ANNUAL PROJECT SUMMARY REPORT

FOR

RICELAND MOSQUITO MANAGEMENT PROGRAM

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Submitted by

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Mosquito Population Ecology Effort (Part could also come under *Survey*):

Mosquitoes of the *Anopheles quadrimaculatus* group were sampled intensively in several sites in Mississippi to determine the sibling species composition of each site. Species composition was determined by means of electrophoresis, following Lanzaro (1986: PhD Diss., Univ. of Florida) and Narang et al. (1989: Ann. Ent. Soc. Am. 82: 508). The aim was in part to determine if there were any ecological differences between the species in habitat preference, and partly to find which were most likely to affect human health in the Delta, and especially the Cleveland area where an abatement district has just been funded. The results of this survey are given in Table 1. It can be seen that the Delta sites (Stoneville, Cleveland, and Delta National Forest) were almost pure species A. In the Hill Country of Mississippi, the Noxubee Wildlife Refuge has previously been reported to contain only species A and B. (Lanzaro, Narang et al., loc. cit., and pers. comm.). Surprisingly, Noxubee had species A, B, and a third putative species, here nicknamed ?C. Gene frequencies at some diagnostic loci are given in Table 2. The 110 allele at Idh-1 is much commoner in species ?C than in A or B; the 100 allele may be homozygous at Idh-1 in species ?C, as is the case for many species A; however, species ?C is usually homozygous for the 100 allele at Idh-2. The two Got loci usually confirm the diagnosis. Species ?C is awkward to assign at present, since it does not fit the species C extensively studied by Narang et al. Their species C has a diagnostic allele at Had-3, which is not the case here (Table 2). Further work is planned in collaboration with Lanzaro, Narang, Kaiser, and Seawright to establish whether species ?C can be assigned to a known species (perhaps C or D) or whether it is new.

Species ?C forms about 14% of the *An. quadrimaculatus* group individuals collected in Noxubee. Either it was present, but missed by previous workers, or it was much rarer and has perhaps undergone a population explosion. It will be interesting to follow this species in future years.

It is puzzling that so many species of similar mosquitoes should exist sympatrically. One might expect some degree of niche differentiation between these sibling species. Preliminary work by the Arkansas Unit, particularly Dana Bassey, has suggested that species B is relatively more common in undisturbed, forested habitats. In Mississippi this is true for Noxubee; however, species B might also be expected in primary swamp forest habitat like the Delta National Forest. The latter area has ancient forest, as evidenced by the large size of the bald cypresses (*Taxodium*), in whose hollow trunks many of the *An. quadrimaculatus* were collected (Blue Lake Campground). And yet, only one individual of species B was found in this habitat (Table 1).

There is some evidence for microhabitat preferences of the sibling species at Noxubee (Table 1). In particular relatively more species A and less species B and ?C were found in the Boat Dock area than the HQ area. Differences in species composition between Noxubee subsites were significant. It is also possible that there

is some seasonality in the occurrence of these species (Lanzaro, pers. comm.). This has not yet been tested, but could be responsible for some of the heterogeneity of subsites, since the samples were collected at different times of year. Future work should indicate whether seasonality or microhabitat preferences are involved. In particular, mating will be investigated to find if they are localized in different areas for the different species.

A major effort was expended on rearing broods from eggs laid by single wild females collected from natural resting sites. The purpose of this work was threefold. (1) This work will indicate the likelihood of multiple insemination. (2) Any gene flow between the sibling species can be detected in the broods. This data will complement ongoing investigations into linkage disequilibria within each species. (3) A proportion of the progeny in each brood is preserved as eggs, larvae, pupae, and adults enabling detailed morphological work to proceed on all of the life stages, while another portion of the progeny can be analysed genetically in order to identify species.

Analysis of the data for objectives 1 and 2 has not yet been analysed. However, multiple insemination and interspecific gene flow both appear rare or absent.

Morphological work involving the scanning electron microscope is currently being performed by Mark Beisler, a student in the department. To date no morphological characteristics which can be used to separate the species have been found. However, the search does not seem an impossible one. Narang et al. noted that the larvae of species B are often green, whereas those of species A are usually brown. The emerging adults of species B also show the green body trait, so the colour of each brood run on the gels were scored green, greenish brown, or brown. Table 3 shows that a green or greenish colour is significantly associated with species B, and that species A is more usually brown. Nonetheless, the colour is not easy to score, and scoring just on the basis of larval colour could lead to errors. Larvae were mostly reared in white enamel trays. However, one brood which was reared in a black enamel tray produced extremely darkly-pigmented larvae. If facultative colour change is normal, it could be that quite different larval colours are present in the field. The search for diagnostic morphological markers continues.

Economic Impact Assessment Effort:

Knowledge of insecticide efficacy is important for mosquito abatement districts. If there is evidence of insecticide resistance, insecticides to which resistance has evolved should be avoided. Adult *Anopheles quadrimaculatus* were therefore taken from two sites, a horse barn in Boyle (near Cleveland), and Noxubee, MS. In Cleveland, mosquito control and agricultural practices might be expected to lead to exposure to insecticides. Mosquitoes from Noxubee Wildlife Refuge are assumed to have rarely come into contact with insecticides. Adult *Anopheles* were tested for two hours in vials coated internally with known quantities of permethrin. The results are

summarized in Fig. 1. Essentially, *An. quadrimaculatus* from Noxubee were as tolerant to permethrin as were those from Cleveland.

Biting habits of *Anopheles quadrimaculatus* in Cleveland did seem different to those in Noxubee. Whereas no bites from *Anopheles* during the daytime were ever received in Noxubee, the Cleveland populations were much more agressive to man; many bit while the collections were being made. Again, it seems possible that this is an environmental rather than genetic difference, and needs more investigation.

Publications:

Mallet, J. 1989. The evolution of insecticide resistance: have the insects won? Trends in Ecology and Evolution 4: 336-340.

Mallet, J. (1989) Modeling: mixtures vs. rotations. Pesticide Resistance Management (WRCC-60 Newsletter) 1(2):26-27.

Meetings attended:

S-230 Annual meeting, San Diego, CA. February 13-15, 1989.
Mississippi Mosquito Control Workshop, Jackson, MS. March 9, 1989.
Mississippi Mosquito Control Commission, Cleveland, MS. July 19, 1989.
S-230 informal meeting and collaborative fieldwork, Stuttgart, AR. July 17-18, 1989.
City Mosquito Control Meetings, Clarksdale, Cleveland and Greenville, MS. July 20-21, 1989.
S-230 Meeting, Shreveport, LA; in conjunction with Louisiana Mosquito Control Association. October 16, 1989.
Mississippi Mosquito Control Commission, Jackson, MS. November 28, 1989.
Entomological Society of America, Annual Meeting, San Antonio, TX. December 10-14, 1989

TABLE 1. Biochemical identification of sibling species of the *Anopheles quadrimaculatus* group from sites in Mississippi. Sample size = 329.

Site	Resting site	Numbers of individuals		
		Species A	Species B	Species ?C
Noxubee	HQ Hut A	23	70	13
	HQ Hut B	8	24	7
	HQ Tree Holes	0	13	4
	Campground	2	1	0
	Boat Dock restrooms	14	9	3
	Totals	47	117	27
	Percent	25%	61%	14%
Stoneville		29	0	0
	Percent	100%		
Cleveland	W.Cleveland, Church	26	0	0
	Boyle, Horse Barn	18	0	0
	Totals	44	0	0
	Percent	100%		
Delta National Forest, Blue Lake Area				
	Under bridge	29	0	0
	Campground	35	1	0
	Totals	64	1	0
	Percent	98%	2%	

Species ?C may be the same as species C or D of Seawright, Narang, Kaiser, et al., or it may be entirely new. A G-test of homogeneity revealed significant differences in species composition between resting subsites at Noxubee ($G = 24.74$, d.f.$= 8$, $P < 0.005$).

TABLE 2. Frequencies of alleles at various loci in the three species found at Noxubee Wildlife Refuge.

	Species A	Species B	Species ?C
Idh-1			
110	.03	0	.63
100	.75	.02	.37
86	.17	.98	0
83	.05	0	0
Idh-2			
209	.02	0	0
181	.02	0	0
162	.19	1.00	.88
132	.66	0	.12
100	.11	0	0
Got-1			
130	0	0	0
122	.09	.03	0
109	0	.11	0
100	.87	.75	.38
98	.04	.11	0
89	0	0	.44
83	0	0	.13
69	0	0	.06
Got-2			
170	.01	.02	0
138	0	0	0
115	.02	.02	0
100	.92	.86	.06
80	.01	0	.06
38	.04	.10	.88
Had-3			
156	.06	.07	.07
100	.92	.91	.90
45	.02	.02	.03
25	0	0	

Notes: Data for species A and B taken from Narang et al., loc. cit. Data for species ?C taken from a small sample of 26 individuals, including brood parents.

TABLE 3. Colour of abdomen in broods in relation to sibling species in *Anopheles quadrimaculatus* species group from Noxubee, MS. Biochemical species identification is about 99% accurate, and is based on two isocitrate dehydrogenase loci. Broods heterozygous or ambiguous for diagnostic alleles at either of these loci have been omitted. (Additional broods tentatively identified to species if one locus was polymorphic are in parentheses).

Colour:	Brown	Green or Greenish Brown	Totals
Species A	6(+4)	0(+2)	6(+6)
Species B	7(+0)	33(+6)	40(+6)
Species ?C	1	3	4

Species A and B have significantly different fractions of green and brown: $G = 17.68$, $P < 0.001$. This is also true if broods with one Idh locus polymorphic are included: $G = 20.12$, d.f.=1, $P < 0.001$.

IGURE 1: DOSAGE-MORTALITY DATA FOR NOPHELES QUADRIMACULATUS: OXUBEE, MS., AND CLEVELAND, MS.