ASCOGREGARINE PARASITES AS POSSIBLE BIOCONTROL AGENTS OF MOSQUITOES

Michelle Tseng
Department of Zoology, University of British Columbia, 2370-6270 University Blvd,
Vancouver, British Columbia, V6T 1Z4

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INTRODUCTION

The seemingly ubiquitous presence of gregarine parasites in numerous mosquito taxa (Table 1; Chen 1999) suggests that consideration of these parasites as biological control agents may be a worthwhile endeavor. However, because most studies prior to 1985 did not demonstrate significant fitness (e.g., mortality, adult size) differences between infected and non-infected mosquitoes, Beier and Craig (1985) deemed in their overview of gregarine parasites of mosquitoes that

> "There is no evidence that gregarines can be used to control mosquitoes, and no evidence that gregarines in their natural habitat have a significant negative impact on populations of their normal host." (p 182)

However, the authors did suggest that because "conventional strategies for controlling container-breeding mosquitoes are not effective, the possibility of using gregarines in unnatural mosquito hosts should not be ruled out."

A number of additional studies have since been published that have examined the pathogenicity of gregarine parasites both for natural and non-natural hosts, as well as for hosts reared in stressful vs. non-stressful environments. This paper reviews the outcomes of these studies and addresses whether the prospects of using gregarines as mosquito biological control agents have changed with these recent findings.

GREGARINE LIFE CYCLE

Much progress has been made in understanding the details of the gregarine life cycle in the last 20 years. These studies have mainly focused on the development and within-host movement of *Ascogregarina taiwanensis* (Chen and Yang 1996, Chen et al. 1997a, Chen 1999, Chen and Fan-Chiang 2001), the gregarine commonly found in *Aedes albopictus*. The life cycle is similar to other species in the same genus, and it is described briefly here (Fig. 1). Oocysts ingested by early mosquito instars release sporozoites, which then enter into host epithelial cells and develop into trophozoites. Prior to mosquito pupation, trophozoites migrate from the midgut into the Malphighian tubules, where they transform into either macro- or microgametes. During the pupal stage, 2 gametes fuse to form a gametocyst, within which hundreds of oocysts are formed (Chen 1999). Oocysts are shed into rearing containers by metamorphising adults as well as by any adults that happen to die in the containers. Greater detail on gametocyst formation, trophozoite migration, and sporogonic development can be found in Chen et al. (1997a), Chen and Fan-Chiang (2001) and Chen et al. (1997b) respectively.

IMPACT OF GREGARINE INFECTION ON MOSQUITO FITNESS

ASCOGREGARINA TAIWANENSIS

The colonization and rapid expansion of *Aedes albopictus* in North and South America from Asia in the mid-1980s generated renewed interested in the ecology, population genetics, and breeding structure of this mosquito (Black et al. 1988, Kambhampati and Rai 1991, Kambhampati et al. 1991, Rai 1991, Ayres et al. 2002, Birungi and Munstermann 2002, de Oliveira et al. 2003). Accompanying these were studies detailing the fitness effects of *A. taiwanensis* infection on *Ae. albopictus* as well as on other mosquito species (Garcia et al. 1994, Comiskey et al. 1999a, 1999b; Tseng 2004). Three of these studies found that the severity of *A. taiwanensis* on *Ae albopictus* was often dependent on the environment of the mosquito. For example, Comiskey et al. (1999a) found that when given high nutrients, post blood-feed mortality was equal between mosquitoes that were infected or uninfected with *A. taiwanensis*, but when given low nutrients, post blood-feed mortality was 4 times higher in gregarine-infected mosquitoes. Similarly, Comiskey et al. (1999b) reported that mortality of infected larvae and pupae reared under low nutrients was 7 times higher than mortality of uninfected larvae reared at the same food level, but that no difference in mortality was observed between infected and uninfected larvae reared at high food levels.

Table 1. Known *Ascogregarine* parasites of mosquitoes (modified from Chen 1999).

Host	Ascogregarine species	Reference
Ae. aegypti	*A. culicis*	Ross 1895
Ae. albopictus	*A. taiwanensis*	Lien and Levine 1980
Ae. alcasidi	*A. lanyuensis*	Lien and Levine 1980
Ae. polynesiensis	*A. polynesiensis*	Pillai et al. 1976
Armigeres subalbatus	*A. armigerei*	Lien and Levine 1980
Oc. triseriatus	*A. barretti*	Vavra 1969
Oc. sierrensis	*A. clarki*	Sanders and Poinar 1973
Oc. geniculatus	*A. geniculati*	Mustermann and Levine 1983
Oc. hendersoni	*A. sp.*	Rowton et al. 1987
Tripteroides dolfleini	*A. tripteroidesi*	Vavra 1969

Tseng (2004) also demonstrated significant interactions between *Ae. albopictus* rearing conditions and *A. taiwanensis* infection. Specifically, gregarine infection reduced the size of emergence of male mosquitoes by 5.5% when mosquitoes were kept in crowded or uncrowded conditions.

However, this pattern was not seen in females. For females reared in crowded conditions, both infected and uninfected females emerged at approximately the same small size (mean wing length ~2.85 mm). When uncrowded, uninfected females emerged at a much larger size than did infected females (mean wing lengths ~3.1 mm, and 2.78 mm respectively). Thus, infection actually had relatively larger effect when females were reared in uncrowded conditions, namely because uninfected females were able to attain much larger sizes in uncrowded versus crowded conditions.

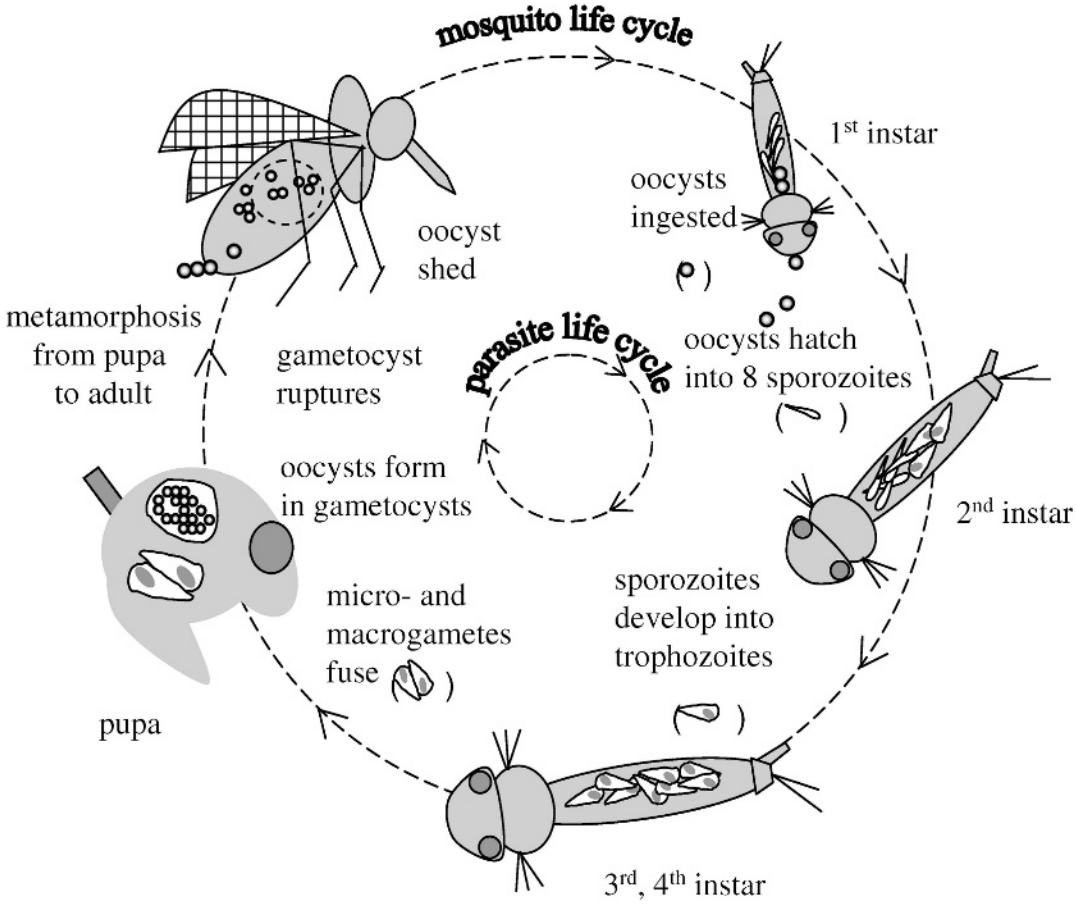

Fig. 1. Life cycle of *Ascogregarina taiwanensis* in *Aedes albopictus*.

No effect of infection on mortality was observed. Together, these 2 groups of studies suggest that both nutrient level and larval crowding can mediate the effect of gregarine infection, and that these effects may be host-sex specific.

The pathogenicity of *A. taiwanensis* was also tested on several other species of mosquitoes (Garcia et al. 1994). *Culex quinquefasciatus*, *Cx. nigripalpus*, *Cx. territans* and *Anopheles quadramaculatus* were all found not susceptible. *Aedes aegypi* and *Ochlerotatus triseratus* were found to be susceptible, but no *A. taiwanensis* oocysts were produced in these species. Pathogenicity was highest in *Oc. taeniorhynchus*, where individuals showed 76% mortality when infected, but only 20% mortality when uninfected. Infected *Ae. albopictus* were 7.5% more likely to die than uninfected. These results are in accord with earlier studies demonstrating increased infection severity in some gregarine – 'non-natural' host combinations (Walsh and Olson 1976, Spencer and Olson 1982).

ASCOGREGARINA BARRETTI

The overall pathogenicity of *A. barretti* for its natural host *Oc. triseriatus* appears to range from negligible to mild. Copeland and Craig (1992) reported that *A. barretti* had no effect on *Oc. triseriatus*, but was pathogenic for the non-natural host *Oc. hendersoni*. *Ochlerotatus hendersoni* also competed more poorly against *A. triseriatus* when infected, but not when uninfected. In contrast, Siegel et al. (1992) documented that *A. barretti*-infected *Oc. triseriatus* pupae were 3.53 and 2.76 times more likely to die in 2 consecutive years. These authors concluded that "*A. barretti* infection was deleterious to *Oc. triseriatus* and that the effects of this pathogen may be moderated by environmental factors" (Siegel et al. 1992).

ASCCOGREGARINA CULICIS

Sulaiman (1992) demonstrated that the severity of *A. culicis* infection on its natural host *Ae. aegypti* varied both among 4 different geographic origins of the parasite, and among 3 different populations of *Ae. aegypti*. Mortality of infected mosquitoes also increased with parasite dose. Mortality induced by the 4 parasite strains ranged from 14.4–37.8% at the lowest dose (50 oocysts per larva), to 51.6–99.6% at the highest dose (1600 oocysts per larva). When infected with 1 strain of *A. culicus*, mortality of 3 populations of *Ae. aegypti* ranged from 43.1–90.3%. The high level of mortality seen in this study suggests that the use of gregarine parasites as a biocontrol agent may be feasible in certain host-parasite strain combinations.

CONTROL PROSPECTS

Recent investigations on the fitness effects of gregarine parasites suggest that under some conditions (e.g., high dosage, low nutrients, crowding), parasite infection does increase mortality of the natural host, as well as non-natural hosts. Biocontrol strategies aim to reduce the population sizes of target organisms to a level at which they no longer constitute a biting nuisance or pose a major health problem (Service 1985). Given what is currently known about the pathogenicity of gregarines for mosquitoes, what is the likelihood that gregarines might be a useful biocontrol agent? With respect to using gregarines to increase mortality of non-natural hosts, because oocysts are typically not formed in these hosts, it may be difficult to devise a sustainable long-term strategy to this effect. Oocysts would need to be artificially disseminated every generation, and given the difficulty of finding the numerous natural and artificial containers used as breeding sites for many of these mosquitoes, this task would be challenging at best.

With respect to using gregarines to increase mortality of natural hosts, it may be useful to examine conditions (other than high doses and stressful larval environments) that may elevate the pathogenicity of these parasites. For example, what is the effect of infection by 2 or more species of gregarines on mosquito fitness? Additionally, theoretical studies of the evolution of parasite virulence often note that the harm inflicted on the host by the parasite should increase with elevated parasite transmission rates (Anderson and May 1981). Might it be possible to artificially select for gregarines of increased virulence in the laboratory, and then release these "high virulence" oocysts into nature? The potential for this type of strategy to work would depend on whether these "high virulence" strains could maintain themselves in the wild. Following Sulaiman (1992), a simpler strategy may be to infect natural hosts with oocysts from distant locations, since they seem to inflict higher mortality on hosts on which they have not co-evolved. Dead infected adults could be collected from one location, transported to another locale, ground up to release retained oocysts, and then distributed into known breeding sites, such as tree holes, tire piles or cemetery vases. As mentioned above, distributing oocysts into *all* possible natural and artificial containers is likely not possible. Multiple introductions of oocysts may be necessary if the pathogenicity of these oocysts is so high that hosts do not survive long enough for parasites to successfully reproduce. Additionally, if natural selection in this new environment favors parasite strains that are less virulent to local hosts, the source of the foreign introduction may need to be varied. Lastly, studies comparing the pathogenicity

of gregarines from multiple locations need to be done to assess the frequency with which foreign parasites are more virulent than local parasites.

CONCLUSION

Although gregarine infection can reduce the fitness (mortality, adult size) of both natural and non-natural hosts, the fact that gregarines require their host to live to adulthood in order for parasites to be transmitted reduces the efficacy of these parasites as a sustainable biocontrol agent. However, short-term introductions of oocysts from other locations, or introduction of artificially selected oocysts may result in temporary reductions in mosquito population size.

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REFERENCES

Ayres C, Romao TPA, Melo-Santos MAZ, Furtado AF. 2002. Genetic diversity in Brazilian Populations of *Aedes albopictus*. *Memorias Do Instituto Oswaldo Cruz* 97:871–875.

Anderson RM, May RM. 1981. The population-dynamics of micro-parasites and their invertebrate hosts. *Philos Trans R Soc Lond B Biol Sci* 291:451–524.

Beier JC, Craig GB Jr. 1985. Gregarine parasites of mosquitoes. In: Laird M, ed. *Integrated Mosquito Control Methodologies,* Volume 2. London: Academic Press. p 167–184.

Black WC, Ferrari JA, Rai KS. 1988. Breeding structure of a colonizing species – *Aedes albopictus* (Skuse) in the United States. *Heredity* 60:173–181.

Birungi J, Munstermann LE. 2002. Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial ND5 sequences: Evidence for an independent invasion into Brazil and United States. *Ann Entomol Soc Am* 95:125–132.

Chen WJ. 1999. The life cycle of *Ascogregarina taiwanensis* (Apicomplexa : Lecudinidae). *Parasitol Today* 15:153–156.

Chen WJ, Fan-Chiang MH. 2001. Directed migration of *Ascogregarina taiwanensis* (Apicomplexa : Lecudinidae) in its natural host *Aedes albopictus* (Diptera: Culicidae). *J Eukaryo Microbio* 48(5):537–541.

Chen WJ, Chow CY, Wu ST. 1997a. Ultrastructure of infection, development and gametocyst formation of *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae) in its mosquito host, *Aedes albopictus* (Diptera: Culicidae). *J Eukaryo Microbio* 44:101–108.

Chen WJ, Wu ST, Chow CY, Yang CH. 1997b. Sporogonic development of the gregarine *Ascogregarina taiwanensis* (Lien and Levine) (Apicomplexa: Lecudinidae) in its natural host *Aedes albopictus* (Skuse) (Diptera: Culicidae). *J Eukaryo Microbio* 44:326–331.

Chen WJ, Yang CH. 1996. Developmental synchrony of *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae) within *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol* 33:212–215.

Comiskey NM, Lowrie RC, Wesson DM. 1999a. Effect of nutrient levels and *Ascogregarina taiwanensis* (Apicomplexa : Lecudinidae) infections on the vector competence of *Aedes albopictus* (Diptera: Culicidae) for *Dirofilaria immitis* (Filarioidea: Onchocercidae). *J Med Entomol* 36:55–61.

Comiskey NM, Lowrie RC, Wesson DM. 1999b. Role of habitat components on the dynamics of *Aedes albopictus* (Diptera : Culicidae) from New Orleans. *J Med Entomol* 36:313–320.

Copeland RS, Craig GB. 1992. Interspecific competition, parasitism, and predation affect development of *Aedes hendersoni* and *A. triseriatus* (Diptera, Culicidae) in artificial treeholes. *Ann Entomol Soc Am* 85:154–163.

de Oliveira RL, Vazeille M, de Filippis AMB, Failloux AB. 2003. Large genetic differentiation and low variation in vector competence for dengue and yellow fever viruses of *Aedes albopictus* from Brazil, the United States, and the Cayman Islands. *Am J Trop Med Hyg* 69:105–114.

Garcia JJ, Fukuda T, Becnel JJ. 1994. Seasonality, prevalence and pathogenicity of the gregarine *Ascogregarina taiwanensis* (Apicomplexa, Lecudinidae) in mosquitoes from Florida. *J Am Mosq Control Assoc* 10:413–418.

Kambhampati S, Black WC, Rai KS. 1991. Geographic origin of the United States and Brazilian *Aedes albopictus* inferred from allozyme analysis. *Heredity* 67:85–94.

Kambhampati S, Rai KS. 1991. Mitochondrial DNA variation within and among populations of the mosquito *Aedes albopictus*. *Genome* 34(2):288–292.

Lien SM, Levine ND. 1980. Three new species of *Ascocystis* (Apicomplexa: Lecudinidae) from mosquitoes. *J Protozool* 27:147–151.

Mustermann LE, Levine ND. 1983. *Ascogregarina geniculati* sp. n. (Protozoa, Apicomplexa) from the mosquito *Aedes geniculatus*. *J Parasitol* 69:769–772.

Pillai JS, Neill HJC, Sone PF. 1976. *Lankesteria culicis* a gregarine parasite of *Aedes polynesiensis* in Western Samoa. *Mosq News* 36:150–154.

Rai KS. 1991. *Aedes albopictus* in the Americas. *Annu Rev Entomol* 36:459–484.

Ross R. 1895. Some observations on the crescent-sphere flagella metamorphosis of the malaria parasite within the mosquito. *Trans South Ind Br Med Assoc* 6:334–350.

Rowton ED, Copeland RS, Craig GB. 1987. Isolation of *Ascogregarina* sp. (Eugregarinida: Lecudinidae) from *Aedes hendersoni*. *J Am Mosq Control Assoc* 3:645–646.

Sanders RD, Poinar GO Jr. 1973. Fine structure and life cycle of *Lankesteria clarki* sp. n. (Sporozoa: Eugregarinida) parasitic in the mosquito *Aedes sierrensis* (Ludlow). *J Protozoo.* 20:594–602.

Service MW. 1985. Some ecological considerations basic to the biocontrol of Culicidae and other medically important insects. In: Laird M, ed. *Integrated Mosquito Control Methodologies* Volume 2. London: Academic Press. p 9–30.

Siegel JP, Novak RJ, Maddox JV. 1992. Effects of *Ascogregarina barrettii* (Eugregarinida, Lecudinidae)

infection on *Aedes triseriatus* (Diptera, Culicidae) in Illinois. *J Med Entomol* 29:968–973.

Spencer JP, Olson JK. 1982. Evaluation of the combined effects of methoprene and the protozoan parasite *Ascogregarina culicis* (Eugregarinida, Diplocystidae), on *Aedes* mosquitoes. *Mosq News* 42:384–390.

Sulaiman I. 1992. Infectivity and pathogenicity of *Ascogregarina culicis* (Eugregarinida, Lecudinidae) to *Aedes aegypti* (Diptera, Culicidae). *J Med Entomol* 29:1–4.

Tseng M. 2004. Sex-specific response of a mosquito to parasites and crowding. *Proc R Soc Lond B Bio Sci*, S186–S188.

Vavra J. 1969. *Lankesteria barretti* n. sp. (Eugregarinida, Diplocystidae), a parasite of the mosquito *Aedes triseriatus* (Say) and a review of the genus *Lankesteria mingazzini*. *J Protozool* 16:546–570.

Walsh RD, Olson JK. 1976. Observations on the susceptibility of certain mosquito species to infection by *Lankesteria culicis* (Ross). *Mosq News* 36:536–545.