



Escape from gregarine parasites affects the competitive interactions of an invasive mosquito

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Abstract

When a species is introduced into a new location, it may escape, at least temporarily, from its natural enemies. In field surveys, we found that when the exotic, invasive mosquito, *Aedes albopictus*, invades new sites, it initially experiences reduced infection by its gut parasite, *Ascogregarina taiwanensis*. To determine the effect of this escape from parasitism on the competitive ability of *A. albopictus*, we performed a laboratory competition experiment in which infected and uninfected *A. albopictus* larvae were reared in microcosms alone and in competition with larvae of the native mosquito, *Ochlerotatus triseriatus*. We analyzed the effect of parasitism by *A. taiwanensis* on *A. albopictus* performance when subjected to intra- and interspecific competition across a range of larval densities, as well as the effect of *A. albopictus* parasitism by *A. taiwanensis* on the competitive impact of *A. albopictus* on *O. triseriatus*. At a density of 30 *O. triseriatus* larvae, *O. triseriatus* survivorship was significantly reduced by the addition of 30 uninfected *A. albopictus*, but not by addition of 30 infected *A. albopictus*, and not by addition of 15 *A. albopictus* whether infected or uninfected. Although estimated finite rate of population increase (λ') showed similar trends, and was significantly affected by treatments, no pairwise differences in rate of increase were significant. Infection by *A. taiwanensis* also significantly prolonged *A. albopictus* female development time and reduced the intraspecific competitive effect of increased density of *A. albopictus*, but did not affect *A. albopictus* survivorship, mass, or estimated finite rate of population increase. Thus, when *A. albopictus* escapes from this parasite as it colonizes new sites, this escape may give it a small, but significant, added competitive advantage over *O. triseriatus*, which may facilitate range expansion of *A. albopictus* and enhance *A. albopictus*'s initial impact on resident species.

Introduction

Introduction of exotic species can have profound effects on native species and communities. Invasion by exotics often leads to altered native ecosystems, introduction of diseases, and reduced growth and survival of native species (Ehrlich 1986; Vitousek et al. 1996). Invasion may also lead to the competitive displacement of native species. Successful exotic species typically are habitat generalists, have genetic variability, and are often aided by human activity and disturbance

(Ehrlich 1986; Drake et al. 1989). In addition, escape from their natural enemies (predators and parasites) may enhance the ability of invading species to impact native species via competition (Lugo 1994; Tilman 1999).

The Asian tiger mosquito, *Aedes albopictus* (Skuse), was first discovered in North America in Houston, Texas in 1985 (Sprenger and Wuithiranyagool 1986), having likely been introduced from northern Asia via used tires containing its eggs (Hawley et al. 1987; Hawley 1988; Craven et al. 1988; Focks et al. 1994).

Since then, *A. albopictus* has spread rapidly across the United States and the globe (see Reiter 1998). Like many exotic, invasive species, *A. albopictus* is an ecological generalist, exploiting a wide range of tropical and temperate habitats, including disturbed ones (Rai 1991). It is clear that *A. albopictus*'s successful invasion is facilitated by its ability to succeed in competition with native and resident species (Peacock et al. 1988; Ho et al. 1989; Livdahl and Willey 1991; Hobbs et al. 1991; O'Meara et al. 1992; Edgerly et al. 1993; Novak et al. 1993; Hornby et al. 1994; O'Meara et al. 1995; Juliano 1998). Invasion by *A. albopictus* is associated with declines in resident *Aedes aegypti* (L.) populations in Florida (Peacock et al. 1988; O'Meara et al. 1992; Hornby et al. 1994; O'Meara et al. 1995) and Alabama (Hobbs et al. 1991).

Because of their similar use of habitat, in the mid-western United States *A. albopictus* is likely to compete with the native tree-hole mosquito, *Ochlerotatus* (formerly *Aedes*) *triseriatus* (Say) (Grimstad et al. 1989; Swanson et al. 2000). In laboratory (Ho et al. 1989; Livdahl and Willey 1991; Novak et al. 1993) and field (Ho et al. 1989; Juliano, unpublished data) studies, *A. albopictus* is a superior competitor against *O. triseriatus*. Livdahl and Willey (1991) predicted that *A. albopictus* will coexist with *O. triseriatus* in tree-holes, but will displace it in tire habitats. Teng and Apperson (2000) determined that although *A. albopictus*'s seasonal rate of population growth may be affected by the presence of *O. triseriatus* in container habitats, its establishment in those habitats would not be. *Aedes albopictus* larvae are known to prevent egg hatching of *O. triseriatus* and *A. aegypti*, whereas the presence of larvae of these species does not inhibit *A. albopictus* eggs from hatching (Edgerly et al. 1993).

Aedes albopictus was first recorded in the central Illinois city of Peoria in 1997 (Kitron et al. 1998). *Aedes albopictus*'s readiness to breed in man-made containers makes Peoria an ideal location for the study of *A. albopictus* during the early phases of invasion, as the city consists of an unusual array of alleys with densely-packed houses, which often have discarded water-holding containers such as buckets, tires, and trash cans present in the yards. Since 1997, *A. albopictus* has expanded its range in Peoria from a single tire site to an area covering ~ 2 km² (M. Lancaster, personal communication).

Aedes albopictus is often heavily parasitized (67–100% of larvae – Munstermann and Wesson 1990; Garcia et al. 1994; Blackmore et al. 1995) by the

protozoan gut parasite, *Ascogregarina taiwanensis* (Lien and Levine) (Lien and Levine 1980; Chen and Kuo 1990; Fukuda et al. 1997; see Beier and Craig 1985 for details on the life cycles of *Ascogregarina*). Effects of *Ascogregarina* parasites on *Aedes* spp. are well studied (Walsh and Olson 1976; Lien and Levine 1980; Spencer and Olson 1982; Beier and Craig 1985; Walker et al. 1987; Copeland and Craig 1992; Siegel et al. 1992; Garcia et al. 1994; Yeh et al. 1994; Comiskey et al. 1999; Van Rhein 1999). Gregarine parasites are reported as having low or no pathogenicity to their natural hosts (Walsh and Olson 1976; Beier and Craig 1985; Copeland and Craig 1992; Yeh et al. 1994). Van Rhein (1999) found that *A. barretti* (Vavra), the gregarine parasite of *O. triseriatus*, reduces female fecundity and size. *Ascogregarina taiwanensis* increases larval mortality under stressful conditions, decreases female fecundity and prolongs male and female development under stressful or non-stressful conditions (Comiskey et al. 1999), and reduces *A. albopictus* oviposition and hatch rate (Yeh et al. 1994). Garcia et al. (1994), in contrast, reported that *A. taiwanensis* had little effect on *A. albopictus* mortality. It is clear from a number of studies mentioned above that negative effects of the gregarines on host fitness can be exaggerated when the hosts are crowded, underfed, or in competition. This may result from consumption of available resources by the parasite, or diversion of those resources to use by the host to repair damaged tissue and to elicit immune responses against the parasite (Comiskey et al. 1999).

If an invasive species, like *A. albopictus*, is able to escape infection by a parasite as it invades a new location, like Peoria, that species should have a competitive advantage over other species that cannot escape detrimental effects of parasitism (Tilman 1999). This relationship may be particularly important if there is a time lag between establishment of *A. albopictus* and establishment and population increase of *A. taiwanensis*. The gregarine's rate of spread can be no greater than that of its host, which is its only means of dispersal (Beier and Craig 1985). Because the invasion of *A. albopictus* may entail its establishment in new artificial containers and tree-holes where *A. taiwanensis* is initially rare or absent (Blackmore et al. 1995), this escape from (or reduction of) infection may give *A. albopictus* an initial competitive advantage in new habitats over native, established *O. triseriatus*, which would likely be parasitized by resident, possibly long-established populations of *A. barretti* (Van Rhein et al.

2000). Thus, this system provides us an opportunity to test the hypothesis that escape from parasites enhances invasion by and impact of *A. albopictus*.

We performed a two-part study to determine: (1) if *A. albopictus* escapes infection by *A. taiwanensis* in recently-established *A. albopictus* sites relative to long-established sites; and (2) the effect such an escape would have on competitive interactions between *A. albopictus* and *O. triseriatus*. The first part of our study was a series of field surveys conducted in East St Louis and Peoria, Illinois, that compared parasite intensity and prevalence in larvae from tires that had been colonized by *A. albopictus* for about 14 years (East St Louis), 2 years (Peoria), and less than 1 year (Peoria). We predicted that intensity and prevalence of *A. taiwanensis* would increase with increased time since *A. albopictus* colonization. We also sampled oviposition traps in Peoria to determine if parasites are present when *A. albopictus* first oviposits in a container.

The second part of the study was a laboratory experiment that tested the hypothesis that when *A. albopictus* is not infected by *A. taiwanensis*, it has a considerable competitive advantage over *O. triseriatus* infected with *A. barretti*, an advantage that may be reduced or eliminated when *A. albopictus* is infected. This hypothesis predicts that a high density of uninfected *A. albopictus* should have a greater negative effect on *O. triseriatus* than a comparable density of infected *A. albopictus* (perhaps due to greater ability to harvest and to deplete resources – Werner 1994). In addition, we evaluated the competitive response of *A. albopictus* to competition from *O. triseriatus*, expecting that response to be worse when *A. albopictus* is infected than when it is not (perhaps due to greater minimum resource requirements and lower physiological efficiency – Werner 1994). Thus, we expected that *O. triseriatus* should have a greater negative effect on infected than on uninfected *A. albopictus*.

Methods

Field surveys: escape from infection

Ascogregarina taiwanensis in tires

We sampled tires in Peoria, Illinois, during summers 1999 and 2000, and in East St Louis, Illinois, during summer 2000, to determine intensity and prevalence of *A. taiwanensis* infection at sites with varying times since *A. albopictus* colonization. The East St Louis area

was colonized by *A. albopictus* as early as 1986 (Centers for Disease Control 2000), and we thus designated it a ‘long-established’ *A. albopictus* site (≥ 14 years). When we sampled larvae in Peoria in 1999, the bulk of the *A. albopictus* population had resided there for two years (Kitron et al. 1998) in a single tire pile behind an automotive garage. By the end of the summer, the tire pile had been removed. At the start of summer 2000, a new pile of tires, previously uninhabited by *A. albopictus*, was present in the same location behind the garage. We therefore designated the tire pile from summer 1999 ‘recently established’ (2 years), and the summer 2000 tire pile ‘newly established’ (< 1 year).

Larvae were collected in Peoria on two dates during summer 1999 (June 22 and August 17), on three dates during summer 2000 (July 3, July 31, and August 7), and in East St Louis on two dates during summer 2000 (June 2 and July 3). Because infection by gregarine parasites takes place during the early larval stages (Beier and Craig 1985), we collected only at later stages (3rd and 4th instars). After removing larvae, water was returned to the tires to prevent removal of parasite oocysts. Larvae were transported to the laboratory, reared to 4th instar if necessary, and identified by species under a dissecting microscope. We then dissected the larval midguts under a dissecting microscope using procedures described by Munstermann and Wesson (1990).

We determined intensity of infection by counting the number of *A. taiwanensis* gamonts or trophozoites in each larval midgut under a compound microscope. We analyzed infection intensity (log-transformed to meet assumptions of normality and homogeneity of variances) among the three site–year combinations using a one-way analysis of variance (ANOVA) (PROC GLM, SAS Institute 1995). Significant pairwise differences were determined using Bonferroni multiple comparisons. Prevalence of *A. taiwanensis* was defined as the proportion of larvae infected within a sampling date or within each site–year combination. We analyzed contingency tables using a two-tailed Fisher’s Exact Test (PROC FREQ, SAS Institute 1995) to determine if prevalence was independent of date sampled within a site–year combination, and then pooled samples within a site–year combination and compared prevalence of the parasite across site–year combinations, again by analyzing contingency tables with a two-tailed Fisher’s Exact Test. Bonferroni adjustments were made for multiple tests. Our prediction that *A. albopictus* escapes its parasite when it invades new

sites would be supported with an increase in intensity and prevalence of parasitism from newly, to recently, to long-established sites.

Ascogregarina taiwanensis in oviposition traps

Twice a week from June 1–September 19, 2000, we sampled 25 oviposition traps (design described by Swanson et al. 2000) placed within the vicinity of 1999 *A. albopictus* activity (Lancaster et al., unpublished data) for the presence of *A. albopictus* larvae. Of these traps, 17 had yielded *A. albopictus* in 1999, and 6 had not (2 traps were new in 2000). Only 10 of these 25 traps yielded *A. albopictus* in 2000 (see Figure 1). Three of the traps where *A. albopictus* larvae occurred were located near the tire pile behind the automotive garage (the focal point of 1999 *A. albopictus* activity) described above, and the others were located at varying distances from the garage (Figure 1). Each sampling period, we collected any larvae (>2nd instar) present and refilled the traps with oviposition fluid (10% oak leaf infusion, 90% water). Larvae were dissected as described above. We pooled samples for each oviposition trap and determined mean infection intensity and prevalence for each trap. We used these data to quantify

parasitism in individual containers newly colonized by *A. albopictus*. We expected that traps in areas nearest the 1999 focal point of *A. albopictus* activity would yield higher intensity or prevalence of infection than would traps located further from the focal point, at locations where *A. albopictus* had not been recorded in 1999.

Laboratory experiment: escape from infection and competitive ability

Establishment of parasite colonies and experimental infection

Prior to the start of the experiment, we established colonies of *A. taiwanensis* and *A. barretti* using methods described by Beier and Craig (1985). Field-collected larvae of each mosquito species were reared to adulthood in plastic containers with screened lids and allowed to die inside the containers. Once all adults had died, we ground the bodies using a Tissue Tearor™ (Biospec Products, Inc.), passed the water through a series of vacuum filters (70, 60, and 20 μm), and then centrifuged the water at 2500 rpm for 12 min to concentrate oocysts. Oocysts per 1 μl

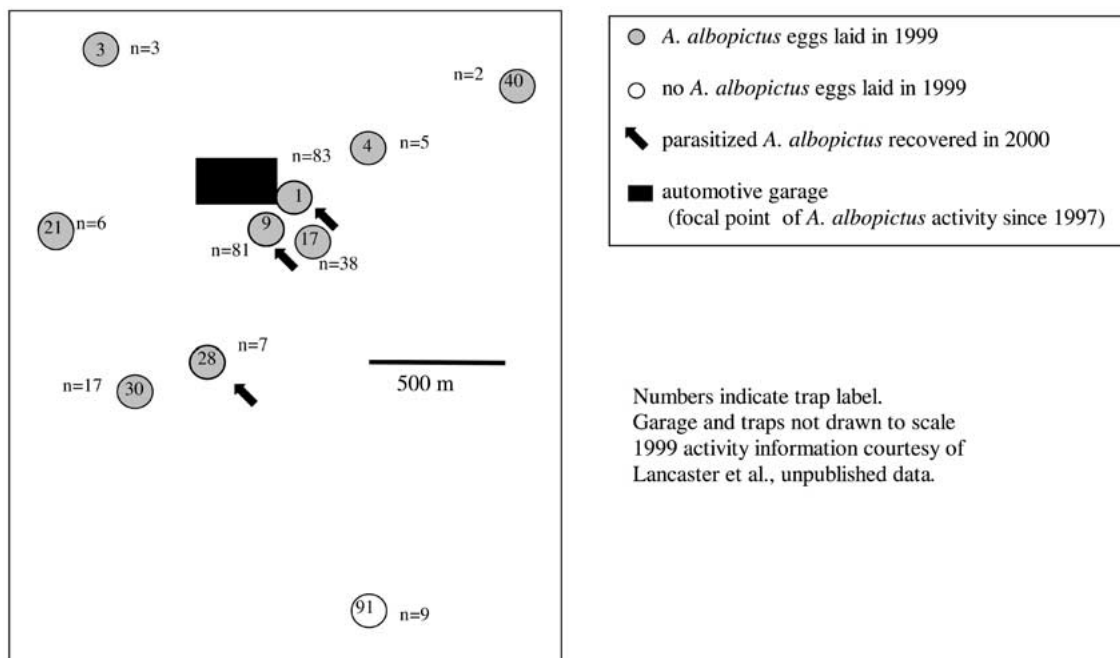


Figure 1. Oviposition traps yielding *Aedes albopictus* and sampled for *Ascogregarina taiwanensis* in Peoria, summer 2000. Traps are mapped in relation to their proximity to the focal point of 1999 *A. albopictus* activity (Lancaster et al., unpublished data). The number of *A. albopictus* larvae dissected from each trap (*n*) is given. Presence of *A. albopictus* in the previous year and presence of the parasite are indicated.

were counted using a hemocytometer. To amplify parasite colonies, each *Ascogregarina* species was passed through three generations of their respective host mosquitoes. Oocyst suspensions were stored at 4 °C in darkness to prevent algal growth (Comiskey et al. 1999). *Ascogregarina* oocysts remain viable at 26 °C for up to 11 months, and at 4 °C, may survive even longer (Beier and Craig 1985).

To start the experiment, we synchronously hatched (Novak and Shroyer 1978) approximately 2000 eggs of each mosquito species (*A. albopictus*: F₁ colony from Peoria, IL; *O. triseriatus*: F₃ laboratory colony from Bloomington, IL), and then infected approximately 600 1st instar *A. albopictus* larvae and approximately 600 1st instar *O. triseriatus* larvae with approximately 10,400 oocysts/larva of their respective parasites. Preliminary tests showed that this dose produced relatively uniform infections, with infection intensity similar to that observed in nature. For infection, newly-hatched larvae of each species were housed separately in batches of 100 in 50 ml Fisherbrand® tripour beakers containing 15 ml deionized water. Each beaker received 5.75 ml of *A. taiwanensis* oocyst suspension (181.25 oocysts/ μ l) for *A. albopictus* infection, or 8.23 ml of *A. barretti* oocyst suspension (126.28 oocysts/ μ l) for *O. triseriatus* infection. Larvae were reared with oocysts for 40 h in an environmental chamber (14 h : 10 h L : D, 26 °C) with 6 mg bovine liver powder as food. After this initial exposure period, there was no further risk of parasite transfers among larvae, as larvae are only susceptible to infection during the early instars (Beier and Craig 1985). For the control (uninfected) treatment, approximately 600 uninfected *A. albopictus* larvae were reared identically to the infected ones, except with 5.75 ml deionized water added in place of the 5.75 ml of oocyst suspension. Twenty infected and twenty uninfected larvae of *A. albopictus* and twenty infected *O. triseriatus* were reared to 4th instar and dissected to confirm infection status and to determine intensity of infection using methods similar to those of Munstermann and Wesson (1990).

Experimental design

We established 17 treatment groups with 3 replicates per treatment (the 30 infected *A. albopictus* with zero *O. triseriatus* treatment had 2 replicates). All treatments were applied to larvae in 400 ml tri-cornered polypropylene beakers (Fisherbrand®). Each beaker held 200 ml deionized water and 1.0 g dried

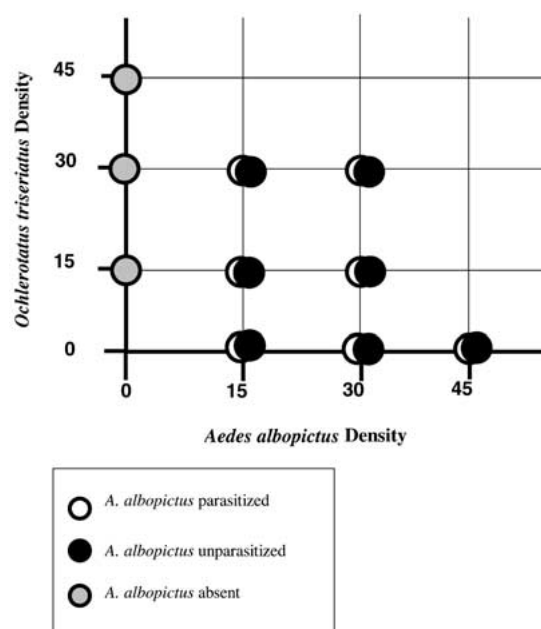


Figure 2. Schematic of the experimental design, showing the density-parasitism treatment combinations for *A. albopictus* and *O. triseriatus* larvae in experimental microcosms.

red oak (*Quercus rubra*) leaves as a resource substrate (leaves had been dried for 48 h at 50 °C and then soaked in deionized water for 3 days immediately prior to the start of the experiment). Second instar larvae were placed in these microcosms at varying density-parasitism treatments as shown in Figure 2. All *O. triseriatus* larvae were infected in order to mimic field conditions in Peoria, Illinois (Aliabadi and Juliano, unpublished data). We maintained water levels at 200 ml and checked containers daily for pupae. Every 2 weeks we added an additional 0.2 g pre-soaked leaves. Pupae were removed as they appeared and placed individually in 14.8 ml vials and allowed to eclose. Adults were dried at 50 °C for 24 h, identified by species and sex, and weighed. We also measured wing lengths of females. Microcosms were housed at 26 °C and 14 h : 10 h L : D.

Analyses

We determined effects of infection of *A. albopictus* by *A. taiwanensis* on survivorship of each species, mean adult mass (each sex), and median days to eclosion (each sex). We also calculated an estimate of the finite rate of population increase (λ') for each species (Livdahl and Sugihara 1984; Livdahl and Willey 1991; Léonard and Juliano 1995; Juliano 1998; Nannini and

Juliano 1998). This index allows multiple performance variables to be incorporated into a single value related to population growth. This index was calculated as:

$$\lambda' = \exp(r'),$$

where r' is an estimate of the per capita rate of population change (Livdahl and Sugihara 1984), calculated for each replicate as:

$$r' = \ln \left[\frac{(1/N_0) \sum A_x f(w_x)}{[D + \sum x A_x f(w_x)] / \sum A_x f(w_x)} \right],$$

where N_0 is the number of females in the cohort (assumed to be 50%), A_x is the number of females eclosed on day x , w_x is a measure of mean size of eclosed females on day x (dry mass for *O. triseriatus*, wing length for *A. albopictus*), D is the time between eclosion and reproduction, and $f(w_x)$ is the size-dependent fecundity (Livdahl and Sugihara 1984; Livdahl and Willey 1991; Léonard and Juliano 1995; Juliano 1998; Nannini and Juliano 1998). We assumed $D = 12$ days for *O. triseriatus* (Livdahl and Willey 1991; Léonard and Juliano 1995; Grill and Juliano 1996; Nannini and Juliano 1998), and $D = 14$ days for *A. albopictus* (Livdahl and Willey 1991; Juliano 1998). For size-dependent fecundity, we used the relationship $f(w_x) = (1/2) \exp[4.5801 + 0.8926(\ln w_x)] - 1$ for *O. triseriatus*, ($r^2 = 0.5377$, Nannini and Juliano 1998), and $f(w_x) = 17.2w_x - 14$ for *A. albopictus* ($r^2 = 0.03$, Livdahl and Willey 1991; Juliano 1998). We used λ' instead of r' because r' cannot be estimated for a replicate yielding no adults, whereas λ' can be estimated (Léonard and Juliano 1995; Grill and Juliano 1996; Juliano 1998; Nannini and Juliano 1998).

For *A. albopictus*, we analyzed all performance variables using linear models (PROC GLM, SAS Institute 1995), with *A. albopictus* infection status (infected or uninfected by *A. taiwanensis*) as a categorical variable, and *A. albopictus* and *O. triseriatus* densities as continuous variables. In these analyses, we first tested models involving interactions between the continuous and class variables (i.e. infection**A. albopictus* density interaction). Our primary interest was in interactions of parasitism status of *A. albopictus* and densities of each of the competitors. Significant interactions indicate that the effect of inter- or intraspecific density on *A. albopictus*'s population performance is altered by parasitism of *A. albopictus*. When those interactions were not significant, we tested reduced models (analysis of covariance models), omitting those

interactions to determine if competition was present (indicated by significant density effects) and whether there was any effect of parasitism by *A. taiwanensis* (indicated by a significant INFECTION effect). We also tested for interactions between *A. albopictus* and *O. triseriatus* density. Interpretations of biological implications of significant effects were based on graphical analysis of the fitted response surface of performance vs densities for the two parasitism treatments or on least squares means. When necessary, data were transformed in order to meet assumptions of normality and homogeneity of variance.

For *O. triseriatus*, we tested for effects on survivorship (square root-transformed to meet assumptions of normality and homogeneity of variance) and λ' means using one-way ANOVAs (PROC GLM, SAS Institute 1995), with species densities and *A. albopictus* parasitism status combined into a single categorical variable. The use of ANOVA eliminated the problems of nonlinear relationships of these variables to species densities, and the undefined nature of INFECTION when *A. albopictus* was absent from a treatment (the intraspecific *O. triseriatus* treatments).

Results

Field surveys

Tire data

Intensity of infection. Intensity of *A. taiwanensis* infection in *A. albopictus* larvae increased significantly (Table 1) over the course of the sampling season within

Table 1. ANOVA table with contrasts comparing mean intensity (log-transformed) of *A. taiwanensis* infection within and among three site-year combinations (ESL00 is a long-established *A. albopictus* site, P99 is a recently established site, and P00 is a newly established site). Bonferroni-adjusted comparisonwise significance criterion was $P < 0.01$.

Source	df	F	P
Site-year combination	6	51.78	0.0001
Error	149		
$R^2 = 0.6759$			
<i>Contrast</i>			
ESL00 vs P99	1	84.24	0.0001
ESL00 vs P00	1	169.76	0.0001
P99 vs P00	1	12.12	0.0007
ESL00 1st vs 2nd sample	1	24.16	0.0001
P99 1st vs 2nd sample	1	27.88	0.0001
P00 1st vs 2nd sample	1	0.57	0.4530
P00 1st vs 3rd sample	1	14.70	0.0002
P00 2nd vs 3rd sample	1	13.39	0.0004

each site–year combination, with the exception of the first two samples collected in Peoria in 2000 (Figure 3). When samples within a site–year combination were pooled, all three site–year combinations differed significantly in mean infection intensity (Table 1). The long-established site (ESL00) had significantly higher intensity of infection (an order of magnitude greater – Figure 3) than did the recently established (P99) site, which in turn had significantly higher infection intensity than the newly established (P00) site.

Prevalence of infection. Contingency table analysis using Fisher’s Exact Test showed that for P99 and P00 (ESL00 could not be analyzed for independence of prevalence and sampling date because all larvae from this site were infected), prevalence of parasites was dependent on the sampling date (for P99, $P \ll 0.0001$, for P00, $P \ll 0.0001$), with samples collected later in the season having a higher prevalence of *A. taiwanensis* (Figure 4). We then pooled samples within a site–year combination, and found that the proportion of

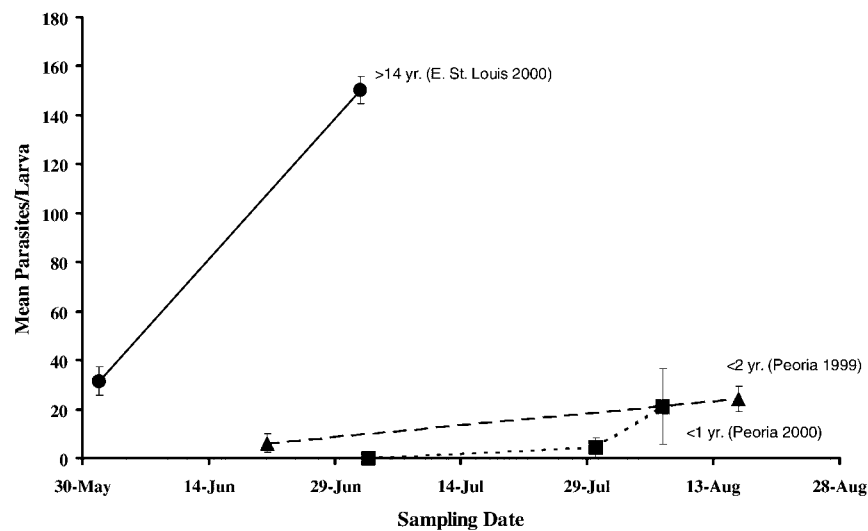


Figure 3. *Ascogregarina taiwanensis* infection intensities (mean \pm SE, back-transformed) in *A. albopictus* larvae from tires from long-established (ESL00), recently established (P99), and newly established (P00) sites. Statistical results given in Table 1.

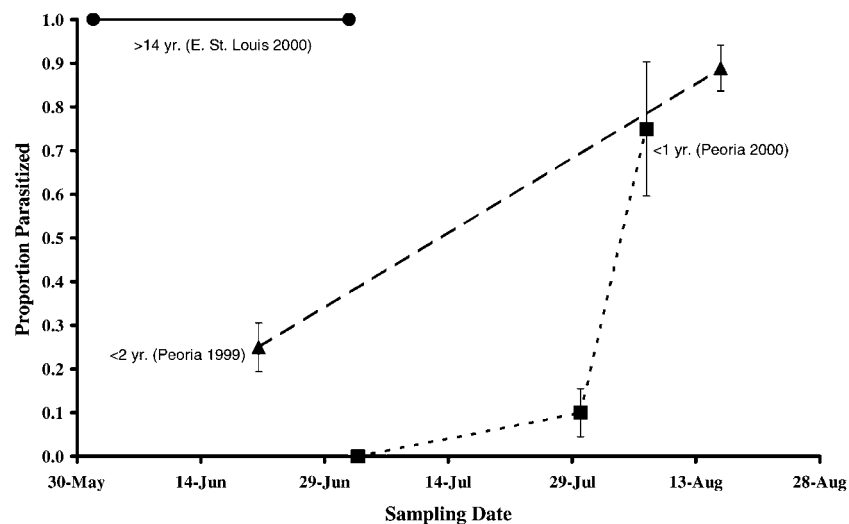


Figure 4. *Ascogregarina taiwanensis* infection prevalence (mean \pm SE) in *A. albopictus* larvae from tires from long-established (ESL00), recently established (P99), and newly established (P00) sites.

A. albopictus larvae infected differed significantly ($P \ll 0.0001$) among the three site-year treatments. Infection prevalence was significantly (experimentwise $\alpha = 0.05$) higher in larvae from ESL00 (55/55 larvae parasitized) than in larvae from P99 (35/49 larvae parasitized) or P00 (9/52 larvae parasitized) (see also Figure 4). Similarly, prevalence of *A. taiwanensis* was significantly higher in larvae from P99 than in larvae collected from P00.

Oviposition trap data

Ascogregarina taiwanensis infection intensity in 2000 was extremely low in all oviposition traps from which we dissected *A. albopictus*. Most (7 out of 10) traps with larvae yielded no parasitized larvae ($n = 251$ total *A. albopictus* larvae dissected). Mean intensity of infection was moderate only in traps #1 and #9 (mean \pm SE: 5.58 ± 1.70 and 22.04 ± 6.15 parasites/larva, respectively). These two traps were located nearest the automotive garage, the focal point of 1999 *A. albopictus* activity (Figure 1). Trap #28, farther from the garage, had a mean intensity of 0.43 ± 0.43 parasites/larva. For traps #1, 9, and 28, prevalence of infection was 20% ($n = 83$ larvae), 48% ($n = 81$ larvae), and 14% ($n = 7$ larvae), respectively. Only one of the traps yielding *A. albopictus* (#91) was newly colonized in 2000 (i.e. had yielded no *A. albopictus* in 1999), and this trap yielded only unparasitized ($n = 9$) larvae in 2000.

Laboratory experiment

Because many replicates yielded no adult *O. triseriatus*, we could not analyze mass or development time for either sex of this species. There was slight contamination of some microcosms containing *O. triseriatus* by *A. aegypti*. A total of 15 adult *A. aegypti* individuals emerged in our experiment, with 1–2 individuals occurring in 13 different microcosms spread throughout 9 different treatments. *Aedes aegypti* and *O. triseriatus* colonies were maintained in the same room and it is likely one or more *A. aegypti* females oviposited on the *O. triseriatus* egg papers used in this experiment. Including or excluding these contaminated replicates did not change our conclusions for any variable. We therefore report results from analyses of the entire data set.

Survivorship. Treatment significantly affected *O. triseriatus* survivorship ($F_{10,22} = 17.05$, $P = 0.0001$). Survivorship of *O. triseriatus* was high only

when they were alone at low density (15 larvae – Figure 5). For *O. triseriatus* at low density (15 larvae), additions of 15 or 30 *A. albopictus*, either infected or uninfected, significantly reduced survivorship of *O. triseriatus* compared to the 0 *A. albopictus* treatment (Figure 5, Table 2). In contrast, for *O. triseriatus* at intermediate density (30 larvae) only addition of 30 uninfected *A. albopictus* significantly lowered

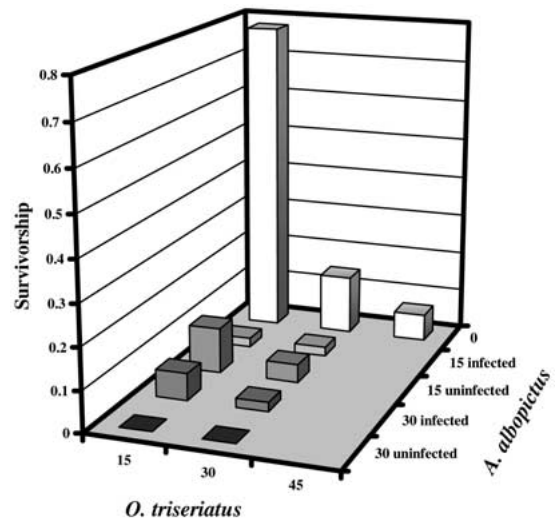


Figure 5. Means for density-parasitism treatments for *O. triseriatus* survivorship. Data shown are back-transformed. Statistical result given in Table 2.

Table 2. Comparison of interspecific competition treatments with two single species treatments for *O. triseriatus* survivorship.

Effect of adding	Intraspecific competition treatment	
	0 <i>A. albopictus</i> : 15 <i>O. triseriatus</i>	0 <i>A. albopictus</i> : 30 <i>O. triseriatus</i>
15 <i>A. albopictus</i> infected	*	n.s.
15 <i>A. albopictus</i> uninfected	*	n.s.
30 <i>A. albopictus</i> infected	*	n.s.
30 <i>A. albopictus</i> uninfected	*	*

Asterisks indicate that the addition of the number of infected or uninfected *A. albopictus* for that row yielded significantly lower survivorship than did the intraspecific treatment in that column; 'n.s.' indicates the comparison was not significant (Bonferroni-adjusted for experimentwise $\alpha = 0.05$). Numbers (0, 15, 30) indicate the number of *A. albopictus* : *O. triseriatus* larvae in the microcosm. Associated means are given in Figure 5.

survivorship (Figure 5, Table 2). Addition of 30 uninfected *A. albopictus* always resulted in 0% survivorship to adulthood for *O. triseriatus* (Figure 5). Survivorship of *O. triseriatus* was significantly greater when 15 *O. triseriatus* larvae were alone in a microcosm than when 30 or 45 *O. triseriatus* larvae were alone (Figure 5), but there was no significant difference in survivorship between intraspecific densities of 30 and 45 (Figure 5).

INFECTION had no significant effect on survivorship of *A. albopictus* (Table 3). However, both ALBOPICTUS and TRISERIATUS densities significantly affected *A. albopictus* survivorship (Table 3). Slopes of these relationships were both negative (Table 4) indicating both intra- and interspecific competition occurring. The ALBOPICTUS*INFECTION, ALBOPICTUS*TRISERIATUS, and TRISERIATUS*INFECTION interactions were all not significant and were removed from the analysis.

Estimated finite rate of population increase (λ'). Although the treatment effect was significant for *O. triseriatus* estimated finite rate of population

Table 3. ANOVA table showing the effects of intra- (ALBOPICTUS) and interspecific (TRISERIATUS) densities and INFECTION status of *A. albopictus* by *A. taiwanensis* on survivorship and estimated finite rate of population increase (λ') of *A. albopictus*. Assumptions of normality and homogeneity of variance were not met with any transformations for λ' . Significance criterion was $P < 0.05$.

Source	Survivorship			(λ')		
	df	F	P	df	F	P
ALBOPICTUS	1	59.17	0.0001	1	2.02	0.1641
TRISERIATUS	1	86.39	0.0001	1	1.55	0.2203
INFECTION	1	0.02	0.8806	1	0.66	0.4231
Error	37			37		
		$R^2 = 0.7519$			$R^2 = 0.0833$	

Table 4. Summary statistics for analysis of *A. albopictus* performance variables for which there were no interactions of INFECTION and densities of *O. triseriatus* or *A. albopictus*.

Variable	Least squares mean \pm SE		Slopes \pm SE	
	Infected	Uninfected	<i>A. albopictus</i> density	<i>O. triseriatus</i> density
Survivorship	0.4600 \pm 0.0365	0.4677 \pm 0.0356	*-0.0195 \pm 0.0025	*-0.0200 \pm 0.0022
λ'	1.0468 \pm 0.0374	1.0044 \pm 0.0365	-0.0037 \pm 0.0880	-0.0027 \pm 0.0022
Mean female mass	0.2035 \pm 0.0109	0.2130 \pm 0.0109	*-0.0021 \pm 0.0008	-0.0009 \pm 0.0006
Mean male mass	0.1313 \pm 0.0065	0.1359 \pm 0.0061	*-0.0088 \pm 0.0030	*-0.0109 \pm 0.0016
Median male development time	21.6344 \pm 3.5076	23.3351 \pm 3.3355	*0.0687 \pm 0.0231	*0.0429 \pm 0.0196

Least squares means (\pm SE) for infected and uninfected groups, and slopes (\pm SE) were obtained from the final reduced models (see text and Tables 3, 5, and 6). Slopes marked with "*" are significantly different from 0.

increase ($F_{10,22} = 2.46$, $P = 0.0378$), after adjusting for multiple tests, we could not identify any significant pairwise differences in λ' (Figure 6). Figure 6 shows that λ' for *O. triseriatus* was always 0 when 30 uninfected *A. albopictus* were added. For *A. albopictus*, neither INFECTION, nor intra-, nor interspecific competition, nor any of the interactions, significantly affected λ' (Tables 3 and 4).

Mass. For both male and female *A. albopictus*, there was no significant interaction of density of either species with INFECTION. For females, only the effect of intraspecific competition was significant (Table 5), with increasing *A. albopictus* density lowering mass (Table 4). For males, INFECTION did not significantly affect mass (Table 5). Effects of both ALBOPICTUS and TRISERIATUS densities, and their interaction, were significant for males (Table 5), with increasing density lowering mass (log-transformed) (Table 4). Thus, there were effects of both intra- (females and males) and interspecific (males) competition on *A. albopictus* mass, but the parasite did not alter these competitive interactions.

Development time. For *A. albopictus* females, INFECTION, ALBOPICTUS, and TRISERIATUS densities, and the ALBOPICTUS*INFECTION interaction were all significant (Table 6). Median development time increased with increased inter- and intraspecific competition, but when *A. albopictus* was infected, the effect of conspecifics on development time was significantly lowered (Figure 7, note shallower slope, 0.90 ± 0.50 , for regression for the infected group vs 1.56 ± 0.21 for the uninfected group). Because of this difference in the relationship of development time to density of infected vs uninfected conspecifics, the apparent effect of infection on development time of

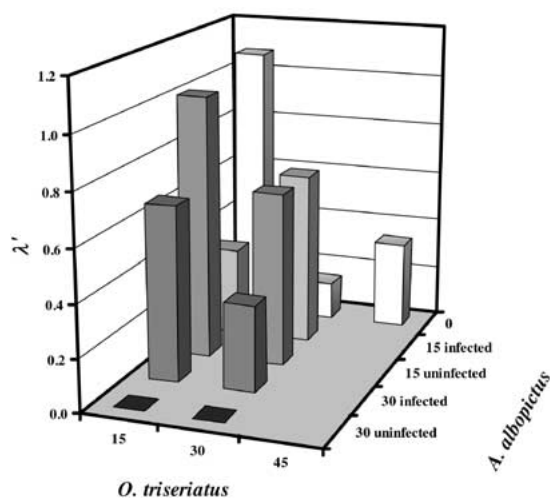


Figure 6. Means for density-parasitism treatments for *O. triseriatus* estimated finite rate of increase, λ' .

Table 5. ANOVA table showing the effects of intra-(ALBOPICTUS) and interspecific (TRISERIATUS) densities, INFECTION status of *A. albopictus* by *A. taiwanensis*, and the ALBOPICTUS*TRISERIATUS interaction on mean masses of male (log-transformed) and female *A. albopictus*. Significance criterion was $P < 0.05$.

Source	Females			Males		
	df	F	P	df	F	P
ALBOPICTUS	1	7.83	0.0082	1	34.66	0.0001
TRISERIATUS	1	1.97	0.1687	1	13.95	0.0007
INFECTION	1	0.38	0.5420	1	0.18	0.6781
ALBOPICTUS*TRISERIATUS	—	—	—	1	5.37	0.0265
Error	36			35		
		$R^2 = 0.1921$			$R^2 = 0.5528$	

A. albopictus was reversed at high density relative to that at low density (Figure 7); at low conspecific density, development times of uninfected *A. albopictus* females were less than those of infected females (Figure 7), but at high conspecific densities, development times of uninfected females were greater than those of infected females (Figure 7). INFECTION had no effect on median development time of *A. albopictus* males (square root-transformed), and interactions with INFECTION were also not significant (Table 6). Both ALBOPICTUS and TRISERIATUS density significantly affected male development time (Table 6), with increasing inter- and intraspecific density increasing the median number of days it took male *A. albopictus* to eclose (Table 4).

Table 6. ANOVA table showing the effects of intra-(ALBOPICTUS) and interspecific (TRISERIATUS) densities, INFECTION status of *A. albopictus* by *A. taiwanensis*, and the ALBOPICTUS*INFECTION interaction on median development time (time to eclosion) of male (square root-transformed) and female *A. albopictus*. Significance criterion was $P < 0.05$.

Source	Females			Males		
	df	F	P	df	F	P
ALBOPICTUS	1	67.95	0.0001	1	8.87	0.0052
TRISERIATUS	1	31.76	0.0001	1	4.81	0.0349
INFECTION	1	6.66	0.0142	1	0.03	0.8621
ALBOPICTUS*INFECTION	1	5.55	0.0242	—	—	—
Error	35			36		
		$R^2 = 0.7085$			$R^2 = 0.2286$	

Discussion

The results of this study demonstrate that escape from a parasite can affect competitive interactions of an invasive species with native species. A high density of unparasitized *A. albopictus* appears to have a slightly, but significantly, greater competitive impact on survivorship and perhaps population growth of *O. triseriatus* when compared to the effect of a high density of parasitized *A. albopictus* (Figure 5 and 6). Parasitized *A. albopictus* larvae also appear to produce a slightly but significantly greater intraspecific competitive effect on conspecific development time than do unparasitized *A. albopictus* (Table 6, Figure 7). These results are consistent with our predictions. Escape from parasitism, if it occurs in nature, may contribute to the competitive advantage of *A. albopictus* by making the native species, which typically harbors its own *Ascogregarina* species, a relatively poorer competitor against the invader than it would have been had the invader's parasite been present. The effects of *A. taiwanensis* infection of *A. albopictus* on *O. triseriatus* represent an indirect effect of this parasite on another species. Our design and method of infection precludes infection of *O. triseriatus* larvae by *A. taiwanensis*. Such cross infections have been created in the laboratory, but have a low success rate (Munstermann and Wesson 1990; Garcia et al. 1994).

Our field surveys revealed that when *A. albopictus* colonizes new sites and containers, it escapes (or experiences reduced) infection by *A. taiwanensis* (Figures 2 and 3). Low prevalence and intensity of

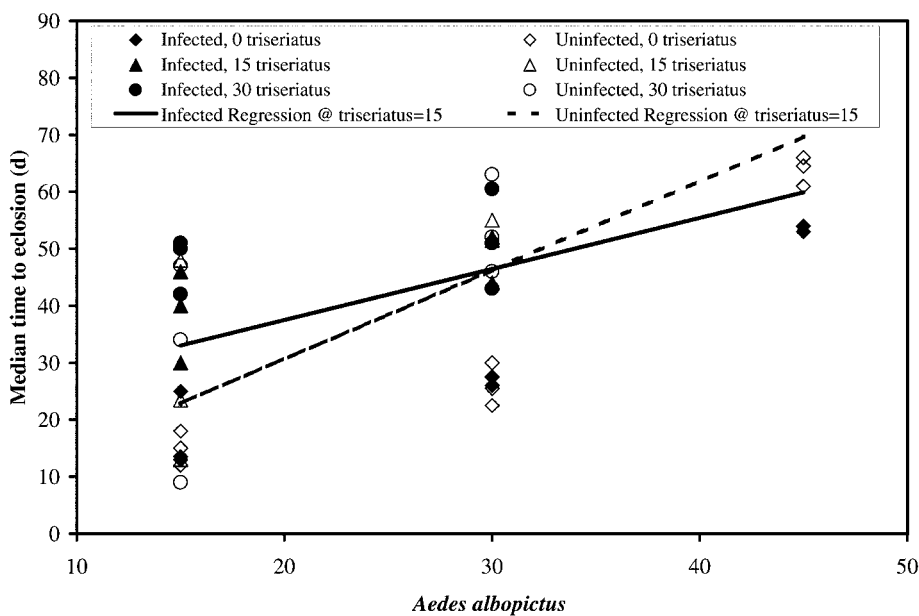


Figure 7. Effect of infection by *A. taiwanensis* on female *A. albopictus* median days to eclosion across varying larval densities, and with or without competition with *O. triseriatus*. Regression equations are: unparasitized – days = $11.87 + 1.56(A. albopictus) + 0.71(O. triseriatus)$; parasitized – days = $8.87 + 0.90(A. albopictus) + 0.71(O. triseriatus)$. The difference in slopes relative to density of *A. albopictus* are significant (Table 6), indicating that development time increases faster with the density of uninfected vs infected *A. albopictus*. Regression lines plotted on the graph are for a density of *O. triseriatus* = 15.

infection is also observed in newly colonized oviposition traps at the relatively new Peoria site. Low levels of infection by *A. taiwanensis* in *A. albopictus* at recently (<3 years) colonized sites have also been observed in other studies (Munstermann and Wesson 1990; Blackmore et al. 1995; Fukuda et al. 1997). Thus, because invading *A. albopictus* experience reduced infection by *A. taiwanensis* when they colonize new containers and sites, we predict that the initial competitive advantage of *A. albopictus* over resident *O. triseriatus* will be slightly enhanced. Further, at the initially low parasite densities, uninfected *A. albopictus* (at least females) may have development times that are shorter than those in habitats where this parasite is well established. This more rapid development may increase the likelihood that the colonizing population of *A. albopictus* will grow rapidly to a size sufficient to be relatively safe from stochastic extinction. Though the effects of reduced parasitism at newly colonized sites are not large, they all may contribute to enhancing the likelihood of successful establishment of *A. albopictus* and its impact on native species.

Although the impact of escape from parasitism on the spread of *A. albopictus* in the United States may

be temporary, our data suggest that whether or not *A. albopictus* is parasitized by *A. taiwanensis* may affect the early phases of invasion. Although it is apparent from our laboratory experiment that this escape can affect competitive interactions with *O. triseriatus*, the actual importance of this escape in the invasion and spread of *A. albopictus* may depend on the initial population size of *A. albopictus* at the site. Because competition with *A. albopictus* (regardless of parasite status) may affect *O. triseriatus*'s performance more than *A. albopictus*'s escape from its parasites, the effect on *O. triseriatus* of escape of *A. albopictus* from *A. taiwanensis* may only be important when *A. albopictus* are abundant at a new site. If a new site is initially colonized by only one or a few mated females, as would be the case if a single vehicle transported a few adult 'stow-aways' to the new area, then the initial population size of *A. albopictus* would be small, and thus the competitive impact on *O. triseriatus* would be small, even though *A. taiwanensis* infection would be low or absent. However, *A. albopictus* populations are probably often established when multiple tires or other containers carrying *A. albopictus* eggs or larvae are moved into a new area. In such cases, the initial

population of *A. albopictus* may be quite large, and the absence or scarcity of *A. taiwanensis* (e.g. if the source location of *A. albopictus* had a low parasite population due to recent establishment – Blackmore et al. 1995), may enhance *A. albopictus*'s competitive impact on *O. triseriatus*.

The effect of escape from parasitism may also be important if the *A. albopictus* population is able to grow rapidly, before the parasite population builds up. This possibility has been observed in areas of Florida where, in less than 1 year, *A. albopictus* has often gone from being absent to being the dominant *Aedes* species (O'Meara et al. 1995), while *A. taiwanensis* infections in those areas during the same time period remained very low (Fukuda et al. 1997). Despite the recency of colonization of Peoria, in 1999, hundreds of *A. albopictus* larvae could be observed in a single tire (B.W. Aliabadi, personal observation), but infection intensity was still very low compared to the long-established site in East St Louis (Figure 3). Further, prevalence of infection at the Peoria site was very low early in both 1999 and 2000, in marked contrast to high prevalence of infection at all times in East St Louis (Figure 4). Clearly, many larvae are unparasitized, and those that are parasitized have low parasite loads at the most recently colonized sites. Our data do not address another important question about the role of *A. taiwanensis*: What may be the role of spatial structure (patchiness) in a location that is undergoing invasion by *A. albopictus* and its parasite *A. taiwanensis*? If breeding sites for *A. albopictus* are patchily distributed, there may be important temporal and spatial variation among local patches in the prevalence of parasitism, which may contribute to temporal and spatial patterns of invasion and increase by *A. albopictus*. Though our data do not address this issue, it remains an important area in need of investigation.

Although our field data and those of others (Munstermann and Wesson 1990; Blackmore et al. 1995; Fukuda et al. 1997) suggest that it may take several years before large *A. taiwanensis* populations become established, sites may vary in length of time for parasite establishment due to the routes by which parasites are dispersed to new sites. Although most *A. taiwanensis* oocysts are defecated into the water from which an adult ecloses (Beier and Craig 1985), infected adult mosquitoes continue to defecate oocysts throughout their lives (Beier and Craig 1985). Thus, the primary dispersal route of the parasite in most new containers may be via an infected dispersing female

defecating into the container into which she oviposits, which may deposit relatively few oocysts in that container. Although transovarial transmission of gregarine parasites has never been observed (Fukuda et al. 1997), mosquito eggs can become contaminated on their surfaces with parasite oocysts defecated during oviposition (Vavra 1969). This transovum transmission may be the primary dispersal route of the parasite in certain situations, and may lead to more oocysts deposited into a new container than would be deposited if the ovipositing mosquito simply defecated. Thus, containers where transovum transmission occurs may exhibit a more rapid establishment of *A. taiwanensis*. Further investigation is needed to assess the relationship between route of oocyst transmission and the rate at which parasite populations increase.

In addition to invading *A. albopictus* having a small competitive advantage over *O. triseriatus* due to escape from *A. taiwanensis*, *A. albopictus* may intrinsically exhibit greater competitive ability than the native species, a trait common to many successful invasive exotics (Drake et al. 1989). *Ochlerotatus triseriatus* develop more slowly than do *A. albopictus* (Teng and Apperson 2000), and this slow development is exaggerated in competitive interactions (Ho et al. 1989). In addition, *O. triseriatus*'s slower palatal mouth-brush movements (152 strokes/min versus 221 strokes/min for *A. albopictus*; Ho et al. 1989) and high mortality in interspecific encounters may contribute to its poor competitive ability. Lower metabolic rate and poorer adaptation to tire habitats have also been postulated as mechanisms by which *O. triseriatus* is easily out-competed by other *Aedes* mosquitoes (Ho et al. 1989). Barrera (1996) reported that *O. triseriatus* has a weaker ability to withstand starvation compared to *A. albopictus*. In general, interspecific competition may be more detrimental than intraspecific competition to *O. triseriatus* (Ho et al. 1989).

Because survivorship of *O. triseriatus* was so low in our experiment, we were unable to analyze data on *O. triseriatus* mass or development time. However, the impact of *A. taiwanensis* parasitism on *A. albopictus*'s competitive effect on survivorship and estimated rate of population increase of *O. triseriatus* provide the clearest indication of differential competitive ability of *O. triseriatus* against infected and uninfected *A. albopictus* (Grill and Juliano 1996; Juliano 1998). Development of *A. albopictus* females was prolonged by infection with *A. taiwanensis*, but only at low larval densities. The apparent reversal of this parasite effect

at high densities (Figure 7) may seem counterintuitive, but at high densities, *A. albopictus* development may be maximally prolonged due to intraspecific competition, so that parasitism of larvae becomes relatively unimportant. Indeed, at the highest combined density, median eclosion time for unparasitized females was 52 days, compared to 23.5 days at the lowest combined density. In addition, when host development is greatly prolonged, parasites may fail to complete development (Chen and Yang 1996), and so may have their impact reduced.

We detected no significant effect of *A. taiwanensis* infection on any aspect of *A. albopictus* male fitness. Numerous investigators have shown that most environmental challenges are more detrimental to female than to male *Aedes* (Walker et al. 1987; Lounibos et al. 1993; Juliano and Stoffregen 1994; Ball and Baker 1996; Hechtel and Juliano 1997; Comiskey et al. 1999). This is likely due to the extra nutritional requirements of females due to larger mean size, and because females may be more sensitive to mortality sources in general (Juliano and Stoffregen 1994). In addition, males and females may have different susceptibilities to infection when exposed to *A. taiwanensis*, although field data in New Orleans by Comiskey et al. (1999) suggest any differences are minor.

Successful invasion of the Midwest by *A. albopictus* may have practical consequences, as parts of the Midwest are focal regions for La Crosse encephalitis (Clark et al. 1983a, b). La Crosse virus has recently been isolated from field-collected *A. albopictus* in North Carolina and Tennessee (L.P. Lounibos, personal communication), and *A. albopictus* can transmit the virus in the laboratory equally or more efficiently than the natural vector, *O. triseriatus* (Grimstad et al. 1989; Cully et al. 1992). Thus, as *A. albopictus* spreads, it may become an additional, or even the primary, vector. In addition, competition with *A. albopictus* is likely to lead to reduced size of *O. triseriatus* adults (Novak et al. 1993). This reduction in size of *O. triseriatus* adults may enhance *O. triseriatus* transmission of La Crosse, as reduced adult size is associated with increased efficiency of transmission (Grimstad and Haramis 1984; Paulson and Hawley 1991). Thus, escape from *A. taiwanensis* infection, which may give *A. albopictus* an additional competitive advantage over *O. triseriatus*, may also affect La Crosse virus transmission. However, the overall impact of successful invasion by *A. albopictus* on La Crosse transmission will depend on both the

ability of *A. albopictus* to act as a vector and on the impact of this invasion on the native vector *O. triseriatus*. Predicting such complex effects probably requires explicit modeling of the vector-host-parasite system.

There are many cases where introduction of natural parasites or predators of invasive, exotic species has led to their control (see reviews by Clausen 1978; Coulson and Soper 1989; Rosen 1991). However, although it is clear from these studies that natural enemies have significant impacts on an exotic's ability to invade, few empirical studies have been conducted that test experimentally the mechanisms by which escape from a natural enemy can enhance exotic species invasion and establishment. This study has shown that escape from parasite-induced reduction in competitive ability may be one mechanism by which escape from a natural enemy may facilitate invasion and enhance impact on a native species. Researchers studying invasion biology should consider this type of effect of escape from natural enemies when investigating the success and impact of introduced species.

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