses were used. Unidirectional reproductive incompatibilities preclude certain backcrosses, which could have been used to test alternative models for genetic control of susceptibility.
from 8 to 41.2%; in both colonies, the highest infection rates were in the offspring of field-collected mosquitoes. There was a significant correlation between susceptibility to yellow fever virus and the frequency of one malate dehydrogenase allele (MdH 100), indicating a genetic basis for the fluctuations in susceptibility to the virus (88). The transmission rate for La Crosse virus rose in a colony of *Aedes triseriatus* that was established from a population within the La Crosse area of endemicity (51). This may have been due to a founder effect, genetic drift, or selection at a locus that is closely linked to the loci for La Crosse vector competence. Alternatively, the genes for refractoriness may have pleiotropic effects that reduce fitness, and in the absence of selective pressure for these genes, their frequency declined in the laboratory colony.

Two colonies of *Culicoides variipennis*, established with small numbers of field-collected adults (50 and 95 adults), differed significantly from the populations from which they were obtained, with respect to susceptibility to bluetongue virus; one colony was highly susceptible, whereas the other was almost completely refractory (70). A third colony, established with ca. 6,400 adults, maintained considerable heterogeneity for infection with bluetongue virus and had a susceptibility rate similar to that of the population from which it was founded. Results with these colonies and with a large, well-established colony indicate that the number of individuals used to establish the initial colony and the magnitude of the initial increase in the size of the colony are good predictors of whether the colony will be representative of the field population (70). No doubt the predictive parameters indicate the adequacy of sampling the genetic diversity in the natural population.

Are there any patterns in interpopulation variation? Some evidence suggests that in a species with a relatively stable geographic distribution there is a difference in susceptibility of populations within and outside the area where the pathogen is endemic. *Aedes triseriatus* mosquitoes from within the area where the La Crosse virus is endemic are significantly less likely to become infected with or to transmit La Crosse virus, isolated from *Aedes triseriatus* mosquitoes that were collected within this area, than are mosquitoes from outside the area (51). This is consistent with the hypothesis that there is selection for resistance to pathogens within areas where the pathogens are endemic. However, there are no such differences in the numbers of *Brugia malayi* and *Brugia pahangi* developing in various strains of *Aedes polynesiensis* (27).

The second pattern is one of patchiness in the occurrence of high levels of susceptibility. Although there are large interpopulation variations in the susceptibility rates and in the dissemination rates in 10 strains of *Aedes albopictus* and 7 strains of *Aedes aegypti* that had fed on a monkey infected with Chikungunya virus, there is no geographic pattern to susceptibility or dissemination rates (169). This lack of a relationship may be attributed to recent, multiple introductions of mosquitoes into New World locations, from which some of the strains were established, and to founder effects and genetic drift within the colonies tested (169).

The third pattern is one of having differences in susceptibility parallel other genetic differences between populations. An analysis of *Aedes aegypti*, from recently established (F 3 or earlier) and multiple isofemale line colonies from 22 locations in Asia, the Asian Pacific, Africa, and the New World showed that mosquitoes from sylvan populations in West Africa are significantly less susceptible to yellow fever virus than are mosquitoes from all other populations, except three from the southern United States (161). These findings correspond well to the genetic relatedness (based on biochemical genetic data) of the strains (161). Wallis et al. [179] suggest that genes for vector competence may be among the genes that are partitioned among the seven genetic-geographic groups of *Aedes aegypti* and that such partitioning may have epidemiological implications.

**BIOCHEMICAL GENETICS**

In the above sections, I reviewed evidence that vector-pathogen interactions are influenced by the genetics of vectors and that the susceptibility to pathogens may vary among populations of vectors. Although these genetic factors are important, they are not the only aspects of vector genetics that influence the epidemiology of arthropod-borne diseases or our efforts to control such diseases through vector control. The vertebrate-vector-pathogen system is a dynamic one, and its rate of evolution depends upon the selective pressures applied to its components and upon the amount of genetic variation within each component. Although these components are interacting and are to a large measure inseparable, only genetic variation in the vectors is covered here.

**Rationale for Using Biochemical Genetic Measures of Variability**

Genetic variation has been estimated by several techniques including morphological studies, cytogenetics, protein electrophoresis, and direct measurement of DNA variability. At present, the most extensive data are from protein electrophoresis. This is a relatively inexpensive technique, and since the alleles are codominant, heterozygotes can be detected directly. There is a sound theoretical basis for analyzing the results of electrophoretic studies, and there are several techniques to use such data to estimate genetic variation within a population, differences between populations, and the number of migrants between populations. For these and other practical reasons, protein electrophoresis is probably the most widely used technique to estimate genetic variation.

The significance of the genetic diversity that is revealed by protein electrophoresis is still a subject of debate, with one school maintaining that the variation is largely or almost entirely selectively neutral while others maintain that each allele makes a unique contribution to the fitness of its bearer. The subject has been reviewed many times (120) and is outside the scope of this review. Although this controversy is unresolved, the allele frequency data used to calculate levels of heterozygosity have been used to elucidate the genetic structuring (i.e., division of a species or population into groups that have different allele or genotype frequencies) of many vectors on a global, regional, or local scale. Two common ways to study the genetic structure of populations are to test for conformity to Hardy-Weinberg equilibrium (HWE) and to look for a Wahlund effect (i.e., a deficiency in the number of heterozygotes that results from combining samples from two noninterbreeding populations that differ in allele frequencies). Failure of the sample to conform to HWE or the presence of a Wahlund effect may indicate, but does not prove, the coexistence of populations that do not interbreed. Another method is the use of Wright’s F statistics. These are the reductions in heterozygosity of a subpopulation due to genetic drift, F ST, the reductions in heterozygosity of an individual due to inbreeding within the subpopulation, F IS, and an overall inbreeding coefficient, i.e., the reductions in heterozygosity of an individual relative to the total population, F FT.

Electrophoretic techniques are used to provide three measures of variation within a population or species: effective number of alleles per locus, proportion of polymorphic loci, and
average heterozygosity per locus. The first of these parameters is the least often reported and is not summarized here. There is a strong correlation between the magnitudes of the second and third parameters (120), and therefore I have summarized only the data on the average heterozygosity per locus. I have restricted this portion of the review to those papers in which allele frequencies are reported for 10 or more loci. The mean heterozygosity per locus values of field populations and laboratory colonies are tabulated in Tables 2 and 3. Below, I comment on the genetic variation within and between populations and on the biological questions that have been addressed by using these data.

**Variation in Natural Populations**

Although electrophoretic data are available for most vectors, I found no recent papers in which allele frequencies were reported for 10 or more loci in any species of blackflies. There are, however, reports that populations of blackflies can be distinguished on the basis of allele frequencies. For example, it is possible to distinguish between *Simulium damnosum* flies from western Kenya and those from the Mount Kenya area by using hexokinase (*Hk*) or phosphoglucomutase (*Pgm*) alleles (104, 105), but these studies only serve to confirm that there is little or no gene flow between members of this species complex.

There is considerable variation in the mean heterozygosity per locus among natural populations of vectors (Table 2), ranging from no demonstrable variation in a population of *Culex pipiens pipiens* in Egypt (32) to a high of 0.354 ± 0.057 (i.e., 35.4% ± 5.7%) of the individuals are heterozygous at a randomly chosen locus) in a population of *Anopheles minimus* in Thailand (80). Caution must be exercised when comparing heterozygosities, because these are influenced by the technique used and by the enzymes examined. The most reliable differences are probably those reported within a publication or those reported from a laboratory in which a standardized technique is used. With these reservations in mind, the data summarized in Table 2 illustrate that although there is significant interpopulation variation in at least 10 species of vectors, the amount of intraspecific heterozygosity is similar to that which occurs among other invertebrates (120).

The above generalization notwithstanding, low levels of heterozygosity are found in some populations, and these have been used to suggest that a variety of biological, environmental, or historical factors have influenced these populations. Low levels of arthropod mobility, in combination with host territoriality, may contribute to low levels of heterozygosity in members of the tick genera *Aponomma* and *Amblyomma* (14). However, these findings must be viewed cautiously, because fewer than 10 ticks were examined from most populations. Low levels of heterozygosity in *Anopheles albimanus* in southern Colombia (116) and in *Glossina swynnertoni* in northern Tanzania (44) may indicate small breeding populations or selection of certain alleles in these particular environments. Populations of *Aedes aegypti* from large Caribbean islands (Jamaica and Puerto Rico) have significantly higher heterozygosity (0.152 ± 0.016) than those from small islands (0.101 ± 0.006) (180). Although this may be related to population size, there are many other differences between islands (180).

Historical events, such as genetic bottlenecks created by population reduction during mosquito control, may have reduced the variability in three U.S. populations of *Aedes albopictus* (at Memphis, Evansville, and Jacksonville) (9, 71), reduced the heterozygosity in *Aedes aegypti* in Houston (59), and contributed to differences between *Aedes aegypti* populations in the West Indies (180). Similarly, the movement of *Glossina morsitans* from one population to another in Tanzania may have contributed to changes in Wright’s inbreeding coefficient, *F* in the recipient population (44). In samples that were taken 18 months apart, these values changed from relatively large values indicative of an earlier genetic bottleneck or current inbreeding to negative values due to an excess of heterozygotes (44).

### Population Structure of Vectors at a Geographic Level

Allele frequencies at 11 loci in *Aedes aegypti* collected at 63 localities in 21 countries placed these populations into seven genetic-geographic groups (179). The data indicate that *Aedes aegypti* from west Africa is more closely related to the feral form (*Aedes aegypti formosus*) from east Africa than is it to the domestic east African form (*Aedes aegypti aegypti*). New World *Aedes aegypti* mosquitoes (except those from southeastern United States) are closely related to east African domestic forms, not to the west African form. *Aedes aegypti* in the southeastern United States are genetically similar to Asian populations (179), but it is not clear whether this similarity represents convergence that occurred following a genetic bottleneck in the U.S. populations or whether these populations descended from recent introductions from Asia.

Within two villages in eastern Kenya, *Aedes aegypti* populations were panmictic and allele frequencies were generally stable over a 1-year period (159). However, mosquitoes from the villages, which were separated by 2 km, had significantly different allele frequencies at four polymorphic loci, indicating that neither migration between villages nor gene flow through the intervening *Aedes aegypti formosus* population(s) was sufficient to prevent genetic differentiation (159). Similarly, differences in allele frequencies at 2 of 11 loci suggest that *Aedes aegypti* on Anguilla, West Indies is genetically structured, either geographically or on the basis of larval habitats (178).

A stepwise multiple-discriminant analysis clustered populations of *Aedes albopictus* on the basis of country of origin, with Brazil, China, Japan, and the United States forming a closely associated group of clusters (71). In a second discriminant analysis, with the U.S. and Brazilian populations treated as “unknowns,” 57, 25, and 18% of the U.S. populations were distributed among populations from Japan, China, and Malaysia, respectively; three Brazilian populations were placed with Japanese populations, and one was placed with the Madagascan population. These results suggest that the U.S. and Brazilian populations of *Aedes albopictus* were of Japanese origin (71). There was no correlation between genetic and geographic distances among 204 Asian populations of *Aedes albopictus* studied at eight polymorphic loci (71). However, if two Bornean populations were excluded (because allele frequencies at the locus for α-glycerophosphate dehydrogenase made these populations very different from other populations), there was a good correlation (*r* = 0.34; *P* < 0.001) between genetic and geographic distances (71). This example illustrates one limitation in studies that address few loci: results at a single locus may have a profound effect upon any pattern that is observed. About 45% of the genetic variation that occurs in *Aedes albopictus* is attributable to variation between locations within cities, 33% is attributable to variation between cities within countries, and 22% is attributable to variation between countries (71). In U.S. populations, most variance in allele frequencies is attributable to variation between populations within a location (86.3% in 1986, 85.6% in 1988) and only 13.7 to 14.4% is attributable to variation between locations (72). The parti-
### TABLE 2. Electrophoretic variation in natural populations of vectors

| Taxon | No. of loci | Sampling site | Mean heterozygosity ± SEM | Reference |
|-------|-------------|---------------|----------------------------|-----------|
| **Ticks** | | | | |
| Amblyomma albolimbatum | 16–20 (cq) | 21 southern Australian populations | 0.023 ± 0.004 | 14 |
| Amblyomma americanum | 21 (ns) | 9 U.S. populations | (0.101 ± 0.047 to 0.136 ± 0.046) | 63 |
| Amblyomma limbatum | 16–20 (cq) | 15 southern Australian populations | 0.016 ± 0.007 | 14 |
| Aponomma hydrosauri | 16–20 (cq) | 11 southeastern Australian populations | 0.040 ± 0.006 | 14 |
| | | 12 southwestern Australian populations | 0.010 ± 0.004 | 14 |
| Boophilus microplus | 24 (sg) | 4 southern Mexican populations | (0.100 ± 0.029 to 0.128 ± 0.035) | 139 |
| Ornithodorus erraticus | 9 (sg) | Abu Ghalib, Egypt | 0.055 ± (0.055) | 177 |
| | 9 (sg) | El Baragil, Egypt | 0.033 ± (0.028) | 177 |
| Ornithodorus sonrai | 9 (sg) | Bandia, Senegal | 0.027 ± (0.027) | 177 |
| **Triatoma infestans** | | | | |
| | 19 (ca) | Cochabamba, Bolivia | (0.024 ± 0.019 to 0.047 ± 0.032) | 28 |
| **Ceratopogonid midges** | | | | |
| Culicoides variipennis | 21 (sg) | “Yungas” La Paz, Bolivia | (0.011 ± 0.072) | 15 |
| | 21 (sg) | Guayama, Puerto Rico | 0.185 ± 0.051 | 152 |
| | 21 (sg) | Béisbro (Fla.) | 0.199 ± 0.059 | 152 |
| | 21 (sg) | Beachville, Md. | 0.231 ± 0.053 | 152 |
| Culicoides variipennis occidentalis | 21 (sg) | 6 North American populations | 0.127 ± 0.006 | 155 |
| Culicoides variipennis sonorensis | 21 (sg) | 31 North American populations | 0.171 ± 0.009 | 155 |
| | | | | |
| **Phlebotomine sandflies** | | | | |
| Latzomyia caprata | 11 (ca) | Yucomo, Bolivia | 0.145 ± (0.079) | 15 |
| Latzomyia longipalpis | 10 (ca) | “Yungas” La Paz, Bolivia | (0.027 ± 0.019 to 0.047 ± 0.032) | 11 |
| Latzomyia yucumensis | 11 (ca) | Yucomo, Bolivia | 0.145 ± (0.079) | 15 |
| Phlebotomus papatasi | 17 (ca) | Aswan, Egypt | 0.19 ± 0.03 | 77 |
| | 17 (ca) | Sinai, Egypt | 0.17 ± 0.04 | 77 |
| | 17 (ca) | Alexandria, Egypt | 0.21 ± 0.05 | 77 |
| | 17 (ca) | Sharm el-Sheik, Egypt | 0.15 ± 0.03 | 77 |
| | 17 (ca) | Giza, Egypt | 0.17 ± 0.03 | 77 |
| **Mosquitoes** | | | | |
| Aedes aegypti | 11 (ns) | 7 Anguilla, West Indies, populations | 0.083 ± 0.055 to 0.143 ± 0.053 | 178 |
| | 11 (sg) | 2 Jamaican populations | 0.113 ± 0.050 to 0.206 ± 0.069 | 180 |
| | 11 (sg) | 4 Puerto Rican populations | 0.108 ± 0.045 to 0.165 ± 0.071 | 180 |
| | 11 (sg) | 8 small Caribbean Island populations | 0.057 ± 0.038 to 0.130 ± 0.082 | 180 |
| | 11 (sg) | 4 Trinidad Island populations | 0.078 ± 0.044 to 0.111 ± 0.058 | 180 |
| | 10 (sg) | 16 Houston, Tex., populations | 0.070 ± 0.045 to 0.127 ± 0.066 | 59 |
| | 11 (sg) | 15 West African populations | 0.107 ± 0.004 | 179 |
| | 11 (sg) | 6 Asian populations | 0.081 ± 0.007 | 179 |
| | 11 (sg) | 8 southeastern U.S. populations | 0.137 ± 0.008 | 179 |
| | 11 (sg) | 11 Mexican and southwestern U.S. populations | 0.130 ± 0.012 | 179 |
| | 11 (sg) | 9 South and Central American populations | 0.102 ± 0.010 | 179 |
| | 11 (sg) | 5 Caribbean populations | 0.179 ± 0.017 | 179 |
| Aedes aegypti formosus | 11 (sg) | 3 East African populations | 0.209 ± 0.014 | 179 |
| Aedes bimaculatus | 10 (pg) | Los Angeles County, Calif. | 0.126 ± 0.060 | 10 |
| Aedes cuneus | 17 (pg) | Gogebic County, Mich. | 0.193 ± 0.058 | 29 |
| Aedes excrucians | 17 (pg) | Gogebic County, Mich. | 0.209 ± 0.051 | 29 |
| Aedes fitchii | 17 (pg) | Gogebic County, Mich. | 0.214 ± 0.052 | 29 |
| Aedes hensleri | 23 (pg) | 8 Indiana populations | 0.117 to 0.144 | 98 |
| Aedes laguna | 10 (pg) | Baja Calif. Sur, Mexico | 0.047 ± 0.032 to 0.152 ± 0.062 | 10 |
| Aedes mississippiensis | 10 (pg) | Santa Cruz County, Ariz. | 0.091 ± 0.003 | 15 |
| Aedes mystacinus | 10 (pg) | Arizona | 0.116 ± 0.070 to 0.198 ± 0.090 | 10 |
| Aedes purpuripennis | 10 (pg) | Yavapai County, Ariz. | 0.074 ± 0.050 | 10 |
| Aedes sierrensis | 10 (pg) | King County, Wash. | 0.104 ± 0.055 | 10 |
| Aedes stimulans | 10 (pg) | 9 California populations | 0.042 ± 0.023 | 10 |
| Aedes triseriatus | 17 (pg) | 4 Mich., Ind., N.Y. populations | 0.091 ± 0.054 to 0.145 ± 0.044 | 29 |
| Aedes triseriatus | 17 (pg) | 10 Mich. and Ind. populations | (0.167 ± 0.049 to 0.212 ± 0.053) | 93 |
| Aedes varipalus | 10 (pg) | Washington County, Utah | 0.090 | 10 |
| Anopheles albimanus | 25 (sg) | 3 northern Columbia populations | 0.20 ± 0.04 to 0.22 ± 0.04 | 116 |
| Anopheles albimanus | 25 (sg) | 8 southern Columbia populations | 0.05 ± 0.03 to 0.16 ± 0.04 | 116 |
| Anopheles balabacensis | 21 (pg) | Banggils, Malaysia | (0.007 ± 0.005 to 0.026 ± 0.017) | 62 |
| Anopheles deaneorum | 24 (sg) | Costa Marques, Rondonia, Brazil | 0.11 ± 0.02 | 114 |
| Anopheles manjoana-CM | 24 (sg) | Costa Marques, Rondonia, Brazil | 0.08 ± 0.03 | 114 |

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tioning of variation is consistent with the observation that in 333 locus-population combinations tested, there were 61 deviations from HWE and 55 of these had heterozygote deficiencies. These results suggest inbreeding (71), which is consistent with the limited flight dispersal of *Aedes albopictus*.

Allele frequencies at seven (9) or eight (73) polymorphic loci in *Aedes albopictus* from five U.S. states indicate that there is a great deal of genetic variation in this species in North America and that differences between nearby populations are as great as those between remote populations (9, 73). These data suggest either that each founder population was large or that there were multiple introductions at each site. In contrast, the Brazilian populations are monomorphic at two of the eight (normally) polymorphic loci, and it has been argued that mosquitoes from a single Japanese location were introduced to one site in Brazil and that this was followed by dispersal to other locations (71).

The high levels of interpopulation variation in *Aedes albopictus*, outlined above, differ from the molecular genetic data obtained with mitochondrial DNA. *Aedes albopictus* has very little variation in mitochondrial DNA, with only 3 of 19 populations having more than one haplotype (74). The most likely explanation for this is that the present distribution of this species is the result of a recent expansion, most probably attributable to human activities (74). It may, however, be the result of cytoplasmic incompatibility that has maintained a single dominant mitochondrial DNA haplotype (75).

Genetic distances between populations indicate that there is little genetic differentiation among 12 populations of *Culex pipiens pipiens* in Egypt and Israel (176). Geographic distances and genetic distances were not strongly correlated, possibly because of transport of mosquitoes by humans (176). Similarly, there is very little genetic distance (mean $D = 0.006$) between any of four populations of *Culex pipiens quinquefasciatus* from west Africa and one from southeastern Africa (171). Comparison of the African populations with those in the United States demonstrated that each of the latter is as similar to any African population as it is to any other U.S. population, thus providing strong evidence for an African origin for *Culex pipiens quinquefasciatus* in the United States (171).

Interpretation of interpopulation differences must be treated cautiously, especially if there are intervening populations that have not been sampled. Clines in allele frequencies have been demonstrated and may provide evidence for gene flow between populations as well as evidence of environmental pressures for selection of certain alleles.

| Taxon                        | No. of loci$^a$ | Sampling site                      | Mean heterozygosity ± SEM | Reference |
|------------------------------|-----------------|-----------------------------------|---------------------------|-----------|
| *Anopheles marajoua-IG*      | 24 (sg)         | Iguape, Sao Paulo State, Brazil   | 0.22 ± 0.04               | 114       |
| *Anopheles marajoua-MA*      | 24 (sg)         | Island of Marajo, Para State, Brazil | 0.08 ± 0.03               | 114       |
| *Anopheles minimus*          | 12–20 (pg)      | 3 northern Thailand populations   | (0.250 ± 0.050 to 0.303 ± 0.076) | 80       |
| *Anopheles quadrimaculatus A* | 35–34 (pg)     | 3 southern U.S. populations       | (0.195 ± 0.039 to 0.241 ± 0.039) | 117      |
| *Anopheles quadrimaculatus B* | 30 (sg)        | 10 southeastern U.S. populations  | 0.127 ± 0.037 to 0.180 ± 0.042 | 85       |
| *Anopheles quadrimaculatus C* | 34 (sg)        | 2 southern U.S. populations       | (0.182 ± 0.042 to 0.221 ± 0.045) | 117      |
| *Anopheles quadrimaculatus D* | 20 (sg)        | 3 southeastern U.S. populations   | 0.102 ± 0.042 to 0.105 ± 0.041 | 85       |
| *Anopheles quadrimaculatus E* | 34 (sg)        | Levy County, Fla.                 | (0.235 ± 0.040)           | 117      |
| *Anopheles quadrimaculatus F* | 29 (sg)        | Northwestern Fla.                | 0.22 ± 0.05 to 0.27 ± 0.06 | 115      |
| *Anopheles quadrimaculatus G* | 29 (sg)        | Northwestern Fla.                | 0.17 ± 0.04 to 0.20 ± 0.05 | 115      |
| *Anopheles quadrimaculatus H* | 29 (sg)        | Near Savannah, Ga.               | 0.16 ± 0.04 to 0.19 ± 0.05 | 115      |
| *Culex pipiens s.l.*         | 10 (sg)         | 13–16 Texan and Californian populations | 0.152 ± 0.048             | 160      |
| *Culex pipiens quinquefasciatus* | 17 (sg)      | 12 Egyptian and Israeli populations | (0.071 ± 0.033 to 0.147 ± 0.043) | 176      |
| *Tabanus confinis*            | 12 (sg)         | 5 eastern U.S. populations        | (0.042 ± 0.042 to 0.089 ± 0.048) | 149      |
| *Tabanus lineola*             | 11 (sg)         | 4 U.S. populations                | (0.042 ± 0.026 to 0.087 ± 0.050) | 145      |
| *Tabanus nigrovittatus*       | 12 (sg)         | 11 eastern U.S. populations       | (0.061 ± 0.031 to 0.125 ± 0.057) | 149      |
| *Tabanus confinis*            | 11 (pg)         | Nalusanga, Zambia                | 0.137 ± 0.068 to 0.162 ± 0.075 | 40       |
| *Tabanus lineola*             | 11 (pg)         | Keembe, Zambia                   | 0.093 ± 0.063             | 40       |
| *Tabanus nigrovittatus*       | 12 (pg)         | Ngaruman, Kenya                  | 0.171 ± 0.063             | 43       |
| *Glossina morsitana centralis* | 13 (pg)       | Rekometje, Zimbabwe              | (0.090 ± 0.061)           | 1        |
| *Glossina maruoana*           | 12 (pg)         | Lambwe, Kenya                    | 0.092 ± 0.063             | 162      |
| *Glossina stenomaculata*      | 17 (pg)         | Makuyuni, Tanzania               | 0.061 ± 0.037             | 44       |

$^a$ The medium used for electrophoresis is given in parentheses: ca, cellulose acetate; ns, not stated; pg, polyacrylamide gel; sg, starch gel.

$^b$ Values given in parentheses were calculated by R. H. Gooding, using data from the reference cited.
**TABLE 3. Electrophoretic variation in laboratory colonies of vectors**

| Taxon                      | No. of loci | Geographic origin of colony | Mean heterozygosity ± SEM | Reference |
|---------------------------|-------------|-----------------------------|--------------------------|-----------|
| **Ticks**                 |             |                             |                          |           |
| *Boophilus annulatus*     | 22 (sg)     | Maverick County, Tex.       | (0.052 ± 0.029)          | 139       |
| *Boophilus microplus*     | 24 (sg)     | Cameron County, Tex.        | (0.125 ± 0.040)          | 139       |
|                           | 23–24 (sg)  | Starr County, Tex.          | (0.115 ± 0.036 to 0.119 ± 0.040) | 139       |
|                           | 24 (sg)     | Gurabo, Puerto Rico         | (0.098 ± 0.034)          | 139       |
| **Triatoma bugs**         |             |                             |                          |           |
| *Rhodnius prolitis*       | 22 (sg)     | Not stated                  | 0.096 (± 0.039)          | 60        |
|                           | 22 (sg)     | Lara State, Venezuela       | 0.044 (± 0.022)          | 60        |
|                           | 22 (sg)     | Trujillo State, Venezuela   | 0.052 (± 0.031)          | 60        |
| **Ceratopogonid midges**  |             |                             |                          |           |
| *Callicoides varitipennis*| 21 (sg)     | Edwards County, Tex.        | 0.125 ± 0.041 to 0.140 ± 0.042 | 152       |
|                           | 21 (sg)     | Owyhee County, Ind.         | 0.147 ± 0.046 to 0.164 ± 0.050 | 152       |
|                           | 21 (sg)     | Lincoln County, Neb.        | 0.121 ± 0.049 to 0.147 ± 0.046 | 152       |
| **Phlebotomine sandflies**|             |                             |                          |           |
| *Lutzomyia longipalpis*   | 27 (sg)     | Minas Gerais, Brazil        | 0.057 ± 0.028            | 86        |
|                           | 27 (sg)     | Tolima, Colombia            | 0.071 ± 0.027            | 86        |
|                           | 27 (sg)     | Liberia, Costa Rica         | 0.058 ± 0.026            | 86        |
| **Phlebotomine papatasi** | 17 (ca)     | Alexandria, Egypt           | 0.06 ± 0.02              | 77        |
| **Mosquitoes**            |             |                             |                          |           |
| *Aedes atropalus*         | 17 (pg)     | FitzRoy Bay, Ont., Canada   | 0.033 (± 0.026)          | 111       |
| *Aedes epatia*            | 17 (pg)     | Col. R. Grand County, Utah  | 0.033 (± 0.023)          | 111       |
|                           | 17 (pg)     | Austin, Tex.                | 0.104 (± 0.047)          | 111       |
|                           | 17 (pg)     | Metapan, El Salvador        | 0.087 (± 0.036)          | 111       |
| **Anopheles stephensi**   | 16 (sg)     | 6 unselected lines from     | 0.066 ± 0.038 to 0.110 ± 0.057 | 173       |
|                           |             | Punjab, Pakistan            |                          |           |
|                           | 16 (sg)     | *P. falciparum* refractory  | 0.046 ± 0.035            | 173       |
|                           | 16 (sg)     | *P. falciparum* susceptible | 0.041 ± 0.024            | 173       |
| **Culex pipiens quinquefasciatus** | 17 (sg) | Koulak, Senegal | 0.01 (± 0.006) | 171 |
| **Culex tarnalis**        | 12 (sg)     | Paso West, Calif.           | 0.05 ± 0.03 to 0.15 ± 0.024 | 76        |
|                           | 13 (sg)     | Breckenridge, Calif.        | 0.10 ± 0.04 to 0.20 ± 0.07 | 76        |
| **Sabethes cyaneus**      | 31 (pg)     | Ipeti, Panama               | (0.119 ± 0.038)          | 113       |
| **Tsetse flies**          |             |                             |                          |           |
| *Glossina brevipalpis*    | 14 (pg)     | Kibwezi Forest, Kenya       | 0.200 ± 0.071            | 46        |
| *Glossina longipalpis*    | 14 (pg)     | Nguruman, Kenya             | 0.082 ± 0.049            | 46        |
| *Glossina moritans morsitans* | 14 (pg) | Kariba, Zimbabwe | 0.167 ± 0.057 | 47 |
|                           | 14 (pg)     | Handeni, Tanzania           | 0.073 ± 0.027            | 47        |
|                           | 13 (pg)     | Kariba, Zimbabwe            | 0.157 ± 0.057            | 43        |
| **Glossina palpalipes**   | 14 (pg)     | Shomba Hills, Kenya         | 0.183 ± 0.058            | 45        |
|                           | 14 (pg)     | Shomba Hills, Kenya         | 0.223 ± 0.063            | 46        |
|                           | 14 (pg)     | Nguruman, Kenya             | 0.185 ± 0.063            | 45        |
|                           | 12 (pg)     | Lambwe, Kenya               | 0.165 ± 0.071            | 162       |

* Values given in parentheses were calculated by R. H. Gooding, using data from the reference cited.
Bay, La., or the presence of nine specimens that had been incorrectly identified as Tabanus lineola (145).

Anopheles quadrimaculatus species C, a species with a patchy distribution in Florida and Georgia, has been further subdivided into C1 (Florida) and C2 (Florida and Georgia) on the basis of allele frequencies at five electrophoretically detectable loci (115). C1 and C2 were separated by a Nei genetic distance of 0.14, a value indicative of sibling species in the genus Anopheles (85). C1 and C2 in Florida have similar amounts of genetic variation. Therefore, the genetic differences between them are unlikely to be due to founder effects, since chance effects would have to produce the same amount of genetic variation independently in both C1 and C2. Differences are likely to be due to limited migration from breeding sites and/or to selection of specific genotypes in different environments (115). Hybridization studies are apparently in progress (115); when available, the results will no doubt help clarify the species status of C1 and C2.

Anopheles gambiae s.l. in western Kenya deviates significantly from HWE at four of seven loci (in three cases, there was a significant deficiency of heterozygotes), and mosquitoes from four villages differed significantly in allele frequencies at five loci (110). The deficiencies of heterozygotes are consistent with the presence of two or more noninterbreeding populations, and the differences in allele frequencies suggest limited mosquito migration between villages or the possibility that different taxa occupy different villages. Similarly, when samples of Anopheles quadrimaculatus from four sites within 15 km of each other in Kruger National Park, South Africa, were pooled, aspartate aminotransferase was not at HWE; also, there was a deficiency of heterozygotes at two sampling sites (17). The data were interpreted as indicating either that there is very limited migration between sites or that two taxa exist within the nominal species Anopheles quadrimaculatus (17). However, it must be borne in mind that the heterozygote deficiency could be due to inbreeding or to selection against heterozygous individuals.

Genetic Structure of Local Populations

Among Phlebotomus papatasi from five widely separated populations in Egypt, there were 73 combinations of loci and locality in which there were polymorphic loci (77). At 30 of these, there were significant deviations from HWE. The deviations occurred among 12 of the 17 loci examined and, in each population, involved from 29 to 40% of the loci. With one exception, deviations from HWE were due to deficiencies of heterozygotes. Despite the deviations from HWE, the populations were genetically very similar, and it would require additional studies to determine whether the heterozygosity deficiencies were due to a Wahlund effect or to local inbreeding.

A Wahlund effect at two isocitrate dehydrogenase loci revealed the sympatric occurrence of two forms of Anopheles quadrimaculatus (forms A and B) at several localities in the southeastern United States (85). A cluster analysis of the genetic distances (based on 20 loci) between 13 populations of Anopheles quadrimaculatus placed the 10 populations of form A in one cluster and the three populations of form B in another (85); the “forms” are actually sibling species.

At two localities (Ban Phu Rat and Taow-Kee) in Thailand, samples of Anopheles minimus s.l. completely lacked one class of the octanol dehydrogenase heterozygotes and there was a deficiency of Mpi and Gcd (glycerol dehydrogenase) heterozygotes, suggesting the existence of sympatric assortatively mating populations (50).

In addition to searching for deviations from HWE, several other methods have been used to search for evidence of structuring of vector populations. One, to date unsuccessful, approach is to compare samples collected by different methods. For studies of tsetse flies, the rationale for this approach is that traps for the savannah-inhabiting species are believed to mimic the fly’s hosts, and it is at or near the hosts that mating occurs. Allele frequencies in samples of Glossina morsitans centralis collected by three methods did not produce any evidence of variation of the population at Nalusanga, Zambia (40), even though these collecting methods result in samples with significantly different sex ratios, age structures, and infection rates. Allele frequencies at Pgm in Phlebotomus perfiliewi perfiliewi that were collected by three methods at Teramo, Italy, did not reveal any subdivision of the population (182).

A second approach is to examine specimens collected from different habitats in one location. Triatoma infestans specimens collected from or near human habitation and those collected from guinea pig habitat did not have significantly different allele frequencies at two polymorphic loci (28). Because so few polymorphic loci were examined, the results provided weak evidence that there is no differentiation of the Triatoma infestans populations into domestic and nondomestic populations. If substantiated, this suggests that there is a single population of bugs involved in transmitting Trypanosoma cruzi from wild animals to humans.

A third approach is based on sampling insects that differ in resting behavior after taking a blood meal (62) or that differ in the times at which mating behavior is displayed (146).

Anopheles balbucensis mosquitoes that consistently remain indoors after feeding differ from those that consistently display early postfeeding departure from buildings, with respect to allele frequencies at loci for an esterase (Est-3) and isocitrate dehydrogenase (Idh-2) but not at seven other loci, nor when data from the nine polymorphic loci are pooled (62). However, the genetic evidence for mosquitoes with the two postfeeding behaviors is equivocal, since the sample of early-egress mosquitoes was not at HWE for Est-3 and may therefore be a sampling error. There also appears to be an error in the χ² value for the Idh-2 data that is supposed to show the statistical difference between the samples.

Allele and genotype frequencies at one (Pgm) of three loci studied in male Tabanus nigrovittatus provided weak evidence for population structuring, on the basis of the time at which hovering (mating behavior) occurred each morning in a population in New Jersey (146). The pooled data from the two samples did not deviate from HWE, and data from the other loci did not support the hypothesis of structuring of the population. It was suggested that the gene products of the various Pgm alleles function optimally at different temperatures (146). No females were examined, and it is possible that females fly when early- and late-hovering males are active and thus preclude genetic isolation based on the time of hovering. There is no a priori reason why two reproductively isolated populations could not have similar allele frequencies at some loci and markedly different frequencies at other loci. To extend this study, we need an examination of a large number of loci to find diagnostic loci that would establish a lack of gene flow between the early- and late-hovering flies and the inclusion of females in the study to establish whether they are partitioned into early and late fliers.

Populations Lacking Apparent Subdivision

Genetic structuring has not been detected in all vector populations studied. There is strong genetic evidence for suppression of structuring through interpopulation migration among
strong fliers such as tabanids (149), moderately strong fliers like mosquitoes (10, 140), weak fliers like biting midges (156), and nonflying vectors such as ticks (63, 139). It therefore appears that flying ability does not predict population structuring.

Local fixation indices ($F_{is}$) and the ratios of between-population genetic diversity to total genetic diversity indicate that within Tabanus nigrovittatus and within Tabanus conterminus, there is a significant amount of gene flow between populations in the eastern United States (149).

In contrast to an earlier finding (140), no correlation exists between habitat (beech or oak forest) and alleles at an esterase locus ($Est-6$) in Aedes triseriatus (94). The lack of population structuring is due to the mixture of tree hole types in each forest in which mosquitoes breed and to the migration rate between habitats (94). With regard to the last point, the distribution of conditional average allele frequencies among 10 populations indicates that Aedes triseriatus has a high rate of migration (94). This conclusion is supported by earlier work that demonstrated very low levels of genetic distance between populations of Aedes triseriatus in Indiana and Michigan (95).

There is very little genetic differentiation, at 10 biochemical loci, among nine populations of Aedes sierrensis over a 2,000-km range (10), suggesting significant gene flow between populations. Nonetheless, there are significant differences in adult ornamentation (i.e., the pattern of scales) between the northern and southern populations (10). This situation is similar to that in Aedes albopictus, where morphological characters group populations on the basis of geographic origin within the United States but the biochemical genetic data do not (73). Perhaps the alleles at biochemical loci are selectively neutral while certain morphological characters are subject to significant local selective pressures in Aedes sierrensis and Aedes albopictus. It is also possible that the allozymes are responding to similar environmental pressures throughout the range of the species whereas morphological characters are responding to environmental factors that are variable throughout the range.

Other possibilities are that the morphological characters are neutral and the differences are due to genetic drift or that the morphological variation is nongenetic and is determined by environmental variation.

A Wahlund effect was not observed among Culicoides variipennis sonorenensis midges at a nonpermanent breeding site (in Wald County, Colo.), suggesting that the site is reinvaded annually by midges that come primarily, if not exclusively, from a single permanent population (156). There is additional evidence indicating significant migration between populations of Culicoides variipennis sonorenensis. Two methods for estimating gene flow between populations (the $F_{st}$ method and the private-alleles method) indicate that an average of 2.15 to 6.96 individuals migrate between populations (in Wald County) per generation (156).

For nine North American populations of Amblyomma americanum, the $F_{ST}$ values (both in absolute terms and relative to $F_{IS}$ and $F_{TT}$) were low, indicating a lack of interpopulation divergence (63). Similarly, there was a high genetic similarity among four natural populations and three laboratory colonies of North American Boophilus microplus, suggesting that the populations have a common gene pool (139). This interpretation is supported by a relatively low $F_{ST}$ value, averaged over the polymorphic loci (139). These findings differ from what has been predicted for parasites (130), i.e., low levels of genetic variability within local populations and high levels of genetic divergence between populations. Although the mobility of ticks is inherently low, that of at least some of their hosts, particularly domestic animals, is high. It is this latter factor that is probably responsible for the lack of significant interpopula-

tion genetic divergence over wide geographic areas (63, 139).

The low level of genetic differentiation of the geographic populations suggests that there will probably be no genetic barriers to biological control operations, if these are implemented against ticks (139).

### Temporal Changes in Colonies and Field Populations

Few vector populations have had population genetic studies conducted on them more than once. In some studies (11, 44), there were significant differences between samples obtained on two dates, but the cause of these differences (cyclic or noncyclic changes in the population, or sampling errors) is not discernible from the data.

Changes in allele frequencies that correlate with seasonal changes are more easily accepted as indicative of real changes within a population. In a population of Culicoides variipennis at Ausman, Colo., there was a consistent temporal change in allele frequencies at the loci Pgl and Mdh. These changes were attributed to differential winter survival of larvae of various genotypes at Pgl, Mdh, or closely linked loci (152, 156). Similarly, the alleles $Hk^A$ and $Pgd^A$ in Culex pipiens s.l. that are known to increase in frequency with latitude and with cooler climates were shown to increase in frequency throughout the summer and into the fall in a Memphis, Tenn., population (133). These changes are consistent with, but not proof of, selection of alleles with products that function better under cool conditions. Furthermore, the frequency of a third allele (the B1 allele of Pgm) that has the same geographiccline as $Hk^A$ and $Pgd^A$ did not increase with the advancing season (133).

It is expected that in small isolated populations, random changes in allele frequencies will occur as a result of genetic drift. Over a 2-year period, significant changes occurred at three or more polymorphic loci in 4 to 11 Aedes albopictus populations in Texas (72). Similar but smaller changes occurred in populations in three other states. Although rare alleles were lost from some populations, there were no statistically significant changes in heterozygosities during the study period (72).

Munstermann (112) reviewed the genetic variability among Aedes spp. and the changes that accompany colonization. As expected, laboratory populations commonly have lower heterozygosities and fewer alleles than do field populations. However, unexpectedly high heterozygosities at biochemical marker genes may be explained by the presence of lethal alleles at loci that are closely linked to the polymorphic genes (96, 97, 112).

Adaptation to laboratory conditions occurred in Culex tarsalis maintained under either constant temperature and humidity in relatively small cages or variable temperature and humidity in relatively large cages (79). Adaptation, as measured by reproductive rate and mating competitiveness, was greater under the former conditions. By the 12th generation, the genetic distance between the parental population and the constant-conditions colony was greater than the distance between the parental population and the variable-conditions colony. However, when tested with wild males and females in outdoor cages, males from the variable-conditions colony were not more competitive than those from the constant-conditions colony, possibly because of assortative mating by the wild mosquitoes (79). Mating competitiveness of male mosquitoes, particularly those that are not associated with human habitation but which breed in large outdoor sites, may change during colonization and may result in assortative mating that is detrimental to the use of these mosquitoes in genetic control programs (135).
There is little evidence to indicate genetic selection of tsetse flies during the early stages of colonization (41). However, one genetic change may be linked to a change in tsetse husbandry. Allele frequencies at the locus for midgut alkaline phosphatase changed in a large colony of Glossina morsitans morsitans at about the time that the colony was switched from an in vivo to an in vitro feeding system (41). Other changes may occur through stochastic processes. For example, two colonies of Glossina palpalis gambiensis, both of which were established with flies collected from the same locality in Burkino Faso, West Africa, but having different colonization histories, differed in susceptibility to and maturation of Trypanosoma brucei gambiense and had different allele frequencies at two X chromosome loci (Hk and Pgm) and one autosomal locus (Psg) (30).

**Differences among Colonies and between Colonies and Field Populations**

Many laboratory studies on arthropods are carried out on the assumption that colony material is similar to material from natural populations. However, it is well recognized that laboratory colonies may have reduced genetic variability as a result of founder effects, genetic drift, selection, etc. (41, 112). For example, significantly lower levels of heterozygosity occur in laboratory colonies of Culicoides variipennis (reduced by 28% [152]) and Phlebotomus papatasi (reduced by 71% [77]). The latter finding is not surprising, since the colony was established with offspring from 30 females and was in its 32nd generation at the time the heterozygosity was estimated (77).

Reduced heterozygosity in laboratory colonies is not invariably the case. Colonies of Glossina morsitans morsitans (43) and Glossina pallidipes (162) do not differ significantly from field-collected flies in mean heterozygosity, indicating that the colonies have not gone through major genetic bottlenecks. However, these colonies differ significantly from field populations in heterozygosity and allele frequencies at some loci (43, 162), and these differences have been interpreted as indicating that rare alleles may be favored under laboratory conditions (162). There was a high level of genetic similarity among four natural populations and three laboratory colonies of North American Boophilus microplus (139). Compared with natural populations, the tick colonies had insignificantly higher heterozygosities and frequencies of some, normally rare, alleles.

Significant differences in heterozygosity occur between what are believed to be conspecific colonies (examples from six species are given in Table 3). There are also examples (Glossina pallidipes [45], Rhodnius prolixus [60]) in which colonies differ significantly in allele frequencies at some loci, and at least one comparison of colonies has revealed the possible existence of cryptic species. There is a great deal of genetic divergence among colonies of Lutzomyia longipalpis from Costa Rica, Colombia, and Brazil, as revealed by “private alleles” in each colony, by the high values of Nei’s genetic distance (which are more typical of different species of Lutzomyia [83] than of populations within a species), and by the large proportion of the loci that could be used to correctly assign an individual to a given colony (86). Intercolony hybridization revealed sterility among F1 males. Overall, the results suggest that Lutzomyia longipalpis is a species complex. Of considerable significance is the unpublished work of A. Warburg (cited by Lanzaro et al. [86]) that indicates that the Costa Rican Lutzomyia longipalpis lacks the salivary vasodilatory peptide that occurs in Brazilian Lutzomyia longipalpis (136), and the finding that Leishmania donovani chagasi transmitted by Lutzomyia longipalpis fails to invade the viscera (86). These examples indicate some of the problems faced when comparing research done on different colonies of vectors.

**IMPLICATIONS OF GENETIC VARIATION**

I have summarized evidence demonstrating a significant amount of genetic variation within and between vector populations. This variation was estimated by using primarily electrophoresis of enzymes, but variation occurs also in traits related to vector competence and in cytogenetic and molecular characteristics that were not covered in this review. Genetic variation in vectors is important. First, variation creates uncertainty with respect to interpretation and application of information. Second, genetic variation provides the raw material for change within populations that are subjected to new pressures, such as control measures and the introduction of new organisms, including pathogens and competitors, into an environment. Third, genetic variation may provide material that is useful for developing disease control strategies. Above, I have alluded to some of these implications; below, I discuss them more specifically.

**Potential Taxonomic Problems**

Are we confident that current information is representative of the taxonomic level (population, subspecies, species) to which it is attributed? Much of what we do in biology is predicated upon the assumption that we understand the taxonomic status of the organism being studied. Implicit in this review is the assumption that the genetic variation being reviewed is intraspecific, not interspecific. However, what constitutes a species changes with time as new information becomes available. Occasionally, our understanding of what constitutes a particular species changes radically, forcing us to reevaluate our knowledge. Females of the Anopheles quadrimaculatus complex in Florida vary in their susceptibility to Brugia pahangi and subperiodic Brugia malayi, with Anopheles quadrimaculatus species A having higher infection rates and higher infection intensities than do Anopheles quadrimaculatus species B and C (118). Prior to the discovery that Anopheles quadrimaculatus is a complex of at least four sibling species, these results would have been viewed as interpopulation, not interspecific, variation. This example raises the question of how much of what we consider to be interpopulation (particularly geographic) variation, documented earlier in this review, will turn out to be interspecific variation.

When subspecies or species status is assigned, the taxa are believed to differ in important characters, not just in the traits that permit identification of individuals as members of one or another taxon. Therefore, it is important to understand the genetic basis of the characters used to separate taxa and to know, with some certainty, that these characters have not arisen, or have been lost, independently in several populations within the putative taxa. In some groups, identification of an individual as being a member of a particular subspecies or of a particular sibling species may be based upon a single character such as scale patterns or the presence or absence of a particular electrophoretic variant. Breeding experiments have established that the scale patterns commonly used to distinguish subspecies of Aedes aegypti are under relatively simple genetic control (61), thus raising doubts about whether biological features attributed to a subspecies of Aedes aegypti, identified by scale pattern alone, are really characters of a subspecies or just of a population or a laboratory colony. Similarly, Anopheles gambari sensu stricto may be distinguished from sibling species by alleles for the enzymes superoxide dismutase and aspartate

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aminotransferase. However, in a sample of 37 mosquitoes, identified cytogenetically as *Anopheles gambiae* species, a superoxide dismutase allele that was thought to exist only in *Anopheles bwambae* and *Anopheles arabiensis* was found in one female and in the offspring of another female (67). Similarly, several *Anopheles gambiae* complex specimens from near Brazzaville, People’s Republic of Congo, and Kruger National Park, South Africa, had variants of aspartate aminotransferase that were thought to be characteristic of and restricted to sibling species (17). These results show the importance of using at least two independent methods for the identification of specimens when dealing with a species complex.

There is also the important issue of the taxonomic status of vector populations, when these do not correspond exactly to the geographic distribution of the pathogens that they transmit. Consider the case when the distribution of a pathogen corresponds to the range of vector A but not to the range of a closely related vector, B. If A and B are distinct taxa, the distribution of the pathogen may be attributed to interspecific differences and to the distribution of its vector, A. However, if A and B are from the same taxon and thus actually or potentially part of the same gene pool, other causes must be sought to explain the distribution of the pathogen. Studies of vector genetics would no doubt contribute to understanding such situations, and they have the potential to reveal potential control strategies.

**Consequences of Temporal Variation**

We like to believe that experimental results can be extrapolated in space and time. Among vector-pathogen interactions, there are several examples that caution us about extrapolation of results based upon a single examination of a vector population. Changes in the susceptibility of *Culex tarsalis* to viruses occur both in laboratory colonies that are held under constant conditions and in some field populations (58). In a semi-isolated population in California, there was significant variation throughout the summer and from year to year in the *per os* susceptibility to western equine encephalomyelitis virus and to *St. Louis* encephalitis virus (58). The changes in susceptibility to the viruses occurred independently. There were indications that susceptibility may covary with environmental factors, but the identities of the factors and the nature of the interactions were not fully established. Whether genetic changes occurred in the vector population was not established.

It is clear that under both laboratory and field conditions, temporal changes occur in vector competence, and there is little to be gained by simply documenting more such changes. The underlying causes of the changes have not been established. What is needed is an understanding of the mechanisms of interaction between pathogens and their vectors and an elucidation of the factors that influence the expression of these interactions.

**Implications of Intercolony Variation**

Given the amount of temporal variation that may occur within a population, it is not surprising that interpopulation, including intercolony, variation is common. The locus for one of the esterases (*Est-6*) in *Aedes aegypti* is highly polymorphic in 27 colonies that were in approximately their 20th generation, with at least 12 and possibly 20 alleles occurring at this locus (93). A comparison of the observed distribution of alleles in the colonies with two assumed global distributions led to the conclusion that the former is unlikely to have arisen from random fluctuations of neutral alleles but, rather, that some selection is responsible for maintaining polymorphism. If certain characters are under selective pressure, then these, as well as those controlled by adjacent loci, may vary between colonies.

These findings imply that if estimates of vector competence are based on studies of laboratory colonies, there is a need to survey many colonies for vector competence before arriving at any conclusions about the vector competence of a species. Indeed, this conclusion had been substantially demonstrated in studies of the susceptibility of *Aedes aegypti* to *Plasmodium gallinaceum* (78). In this model, there is a large amount of variation in susceptibility between colonies, and this variation has several implications for studies conducted with laboratory colonies (78). First, it is possible that a colony is devoid of factors for susceptibility or refractoriness; selection programs initiated with such colonies are doomed to failure. Second, any conclusions based upon vector competence of a species that has been estimated with only one colony is suspect. Even when several recently established colonies are used, the absence of vector competence in all laboratory colonies is not proof that a species lacks vector competence.

**Specificity of Vector-Pathogen Interactions**

Attempts to extrapolate the findings from one vector-pathogen model to others have met with mixed results. It is encouraging that in some cases one relationship has proven to be a good predictor of another (90, 101, 108, 167). One reason for wishing to make such extrapolations is that when they can be made, they provide opportunities for use of the least hazardous pathogen as a model for a more hazardous one.

However, in most cases, the relationship of a vector with one pathogen is a poor indicator of its relationship with others, particularly when genetic variants have been selected for susceptibility or resistance. Two colonies of *Culicoides varipennis sonorensis* have 7.5- to 12.1-fold variation in susceptibility to five strains of bluetongue virus, with markedly different rank orders of susceptibility to these strains (106). A strain of *Culex tarsalis* that is highly resistant to infection with western equine encephalomyelitis virus is readily infected with other viruses, such as *St. Louis* encephalitis virus (81). Three strains of *Anopheles albimanus* from El Salvador differed significantly in their susceptibility to *Plasmidium vivax* but not to *Plasmodium falciparum* (183). A strain of *Glossina morsitans morsitans* with high midgut infection rates for *Trypanosoma congolense* and *Trypanosoma brucei brucei* had a high rate of *Trypanosoma brucei brucei* maturation, but *Trypanosoma brucei gambiense* failed to mature in this strain, indicating that factors controlling susceptibility to midgut infection differ from those controlling maturation (101). The specificity of susceptibility and refractoriness genes in mosquitoes has been extensively documented with regard to the filarioid worms. The *Aedes aegypti* genes for susceptibility and refractoriness to subperiodic *Brugia malayi* do not influence the development of *Dirofilaria immitis* and *Dirofilaria repens* in the Malpighian tubules (90), and the genes controlling the development of *Dirofilaria immitis* affect neither the development of *Brugia pahangi* in the thoracic muscles nor the development of *Dirofilaria cortynodes* in the fat body (103). Similarly, in *Culex pipiens*, the gene that controls susceptibility to *Brugia malayi* and *Brugia pahangi* has no effect upon susceptibility to *Wuchereria bancrofti* (121). These examples provide compelling reasons to be cautious when extrapolating results from one vector-pathogen model to another.

There is a limited amount of data on the influence of the vector upon the virulence of parasites. Strains of *Trypanosoma simiae* vary in their virulence to pigs in eastern Kenya: 13
strains isolated from *Glossina brevipes* were highly virulent, 2 strains from *Glossina austeni* were moderately virulent, and 1 strain from *Glossina pallidipes* caused a chronic infection (69). Passage of a “*G. brevipalpis* strain” through *Glossina pallidipes* reduced its virulence, whereas passage of the “*G. pallidipes* strain” through *Glossina brevipalpis* increased its virulence (69). Unfortunately, comparable studies have not been done to determine whether intraspecific genetic variation in a vector influences the virulence of parasites. Furthermore, we have no information on the mechanisms involved in the apparent changes in virulence. Did the changes affect all of the trypanosomes that were in the tsetse flies, or was there selection for virulent or attenuated strains? If these observations can be substantiated, they may provide an interesting model for studying the interactions between pathogens and vectors.

**Risk Assessment Based upon Vector Susceptibility to Pathogens**

Given the large amount of information on the genetics of vector susceptibility to pathogens, it is reasonable to ask whether we can assess the risk of a vector-borne disease in any locality. Curtis (24) pointed out that there may be only one example of the successful application of knowledge of the vector potential of an insect. That example is an unpublished account of the successful selection, during the 1940s, of the sites for military camps in Italy, based upon the distribution of populations of *Anopheles maculipennis* that bite humans and those that do not.

Our past record notwithstanding, it ought to be possible to foresee some risks. Three North American (mainly U.S.) examples involving malaria, dengue, and La Crosse virus illustrate this point. First, the widespread occurrence of potential malaria vectors such as *Anopheles quadrimaculatus* s.l. serve as a reminder that the occasional introduction of malaria carriers creates opportunities for local outbreaks of malaria in a part of the world where the average physician, by training and experience, is ill prepared to diagnose the disease. In a similar manner, *Aedes albopictus* from three U.S. locations (Indianapolis, New Orleans, and Houston) is capable of vertical transmission of a strain of dengue-1 virus from Jamaica (13), as well as horizontal transmission of this virus. These populations may provide a mechanism for overwintering dengue-1 virus. Finally, one interpretation of the data suggests that La Crosse virus could spread outside its present range. If the basis for the present restriction of the area where La Crosse virus is endemic to the north-central part of the *Aedes triseriatus* range in the eastern United States and adjacent parts of Canada is historical rather than ecological, there is considerable potential for its expansion, because (i) *Aedes triseriatus* mosquitoes from other areas have higher levels of vector competence than do those from within the area where the pathogen is endemic (51) and (ii) La Crosse virus can be transmitted by *Aedes albopictus* (147).

A problem with assessing local risk factors is that the classical procedures for determining vector competence are time-consuming and expensive. In the absence of a perceived imminent threat, they are unlikely to command substantial resources. It is possible that new molecular techniques will provide methods for rapidly assessing risk factors. Indeed, it may already be possible to assess the risk of trypanosome transmission, posed by any tsetse fly population, by determining through immunological procedures the prevalence of maternally inherited factors that control the susceptibility of tsetse flies to trypanosomes (100). Similarly, DNA probes have been developed to distinguish between females of *Anopheles gambiae* and *Anopheles arabiensis* (36).

**Potential Uses of Refractory and Susceptible Colonies**

By using standard selection procedures, colonies of vectors that are highly susceptible or highly resistant to various pathogens have been established. For the foreseeable future, the most important use of such colonies is as a source of material for establishing the mechanisms by which vectors and pathogens interact. The successful application of genetic techniques to control arthropod-borne diseases rests upon a thorough understanding of such mechanisms.

It is likely that classical genetic approaches will continue to produce colonies that are susceptible or resistant to pathogens. Arthropods from colonies that are highly susceptible to pathogens may be useful in producing the vertebrate-infective form of a pathogen for use in testing vaccines and prophylactic agents. Highly refractory strains may be established either by introducing foreign genes into the germ line of a vector (22) or by establishing vector strains that carry maternally inherited, transformed symbionts that produce antipathogen agents (4, 5). Such colonies may be useful as sources of males for sterile-male release programs, for the introduction of deleterious genes into natural populations, or as replacements for a population having high vector competence.

The feasibility of population replacement has been demonstrated under laboratory conditions. In caged populations of *Anopheles gambiae*, susceptibility to *Plasmodium yoelii nigeriensis* has been reduced by a release of males from a strain with low susceptibility, even though mosquitoes of the low-susceptibility line had lower fitness than those of the high-susceptibility line. Following termination of the release program, the susceptibility of the population remained at half its original level, indicating recombination of genes for refractoriness with those for fitness (49). Similar results were obtained with a caged population of *Anopheles atroparvus* that was susceptible to *Plasmodium berghei berghei*, by introduction of males from a line that had low susceptibility to this malaria parasite (172).

Reduction of a vector population may be accomplished by introduction of deleterious genes from a population which is ill adapted to certain climatic conditions. For example, genes from a tropical strain of *Aedes albopictus*, which has neither egg diapause nor cold hardiness, were introduced into a temperate-zone strain of *Aedes albopictus* in Illinois, with a resulting increase in egg mortality during the winter (55). The absence of a tropical-strain biochemical marker gene from the population during the following year indicates that all offspring sired by males from the tropical strain died during the winter (55).

However, not all deleterious genes may be so readily accepted by the target population, and a knowledge of the genetics of the target population is highly desirable, if not essential, before a genetic control program is implemented. For example, in principle it ought to be possible to reduce a mosquito population, or at least the proportion that serves as vectors, by significantly disrupting the sex ratio in favor of males (21). A sex-linked gene causing production of excess males in *Aedes aegypti* may have such potential (21), but its usefulness may be limited by the occurrence of three genes (two major genes on the X chromosome and a minor autosomal gene) that confer resistance to, or tolerance of, the distorter gene (192).

Although laboratory colonies may have certain desired characteristics, they may have characters of dubious value. For example, in a double-translocation line of *Anopheles stephensi*, 10 of 16 enzymes showed markedly reduced band intensity on
starch gel electrophoresis (173). The cause and significance of this have not been investigated, but van Driel et al. (173) suggested that it may have resulted from breaking up “functional gene associations” and cautioned that if the decreased enzyme activities have physiological significance, it could affect the performance of mosquitoes under field conditions.

Finally, in considering a colony as the source of material for population replacement, it must be borne in mind that the replacement population must have lower vector competence for all pathogens in the area. This may not be easy to determine or to predict, as indicated above in discussion of the specificity of vector-pathogen interactions.

Implications of Temporal Changes in Colonies

To be useful as a model or as a source of arthropods for release during genetic control programs, each colony must be genetically similar to the population from which it was derived. Therefore, founder effects, genetic drift, and selection for laboratory conditions, as opposed to field conditions, are of concern to those maintaining colonies of vectors. The subject has been reviewed with respect to mosquitoes (112) and tsetse flies (41), and in earlier sections I have summarized temporal changes in several colonies. Suffice it to say that genetic changes do occur in colonies, particularly small colonies maintained for many generations and colonies in which the maintenance regime permits inbreeding. The significance of these changes will depend upon the uses made of the colonies and the information derived from them. The relative sizes of laboratory colonies and natural populations ensure that the probability of maintaining rare alleles, or particular combinations of alleles, in a laboratory colony is much lower than it is in natural populations. It therefore follows that studies on laboratory populations will almost certainly underestimate the potential of natural populations to confound almost any control method. For any refractory strain of vector that we develop, there will probably be a parasite with genetically determined mechanisms that circumvent the resistance. For any insecticide that we develop, there will probably be some proportion of the vector population for which it is innocuous.

Possible Responses of Natural Populations to Control Measures

There is a vast literature (not covered here) demonstrating that the response of many vectors to insecticidal pressures often results in selection of resistant populations that differ from susceptible ones in behavior, reduced uptake of insecticides, increased storage, excretion or metabolism of xenobiotics, or reduced sensitivity of the target molecule in the vector. It would be naive to believe that noninsecticidal methods of control will be immune to an evolutionary response by the target species.

Samples of Anopheles arabiensis collected from indoors and from out of doors in Nigerian villages differed from each other in the frequencies of certain chromosome 2 inversions, indicating structuring of the population on the basis of resting (and possibly feeding) behavior (20). It has been suggested that under the pressure of insecticide applications, chromosomal inversions could provide the genetic basis for selection of behavioral resistance to the insecticide (20). Indeed, in a sibling species under nearly natural conditions, it has been possible to achieve significant changes in host preferences (human or bovine) in a population of Anopheles gambiae within less than six generations (37). These experiments demonstrate that there must be considerable polymorphism for feeding preference in natural populations of Anopheles gambiae and that selection is effective, even though the selective pressure can be applied to only one sex (since only female mosquitoes take blood) (37).

The experiments raise questions about the genetic plasticity of target populations with respect to other traits.

In view of the rapid and widespread evolution of insecticide resistance, one must acknowledge that it may be possible for resistance to genetic control programs to evolve through selection for mating behavior that discriminates against laboratory-reared insects (124). After considering this possibility, Pal and LaChance (124) suggested that the ideal genetic system for replacement of a vector strain with a nonvector strain “…would be one causing complete sterility in crosses and full fertility in intrastain matings and having the capacity to equip the strain for release with the same genes controlling mating behavior and adaptation to local conditions as are carried by the target population. All these conditions are met by the cytoplasmic incompatibility system.…”

The use of cytoplasmic incompatibility (CI) to drive the desired characteristics into a population is based upon unidirectional reproductive incompatibility; females from the strain carrying the CI factor (and the desired characteristics) can be fertilized by males of their own type and males of the target population, whereas females in the target population can be fertilized by males from the target population but are sterile if mated to males of the other cytoplasmic type. (The ability of CI to carry a mitochondrial DNA haplotype to fixation has been demonstrated in a caged population of Aedes albopictus [75].) The system may work best if the CI factor and the desired trait were introduced into a species that has no CI factors. If CI factors exist in the target population, the choice of a new driving CI factor is more limited and there is always the possibility that members of the target species, immigrating from another population, will bring a new CI factor and undesirable traits that will replace the “desired CI/desirable trait” population. Furthermore, crossing types for CI are not immutable and, as has been found in Culex pipiens pipiens (91) and Culex pipiens quinquefasciatus (122), they may change with time. Therefore, there is the possibility that the introduced “desired CI/desirable trait” propagule will be superseded by a newly emerging CI factor that carries it with genes for undesirable traits. There is also the possibility that the “desired CI/desirable trait” propagule will encounter more than one type of CI factor in the target population (as occurs in two Australian populations of Culex pipiens quinquefasciatus [122]) and may be effective against only one of these.

CONCLUDING REMARKS

Genetic mechanisms of susceptibility and refractoriness to pathogens have been established in several major vector-pathogen models, but a few of the most thoroughly studied models are artificial associations that have no epidemiological
significance. Much remains to be done with models that will be of direct benefit to public health. The field is now opening to studies on the molecular aspects of vector-pathogen interactions and on the application of molecular genetic techniques for creation of strains of vectors that are incapable of transmitting pathogens. The usefulness of such information will depend upon a thorough understanding of the mechanisms that control genetic variation (particularly variation in vector competence) in natural populations of vectors.

Biochemical genetics, particularly studies of electrophoretic variation in enzymes, have been widely applied to vectors and have revealed that a significant amount of genetic variation exists in most vector populations. These techniques have demonstrated, or confirmed, the existence of sibling species and genetic structuring within several species of vectors. The application of biochemical and population genetics to colonies of vectors has raised questions of how well some colonies represent natural populations. These questions must be addressed (i) to reassure us of the validity of information obtained from experiments with these colonies, (ii) to determine for which goals the work with vectors is appropriate and useful, and (iii) to establish the usefulness of these colonies as sources of material for genetic control of vectors.

There appear to be no end of suggestions for how vectors might be controlled, or replaced, in an effort to reduce the prevalence of diseases that are caused by vector-borne organisms. When attempting the application of any new technique, it is advisable to bear in mind the significant amount of genetic variation that exists in vectors, the complex relationships that exist in all vertebrate-vector-pathogen systems, and the resilience of such systems to perturbations.

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