OBSERVATIONS ON THE SUSCEPTIBILITY OF CERTAIN CULICINE MOSQUITO SPECIES TO INFECTION BY *LANKESTERIA CULICIS* (ROSS) [1]

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ABSTRACT. Several culicine mosquito species not previously recorded as being naturally infected by the protozoan parasite, *Lankesteria culicis*, were exposed to this parasite under controlled laboratory conditions. In addition to *Aedes aegypti* (the natural host of *L. culicis*), infections were established in test populations of *Aedes epactius, A. sollicitans, A. stimulans, A. vexans, Culiseta inornata* and *Psorphora columbiae*. *L. culicis* failed to infect *Culex pipiens quinquefasciatus* and *C. salinarius*. Host responses to the infections are described and discussed.

INTRODUCTION. The protozoan genus *Lankesteria* includes at least 4 species that are known to parasitize mosquitoes. These species are *L. culicis* (Ross) first observed by Ross (1895) in larvae of *Aedes aegypti* (Linnaeus) collected in India; *L. tripteroides* Guenther described by Guenther (1914) as parasitizing larvae of *Tripteroides dofleini* Guenther in Ceylon; *L. barretti* Vàvra isolated from larvae of *A. triseriatus* Say in the United States by Vàvra (1969); and *L. clarki* described by Sanders and Poinar (1973) as a parasite of larvae of *A. sierrensis* (Ludlow) collected in the United States.

Among the mosquito-infecting lankesterian species described thus far, *L. culicis* appears to be the most cosmopolitan in its distribution. In addition to various isolations of *L. culicis* from *Aedes aegypti* collected in India (Ross 1895; Hati and Gosh 1963), this protozoan species has been reported parasitizing larvae of *A. aegypti* in South America (Marchoux et al. 1903); *A. koreicus* Edwards in China (Feng 1930); *A. geniculatus* Oliver in Czechoslovakia (Kramer 1957); *A. ingrami* Edwards in Africa (Garnham 1958); and *A. aegypti* in a large portion of the southeastern United States (Barrett 1968; Gentile et al. 1971). On the basis of these records, it seems that *L. culicis* is capable of parasitizing a wide range of mosquito species. However, there are conflicting reports on the ability of this protozoan to infect various mosquito species. Garnham (1958) stated that *L. culicis* is capable of parasitizing a variety of species belonging to the genera *Armigeres, Anopheles, Culex,* and *Aedes*. Feng (1933), on the other hand, determined that, in addition to *Aedes* mosquitoes, only *Armigeres obturans* (Walker) can be infected by *L. culicis* in nature. This same author was unable to infect *Culex*

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pipiens Say with *L. culicis* isolated from *Aedes koreicus* (Feng 1930). Similarly, Ray (1933) was not able to infect a *Culex* species with *L. culicis* from *Aedes albopictus;* Kramer (1957) could not induce infections of *A. communis* (DeGeer), *A. cantans* (Meigen), or *Culex pipiens* with *L. culicis* from *A. geniculatus;* and Barrett (personal communication) was unable to infect *A. triseriatus* Say with *L. culicis* from *A. aegypti.* On the other hand, Sanders and Poinar (1973) were able to infect 53 percent of an *A. sierrensis* test population with *L. culicis* from *A. aegypti.*

To examine further the ability of *Lankesteria culicis* to parasitize different mosquito species, several culicine species not previously recorded as being naturally or experimentally infected with this protozoan were exposed to *L. culicis* populations from *A. aegypti* under controlled laboratory conditions. These preliminary exposure tests were conducted as part of a comprehensive study of the biology of *L. culicis* in unnatural mosquito hosts and involved mosquito species belonging to the genera *Aedes, Culex, Culiseta,* and *Psorophora.*

METHODS. The mosquito species utilized in the study described herein were *Aedes aegypti, A. epactius* (Dyar and Knab), *A. sollicitans* (Walker), *A. stimulans* (Walker), *A. vexans* Meigen, *Culiseta inornata* (Williston), *Culex pipiens quinquefasciatus* Say, *C. salinarius* Coquillett and *Psorophora columbiae* (Dyar and Knab).

Specimens of *Aedes aegypti* and *A. epactius* used in this study were from laboratory colonies maintained at Texas A&M. Specimens of *A. stimulans* were hatched from eggs collected by W. R. Horsfall near Champaign, Illinois. The other mosquito species were hatched from eggs deposited by wild females caught in the vicinity of College Station, Brazos County, Texas. One hundred individual larvae of each mosquito species were exposed to sporocysts of *L. culicis.* The sporocyst stage of the parasite infects mosquito larvae through ingestion. The sporocysts used in these tests were harvested from an infected laboratory colony of *A. aegypti* maintained at Texas A&M.

Each group of 100 1st instar larvae was placed in 18 x 28.5 x 5 cm enamel pans each of which contained 1 liter of deionized water seeded with ca. 10,000 mature sporocysts of *L. culicis.* The sporocysts were counted out from the stock supply under the optics of a binocular compound microscope and introduced into the larval rearing medium by means of a fine-tipped pipet. A slurry of ground Tetramin® fish food was pipetted onto the bottom of each pan and served as a food source for the mosquito larvae. Control populations of each species were set up at the same time as were the test populations. Each control population consisted of 100 1st-instar larvae treated exactly as were the ones used in the tests except that sporocysts of *L. culicis* were not added to the rearing medium. The amount of water placed in the enamel pans containing larvae of *Culex salinarius* and *C. pipiens quinquefasciatus* was reduced so as to force the larvae to feed off the bottom of their respective pans where sporocysts tended to be most concentrated. The test pans containing larvae and sporocysts as well as the control pans containing only larvae of each mosquito species were subsequently placed in an incubator set at 27° C and 80 percent relative humidity where the mosquitoes were held throughout the remainder of their development.

Each day the test and control pans were checked for larval death and a fresh slurry of ground fish food was added. Dead larvae were dissected immediately (i.e., before decomposition commenced) in Ringer's solution. Each larva was carefully opened along the dorsal midline so as not to disturb the digestive tract and associated organs and the hemocoel was examined for the presence and distribution of parasites. Tissue and organs were then removed from each dissected specimen, placed on slides as wet mounts and observed for the presence of *L. culicis* under

the optics of a compound microscope with a resolving power of 430x. Also, approximately one-half of the larvae that survived to the 4th-instar in each pan were dissected and examined for parasites. The rest of the mosquitoes ($\leq$50 specimens) were allowed to continue their development. Mosquitoes reaching the pupal stage were transferred to emergence vials where they were allowed to complete development to the adult stage. All adults emerging in these vials as well as mosquitoes that died in the pupal stage were dissected and observed for the presence of parasites utilizing techniques outlined above.

The major objective of the research described herein was to determine whether or not infections of *L. culicis* could be induced in experimental populations of the various mosquito species. However, the mortality rates for the infected portion of each test population and for all individuals in each control population were also recorded so as to gain some insight into the effect of induced infections on the survival rate of each given mosquito species. The mortality rates for the infected portion of mosquitoes was based on death and survival of only the infected individuals within the population of $\leq$50 specimens not dying before reaching the fourth instar nor sacrificed for examination as fourth instar larvae. The percent mortality realized for each test population was corrected by Abbott's formula, thus, taking into account those mosquitoes dying within the corresponding control population for a given species.

RESULTS. Mosquitoes infected with *Lankesteria culicis* were observed in test populations representing 7 of the 9 species exposed to the parasite throughout larval development (Table 1). The range of infected individuals within each of these 7 test populations was between 98 (98 percent) for *A. aegypti* and 93 (93 percent) for *A. sollicitans*. The corrected mortality level within the infected portion of each given mosquito population not sacrificed for parasite examination was the lowest for *A. aegypti* (11.3 percent). In comparison, the mortality levels within infected populations of the 6 other species ranged between 86.8 percent for *C. in-*

Table 1. Occurrence of infection and mortality rates within populations of ten culicine mosquito species exposed to sporocysts of *Lankesteria culicis* throughout larval development (100 larvae per test).

Mosquito Species Exposed to *Lankesteria culicis*	Normal Breeding Habitat of Mosquito	Percent Infected[1] (%)	Percent Mortality: Unexposed Control Group (%)	Percent Mortality: Infected Portion of the Test Group[2] (%)
Aedes aegypti[3]	Artificial containers	98.0	3.0	11.3
Aedes epactius	Rockpools	96.0	9.0	86.3
Aedes sollicitans	Salt marsh	93.0	15.0	77.4
Aedes stimulans	Temporary pools	94.0	23.0	80.6
Aedes vexans	Floodplain and temporary pools	94.0	8.0	84.1
Culiseta inornata	Ground pools	95.0	3.0	86.8
Psorophora columbiae	Rice fields and grassy pools	95.0	11.0	81.2
Culex pipiens quinquefasciatus	Foul water and ground pools	00.0	00.0	00.0
Culex salinarius	Ground pools and salt marsh	00.0	00.0	00.0

[1] Percent for each species represents the total infected mosquitoes within both the population of 50 exposed individuals that were sacrificed for parasite examination and the population of $\leq$50 exposed individuals that were not sacrificed.

[2] Percent mortality corrected by Abbott's formula and reflects the death of mosquitoes prior to their reaching the adult stage within only the infected portion of the $\leq$50 individuals not sacrificed for parasite examination.

[3] A natural host for *Lankesteria culicis*.

ornata and 77.4 percent for *A. sollicitans*. Mosquito death occurred most commonly during the pupal stage. All of the infected specimens of *A. aegypti* that died had progressed to the pupal stage. Mortality rates within the control populations ranged between 0.0 percent for the *Culex* species and 23.0 percent for *A. stimulans*.

All extracellular stages (gamonts and gametocysts) of *L. culicis* were confined to the lumens of the Malpighian tubules in the infected pupae of *A. aegypti* and no evidence of encapsulation of the parasite in this mosquito species was observed. In contrast, *L. culicis* was never observed in the Malpighian tubules, and encapsulated extracellular stages of the parasite were frequently observed in infected pupae of the other 6 species (Fig. 1). The majority of the extracellular parasite populations within the pupae of each of these 6 species were confined to areas between the peritrophic membrane and epithelium of the midgut of their respective hosts. Extracellular stages of *L. culicis* occurring within sacrificed 4th-instar larval specimens of *A. aegypti* were all found in the posterior regions of their hosts' midguts. In contrast, the extracellular stages of *L. culicis* were found to be distributed throughout the expanse of the midgut in the larvae of the 6 other infected mosquito species (Fig. 2). It should be noted that the intracellular stages of *L. culicis* (the cephalin stage) were always found confined to the midgut epithelium of early larval instars of each of the 7 mosquito species that were infected by the parasite.

None of the mosquitoes comprising the experimental populations of *Culex p. quinquefasciatus* and *C. salinarius* were infected with *L. culicis* (Table 1). Examination of specimens of these 2 species revealed that some had ingested sporocysts and that the sporozoites had emerged from these sporocysts. However, these sporozoites had not penetrated the gut wall of their host. In some cases the parasites had metamorphosed to the cephalin stage of development and numerous young cephalins were observed within the gut contents of their respective hosts (Figs. 3 and 4).

DISCUSSION. The ability of *Lankesteria culicis* to infect 7 of the 9 mosquito species used in these experiments indicates that this parasite may have a rather broad range of potential host species. The reason why *L. culicis* has been found to infect only one of these species (i.e., *Aedes aegypti*) under natural field conditions is uncertain. Part of the explanation may lie in the type of larval breeding habitat preferred by other mosquito species infected by *L. culicis* during the course of our experiments (Table 1). For example, examination of the literature revealed that various species of *Lankesteria* are known to infect at least 8 mosquito species under natural field conditions. These mosquito species are *Aedes aegypti* (Ross 1895), *A. koreicus* (Feng 1930), *A. albopictus* (Ray 1933), *A. geniculatus* (Ganapati and Tate 1949), *A. ingrami* (Garnham 1958), *A. triseratus* (Vàvra 1969), *A. sierrensis* (Sanders and Poinar 1973), and *Tripteroides dofleini* (Guenther 1914). Each of these species characteristically occupies larval habitats which are considered to be quite confining to the larval stages and protected from temperature extremes. Such confined larval habitats would certainly enhance the frequency of contact between the susceptible stage of the host (mosquito larva) and the infective stage of the parasite (sporocyst). Protection from thermal extremes would promote a greater opportunity for infection of these mosquitoes by *Lankesteria* since high temperatures tend to reduce the viability of the sporocyst stage of the parasite (McCray et al. 1970). With the exception of *A. aegypti* and *A. epactius*, the larval habitats of the other 5 species experimentally infected with *L. culicis* during our studies all tend to be less confining in nature, hence, reducing the chances for host-parasite contact (Table 1). Also these habitats tend to be more exposed to environmental extremes which might be detrimental to the parasite. The mosquito species were infected with *L. culi-*

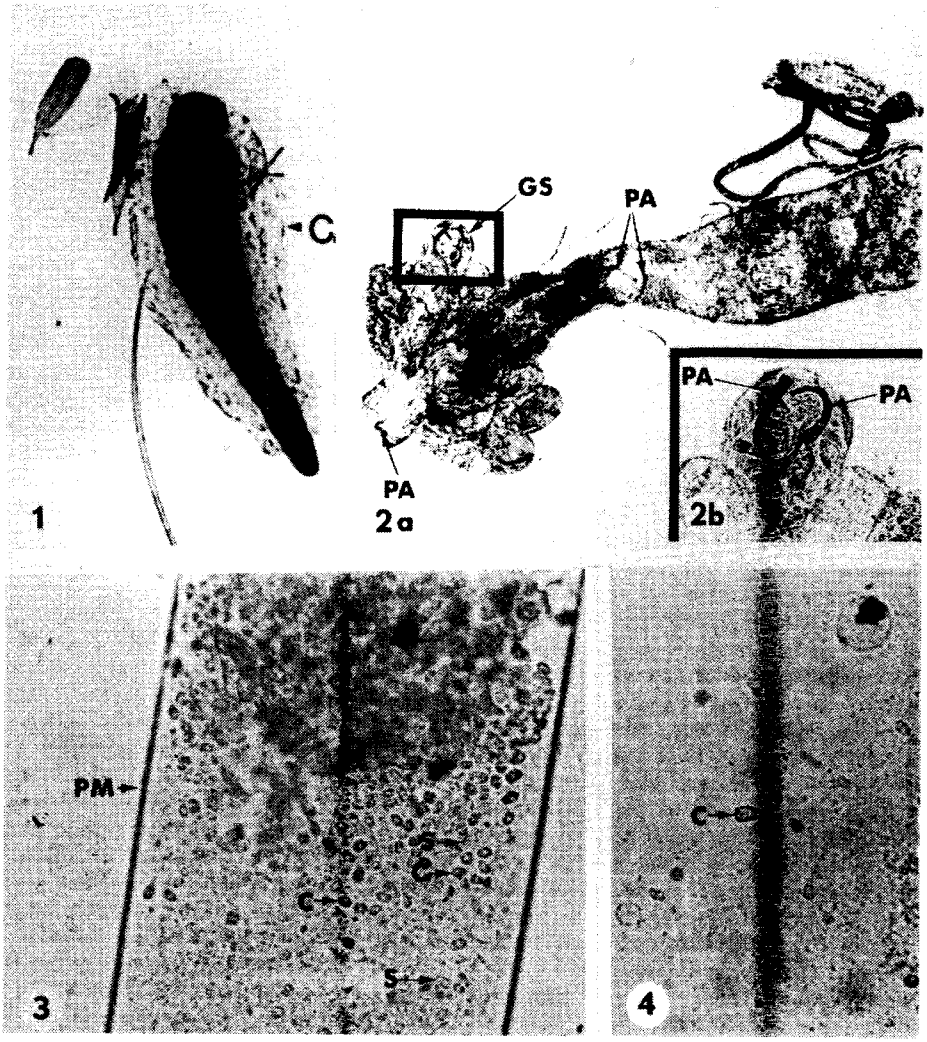

Fig. 1. Light micrograph showing an encapsulated, melanized gamont of *Lankesteria culicis* (C) observed in *Aedes epactius*.

Fig. 2. Light micrograph of the alimentary canal of an *Aedes epactius* specimen infected with *L. culicis:* (a) gamonts (PA) shown present in the interior portions of the midgut lumen and in the gastric caecae (GS), (b) an enlargement of a gastric caecum showing gamonts of *L. culicis* (PA).

Fig. 3. Light micrograph of the midgut contents of a *Culex pipiens quinquefasciatus* specimen infected with *L. Culicis* showing numerous cephalins (C) and sporocysts (5) of the parasite bounded by the peritrophic membrane (PM).

Fig. 4. Light micrograph of the gut contents of a *Culex salinarius* specimen infected with *L. culicis* showing a cephalin (C) of the parasite.

cis in our tests by confining their larval stages in close proximity to the sporocysts of the parasite under moderate temperatures. It would, therefore, appear that these species are at least susceptible to infection if the habitat conditions are favorable. The weakening effects of stress placed on the developing mosquitoes by the laboratory conditions of confinement may account for at least some of the mortality that occurred when they became infected with *L. culicis*.

A given mosquito species' response to infections by *L. culicis* may also be influential in determining whether or not this parasite will be found in association with the mosquito in nature. Infection of *A. aegypti* with *L. culicis* apparently has little or no effect upon the survival rate of this mosquito species (McCray et al. 1970). The results reported herein tend to support this conclusion (Table 1). However, *L. culicis* infections appear to be somewhat lethal to *Culiseta inornata, Psorphora columbiae* and to the other 4 species of *Aedes* used in our experiments. Each of these 6 mosquito species also demonstrated that it has the ability to encapsulate the extracellular stages of the parasite whereas *A. aegypti* did not demonstrate this ability. Hence, even though *L. culicis* is able to infect several other mosquito species besides *A. aegypti* under controlled conditions, host mortality and encapsulation of the parasite might tend to reduce further the chances of this protozoan establishing an association with these other mosquito species under natural conditions.

There appears to be some mosquito species, particularly *Culex* species, which are clearly not susceptible to infection by *L. culicis* even though they can be induced to ingest the infective sporocyst stage of the parasite. Reasons for the inability of the parasite to infect *Culex p. quinquefasciatus* and *C. salinarius* are not known. Results recorded herein indicate that, although the parasites emerge from the sporocysts upon ingestion by larvae of the *Culex* species, they are unable to penetrate the peritrophic membrane (Figs. 3 and 4). Failure to gain access to the epithelial cells of the host's midgut is apparently lethal to the parasite. These parasites appeared to have ceased their development upon entering the cephalin stage, and subsequent examination of pupae and adult stages of *C. salinarius* and *C. p. quinquefasciatus* revealed no evidence of the parasite. The parasite load within the midgut of these 2 mosquito species may well have been flushed out along with the rest of the gut contents at the time of pupation.

Research designed to study further the interaction of *L. culicis* with unnatural mosquito hosts is presently in progress at our laboratories.

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