muscles of *P. terribilis* appear to be less sensitive to this class of sodium channel activators than the muscles of *R. pipiens*.

Although batrachotoxin, veratridine, and grayanotoxin probably do interact with the same regulatory site that controls activation and inactivation of the sodium channel, this site has been modified in *P. terribilis* (and presumably in the other members of the genus) to prevent interaction with batrachotoxin—although the modification still permits partial interaction with veratridine and grayanotoxin. It seems that the regulatory site is essential to the control of activation and inactivation phenomena and that, from an evolutionary perspective, protection from batrachotoxin was achieved in these frogs with minimal alteration of the site.

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References and Notes

1. E. X. Albuquerque and J. W. Daly, in *Receptors and Recognition: The Specificity and Action of Animal, Bacterial and Plant Toxins*, P. Cuatrecasas, Ed. (Chapman & Hall, London, 1977), series B, vol. 1, p. 297.
2. C. W. Myers, J. W. Daly, B. Malkin, *Bull. Am. Mus. Nat. Hist.* **161**, 307 (1978).
3. J. W. Daly, G. B. Brown, M. Mensah-Dwumah, C. W. Myers, *Toxicon* **16**, 163 (1978).
4. M. Neuwirth, J. W. Daly, C. W. Myers, L. W. Tice, *Tissue Cell* **11**, 755 (1979).
5. D. F. Johnson and J. W. Daly, *Biochem. Pharmacol.* **20**, 2555 (1971).
6. Levels of batrachotoxin-homobatrachotoxin in the alkaloid fraction of extracts of *Phyllobates* skin were estimated on the basis of toxic reactions in mice to samples from all five species and on chromatographic isolation of large samples from *P. aurotaenia* and *P. terribilis* (2). Time of death of the mice was dose-dependent, occurring from 4 to 14 minutes after subcutaneous injection of 0.3 to 0.1 μg of batrachotoxin (2). Homobatrachotoxin has a toxicity nearly identical to that of batrachotoxin. The presence and approximate amounts of these toxins were confirmed by thin-layer chromatography followed by specific color reaction with *p*-dimethylaminocinnamaldehyde.
7. E. X. Albuquerque, J. E. Warnick, F. M. Sansone, J. W. Daly, *J. Pharmacol. Exp. Ther.* **184**, 315 (1973).
8. E. X. Albuquerque, J. E. Warnick, J. W. Daly, unpublished data.
9. A very limited program for breeding *P. terribilis* was initiated at the American Museum of Natural History in 1976. The source of the breeding colony was a group of 13 frogs obtained in 1973 from the type locality in western Colombia (2). The two laboratory-reared frogs (F_1) used in the present experiments were 21 and 25 months of age (postmetamorphosis), and although they came from different clutches, they may have had shared parentage since the breeding pairs were not sequestered.
10. Wild-caught *P. terribilis* were fed immature crickets dusted with vitamin (Paltone) and calcium powder. Individuals raised in captivity were fed boiled lettuce while they were tadpoles; after metamorphosis they received calcium- and vitamin-dusted *Drosophila* until they were large enough for small crickets.
11. One observation was inconsistent with the tendency toward decreased production or accumulation of skin toxins in captive *P. terribilis*. A specimen caught at the type locality and maintained for 6 years and 4 months was killed 4 days after its body and limbs became grossly bloated because of water retention. Its skin contained 1150 μg of batrachotoxin-homobatrachotoxin, an amount equivalent to the original average value for the wild population and much higher than that of any other frog kept in captivity for more than a few weeks. The possibility should be considered that physiological stress stimulated toxin production in this individual. All other specimens tested for toxicity were apparently in good health when killed.
12. When mice were given subcutaneous injections of an extract equivalent to 100 mg of the skin of a *P. terribilis* individual reared in captivity (total mass of skin, ~400 mg), they experienced locomotor difficulties, gagging, and, after 5 minutes, repeated episodes of clonic convulsions, followed by recovery in 1 to 2 hours. In contrast, an injection equivalent to only 0.4 mg of the skin of a wild-caught *P. terribilis* maintained in captivity for 6 years caused severe convulsions and death within 8 minutes. Thus this individual was over 1000 times more toxic than the frog that was reared in captivity.
13. W. A. Catterall, *J. Biol. Chem.* **252**, 8669 (1977).
14. T. Narahashi and I. Seyama, *J. Physiol. (London)* **242**, 471 (1974).
15. J. G. Starkus and T. Narahashi, *Am. J. Physiol.* **235**, 204 (1978).
16. We thank M. Neuwirth for making electron microscopic comparisons of granular glands from toxic and nontoxic *P. terribilis* (4). Fieldwork was performed in collaboration with the Gorgas Memorial Laboratory, Panama, and the Museo Departamental de Ciencias Naturales, Cali, Colombia, and was partially financed by the Lincoln Ellsworth Fund of the American Museum of Natural History and by a grant from the Camille and Henry Dreyfus Foundation. This research was also supported by PHS grant NS-12063 to E.X.A.
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Genetics and the Origin of a Vector Population: *Aedes aegypti*, a Case Study

Abstract. *Thirty-four population samples representing the worldwide distribution of the mosquito* Aedes aegypti *were analyzed for variation at 19 to 22 enzyme-coding genes. A multivariate discriminant analysis revealed that the genetic differences among populations in six geographic regions and between two subspecies enable one to determine the regional origin of a population. Such studies of population genetics may have quite general applicability in studying vector-borne diseases.*

Geographic variation in the efficiency of disease transmission has been found among populations of a number of insect vectors (*1*, *2*). Thus in certain situations it may be important to know the geographic origin of a vector population. Such information would enable one to determine the potential hazard, for example, of a reinfestation after a successful eradication program. *Aedes aegypti* is the primary vector to humans of yellow fever and dengue fever virsuses, and can also transmit other diseases. We have conducted extensive population genetic studies on the species (*3*). In this report we show that populations of this vector are sufficiently genetically differentiated to allow strong inference of the geographic origin of a population.

We analyzed between 19 and 22 gene

Table 1. The *Aedes aegypti* populations that were sampled, divided into seven regions and subspecies. Subspecific classification follows Mattingly (*8*). Letters in parentheses refer to points on the graph in Fig. 1.

Country	Location	Country	Location
East African formosus *subspecies (A)*		Upper Volta	Kari village
Tanzania	Dar Es Salaam	Upper Volta	Bwombi village
Kenya	Kombeni forest	*Caribbean (D)*	
Kenya	Shimba hills	Jamaica	Montego Bay
Kenya	Kwa Dzivo village*	Puerto Rico	Mayaguez
Kenya	Mombasa	Puerto Rico	San Juan
East African aegypti *subspecies (B)*		*United States (E)*	
Kenya	Kwa Bendegwa*	United States	Indian County, Fla.
Kenya	Majengo*	United States	New Orleans*
Kenya	Mgandini*	*South America (F)*	
West African formosus *subspecies (C)*		Venezuela	Maracay
Nigeria	Enugu City	Venezuela	Caracas
Nigeria	Enugu center	*Asia (G)*	
Nigeria	Ukana	Indonesia	Jakarta
Nigeria	Mamu River forest	Indonesia	Semarang
Nigeria	Egede village	India	Bangalore
Nigeria	Abor village	Taiwan	Kaohsiung
Upper Volta	Bobo-Dioulasso		

*Two samples were obtained, about a year apart (*11*).

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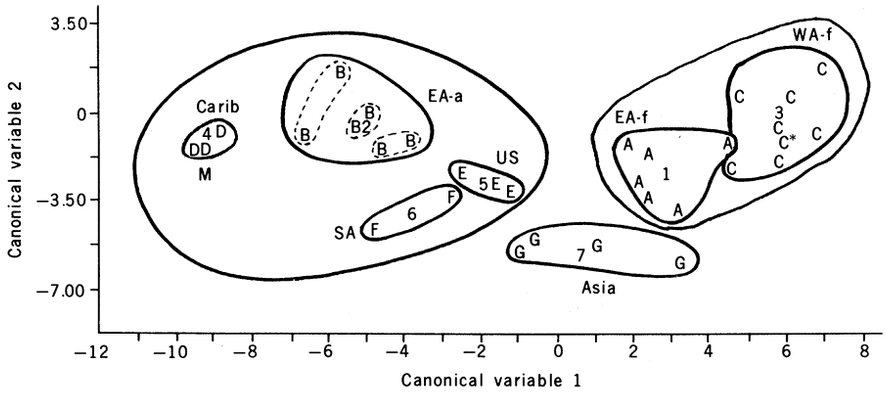

Fig. 1. Two-dimensional graph of first two canonical variables. Letters refer to the 33 samples listed in Table 1. *A*'s are East African *formosus* (*EA-f*); *B*'s, East African *aegypti* (*EA-a*); *C*'s, West African *formosus* (*Wa-f*); *D*'s, Caribbean; *E*'s, United States; *F*'s, South America; and *G*'s, Asia. *M* is the single Mexican collection. The numbers on the graph are the center for that subgroup. The two larger inclusions are *Aedes aegypti formosus* on the right and *A a. aegypti* in Africa and the New World on the left. The dashed inclusions in *EA-a* indicate samples from the same location taken 1 to 2 years apart. The *C** represents two populations that were graphically indistinguishable by this analysis. The contours are drawn in as a visual aid.

loci in 34 samples of *A. aegypti* (Table 1). We obtained two samples, about a year apart, from each of five of the populations; this served to increase the sample size and enabled us to check the temporal stability of patterns. The genes we studied code for enzymes. Variation was detected by electrophoresis of crude homogenates of single mosquitoes and by staining for specific enzyme activities. Genetic studies confirmed that the electrophoretic mobility differences were due to allelic variation at single loci. Most samples were either directly from natural populations or were F_1 offspring of individuals from the field. Between 50 and 250 genes were sampled per locus per population in most instances. For details of the samples, loci studied, and electrophoretic techniques see (*3*).

In Table 2 we present a summary of data for five of the most differentiated loci we studied. Clearly no single locus is diagnostic for subspecies or geographic origin, yet there are discrete frequency differences among the seven subsets of samples. Using a multivariate discriminant analysis we combined the information at all loci simultaneously to maximize our diagnostic powers (*4*).

Figure 1 is a graph showing the genetic differentiation based on the first two canonical variables of the discriminant analysis (*5*). These two variables account for 92 percent of the total dispersion in allele frequencies. From this figure it is apparent that each of the seven subgroups forms clusters separated by convex contours. The probability of assigning a population to its correct region or subspecies, or both, is above .9 in nearly all instances. In two instances, however, a note of caution is warranted. Although the 14 samples of East and West African *Aedes aegypti formosus* show no overlap between them (A's and C's in Fig. 1), they are very close. Further sampling may well reveal overlap. The same can be said about the samples from the United States and South America. Surprisingly, although the U.S. and South American populations are very close, the Caribbean samples cluster quite far away.

Table 2. Mean gene frequencies at five differentiated loci in the seven subsets of populations in Table 1. See (*3*) for more detailed data. Genetics and chromosome map positions may be found in (*12*).

Gene and allele	Subset (see Table 1)						
	A	B	C	D	E	F	G
IDH-2							
100	0.911	0.526	0.932	0.430	0.622	0.660	0.882
116	0.083	0.474	0.067	0.570	0.370	0.340	0.118
PGM							
100	0.778	0.937	0.548	0.985	0.877	0.983	0.968
120	0.124	0.032	0.326	0.0	0.053	0.011	0.0
Others (rare)	0.097	0.031	0.125	0.015	0.070	0.006	0.033
MDH							
84	0.201	0.071	0.170	0.028	0.081	0.036	0.141
100	0.744	0.701	0.795	0.592	0.586	0.722	0.708
120	0.055	0.226	0.035	0.380	0.332	0.252	0.154
6PGD							
100	0.803	0.987	0.976	0.789	0.971	0.932	1.00
116	0.106	0.003	0.008	0.194	0.030	0.069	0.0
Others	0.091	0.0	0.016	0.008	0.010	0.0	0.0
HK-4							
100	0.909	0.552	1.00	0.796	1.00	0.963	0.972
109	0.014	0.128	0.0	0.006	0.0	0.0	0.0
Null	0.045	0.320	0.0	0.198	0.0	0.037	0.028
Others	0.039	0.0	0.0	0.0	0.0	0.0	0.0

During the 1960's attempts were made to eradicate *A. aegypti* from much of the New World. Many countries were declared free of this mosquito, among them Mexico (*6*). Since relaxation of the eradication program, *A. aegypti* has reinfested Mexico. We have obtained one collection from northern Mexico (*7*). The "M" in Fig. 1 shows that this Mexican population is genetically very similar to Caribbean populations and probably represents an introduction from the islands rather than from the United States or South America. As recent epidemics have revealed, Caribbean *A. aegypti* are efficient vectors of dengue fever (*6*). Thus we think this reinfestation represents a potential health hazard.

Figure 1 also confirms our previous conclusions (*3*) concerning Asian populations of *A. aegypti*. Mattingly's (*8*) subspecies *formosus*, which only occurs in Africa south of the Sahara, forms a fairly close genetic group. Although there is considerable morphological variation within populations (*9*), the predominant morphological type outside Africa corresponds to Mattingly's *aegypti* subspecies. This is a truly domestic form closely associated with human habitats. The New World populations and East African *aegypti* subspecies from human habitats form a second major group. The Asian populations, although they morphologically appear to be of the subspecies *aegypti*, fall somewhere between the two major groupings. This occurs because at most genes the subspecies are very close to the *A. a. aegypti* in other parts of the world. However, at the *IDH-2* locus the gene frequencies in the Asian populations resemble those of *A. a. formosus* (Table 2). The fact that yellow fever epidemics have never occurred in Asia has long been the source of speculation concerning the vectorial capacity of Asian *A. aegypti* (*10*). Aitken and col-

leagues (*2*) have recently provided direct evidence that strains of *A. aegypti* from different regions do vary in the efficiency with which they transmit yellow fever virus. The genetic peculiarity of Asian populations, which do not fall easily into either the African *formosus* group or the African and New World *aegypti* group, may somehow be related to their relatively poor ability to transmit yellow fever. We think that some of the isozyme loci we used for the present studies may mark segments of the genome that are related to disease transmission. Thus further studies into the genetics of transmission efficiency in this species are warranted, and the analysis described here suggests a means of characterizing the genetic basis of variation in transmission efficiency.

Note added in proof: Since this report was written we have analyzed a large field sample of *A. aegypti* from Laredo, Texas. The first and second canonical variables are −10.23 and −1.06, respectively. This population is clearly genetically similar to the Caribbean/Mexican populations and is distinct from Southeast U.S. populations.

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References and Notes

1. L. Kartman, *Exp. Parasitol.* **2**, 27 (1953); W. W. Macdonald, *Ann. Trop. Med. Parasitol.* **56**, 368 (1962); W. L. Kilama, *J. Parasitol.* **59**, 920 (1973); P. H. Rodriguez and G. B. Craig, *Am. J. Trop. Med. Hyg.* **22**, 53 (1973); D. J. Gubler and L. Rosen, *ibid.* **25**, 318 (1976); R. B. Tesh, D. J. Gubler, L. Rosen, *ibid.*, p. 326; R. H. Jones and N. M. Foster, *ibid.* **27**, 178 (1978); J. L. Hardy, W. C. Reeves, R. D. Sjogen, *Am. J. Epidemiol.* **103**, 498 (1976).
2. T. H. G. Aitken, W. G. Downs, R. E. Shope, *Am. J. Trop. Med. Hyg.* **26**, 985 (1977); B. J. Beaty and T. H. G. Aitken, *Mosq. News* **39**, 232 (1979).
3. W. J. Tabachnick and J. R. Powell, *Nature (London)* **272**, 535 (1978); W. J. Tabachnick, L. E. Munstermann, J. R. Powell, *Evolution* **33**, 287 (1979); W. J. Tabachnick and J. R. Powell, *Genet. Res.* **34**, 215 (1979).
4. We used the *BMDP Biomedical Computer Programs, P-Series* [Univ. of California Press, Berkeley (1977)], for a stepwise linear discriminant analysis. The measurements $X_1, \ldots, X_p$ on each natural population are allele frequencies [from (*3*)]. The analysis aims to use these measurements to classify populations into different regions. If populations were only from East Africa or West Africa, for example, then a linear discriminant analysis seeks a canonical variable

$$c = a + b_1X_1 + \ldots + b_pX_p$$

to classify populations on the basis of their c values. The procedure used to select $a, b_1, \ldots, b_p$ is to maximize the ratio of the total to within region sums of squares of canonical variable values. If one assumes that the measurements are normally distributed and have common within-region variances and covariances, the canonical variable value of a population can be used to calculate the probability that a population comes from a given region. The classification criterion is to assign a population to the region of most probable origin. Since there are so many measurements ($p = 65$), it is desirable to have a procedure, which searches for measurements providing good classification to enter the canonical variable. A stepwise linear discriminant analysis is a sequence of distinct linear discriminant analyses, in which the analysis at step K is obtained by adding or deleting a measurement to the analysis at step $K - 1$. For the rules for adding or deleting a measurement at each step, see R. I. Jennrich, in *Statistical Methods for Digital Computers*, K. Enstein, A. Ralston, H. S. Wilf, Eds. (Wiley, New York, 1977), pp. 76–95). If a population can be classified into one of several regions, then additional canonical variables are added to the analysis; they are mutually independent within regions. The procedure for constructing the first canonical variable can be used to construct the second canonical variable subject to the independence constraint and so on. The classification criterion is the same, but now depends on additional canonical variables. In our analysis the normality assumption of measurements is reasonable on the basis of the sample sizes used in computing allele frequencies [see (*3*)]. The assumption of common within-group variances and covariances is warranted in that the most important measurements in the canonical variables [see (*5*)] are of moderate frequencies. A variance stabilizing transformation could be used on the measurements, but in previous work we have found that the conclusions reached are not sensitive to the transformations.
5. In the first canonical variable the b coefficients of the allele frequencies for HK-4^{100}, Idh2^{116}, Mdh^{120}, 6Pgd^{100}, and Pgm^{100} were 1.49, −12.51, −13.26, 3.63, and −7.12, respectively. The b coefficients for the second canonical variable were −5.34, 7.57, −1.21, −2.07, and −12.88, respectively. The constant a coefficients for the first and second canonical variables were 6.03 and 15.27.
6. *Pan American Health Organization Scientific Publication No. 228* (1971).
7. The Mexican sample represents a collection from within the city of Piedras Negras, Coahuila, which borders Texas. This sample was forwarded to Yale University by A. Hernandez.
8. P. F. Mattingly, *Ann. Trop. Med. Parasitol.* **51**, 392 (1957).
9. G. A. H. McClelland, *Trans. R. Entomol. Soc. London* **126**, 239 (1974).
10. S. F. Dudley, *J. Trop. Med. Hyg.* **26**, 985 (1934).
11. East African *A. a. aegypti* represent collections from inside houses from three villages in Rabai District Kenya. This represents one of two areas in East Africa where the domestic form is found inside village houses [see M. Trpis and W. Hausermann, *Bull. Entomol. Res.* **68**, 521 (1979)].
12. L. E. Munstermann and G. B. Craig, Jr., *J. Hered.* **70**, 291 (1979).
13. We thank the large number of fellow mosquito biologists who generously contributed to this study by providing mosquito collections, and I. R. Savage for pedagogical comments to J. Arnold. Supported by NIH grants AI 05118 and AI 12016.

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