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REVIEW ARTICLE

Flavivirus Susceptibility in *Aedes aegypti*

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Aedes aegypti is the primary vector of yellow fever (YF) and dengue fever (DF) flaviviruses worldwide. In this review we focus on past and present research on genetic components and environmental factors in *Aedes aegypti* that appear to control flavivirus transmission. We review genetic relationships among *Ae. aegypti* populations throughout the world and discuss how variation in vector competence is correlated with overall genetic differences among populations. We describe current research into how genetic and environmental factors jointly affect distribution of vector competence in natural populations. Based on this information, we propose a population genetic model for vector competence and discuss our recent progress in testing this model. We end with a discussion of approaches being taken to identify the genes that may control flavivirus susceptibility in *Ae. aegypti*. © 2002 IMSS. Published by Elsevier Science Inc.

Key Words: Aedes aegypti, Flavivirus, Vector competence, Population genetics.

The yellow fever mosquito, *Aedes aegypti*, is a well-known vector of a number of debilitating or lethal human arboviruses. Of primary concern are the yellow fever (YF) and dengue fever (DF) flaviviruses. Despite widespread availability of an effective and safe vaccine, YF remains an important public health problem in much of Africa and South America (1–3). Since the early 1990s, major epidemics have occurred annually in West Africa (4–6) and mortality rates have ranged from 25 to 50%. *Ae. aegypti* is also the major vector of DF in tropical and subtropical areas. Nearly 6 million cases of dengue have been reported in South and Central American countries as well in as the Caribbean since 1976. Dengue cases are now reported from virtually every global location in which *Ae. aegypti* occurs (7–15).

There are many components to the epidemiologic and transmission cycle of these and other arboviruses by *Ae. aegypti*. In this review we will focus on past and present research on vector components of transmission and will address the following critical questions: How much variation in vector competence among *Ae. aegypti* populations is attributable to genetic effects and how much to environmental causes? How many genes affect vector competence for flaviviruses in *Ae. aegypti*? How do alleles at these genetic loci interact to determine vector competence? How does knowledge of genetic and environmental components affect distribution of vector competence in natural populations?

Population Genetics of *Aedes aegypti*

Aedes aegypti has a cosmopolitan distribution between the 40°N and 40°S latitudes and is phenotypically polymorphic, varies in gene frequencies as detected by biochemical and molecular genetic markers, and exhibits variation in

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vector competence for arboviruses. In sub-Saharan Africa, *Ae. aegypti* appears as a black sylvan race or subspecies, *Ae. aegypti formosus*, that oviposits primarily in treeholes. A light-colored domestic race, *Ae. aegypti aegypti* is distributed in tropical and subtropical regions outside Africa. This race displays an oviposition preference for artificial containers (e.g., tires and discarded jars) associated with human habitats. Electrophoretic analysis of allozyme variation among populations from throughout the world distribution of *Ae. aegypti* identified eight genetic groups (16,17), including sylvan races (*Ae. aegypti formosus*) in West and East Africa and domestic races (*Ae. aegypti aegypti*) in East Africa, southeast U.S., southwest U.S.-Mexico, Central-South America, the Caribbean, and Southeast Asia-Pacific. Sylvan *formosus* populations from West and East Africa are clearly genetically differentiated from domestic *aegypti* populations. West African *aegypti* populations are genetically homogeneous, whereas *aegypti* populations from southeastern and southwestern U.S. are genetically differentiated. Heterogeneity was also detected among Caribbean island populations (17).

In general, genetic differentiation among worldwide populations is small relative to differentiation found among other insect populations analyzed with allozymes (17). This was suggested as evidence for recent evolutionary origins of sylvatic and domestic subspecies. Slight genetic differences detected among populations within these subspecies also suggest a recent spread and establishment of populations throughout the world. It is reasonable to suggest that much of this spread occurred through human commerce.

Recent studies have focused on more local, regional patterns of gene flow among *Ae. aegypti* populations (18–20). In a study of gene flow conducted in Puerto Rico (18), RAPD-PCR polymorphisms at 57 presumptive loci were used to examine local gene flow among 16 locations in six cities. Average gene heterozygosity was 0.354, more than twice the level detected in earlier allozyme surveys. Nested analysis of variance indicated extensive genetic differentiation among locations within cities. Effective migration rates among cities ranged from 9.7 to 12.2 migrants/generation indicating high dispersal rate over a distance of 40 km.

We have recently expanded the geographic scale of these studies by examining gene flow among locations in Mexico (19,20) (Figure 1). These studies suggest that gene flow among *Ae. aegypti* populations varies a great deal among regions depending upon amounts of human commerce as well as barriers to natural migration through flight. Gene flow is moderate, and genetic diversity is generally low among northeastern populations, which are not strongly isolated by distance. Among Yucatan populations, gene flow is low and populations are genetically isolated by distance. Genetic diversity varies greatly among Yucatan sites. Among Pacific coastal populations, gene flow and genetic diversity are high. These patterns suggest that in northeastern Mexico, *Ae. aegypti* populations may be maintained by a few individuals and experience repeated bottlenecks. Northeastern Mexico is more arid than either Pacific or Yucatan regions. Earlier population genetic studies also found great genetic distances between *Ae. aegypti* collections from southeastern U.S. (including Houston, TX) and northeastern Mexico (21). Southeastern U.S. and Mexico collections arose on separate branches in cluster analysis of allele frequencies (16). Various extrinsic factors could disrupt genetic isolation by distance in *Ae. aegypti*. Human transportation of eggs, larvae, or adults in containers along commercial routes could cause geographically distant populations to become genetically similar. Arid environments or active mosquito abatement practices cause populations to undergo genetic bottlenecks. Thus, populations in proximity could become genetically distinct. A hypothesis of frequent genetic drift is consistent with reduced genetic variability

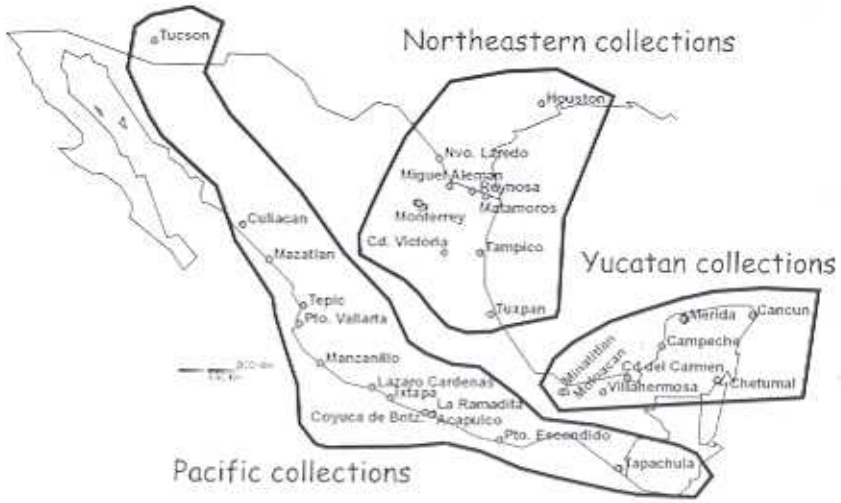

Figure 1. Locations of *Aedes aegypti aegypti* collections throughout Mexico. These collections are subdivided into regions on the basis of genetic relationships.

seen among northeastern collections. Either genetic drift or human commerce is reducing genetic isolation by distance among northeastern populations.

Gorrochótegui-Escalante et al. (20) reported that across all regions of Mexico, populations of *Ae. aegypti* can be expected to remain genetically uniform at distances <150 km. This suggests that in the absence of local selection, genes affecting dengue susceptibility or insecticide resistance should remain uniform in frequency. Furthermore, transgenic mosquitoes or release of genes into populations within 150 km of one another or along most locations of the Pacific coast should spread rapidly. At the same time, results from Nuevo Laredo (Tamaulipas, Mexico), Houston (TX, USA), Miguel Alemán (Tamaulipas, Mexico), Moloacán (Veracruz, Mexico), Minatitlán (Veracruz, Mexico), Culiacán (Sinaloa, Mexico), and Tucson (AZ, USA) populations indicate that *Ae. aegypti* populations may occasionally rapidly shift in genetic composition due either to introduction of foreign populations or genetic drift arising from founder effects.

Physiologic Genetics of Vector Competence

Figure 2 is a generalized diagram showing potential barriers to transmission that an arbovirus must overcome to be transmitted by a mosquito vector (22). Following ingestion in a bloodmeal, the arbovirus must first infect midgut epithelial cells of the vector. Presumably, virions interact with receptors on midgut epithelial cells and penetrate the cells. Uncoating, transcription, and translation of the virus genome is followed by virion maturation. Then, infectious virions must disseminate from the midgut epithelium and infect secondary target organs. If the arbovirus is blocked at early stages of midgut infection (e.g., receptor binding, uncoating, transcription, or translation), this is considered a midgut infection barrier (MIB). If infectious virions do not disseminate to hemocele (or virions to hemocele but do not infect secondary target organs), this is considered a midgut escape barrier (MEB).

Little is known of early events of flavivirus infection of vectors. For other arboviruses (e.g., bunya- and orbiviruses), there is a prerequisite for proteolytic processing of virion surface proteins for efficient vector midgut cell interaction (23–26). Interestingly, this proteolytic cleavage is not a prerequisite for successful infection of cells in secondary target organs, only for midgut epithelial cells exposed to proteolytic milieu of midgut lumen. Laminan has been proposed as a mosquito cell receptor for alphaviruses (27). However, there is no information concerning potential mosquito midgut cell receptors for flaviviruses.

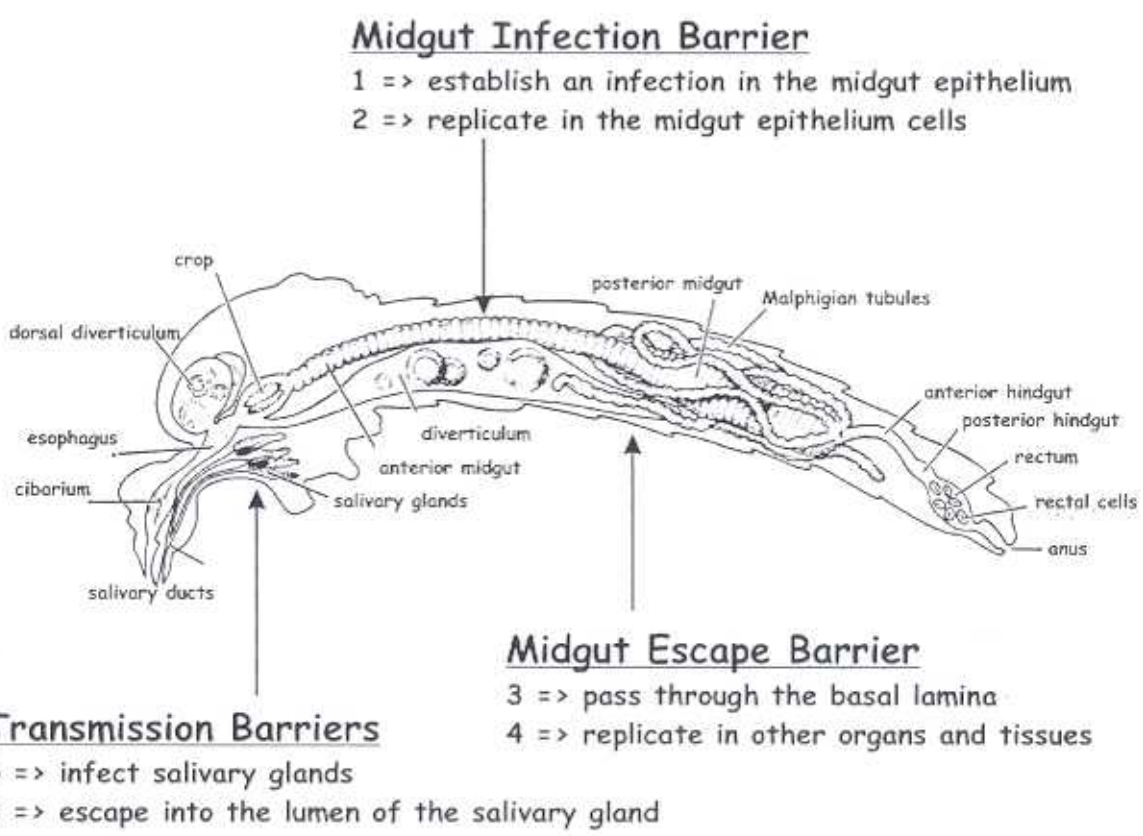

Figure 2. A generalized diagram of the six potential barriers to transmission that an arbovirus faces in being transmitted by a mosquito.

This story is complicated by the effect of arbovirus titer and passage history on vector infection. MIBs can be overwhelmed by high virus titers (22); the basis for this is unknown. Similarly, passage history of the virus can condition vector infection. For example, plaque-purified Sindbis (SIN) and LaCrosse (LAC) viruses, selected for rapid growth and virulence in mice and cell cultures, respectively, poorly infect mosquito midgut cells (28; Borucki, unpublished). Passage history also conditions dengue infection of mosquito midguts. During standardization of protocols to infect *Ae. aegypti* with DEN-2, MI rates depended strongly on the method chosen to amplify virus to include in infectious blood meal (29). Dengue virus was propagated in intrathoracically inoculated mosquitoes (14 days) or in *Ae. albopictus* C6/36 cells (7 or 14 days) (30). Virus titers did not differ dramatically among three meals, but mosquito infections rates did (29). When *Ae. aegypti aegypti* mosquitoes ingested blood meal containing 7.3 $\log_{10}$ by tissue culture infectious dose, 50% endpoint ($TCID_{50}$) per mL of virus propagated in mosquitoes, 54% of mosquitoes had disseminated infections. When blood meal contained 7.7 $\log_{10}TCID_{50}$ per mL of dengue virus propagated in cell culture for 7 days, 24% of mosquitoes had disseminated infections. When blood meal contained 8.1 $\log_{10}TCID_{50}$ per mL, 86% of mosquitoes had disseminated infections. Similarly, when *Ae. aegypti formosus* mosquitoes ingested blood meals containing mosquito-propagated, cell culture 7 day- and cell culture 14 day-propagated viruses, disseminated infection rates were 10, 0, and 86%, respectively. Virus titers did not differ significantly and would not appear to be the determinant of differing infections rates. It is more likely that the quasispecies nature of RNA virus populations could yield viruses with different efficiencies for binding with midgut receptors or for infecting midgut epithelial cells as a result of differing passage histories, the latter perhaps a fruitful area of research.

Most studies of flavivirus vector competence in *Ae. aegypti* indicate that MIB is a major determinant of flavivirus transmission (30–33). Similarly, MIB in *Cx. pipiens* with Western equine encephalitis (WEE) is associated with attachment, penetration, and uncoating of the virus in the midgut (34). MEB is associated with inefficient assembly or maturation of virions in midgut cells or inability of infectious virions to escape from midgut epithelial cells, pass through the basal lamina, and/or infect secondary target organs. MEB is a major determinant of California group virus-productive infections of vectors. Although reassortant LAC and snowshoe hare (SSH) viruses were essentially equivalent in their ability to infect midgut cells of *Ae. triseriatus* mosquitoes, only viruses containing the middle-sized RNA segment (encoding surface glycoproteins) from LAC virus efficiently disseminated from midgut cells to infect secondary target organs (35). Viruses containing the middle-sized RNA segment of SSH did not efficiently disseminate from midgut cells, despite large accumulations of viral antigen in cells. Thus, MEB can be conditioned by inefficient virus assembly or maturation in midgut cells of certain vector strains or species. A salivary gland escape barrier was also demonstrated and was confirmed by others (36). Once infectious virus is produced to sufficient titer, it must escape midgut epithelial cells, enter the hemocele, and subsequently infect secondary target organs. Thus, interruption of any of these events acts as a MEB. Inability of *Cx. tarsalis* to transmit WEE is due in part to a MEB (37). A similar result was observed in a YF refractory strain of *A. aegypti* (38).

Viral infection is disseminated throughout the mosquito body via hemolymph. Eventually, the arbovirus must infect and possibly replicate within salivary gland before it can be shed into the lumen for final transmission in a subsequent bite. Salivary gland infection or escape barriers (SIB or SEB) can prevent transmission (39–42). SIB explained in part the inability of *Cx. tarsalis* to transmit WEE (37). The virus must finally escape into the lumen of the salivary gland, where it can be transmitted to a vertebrate host during the mosquito's normal feeding activities. SEB have been identified in *Cx. tritaeniorhynchus* for Japanese encephalitis (JE) (43), *Ae. triseriatus* for SSH (44), *Ae. hendersoni* for California Group viruses (35), and *Cx. theileri* for Sindbis virus (SIN) (45). SIB was demonstrated in the Palmetto strain of *Ae. hendersoni*, in which 65% had salivary glands infected with LAC but only 5% were capable of transmission (44).

Environmental Factors Also Control Arbovirus Transmission

Biological transmission of arboviruses by a mosquito involves complex interactions among intrinsic biological factors in the mosquito and virus and extrinsic, environmental factors. Vector competence in an individual mosquito is a function of biological barriers discussed previously that an ingested virus must pass through to be replicated and finally transmitted. However, functioning of these barriers on a strictly deterministic basis would fail to explain the large variation in vector competence observed among and within vector species. Consequently, a large number of studies of vector competence during the past 30 years have focused on how genetic and environmental factors influence passage of a virus through these barriers.

The time interval between ingestion of an infective blood meal and oral transmission of virus is defined as the extrinsic incubation period (EIP) in the mosquito. Length of EIP varies among viruses and hosts and is greatly affected by environmental factors, primarily temperature, larval nutrition, and infective dose of virus. Many studies have shown that length of EIP is inversely related to incubation temperature. EIP for YF in *Haemagogus capricornii* was 28 days at 25°C and 12 days at 30°C (46). Similar relationships have been observed with *Ae. triseriatus* infected with Eastern equine encephalitis (EEE) (47), *Cx. tritaeniorhynchus* in-

fected with JE (48), *Cx. tarsalis* infected with WEE (49), and in *Cx. pipiens* and *Ae. taeniorhynchus* infected with Rift Valley fever (RVF) (50). These experiments were carried out at constant temperatures, but exposing populations to cyclical temperature regimes had little impact on EIPs in two of these studies (46,47). EIPs observed under cyclical temperatures approximated EIPs observed at the average temperature experienced during a cycle.

Effects of temperature on EIP are linear only over a limited temperature range. If temperatures are too low, the virus cycle is interrupted and the virus remains dormant. This zero development temperature was estimated as 17.5°C for *Cx. quinquefasciatus* infected with St. Louis encephalitis virus (SLE) (51). High temperatures during EIP also have a deleterious effect on virus replication. Exposure of *Cx. tarsalis* infected with WEE to 32°C caused the infection rate to decline over time (49). Values of these extreme high and low temperatures vary by mosquito and virus (52).

At least two studies demonstrated that larval nutrition has an impact on vector competence. *Cx. tritaeniorhynchus* larvae reared on low-quality diets produced greater titers of JE virus than larvae reared on high-quality diets (48). *Ae. triseriatus* larvae were fed diets that produced small, medium, or large adults (53). Small females from nutritionally deprived larvae were more efficient transmitters of LAC. This effect was attributed to larger blood meals consumed by smaller females (53). A similar result was seen with *Cx. tritaeniorhynchus* females from larvae reared at different densities and infected with West Nile virus (WNV) (54).

Infecting viral dose has been shown to influence subsequent viral titer. Obviously, at low doses the virus may not reach MI threshold. However, once MI threshold has been reached, the infecting dose may influence the number of midgut epithelial cells that become infected. This in turn could affect dissemination of virus into the the salivary gland, which suggests that EIP should be inversely correlated with infecting dose. In support of this, EIP in *Haemagogus capricornii* occurred 13 days following ingestion of a moderate viral dose and 10 days after ingestion of a high titer of virus (46).

Variation for Flavivirus Vector Competence in *Aedes aegypti*

Several studies have documented wide variation among and within populations of *Ae. aegypti* in vector competence for flaviviruses. Geographic variation in oral infection rates with YF was demonstrated among 28 populations of *Ae. aegypti* worldwide (33). Patterns of oral susceptibility correlated with the eight genetic groupings identified by allozyme analysis previously described (17). Least susceptible mosquitoes were from the sylvan *formosus* populations (7–34% with disseminated infection [DI]), whereas greatest susceptibility was found in populations of the Caribbean domestic *aegypti* subspecies (34–53% DI) and East Africa (29–57% DI). A similar set of 32 experiments analyzed 13 geographic populations of *Ae. aegypti* from the South Pacific, Southeast Asia, and East and West Africa for susceptibility to oral infection with the four dengue serotypes. Populations from the South Pacific and Southeast Asia had higher susceptibility (9–62% DI) than populations from East and West Africa (0–12% DI).

Miller and Mitchell (38) presented data on intra-population variation in oral susceptibility to YF within a population of *Ae. aegypti formosus* from Ogbomosho, Nigeria. Investigators determined the proportion of orally infected individuals with DI in 28 families and those transmitting virus into suckling mice. DI rates varied widely among families: in some families no members developed DI, whereas in others up to 70% of siblings exhibited DIs. Transmission rates were also broadly distributed among families, from 0 to 33%. In contrast, among four families from Puerto Rico (*Ae. aegypti aegypti*) DI rates ranged from 27 to 100%, while transmission rates varied from 0 to 44%. In the same study, DI and transmission rates in both subspecies were also examined in the four dengue serotypes and Uganda S and Zika flaviviruses. DI and transmission rates were correlated with those observed with YF, suggesting a generalized response for flaviviruses.

Wallis et al. (55) were able to select refractory (11% DI) and susceptible (29% DI) lines of *Ae. aegypti* from a population with average susceptibility of 15%. In contrast, Miller and Mitchell (38) were able to select completely refractory lines from an *Ae. aegypti formosus* population and a highly susceptible line (>90%) from an *Ae. aegypti aegypti* population from Puerto Rico. F_1 progeny were intermediate in susceptibility, suggesting that alleles at loci conditioning vector competence act additively. Offspring from F_2 backcrosses to susceptible parents approached susceptibility rates found in parents, but offspring from backcrosses to refractory strains remained 50% susceptible. This suggested involvement of multiple loci affecting vector competence in transmission of YF by *Ae. aegypti*.

Intraspecific variation in oral susceptibility is not unique to *Ae. aegypti* or flaviviruses. Similar variation has been identified in a diverse number of other vector species with a variety of arboviruses. These include *Ae. albopictus* for DEN. (56), Chikungunya virus (CV) (57), *Cx. tarsalis* for WEE (58,59), *Ae. triseriatus* for LAC (60), and *Cx. tritaeniorhynchus* to JE (41) and WNV (61).

Quantitative Genetics of Vector Competence in *Aedes aegypti*

Quantitative genetics provides a useful tool for determining the degree to which a phenotypic trait is controlled by genetic and environmental factors. It also provides a means to determine the ways that alleles contribute to the phenotype. We have discussed that susceptibility to arboviruses appears to be under the control of multiple loci and subject to environmental effects. We performed a quantitative genetic study

of the ability of *Ae. aegypti* to propagate DEN-2 in the midgut and in a disseminated infection in the head (31) to test the utility of quantitative genetics in assessing and quantifying genetic and environmental components of vector. This study was conducted with a standard half-sibling breeding design in which *Ae. aegypti aegypti* and *Ae. aegypti formosus* were orally infected. After 14-day EIP, $TCID_{50}$ was determined in midgut (MT) and head tissues (HT).

We showed that genes that act additively to control the ability of *Ae. aegypti* midgut epithelial cells to propagate DEN-2 virus accounted for 41% of total phenotypic variation in both subspecies (31). In *Ae. aegypti formosus*, dominant genes accounted for an additional 9% of phenotypic variation. Genes controlling the ability of DEN-2 virus to propagate in head tissues act additively in *Ae. aegypti formosus* and accounted for 39% of phenotypic variation. In contrast, in *Ae. aegypti aegypti* genes with additive effects, it accounted for only 14% of variation in HT. Instead, dominant genes appeared to control HT in this subspecies and accounted for 54% of phenotypic variation. Genes that control MT acted similarly in both subspecies, while genes that control HT differed. MIB genes in *Ae. aegypti formosus* permitted infections in only 11% of individuals, while MIB genes in *Ae. aegypti aegypti* allowed up to 65% of individuals to become infected.

Once infection of the midgut occurred, the amount of virus propagated in midgut epithelial cells was the same between subspecies. Similarly, once the infection had escaped the midgut, the amount of virus propagated in the head (and presumably other body tissues) was also the same in subspecies. Furthermore, the amount of virus in the midgut did not influence whether or how much virus would escape from the midgut.

These results suggested that at least two genes or sets of genes control vector competence in *Ae. aegypti*, one set controlling MIB, the other controlling MEB. Multigenic control of vector competence for flaviviruses in *Ae. aegypti* had been suggested earlier (32). By crossing strains of *Ae. aegypti* with high and low susceptibility to DEN-2 virus, these investigators reported that the resistant phenotype was dominant. Other authors found that several genes of major effect control flavivirus vector competence in *Ae. aegypti* (55). Miller and Mitchell also concluded that more than one gene was involved, but that there were likely two loci of major effect (38).

Mapping Genes That Control Flavivirus Vector Competence in *Aedes aegypti*

All research to date indicates that level of DEN infection in *Ae. aegypti* is a quantitative rather than a discrete variable appearing to be distributed continuously among individuals and subject to environmental effects. Recent molecular genetic and statistical advances permit mapping of loci affecting expression of quantitative traits, termed quantitative trait loci (QTL). Severson and colleagues (62,63) mapped in *Ae. aegypti* the QTL that condition susceptibility to filarial worms (64) and those conditioning avian malaria susceptibility (65). We mapped and characterized QTL that control midgut infection and escape barriers, thus conditioning vector competence of *Ae. aegypti* for dengue viruses (29). We showed that alleles at primarily two independently segregating loci create MIB (Figure 3). Alleles at these loci act additively both within each QTL and independently among QTL. More recently, Bennett (unpublished information) has identified an MEB QTL on chromosome 1 (Figure 3). Susceptibility alleles at this locus appear recessive relative to those controlling MEB. Thus, in general our results suggest that transmission of dengue is a quantitative genetic trait under control of at least three independently segregating loci.

Genetics of Vector Competence in *Aedes aegypti* Populations

Information presented in this review suggests a general model for the dynamics of flavivirus vector competence in *Ae. aegypti* populations. Our results suggest that variation in dengue infection rates among natural populations of *Ae. aegypti* may be due to segregation of alleles at each of the three QTL. Differences in dengue susceptibility between *Ae. aegypti aegypti* and *Ae. aegypti formosus* populations may reflect differences in frequency of alleles at MIB and MEB loci but may also arise from differences in the presence of specific MIB and MEB loci between populations of both subspecies. However, mapping experiments described previously are artificial because *Ae. aegypti aegypti* and *Ae. aegypti formosus* populations are sympatric in limited regions of Africa. Therefore, we do not know whether the same loci and alleles are segregating within natural populations of *Ae. aegypti*. We are currently mapping MIB and MEB alleles among collections within Mexico (Figure 1), where there is active dengue transmission, to determine whether alleles at the same QTL are segregating within a single population.

Genetic results to date suggest that alleles at the three genetic loci associated with DEN vector competence should vary independently in frequency among populations. Independent segregation in addition to a myriad of environmental factors defined earlier suggest that *Ae. aegypti* population will probably be composed of varying proportions of incompetent and competent mosquitoes. Mosquitoes will range from completely refractory to oral infection, to susceptible to midgut infection but unable to transmit virus, to fully competent to acquire and transmit DEN.

Recently, Bennett and colleagues (66) tested this model by estimating MIB and MEB rates in *Ae. aegypti* populations from throughout Mexico. Mosquitoes from these locations were raised to adults and then fed a blood meal containing 7–8 $\log_{10}$ $TCID_{50}$ of JAM1409 DEN-2. After a 2-week EIP, mosquitoes were frozen at −70°C. The head of the mosquito was removed, crushed, and tested for DEN-2 infection by a standard immunofluorescence assay. If infected, the

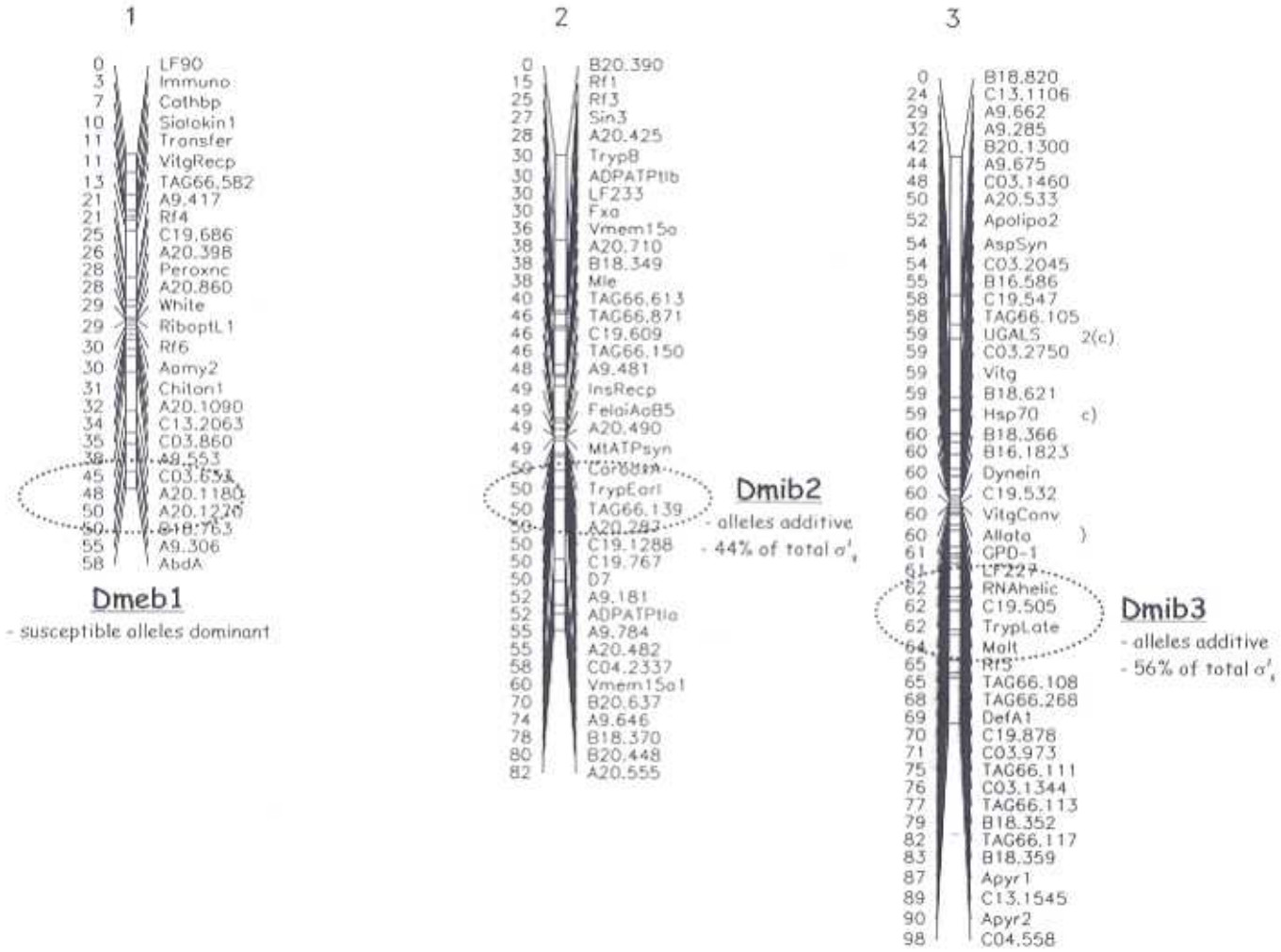

Figure 3. Locations and activities of the three QTL affecting MIB and MEB for flaviviruses in *Aedes aegypti*.

mosquito was designated as MIB−, MEB−. Midgut was dissected in mosquitoes without infected heads. If midgut was infected, the mosquito was designated as MIB−, MEB+; if midgut was uninfected, the mosquito was designated as MIB−, MEB? MIB rate was calculated as number of MIB+, MEB? mosquitoes ÷ total number of mosquitoes analyzed. MEB rate is number of MIB−, MEB− mosquitoes divided by the number of MIB−, MEB+ and MIB−, MEB− mosquitoes.

Figure 4 illustrates that there is a great deal of variation in the frequency of alleles that control DEN-2 vector competence in Mexico. However, Figure 4 also indicates that alleles do not appear to operate independently as predicted; note that there is a weak but significant correlation between MIB and MEB rates. This suggests that MIB and MEB may be controlled in part by similar genes. In other words, a gene that confers an MIB may also confer an MEB. However, the correlation is not strong, supporting our prediction that independent sets of genes control MIB and MEB.

Identification of Genes Controlling Vector Competence in *Aedes aegypti*

Our results suggest that alleles at primarily three independently segregating loci create an MIB or MEB for flaviviruses in *Ae. aegypti*. Alleles at these loci act additively both within each QTL and independently among QTL. Other loci of minor effect may also be involved. The additive genetic pattern observed could reflect differences among genotypes in 1) density of a virus receptor on midgut cells, 2) abundance of intracellular factors needed for viral replication, or 3) abundance of intracellular inhibitors that reduce viral replication. However, very little is known concerning receptors or substances in mosquito midgut cells that condition arbovirus infection and replication.

There is a variety of laboratories developing physical maps of *Ae. aegypti* and many investigators eventually envision an *Ae. aegypti* genome project. However, given the large physical size of the *Ae. aegypti* genome (750–842 Mbp) and low recombinational size (165 cM = 1.1–3.4

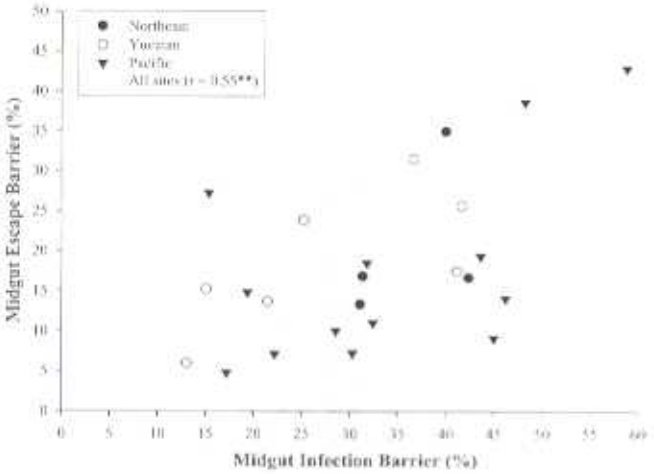

Figure 4. Relationship among percentages of mosquitoes with MIB and MEB in 24 collections obtained throughout Mexico.

Mbp/cM, depending on chromosomal location), other approaches will be essential in identifying candidate genes.

We have been targeting genes expressed in the midgut and whose genome location maps within currently identified MIB and MEB QTL. Note that alleles at the early trypsin locus strongly cosegregate with DEN-2 midgut susceptibility (Figure 3); however, so do any other genes that cosegregate with early trypsin. Early trypsin is the primary part of a unique signal transduction system. A large pool of transcribed message resides in midgut of newly eclosed adults (67,68). Translation of early trypsin is not induced by nectar feeding but by blood feeding. Its function may be to taste the incoming meal to determine whether there is sufficient protein to support a gonadotrophic cycle. If so, the signal transduction pathway activates late trypsin transcription to digest the blood meal.

We conducted a series of experiments to test involvement of trypsins in vector competence. Mosquitoes from the Puerto Rico and a highly DEN-2-susceptible laboratory strain of *Ae. aegypti* (DS3) were split into two groups. One group was fed DEN-2 infectious bloodmeal, while the second was fed infectious bloodmeal laced with soybean trypsin inhibitor (STI). In the Puerto Rico strain, treatment with STI reduced DI by 40%; in the DS3 strain, STI reduced DI by 20%. Thus, inhibition of trypsins in the midgut of *Ae. aegypti* significantly reduces DEN-2-disseminated infection rate (Bennett et al., unpublished). An identical result was obtained by knocking out the early trypsin gene (Sánchez et al., unpublished). Early trypsin, therefore, seems a reasonable candidate gene. We are currently examining involvement of early trypsin in MIB and MEB in field populations of *Ae. aegypti* in Mexico using population genomics approach (69) (Gorrochótegui-Escalante, unpublished). Microscopic investigation of DEN-2-infected midgut epithelial cells may also provide clues to MIB and MEB mechanisms. Preliminary observations suggest that apoptosis may also be involved as a mechanism of MIB and MEB (Bennett et al., unpublished).

Many approaches are underway to identify genes that condition vector competence for flaviviruses in *Ae. aegypti*. It is clear from information presented in this review that no single approach will suffice. Nonetheless, identification of candidate genes using modern genetic reagents (e.g., physical and intensive linkage maps, bacterial artificial chromosomes, express sequence tags), and techniques (e.g., subtractive cDNA libraries) would seem to offer promise in identifying genetic components in *Ae. aegypti* that condition susceptibility to the arboviruses that it transmits.

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