GENETIC ANALYSIS OF ROCK HOLE AND DOMESTIC *AEDES AEGYPTI* ON THE CARIBBEAN ISLAND OF ANGUILLA

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ABSTRACT. Genetic variation was characterized at 11 enzyme coding loci in *Aedes aegypti* collected from 3 rock hole and 4 domestic sites on the island of Anguilla, West Indies. The pattern of gene frequency variation suggests that these mosquito samples do not constitute a single panmictic population, but there are no large consistent differences between rock hole and domestic forms to parallel the East African sylvan-domestic dichotomy. With the exception of one of the domestic populations, two loci did however show some gene frequency differences consistent with genetic differentiation between the 2 habitat types. We conclude that whereas there may be some degree of differentiation between the 2 habitat types, local eradication attempts and sporadic gene flow cause temporal and spatial volatility that is sufficient to swamp these differences.

INTRODUCTION

The probable ancestral habitat of the yellow fever mosquito, *Aedes aegypti* (Linn.), is African forest. Eggs are deposited in suitable tree holes where larval development takes place. From close association with humans, a domestic form of *Ae. aegypti* has come to use a variety of water containers in and around human habitations for breeding. In East Africa, the ancestral dark zoophilic "sylvan" form exists sympatrically with the pale domestic "type" form, and gene flow between them is restricted (Scott and McClelland 1975, Tabachnick et al. 1979). These 2 forms, *Ae. aegypti formosus* Walker and *Ae. aegypti aegypti*, respectively (Mattingly 1957), are otherwise largely allopatric. Some authors believe this to be an artificial division of a biological continuum (McClelland 1974), whereas others adopt the opposite extreme view and regard them as distinct species (Paterson et al. 1976). The existence of an intermediate peridomestic "feral" form in disturbed areas (Trpis and Hausermann 1975) and the absence of isolating barriers in the laboratory (Moore 1979), balanced against genetic discontinuity of the forms in nature (Petersen 1977,[3] Tabachnick et al. 1979, Wallis et al. 1983), suggest that the 2 might be incipient species. The ancestral sylvan form is confined to sub-Saharan Africa; the domestic form is distributed throughout the tropical and subtropical world (Mattingly 1957).

Although *Ae. aegypti aegypti* usually breeds in water containers associated with human habitation, it is highly opportunistic in its use of alternative breeding sites. Eggs are laid in tree holes in New Orleans (Fritchey 1978[4]) and on the Pacific Island of Funafuti (Laird and Mokry 1983), snail shells on the Msasani peninsula (Trpis 1973), half-coconut shells throughout the tropics, and rock holes on Puerto Rico (Fox et al. 1960), the Msasani peninsula (Trpis 1973), Anguilla (Belkin and Heinemann 1976) and the Cayman Islands (Nathan and Giglioli 1982).

The distribution of *Ae. aegypti* on Anguilla in the West Indies is particularly interesting. In addition to typical domestic breeding in a variety of vessels, *Ae. aegypti* makes extensive use of rock holes on the island (Knudsen 1983, Parker et al. 1983). Erosion of the island's limestone substrate has produced numerous small holes and depressions which collect water from the infrequent rain; there may be as many as 10,000 of these karst rock hole pools. This situation is notably distinct from others in terms of the large size of the feral mosquito populations and their distance from human habitation. The rock holes appear to support the vast majority of *Ae. aegypti* on Anguilla, many being more than 1 km from the nearest human habitation. Control of domestic populations on Anguilla is extensive; workers maintain *Lebistes* spp. (guppies) stocks in cisterns and treat containers with 1% Abate (temephos). The thriving rock hole population thwarts attempts to eradicate *Ae. aegypti*; individual rock holes have been sprayed (Parker et al. 1983), but large scale source reduction is clearly impractical. Knudsen (1983) and Parker

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[4] Fritchey, R. S. 1978. *Aedes* (*Stegomyia*) *aegypti* (L.); a comparative study of populations from natural and artifactual breeding sites within New Orleans, Louisiana. M.Sc. thesis, Louisiana State University, New Orleans, LA.

et al. (1983) note that the rock hole form tends to be dark, in particular the 5th hindtarsomere.

We have undertaken an isozyme analysis of samples from both habitats on Anguilla to determine the extent of genetic differentiation. Are the domestic and rock hole forms distinct breeding units with restricted gene flow, as in East Africa (Tabachnick et al. 1979), or is there a single *Ae. aegypti* population being transported around the island in vehicles and water containers, utilizing the available habitats?

MATERIALS AND METHODS

Mosquitoes: Mosquitoes were collected from 7 locations on the island of Anguilla (Fig. 1) representing a range of habitats (Table 1). Collection ANGL+SB represents collections from 2 separate locations that were inadvertently combined in the field. We have emphasized elsewhere the importance of using field-collected (or low colony generation number) material (Lorenz et al. 1984) and follow this here. ANGW is our smallest sample (derived from 14 pupae: 28 haploid genomes); elsewhere we have endeavored to maximize sample size. That these samples are representative of the local population is corroborated by 4 indirect lines of evidence: 1) we have used this method in our worldwide survey of over 100 collections, and clear genetic-geographic structuring is evident (Wallis et al. 1983); 2) repeated sampling of certain regions of interest and anomalous populations seldom reveals significant temporal allele frequency shifts (Tabachnick and Powell 1978, Tabachnick 1982, Wallis et al. 1983); 3) samples from a single egg paper very rarely show deviations from Hardy-Weinberg equilibrium; and 4) *Ae. aegypti* females tend to lay only a few eggs at a time, with oviposition of a batch spread over several days (Christophers 1960).

Eggs were hatched under vacuum in distilled water; larvae were fed on a suspension of liver powder and rabbit chow; adults were provided with raisins as a sugar source and frozen at −70°C until electrophoresed. Insectary ambient conditions were 26°C and 70% RH.

Electrophoresis: Details of electrophoretic procedures used may be found elsewhere (Tabachnick and Powell 1979). For the samples under consideration here, enzyme variation was scored at the following 11 loci (8 enzymes): *Gpd, Hk-2, Hk-3, Hk-4, Idh-1, Idh-2, Mdh, Me, Pgd, Pgm* and *Pgi*. Loci are numbered with respect to increasing anodal migration; approximate anodal mobilities relative to the common allele (termed 100) are used for allozyme nomenclature. Linkage data are available for these loci (Munstermann 1990), all of which behave in a Mendelian manner.

RESULTS

Gpd and *Idh-1* are the only loci that are monomorphic throughout. *Idh-2* and *Mdh* are polymorphic throughout; the other 7 loci are sporadically polymorphic; *Pgi* is highly heterozygous in ANGW (Table 2). Heterogeneity G-tests (Sokal and Rohlf 1981) on gene frequencies for *Idh-2* and *Mdh* reveal significant heterogeneity between samples at the 0.1% level in several cases, and at lower levels of significance in others (Table 3). Hence the samples are not drawn from a single panmictic population. ANGC and ANGW (indoor samples) have high *Idh-2*^{100} frequencies and are highly divergent from the others, the next closest population being ANGGB, a third indoor sample. ANGC and ANGGB have

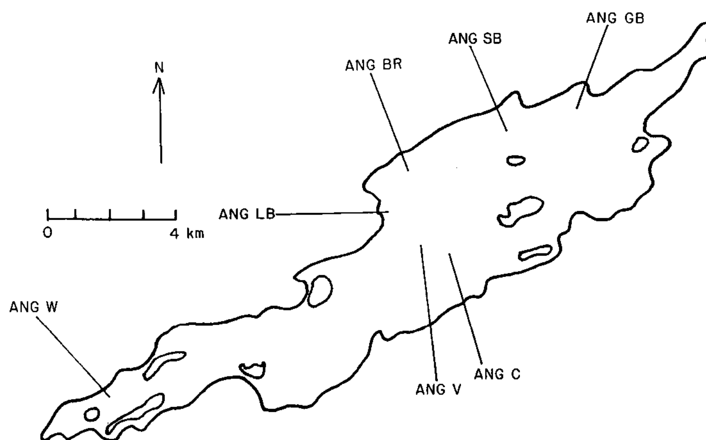

Fig. 1. Map showing locations of *Aedes aegypti* collections on the island of Anguilla.

Table 1. Details of *Aedes aegypti* collections from Anguilla.

Name	ANGC	ANGW	ANGGB	ANGV	ANGLB	ANGL+SB	ANGBR
Location	Central	West End	Gaulin Bottom	Valley	Limestone Bay	Limestone and Shoal bays	Brimogen
Habitat	Garage	Flush box	House	Houses	Rock holes	Rock holes	Rock holes
Date material collected	4–5/82 Eggs	5/82 14 pupae	11/82 Many eggs	5/83 Larvae and pupae	5–6/82 Eggs	9/82 Many eggs	5–6/82 Few eggs
Analyzed	F_0	F_1	F_2	F_1	F_0	F_0	F_0

Table 2. Gene frequencies at 9 enzyme loci in 7 samples of *Aedes aegypti* from Anguilla (*Gpd* and *Idh-1* are monomorphic in all samples. *n* = number of genes sampled).

Collection		ANGC	ANGW	ANGGB	ANGV	ANGLB	ANGL+SB	ANGBR
Hk-2	100	1.000	1.000	1.000	1.000	1.000	0.983	1.000
	113	—	—	—	—	—	0.017	—
	n	108	126	360	94	106	294	30
Hk-3	100	1.000	1.000	1.000	1.000	1.000	0.983	1.000
	110	—	—	—	—	—	0.017	—
	n	108	126	360	94	106	294	30
Hk-4	100	1.000	0.992	0.967	0.894	0.991	0.945	1.000
	109	—	—	—	—	—	0.020	—
	NULL	—	0.008	0.033	0.106	0.009	0.035	—
	n	108	126	360	94	106	256	30
Idh-2	100	0.759	0.881	0.594	0.426	0.462	0.562	0.367
	116	0.241	0.119	0.406	0.574	0.538	0.438	0.633
	n	108	126	360	94	106	292	30
Mdh	80	0.009	—	—	—	—	—	—
	100	0.324	0.524	0.335	0.819	0.594	0.493	0.667
	120	0.667	0.476	0.665	0.181	0.406	0.507	0.333
	n	108	126	352	94	106	276	30
*Me**	95	—	—	0.092	0.182	—	—	—
	100	1.000	1.000	0.908	0.818	1.000	1.000	1.000
	n	108	126	362	90	100	292	30
Pgd	80	0.130	—	0.022	—	—	—	—
	100	0.870	1.000	0.978	1.000	1.000	1.000	1.000
	n	108	62	360	94	100	256	30
Pgm	80	—	—	0.008	—	—	0.003	—
	100	0.972	1.000	0.992	1.000	1.000	0.922	1.000
	120	—	—	—	—	—	0.024	—
	140	0.028	—	—	—	—	0.051	—
	n	108	126	358	92	102	294	30
Pgi	95	—	0.484	0.025	0.181	0.011	—	—
	100	1.000	0.516	0.975	0.819	0.989	1.000	1.000
	n	108	126	360	94	90	294	30

* This locus is in linkage disequilibrium with sex (Tabachnick and Lichtenfels 1978, Wallis and Tabachnick 1982) in the polymorphic samples, and so mean allele frequencies across sexes are given.

low *Mdh*^{100} frequencies and are highly divergent from the other samples. Breaking down the pairwise comparisons into 3 groups: domestic vs. domestic, domestic vs. rock hole and rock hole vs. rock hole, gives total G values of $321.36_{(12)}$,** $332.78_{(24)}$** and $15.14_{(6)}$,* respectively. Thus the domestic samples are extremely divergent from each other and from the rock hole samples, whereas the rock hole samples are more similar to each other.

Expected heterozygosity at a locus is calculated by

$$H_e = 1 - \sum_{i=1} p_i^2$$

where p_i is the frequency of the ith allele at a locus and n = number of alleles. $\bar{H}_e$ is the arithmetic mean of H_e values over all loci (Hedrick 1983). There are no significant differences in $\bar{H}_e$ among the Anguillan populations, which range

from 0.083 ± 0.055 in ANGBR to 0.143 ± 0.053 in ANGV.

Genetic distance (Nei 1972) estimates based on all loci for all pairwise sample comparisons show close relatedness of the 3 rock hole samples ANGLB, ANGL+SB and ANGBR, although ANGL+SB is also genetically close to the domestic samples ANGC and ANGGB (Table 4). ANGW and ANGV are highly divergent from other samples, and ANGV is more similar to the rock hole samples than it is to other domestic ones. The mean genetic distance among Anguillan samples is similar to the average genetic distance among samples within our larger Caribbean islands (Table 5, Wallis et al. 1984).

DISCUSSION

The situation on Anguilla clearly does not parallel that of East Africa, where the 2 sympatric subspecies differ genetically, morphologically and behaviorally. Genetic distances among Anguillan samples are well below the average of 0.0624 between East African indoor and outdoor forms (Wallis et al. 1983). On the present isozyme evidence, Anguillan rock hole *Ae. aegypti* should not be regarded as *Ae. aegypti formosus.*

The allele frequencies do, however, indicate genetic differentiation among samples, some of it consistent with differentiation between rock hole and domestic breeding forms. Although there may be considerable gene flow among sampled populations, Anguilla *Ae. aegypti* do not represent a single panmictic unit. With this number of samples, it is difficult to discriminate between genetic differentiation between 2 habitat types obscured by gene flow, and allele frequency differences resulting from geographic separation unrelated to habitat type.

In contrast to these results, samples of domestic *Ae. aegypti* from New Orleans differ little genetically, suggesting a panmictic population (Tabachnick 1982). Similarly, panmixia has been inferred in East African villages (Tabachnick and Powell 1978) and West African cities (Wallis et al. 1983). We have also found temporal stability of aberrant gene frequencies in Weslaco, TX (Wallis et al. 1983, unpublished data). As these observations are based on the same sampling technique as used here, we feel that the differences that we have observed on Anguilla do not reflect sampling error.

The 3 rock hole populations were sampled where the karstic holes are most numerous. The similarity of ANGL+SB and ANGGB suggests that gene flow occurs between rock hole and domestic sites in this area of the island. The similarity of ANGV with rock hole samples may be a result of an influx of rock hole mosquitoes. As the amount of genetic divergence among populations on Anguilla resembles the divergence observed on larger islands (Table 5, Ta-

Table 5. Genetic distance values with standard errors (D ± SE) between populations of *Aedes aegypti* within 4 Caribbean islands. n = number of collections.

Island	n	D ± SE
Anguilla	7	0.022 ± 0.004
Puerto Rico	4	0.023 ± 0.005
Dominica	3	0.019 ± 0.007
Trinidad	4	0.027 ± 0.007

Table 3. Summary of heterogeneity G-tests for *Igh-2*^{100} (above diagonal) and *Mdh*^{100} (below diagonal) allele frequencies in pairwise comparisons of Anguillan *Aedes aegypti* samples (NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Collection	ANGC	ANGW	ANGGB	ANGV	ANGLB	ANGL+SB	ANGBR
ANGC		*	***	***	***	***	***
ANGW	**		***	***	***	***	***
ANGGB	NS	***		**	*	NS	*
ANGV	***	***	***		NS	*	NS
ANGLB	***	NS	***	***		NS	NS
ANGL+SB	**	NS	***	***	NS		*
ANGBR	***	NS	***	NS	NS	NS	

Table 4. Pairwise genetic distance values over 11 loci for 7 Anguillan *Aedes aegypti* samples.

Collection	ANGW	ANGGB	ANGV	ANGLB	ANGL+SB	ANGBR
ANGC	0.031	0.005	0.047	0.018	0.009	0.029
ANGW		0.035	0.045	0.042	0.036	0.054
ANGGB			0.032	0.010	0.004	0.017
ANGV				0.012	0.021	0.010
ANGLB					0.003	0.001
ANGL+SB						0.007

bachnick and Wallis 1985) where the geographic distance between populations is much greater, we believe that the disparate habitat types play some role in maintaining differentiation.

The loci that suggest genetic differentiation consistent with habitat differences are *Idh-2* and *Mdh*; these and other chromosome II enzyme markers are important in determining worldwide genetic geographic groups (Wallis et al. 1983) and may mark regions having an effect on susceptibility to oral infection with yellow fever virus (Tabachnick et al. 1985). It is interesting that the loci determining color (white abdomen, spot, black tergite and yellow larvae), a character important in distinguishing *Ae. aegypti* and *formosus*, are also on chromosome II (Petersen 1977,[3] Munstermann 1990). In fact, *Idh-2, Pgm* (two loci found to be informative, Wallis et al. 1983), *wa, s, Bt* and *y* all lie within 33 map units of each other in the center of the linkage group. Parker et al. (1983) report that Anguilla rock hole mosquitoes tend to be dark, the 5th hindtarsomere particularly so, but the only known locus controlling this feature is on chromosome III. Preliminary studies have also detected differences in developmental rate and insecticide resistance between the 2 Anguillan forms (Tabachnick 1991).

We conclude that Anguillan *Ae. aegypti* samples show significant genetic heterogeneity, some of which may be attributable to the different habitats available on the island.

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