COMPARATIVE SUSCEPTIBILITY OF SPECIES A, B AND C OF *ANOPHELES QUADRIMACULATUS* COMPLEX TO INFECTION WITH SUBPERIODIC *BRUGIA MALAYI* AND *BRUGIA PAHANGI* (NEMATODA: FILARIOIDEA)[1]

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ABSTRACT. Susceptibilities of natural populations of sibling species A, B and C of the *Anopheles quadrimaculatus* complex and the colonized strain A to subperiodic *Brugia malayi* and *Brugia pahangi* were compared. All 3 sibling species showed varying degrees of susceptibility to both *B. pahangi* and *B. malayi*, and they were considerably more susceptible to *B. pahangi* than to *B. malayi*. The rate and intensity of infection to *B. pahangi* were highest for species A (66.2% and 7.4 L_3/female, respectively) and lowest for species B (21.3% and 1.7 L_3/female). For *B. malayi* these values were higher for species A (29.7% and 1.84 L_3/female) than for species B (13.3% and 0.86 L_3/female) and C (12.6% and 0.75 L_3/female). The colonized strain A of *An. quadrimaculatus* was significantly more susceptible to both *Brugia* species than the natural populations of sibling species A, B and C.

INTRODUCTION

Until recently, *Anopheles quadrimaculatus* Say was considered a single species (Darsie and Ward 1981). However, genetic studies on natural populations from the eastern United States revealed that it is a complex of at least 4 sibling species, A, B, C and D (Narang et al. 1989a, 1989b). All 4 species occur in Florida. *Anopheles quadrimaculatus* is a proven vector of malaria parasites (Boyd 1949), but it is not a natural vector of *Brugia* species. However, colonized strains of *An. quadrimaculatus* have been found to be susceptible to several species of animal filariae, e.g., *Brugia pahangi* (Buckley and Edeson) (Schacher 1962), *Brugia patei* (Buckley, Nelson and Heisch) (Nayar et al. 1984), *Dirofilaria immitis* Leidy (Nayar and Sauerman 1975), *Dirofilaria tenuis* Chandler (Pisty 1958), and *Dirofilaria striata* (Molin) (Nayar et al., unpublished data), but not *Brugia malayi* (Buckley) (Orihel and Pacheco 1966) or *Wuchereria bancrofti* (Cobbold) (Yangco et al. 1984). We recently reported that genetically selected, colonized mutant strains of *An. quadrimaculatus* are susceptible to subperiodic *B. malayi* adapted to and maintained in nude mice, and *B. pahangi* and *B. patei* adapted to and maintained in jirds (Nayar et al. 1989). We subsequently showed that in addition to genetic factors which control susceptibility to *B. malayi*, other factors like vertebrate host adaptation and mosquito nutrition affected the susceptibility of our colonized *An. quadrimaculatus* strain A to *B. pahangi* and *B. malayi* (Nayar et al. 1990, Nayar and Knight 1991).

Most *Anopheles* occur as complexes of sibling species (Narang and Seawright 1990). Natural and laboratory populations of sibling species of the *Anopheles gambiae* Giles complex from Africa show variation in susceptibility to the human filarial parasite, *W. bancrofti* (Mnzava et al. 1989, Hunt and Gunders 1990). Two strains of *Anopheles barbirostris* Van der Wulp, which belong to a species complex in Southeast Asia, also show variations in susceptibility to subperiodic *B. malayi* (Choochote et al. 1984). These studies suggest that the natural populations of sibling species of the *An. quadrimaculatus* complex might also show variations in susceptibility to filarial parasites. In this report, we compared susceptibilities of species A, B and C of the *An. quadrimaculatus* complex to the filarial parasites, *B. pahangi* and subperiodic *B. malayi*. Susceptibility is usually used in the sense of the ability of pathogens to mature in the insect to the stage at which they can be transmitted to the host. In this paper, to measure susceptibility, the rate of infection with third stage larvae and the intensity of infection with third stage larvae per female are considered.

MATERIALS AND METHODS

Natural populations of *An. quadrimaculatus* were collected twice, at 2-wk intervals, from 4 locations in north and central Florida from March 1 to June 15, 1990. The collection sites were: a) Lake Rousseau, Citrus Co.; b) Lake Octahatchee, Hamilton Co.; c) Bear Bay Swamp, Dixie Co.; and d) Lake Panasoffkee, Sumter Co. Members of the 3 sibling species (A, B and C) are present at one or more of these

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Table 1. Rate of infection and intensity of infection of 3 species of *Anopheles quadrimaculatus* complex, on the 11th day after feeding on jirds infected with *Brugia pahangi* and *B. malayi*.

Species	No. of females dissected	Infection rate (%)	Intensity of infection $L_3/♀$ $\bar{x} \pm SE$
		Brugia pahangi	
A	77	66.2 b	7.4 ± 1.2 b
B	61	21.3 d	1.7 ± 0.8 c
C	122	39.3 c	5.5 ± 1.5 b
Colonized strain A	127	94.5 a	19.6 ± 1.3 a
		Brugia malayi	
A	37	29.7 f	1.84 ± 0.79 f
B	98	13.3 g	0.86 ± 0.38 g
C	247	12.6 g	0.75 ± 0.25 g
Colonized strain A	86	64.0 e	4.79 ± 0.79 e

[1] Duncan's (1955) multiple range test. Means followed by the same letters are not significantly different ($P < 0.05$).

locations (Seawright et al., unpublished data). Laboratory colonized strain A of *An. quadrimaculatus*, highly susceptible to *B. malayi* (Nayar and Knight 1991), which was maintained on a 10% sucrose solution containing 0.1% *p*-aminobenzoic acid (PABA), was used as a control for comparison with the natural populations. Field-collected females did not feed readily on *Brugia*-infected jirds in the laboratory. Therefore, they were maintained on a 10% sucrose solution with 0.1% PABA for at least 4 days and starved overnight before they were allowed to blood-feed on jirds infected with *B. pahangi* or *B. malayi*. About 50 females from the colonized strain A were fed during the period of an hour on the same jird as the field-collected females. Since smaller numbers of field-collected females fed on the infected jirds at the time of feeding, 10 colonized females were dissected individually in a drop of Hanks' balanced salt solution and the number of microfilariae ingested were counted. The mosquitoes fed on a jird with a microfilaremia of 250 ± 10 mf/20 μl of blood, and it resulted in a mean ingestion of 80 ± 12 microfilariae per blood meal (range 50–120 mf), which was considered acceptable for our purposes. Only the fully engorged females were separated and maintained on a 10% sucrose solution with 0.1% PABA at 24 ± 1°C. No mortality was observed in blood-fed females during the next 10 days. Preliminary experiments with colonized females showed that developing larvae reached the third stage in the thoracic muscles, but did not start to migrate into the hemocoel or the head until the 12th day at the temperature used. On the 11th day, development of filariae was determined by dissecting the head and thorax of each female; the abdomen of each female was frozen at −20°C for later identification of the species. *Brugia pahangi* is a cogenic species of *B. malayi* and their development is very similar in their mosquito hosts (Beckett and Macdonald 1971). Therefore, different stages of larval development were categorized as described by Schacher (1962). Wild-caught females and the laboratory colonized strain A were identified by electrophoretic methods (Narang et al. 1989a), DNA probes (Johnson 1990)[5], or mitochondrial DNA (Mitchell 1990)[6].

Data were statistically analyzed using an analysis of variance and Duncan's (1955) multiple range test.

RESULTS

Sibling species A, B and C were present in the collections from the 4 locations. The Lake Rousseau collection was composed of species A, Lake Octahatchee species A and B, Bear Bay Swamp species C, and Lake Panasoffkee species A and C. Sibling species D was not present in these collections. Although similar numbers of *B. pahangi* and *B. malayi* microfilariae were ingested by each of the sibling species, based on the number of microfilariae ingested by the colonized strain A, they showed varying degrees of susceptibility to both *B. pahangi* and *B. malayi* (Table 1). All 3 species were significantly more susceptible to *B. pahangi* than to *B. malayi*. Species A and C were not significantly different

[5] Johnson, D. W. 1990. Quick blots and nonradioactive detection systems: improvement on methods for DNA hybridizations using mosquitoes. Ph.D. dissertation, University of Florida. 88 pp.

[6] Mitchell, S. E. 1990. Mitochondrial and ribosomal DNA analysis for the identification of sibling species of the mosquito *Anopheles quadrimaculatus* Say. Ph.D. dissertation, University of Florida. 81 pp.

in susceptibility to *B. pahangi*, but both were significantly more susceptible than species B. The rate of infection and intensity of infection were 66.2% and 7.4 ± 1.2 L_3/female for species A, 39.3% and 5.5 ± 1.5 L_3/female for species C, and 21.3% and 1.7 ± 0.8 L_3/female for species B, respectively (Table 1). Females of all of the natural populations were significantly lower ($P < 0.05$) in susceptibility compared with the colonized strain A (Table 1).

The 3 sibling species ingested almost similar numbers of microfilariae of *B. malayi*. Species A was more susceptible, with significantly higher rate of infection (29.7%) and intensity of infection (1.84 ± 0.79 L_3/female) than both species B and C (13.3% and 12.6% infective rate and 0.86 ± 0.38 and 0.75 ± 0.25 L_3/female, respectively) (Table 1). Species B and C were not significantly different from each other in susceptibility to *B. malayi*. All 3 species were significantly lower ($P < 0.05$) in susceptibility than the colonized strain A (Table 1).

A summary of the susceptibility of the 3 sibling species and colonized strain A in terms of numbers of L_3/female are shown in Table 2. Although filaria larvae developed to the infective stage in some of the mosquitoes of each species, a lack of susceptibility to infection with both *B. malayi* and *B. pahangi* was prevalent among all 3 sibling species of the field-collected mosquitoes. The variable numbers of L_3 developed in individual mosquitoes also indicated considerable genetic differences within each species, with a small percentage of very susceptible mosquitoes in each species.

Table 2. The percentage of individuals of 3 species of *An. quadrimaculatus* complex and colonized strain A recorded with various concentrations of infective third stage *Brugia* larvae.

No. of L_3 larvae/♀	Species A	Species B	Species C	Colonized strain A
		Brugia pahangi		
0	33.8	78.7	60.7	5.5
1–5	29.9	14.8	19.7	18.1
6–10	13.0	1.6	6.6	14.2
11–20	14.3	1.6	4.1	20.5
20–30	5.2	1.6	4.9	16.5
>30	3.9	1.6	4.1	25.2
		Brugia malayi		
0	70.3	86.7	87.4	36.0
1–5	18.9	9.2	8.9	37.2
6–10	2.7	2.0	1.6	11.6
11–20	5.4	1.0	1.6	9.3
20–30	2.7	0	0	4.7
>30	0	1.0	0.4	1.2

DISCUSSION

Sibling species A, B, and C of the *An. quadrimaculatus* complex showed variation in susceptibility to both *Brugia* species. The physiological reasons for variations in susceptibility of the sibling species to either *B. pahangi* or *B. malayi* were not evaluated in this study. They could be either due to damage to ingested microfilariae by cibarial and/or pharyngeal armatures in the alimentary canal as shown by Bryan and Southgate (1988) in sibling species of the *An. gambiae* complex to *W. bancrofti*, or due to the presence of other physiological factors which may inhibit development of ingested microfilariae in the thoracic muscles of these mosquitoes.

The colonized strain A is more susceptible to both species of *Brugia* than the field-caught species A, B and C of *An. quadrimaculatus*, assuming that the females of these species ingested a similar number of microfilariae of each *Brugia* species. This is probably due to the biological characteristics of colony mosquitoes which are genetically very different from those in nature. As pointed out by Hunt and Gunders (1990), only a very small portion of the species gene pool is included when establishing a colony. Theoretically, this "bottleneck" effect may result in genes affecting susceptibility being included or excluded at an unnaturally high frequency. In general, species A from the natural populations were also more susceptible than the other 2 species, but more data should be collected from additional populations to ascertain whether species A is genetically more suitable as a vector for the filaria.

Vernick and Collins (1989) and Vernick et al. (1989) suggested that the joint action of 2 unlinked esterase loci (Est A and Est C) and Pif-B locus controls expression of the susceptible and refractory phenotypes of *Anopheles gambiae* G_3 strain to infection of the malarial parasite, *Plasmodium cynomologi*. Though we have not examined the genetic basis of refractoriness trait or its linkage to other genes in 3 sibling species of the *An. quadrimaculatus* complex, there are significant esterase electromorph differences among the 3 species (Narang et al. 1989a, 1989c).

We used *B. pahangi* and *B. malayi* to study susceptibilities of the sibling species of *An. quadrimaculatus* complex, because these filariae were readily available in animal models. Human infections with naturally occurring *Brugia* species in North America, though rare, have been reported during the last 30 years (Orihel and Beaver 1989). These authors concluded that because *Brugia* species have been reported to infect animals throughout the world and have a demonstrated ability to infect humans as well, it is likely that zoonotic infections will be recognized

with increasing frequency and will be shown to have an even wider geographical distribution. In this context, it is of interest that the sibling species of the *An. quadrimaculatus* complex show variations in susceptibility to the *Brugia* species.

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