creased survival between the egg and pupal stage, therefore at least in later instars, much of the apparent increase in density must be attributed to delayed development, to which increased survival is the key. In other words, with some increase in survival (perhaps limited to earlier instars, perhaps not), density of larvae increases; when density increases this leads to delayed development which causes an apparent further increase in density. This situation cannot continue to amplify without there being a reduction in survival. The reduction in survival may be indicated by the levelling out of the asymptotic curves (as in Fig. 3), or even in the reduction in density in the largest plants which is most strongly evident in instar III larvae. It may thus be that the greatest delay in development is to be found in instar III larvae.

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LABORATORY RESISTANCE OF THE MOSQUITO *ANOPHELES QUADRIMACULATUS* TO THE MERMITHID NEMATODE *DIXIMERMIS PETERSENI*

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ABSTRACT. Larvae of a Lake Charles colony of *Anopheles quadrimaculatus* that has been used for 4 years to produce *Diximermis peterseni* now show resistance to this biological agent. The infection rate by the nematode is only half that of the Gainesville colony from which the Lake Charles colony originated. The mechanism of resistance was observed to be avoidance of attack by larvae when nematode larvae were present.

In the course of routine culturing of the mermithid nematode *Diximermis peterseni* in the mosquito host *Anopheles quadrimaculatus* at the Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana, we observed a decline in nematode production. This laboratory colony, which had been used the past 4 years to produce *D. peterseni* (a possible biological agent) was obtained from the Insects Affecting Man Research Laboratory, Agricultural Research Service, U.S.D.A., Gainesville, Florida, about 12 years ago. The culture method was as fol-

lows: One-month-old damp sand nematode cultures were flooded for 12 hr. Hatched preparasites in an amount to provide an approximate 10:1 ratio to the host, were then poured into rectangular (24 x 40 cm) enamel rearing pans with 3rd- to early 4th-instar *An. quadrimaculatus* larvae. Usually nematodes developed and dropped from hosts in 5 to 7 days. Pupae from surviving uninfected larvae were routinely returned to the mosquito colony to produce adults. Since there had been no change in the rearing procedures, resistance in mosquitoes was suspected. Briggs and Rothenbuhler (unpublished WHO Document, 1975) in their review of resistance of insect hosts to bacterial, fungal, and viral pathogens, concluded that selection for resistance does occur through cellular or humoral responses to pathogens by individual insects in populations. However, resistance of mosquitoes to biological control agents has never been reported. The possibility that *An. quadrimaculatus* had developed resistance to *D. peterseni* was therefore investigated.

MATERIALS AND METHODS. Several methods were used to expose larvae of the Lake Charles colony and also *An. quadrimaculatus* larvae from the Insects Affecting Man Research Laboratory, Gainesville, Florida, that had never been exposed to the preparasitic (infective) stage nematodes. In the 1st series of tests, small groups of early-instar hosts were isolated in spot plates (9 spot depressions per plate) and exposed for 2½ hours to either a 1:1 or 5:1 ratio of 0 to 24-hr-old preparasitic nematodes. Then the larvae were placed in clear plastic containers (9 x 13 cm), reared for 5 days, and dissected to determine the presence and number of nematodes.

In the 2nd series the normal culture method was used. Thus solutions of water and preparasites were poured directly into 24 x 40-cm white enamel rearing pans that contained early 3rd-instar hosts. Ratios of 5:1 and 10:1 (preparasites to hosts) were used; host densities ranged from 300 to 700 larvae per pan. After 5 days, 25 randomly selected larvae from each pan were dissected to determine the presence and number of nematodes.

A 3rd series of tests was made to determine whether a physiological factor was the basis for the observed pattern of resistance. Since it was necessary to obtain single-nematode infections for this purpose, exposures were made in white enamel rearing pans with a 10:1 ratio of preparasites from the Lake Charles strain and a 1:1 ratio of preparasites from the Gainesville strain. After 5 days, individual larvae were isolated in 35 x 10-mm petri dishes and held until the nematode emerged. Twenty-five nematodes from singly infected larvae of each strain obtained in this manner were then weighed individually and measured.

RESULTS AND DISCUSSION. Our results showed clearly that the 2 strains of mosquitoes differed greatly in susceptibility to nematode parasitism. At the ratio of 1:1 used in the 1st tests, infection rates were almost twice as high in the unselected (Gainesville) strain as in the selected (Lake Charles) strain (Table 1). The results of the 2nd series of tests (Table 1) further substantiate this difference. At the 5:1 ratio and normal rearing conditions, the unselected strain had more than twice the number of infected hosts and twice the average number of nematodes than the selected strain; at the 10:1 ratio, the infection level and average number of nematodes per host were closer but still higher in the unselected strain, and 31% more nematodes penetrated. However, the ranges of weights and lengths of nematodes that emerged from the 2 strains of *An. quadrimaculatus* (Table 2) were quite close, though the means were slightly higher in the selected strain. (The t values were significant but not highly so.) These slight differences could result from better adaptation of the selected (Lake Charles) strain to our normal rearing conditions.

The reason for the appearance of resistance in the selected (Lake Charles) strain of *An. quadrimaculatus* is undoubtedly our practice of restocking the colony with pupae from larvae that escape infection

Table 1. Results when selected (Lake Charles) and unselected (Gainesville) strains of *An. quadrimaculatus* were exposed to preparasitic *D. peterseni* in spotplates or in rearing pans.

Strains	Prepara-site to host ratio	No. larvae examined	Percent infection	No. and (%) nematodes penetrating	Average no. nematodes per larva
		Exposed in spotplates			
Selected	1:1	362	31	146 (40)	1.30
"	5:1	252	88	736 (58)	3.31
Unselected	1:1	346	59	270 (78)	1.32
"	5:1	202	96	770 (76)	3.96
		Exposed in rearing pans			
Selected	5:1	50	42	37 (15)	1.76
"	10:1	75	71	151 (20)	2.85
Unselected	5:1	50	90	141 (56)	3.13
"	10:1	75	99	383 (51)	5.17

when they are exposed to the preparasitic nematodes. The exact number of generations it has taken to produce this observed selection is not known. The maximum number of generations based on 1 complete cycle every 2 weeks for 4 years would be 104 generations; however, the number is certainly less than this because every generation was not exposed for a variety of reasons, for example, the press of other duties. In selection experiments with the silkworm, *Bombyx mori,* against a cytoplasmicpolyhedrosis virus, Aizawa, et al (1961) found resistance in the 12th and 13th generations; also, Uzigawa and Aruga (1966) and Watanabe (1967) produced resistant strains in only 5 generations. Kulincevic and Rothenbuhler (1975) obtained noticeable resistance by honey bees, *Apis mellifera,* to a virus in two generations. Resistance in 10 generations in a field-collected strain of house fly, *Musca domestica,* to the beta-exotoxin of *Bacillus thuringiensis* was reported by Harvey and Howell (1965). In view of the number of generations required for such workers to detect resistance, it is more than probable that our population of *An. quadrimaculatus* has been exposed to *D. peterseni* for a sufficent number of generations and subjected to selection pressures which have resulted in resistance.

The nature of resistance can be behavioral, physiological, or a combination. Our observations indicate differences in the behavioral responses of the 2 strains. Larvae of the selected strain were much more active; they moved about and snapped at the attacking nematodes. Those of the unselected strain remained still for longer periods. Even in the spotplate tests, which were very stressful be-

Table 2. Lengths and weights of 25 *D. peterseni* from the selected (Lake Charles and unselected (Gainesville) strains of *An. quadrimaculatus.*

Parameters	Measurements of preparasites of *D. peterseni* from			
	Selected strain		Unselected strain	
	Length (mm)	Weight (mg)	Length (mm)	Weight (mg)
Mean	19.33± 2.52	0.56±0.15	17.93± 2.24	0.44±0.17
Range	13.25—24.0	0.2 —0.9	14.75—24.0	0.2 —0.9
t[1]	2.07	2.61	2.07	2.61

[1] Calculated t values significant.

cause of the forced proximity of host and parasite, larvae from the unselected strain were more often successfully parasitized than were larvae from the selected strain.

It is plain when one compares the data for the rearing pan exposure tests that the selected larvae can avoid attack and therefore have a lower infection rate and a lower nematode-to-host average than larvae from the unselected strain. This is the 1st report of a mosquito developing resistance to a biological control agent.

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EVALUATION OF TWO MOSQUITO-REPELLING DEVICES

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ABSTRACT. Two electronic devices emitting sound waves which, according to label and advertised claims, ward off most female mosquitoes, for a distance of 0.9 to 2.5 ms (3–8 ft) were tested to ascertain their effectiveness as mosquito repellents. Evaluations were conducted in a chamber under practical-use conditions as defined by Soltavatta (1947) to be a distance of 10 in. The results of all evaluations indicated that the devices did not afford protection against the bites of *Aedes aegypti* mosquitoes as claimed by the manufacturer under the conditions used in this study. Only one species of mosquito was used in this study because it has been observed by Soltavatta (1947) that the flight sound pitch is practically the same for all species of mosquitoes.

INTRODUCTION

For about a century, insect control has meant primarily chemical control as well as biological and cultural control. Compared with studies on insecticidal chemicals and means for applying them, research on physical control of insects has not been extensive. Even most of the new non-insecticidal controls such as sterilants or hormones are, with rare exceptions, chemical. Among the many types of energy that could be used in insect control—electricity, heat, light, ionizing radiations, radio-frequencies, pressure, and sound—I tested only sound.

Sound is used by many insects for courtship, mating, and echolocation (Wigglesworth 1969). The acoustical vocabulary of invertebrates is relatively small because of highly specialized emitting organs and limitations of the nervous system. The hearing organ in the mosquito is located in the antennae. One of the specialized organs that responds to pressure oscillations is the Johnston's organ, a chordotonal organ lying in the 2nd segment of the antenna with its distal insertion in the articulation between the 2nd and 3rd segments. The Johnston's organ perceives movements of the antennal flagellum, and most of the sensillae comprising the organ give phasic responses, potentials only developing during and immediately after the