Prospects for an Invasion: Competition Between *Aedes albopictus* and Native *Aedes triseriatus*

Todd P. Livdahl and Michelle S. Willey

Competition between larval populations of the native North American treehole mosquito *Aedes triseriatus* and *Aedes albopictus*, recently introduced from Asia to North America, was assessed by comparing per capita growth rate estimates for experimental cohorts of larvae developing under a variety of initial density combinations in fluid obtained from tires or from treeholes. Estimates of carrying capacities and competition coefficients indicate that competition between the two species will result in stable coexistence in treehole communities but local extinction of *A. triseriatus* in tire habitats.

AEDES ALBOPICTUS, A CONTAINER-breeding mosquito, has begun to colonize the North American continent during the last several years, after its introduction within imported used automobile tires. Successful colonization by this potential vector for disease transmission, most notably dengue fever (*1*), and which occupies much of Asia and the Pacific region, was first observed in Houston in 1985, where it had already achieved dominance in artificial containers (*2*). *Aedes albopictus* has since become widespread, occurring in much of the southeastern and into the midwestern United States (*3*). The introduction of *A. albopictus* has public health significance, and the ecological outcome of this introduction will enhance our understanding of community structure. Successful establishment of *A. albopictus* without the displacement of native filter-feeding and browsing species will imply that evolutionary niche diversification in response to prolonged competition between larval stages is not necessary for the coexistence of ecologically similar species.

Aedes albopictus has demonstrated an aptitude for rapid colonization of artificial aquatic larval habitats, such as discarded automobile tires (*3*). Egg diapause responses to photoperiod and freezing tolerance indicate that *A. albopictus* immigrated from temperate Japanese populations (*4*) preadapted for physical conditions in much of North America. Despite its colonizing ability and tolerance of physical conditions, the eventual success of *A. albopictus* may hinge on its ability to interact with native species within communities under invasion, particularly the mosquitoes that occupy treeholes and tires in eastern North America.

This experiment addresses the potential for competitive success of *A. albopictus* as a colonist of two habitats, tires and water-filled treeholes, both of which maintain well-developed native mosquito fauna. We examined competition between *A. albopictus* and *A. triseriatus*, a common filter-feeding and browsing inhabitant of treeholes and tires throughout the eastern United States (*5*), to determine whether *A. triseriatus* can prevent the establishment of this ecologically similar species. Previous comparisons of separate aspects of single and mixed species cohorts using artificial media in jars, tires, and cartons enable predictions that interactions of *A. albopictus* with resident species may result in a long-term establishment in tires (*6*).

Eggs obtained from laboratory colonies of *A. albopictus* and *A. triseriatus* (*7*) were hatched 24 hours before the experiment. Larvae of each species were disbursed, in all density combinations of 7, 14, 28, and 42 with single species controls for each density, into uncovered 120-ml plastic cups containing 0.5 g of dried leaf litter and 100 ml of treehole or tire fluid, depending on the habitat treatment (*8*). For treehole simulations, 25 ml of fluid was replaced weekly with fresh tree drainage water (stemflow), collected with a slit hose caulked about a maple tree. Both habitat simulations received weekly replacement of evaporated water with distilled water. Experimental populations were maintained in an insectary (22° ± 1°C, ≈70% relative humidity, and 16 hours light:8 hours dark photoperiod). Three replicates were raised for each two-species density combination and five replicates of all single-species density levels. Pupae were removed from experimental habitats and placed within vials of water topped with inverted vials. Adults were removed daily; female wings were measured at magnification ×25.

Per capita growth rates were estimated for all larval cohorts from the composite index (*9*)

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

in which N_0 represents the initial number of females, A_x the number of females emerging on day x, and w_x the mean winglength of females emerging on day x; $f(w_x)$ predicts numbers of female offspring based on female body size; and D is an estimate of the delay between female emergence and first oviposition. Separate studies of individual females, offered blood daily after emergence, provide estimates of $D = 11.9$ days for *A. triseriatus* and $D = 14.2$ days for *A. albopictus*. Linear functions describe size-dependent fecundity: $f(w_x) = 23.2w_x - 51$ for *A. triseriatus* ($r^2 = 0.38$) and $f(w_x) = 17.2w_x - 14$ ($r^2 = 0.03$) for *A. albopictus* (*10*).

Regression of r' on the initial densities of *A. triseriatus* and *A. albopictus* can be expressed in the form

$$r'_i \approx \frac{dN_i}{N_i dt} = r_{m_i} + b_i N_i + b_j N_j \qquad (2)$$

When $(r_{mi} + b_i N_i + b_j N_j)$ is set equal to zero and N_j is plotted as a function of N_i, the result is a line that defines the combinations of initial densities that result in no population growth for species i, the zero growth isocline (Fig. 1). Density combinations falling below an isocline permit continued growth of the species associated with that isocline; density combinations above an isocline will result in the species' decline.

In treehole fluid (Fig. 1A), the isoclines intersect at positive densities for both species; long-term interaction between these species should therefore result in their continued coexistence in treeholes. In tire fluid (Fig. 1B), the isoclines do not intersect at a positive density for *A. triseriatus*; long-term association between these species should result in the competitive exclusion from tires of *A. triseriatus* by *A. albopictus*.

The Lotka-Volterra model of two-species competition provides a useful starting point for the analysis of this interaction, in which the per capita rate of change for species i is a direct analog of Eq. 2

$$\frac{dN_i}{N_i dt} = r_{m_i} - \frac{r_{m_i}}{K_i} N_i - \frac{r_{m_i}\alpha_{ij}}{K_i} N_j \qquad (3)$$

Within this framework, the stability of the interactions depends on a combination of the competition coefficients (α_{ij}), which convert the per capita influence of species j on the growth rate of species i into the equivalent number of species i and carrying capacities (K_i). These quantities are calculated indirectly from the intercept and regression coefficients of Eq. 2.

The difference in the predicted outcome for the two fluids is clarified by comparing

---

Department of Biology, Clark University, Worcester, MA 01610.

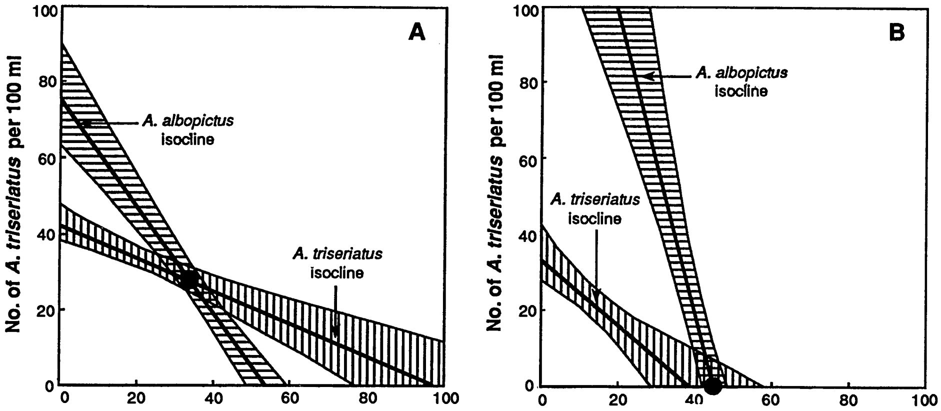

Fig. 1. Density combinations that permit equilibrium for *Aedes triseriatus* and *A. albopictus*. Hatched regions overlay combinations for each species for which the 95% confidence interval of the predicted per capita rate includes zero in (**A**) treehole and (**B**) tire-simulated habitats. The equilibrium combination of densities is circled for each fluid treatment. Differences between the intercepts of the two isoclines are statistically significant on both axes in treehole fluid and on the vertical axis in tire fluid ($P < 0.01$ in each case); the difference between intercepts on the horizontal axis in tire fluid is not significant. Standard errors for these t tests were obtained with the jackknife technique (*11*).

K_i and α_{ij} in each fluid for each species (Fig. 2). The difference does not result from altered K_i's alone, which are significantly lower in tire fluid for both species, reduced by nearly the same amount for each. The weekly addition of nutrients and removal of metabolic wastes by replacing 25 ml with stemflow in treehole fluid may account for much of this difference in K_i.

The differential competitive outcome in the two fluid types results primarily from significant and opposite differences in α_{ij}'s for the two species. If these coefficients reflect overlap of resource use, the differences suggest that resources exist within tires that can be used much more effectively by *A. albopictus* than *A. triseriatus*, and that treehole fluid contains resources that each species exploits differentially. Detailed studies of the larval diets of these two species in the two habitat types should be informative.

This analysis could be enhanced by the incorporation of a wider variety of initial densities of the two species, which would permit tests for nonlinear concave responses to density, known to occur in *A. triseriatus* growing in an artificial medium (*11*), as well as nonadditive interactions between the competitors. However, the narrow isocline regions defined by the linear model for *A. triseriatus* in treehole fluid suggest that nonlinear isoclines resulting from such nonlinear models should not vary substantially from this linear case, leaving intact the prediction of coexistence. More likely, our prediction of competitive exclusion of *A. triseriatus* in tire fluid may require modification to include the possibility of its coexistence with *A. albopictus* at very low densities.

Sufficient time may not have elapsed since the introduction of *A. albopictus* to enable the test of these predictions in field populations, but coexistence between *A. albopictus* and *A. triseriatus* within treehole communities is suggested by the appearance of *A. triseriatus* and *A. albopictus* as the first and second most abundant filter-feeding species in glass jars attached to trees in a Louisiana forest (*14*), making up 81% and 17% of the filtering and browsing larvae collected. The co-occurrence of these two species has also been noted in other studies of treehole communities (*15*). Field studies of *A. albopictus* with *A. aegypti* in artificial habitats, tires in Texas (*2*) and cemetery vases in Florida (*15*), suggest success by *A. albopictus* as a competitor in such containers, with a concomitant reduction of *A. aegypti* populations. *Aedes triseriatus* is only abundant in tire habitats located within shaded areas, and few detailed census data are available for such habitats in areas that have been reached by dispersing *A. albopictus*. However, the potential for *A. albopictus* to dominate artificial habitats has been clear since its discovery in Texas, where *A. albopictus'* abundance was three times that of *A. aegypti* and 20 times that of *A. triseriatus* (*2*).

Although *A. triseriatus* may be the most likely potential treehole competitor, other treehole species might interact with *A. albopictus*. Differences among filter-feeding species in vulnerability to the predatory mosquito larva *Toxorhynchites rutilus* in the Southeast could influence the outcome of the *A. albopictus* invasion. Additional species of potential importance to the establishment of *A. albopictus* in treehole communities include *Anopheles barberi*, a potential filter-feeding competitor that becomes a facultative predator on small larvae during its fourth instar (*16*), and three additional filter-feeding and browsing species: *Aedes hendersoni*, *Orthopodomyia signifera*, and *O. alba*. However, all four of these potential competitors have marked preferences for restricted types of treeholes (*5*), and are therefore unlikely to present consistent ob-

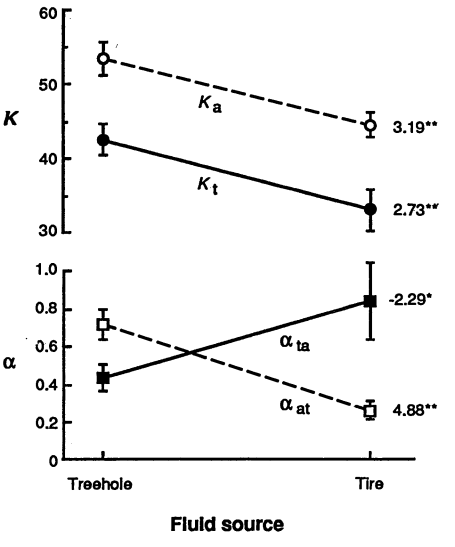

Fig. 2. Comparisons of parameters of competition in treehole and tire fluid. K_t and K_a depict carrying capacities for *A. triseriatus* and *A. albopictus*. Competition coefficients translate the competitive effect of an *A. albopictus* individual on *A. triseriatus* into the equivalent number of *A. triseriatus* individuals (α_{ta}), and the reverse translation (α_{at}). Standard errors for each estimate, obtained with the jackknife technique (*11*), are shown as vertical bars. The t statistics for differences of parameters estimated for treehole and tire fluid treatments are shown (*$P < 0.05$, **$P < 0.01$).

Table 1. Responses of per capita growth rate estimates for each species to the initial density of *Aedes triseriatus* and *A. albopictus* larval cohorts in two types of fluid. Regression analyses are based on Eq. 2. Standard errors for each regression term and the coefficient of determination (r^2) are shown. All regression coefficients are statistically significant ($P < 10^{-4}$). Units are as follows: r_m, days^{-1}; b_1 and b_2, days^{-1} × (individuals/100 ml)$^{-1}$. The numbers of r' values obtained for each regression (n) differ because some cohorts produced no survivors, especially *A. triseriatus* growing at high densities in tire fluid (*10*).

Fluid	Species	n	r_{m_i}	b_1	b_2	r^2
Treehole	*triseriatus*	65	0.0514 ± 0.0029	−0.0012 ± 0.0001	−0.0005 ± 0.0001	0.74
	albopictus	64	0.0798 ± 0.0036	−0.0015 ± 0.0001	−0.0011 ± 0.0001	0.80
Tire	*triseriatus*	35	0.0591 ± 0.0070	−0.0018 ± 0.0003	−0.0015 ± 0.0003	0.66
	albopictus	65	0.0904 ± 0.0036	−0.0020 ± 0.0001	−0.0005 ± 0.0001	0.82

stacles to the establishment of *A. albopictus*; a large fraction of treeholes contain only *A. triseriatus* as potential competitors (*5*). Our results indicate that competition with the predominant species in treehole communities, *A. triseriatus*, will be insufficient to prevent the establishment of *A. albopictus*.

REFERENCES AND NOTES

1. D. Shroyer, *J. Am. Mosq. Control* **2**, 424 (1986).
2. D. Sprenger and T. Wuithiranyagool, *ibid.*, p. 217.
3. *Am. Mosq. Control Assoc. Newsl.* **16** (no. 3), 13 (1990); *ibid.* **16** (no. 2), p. 5; *ibid.* **15** (no. 1), 15 (1989).
4. W. Hawley, P. Reiter, R. Copeland, C. Pumpuni, G. Craig, *Science* **236**, 1114 (1987).
5. W. Bradshaw and C. Holzapfel, *Oecologia* **57**, 239 (1983); *ibid.* **74**, 507 (1988); R. Copeland and G. Craig, *Ann. Entmol. Soc. Am.* **83**, 1063 (1990).
6. W. Black, K. Rai, B. Turco, D. Arroyo, *J. Med. Entomol.* **26**, 260 (1989); C. Ho, A. Ewert, L. Chew, *ibid.*, p. 615.
7. *Aedes albopictus* and one *A. triseriatus* population were established by mosquitoes collected in New Orleans, LA. Additional *A. triseriatus* populations were established by females from central Massachusetts. A three-way analysis of variance of the interactions of Louisiana and Massachusetts *A. triseriatus* with *A. albopictus* reveal no statistically significant differences between *A. triseriatus* strains (T. Livdahl, unpublished data), and the results presented here combine the results of both strains.
8. After removing mosquito larvae, without removing detritus, fluid contents of ten treeholes and ten tires were pooled and homogenized with a canoe paddle during fluid disbursement.
9. T. Livdahl and G. Sugihara, *J. Anim. Ecol.* **53**, 573 (1984).
10. Some cohorts failed to produce adult females. In such cases, the cohort was pooled with emergence data from another cohort within the same experimental cells, with r' calculation based on the total number of individuals for the pooled cohorts.
11. R. Sokal and F. Rohlf, *Biometry* (Freeman, New York, 1981), pp. 795–799.
12. J. Hard, W. Bradshaw, D. Malarkey, *Oikos* **54**, 137 (1989).
13. E. Walker and R. Merritt, *Environ. Entomol.* **17**, 199 (1988).
14. E. Schreiber, C. Meek, M. Yates, *J. Am. Mosq. Control* **4**, 9 (1988).
15. G. O'Meara, "The spread of *Aedes albopictus* and *Aedes bahamensis* in Florida," paper presented at the 1990 Annual Meeting of the Entomological Society of America, New Orleans, 2 to 6 December 1990.
16. J. Peterson, H. Chapman, O. Willis, *Mosq. News* **29**, 134 (1969).

17. We thank J. Edgerly-Rooks, S. Gaimari, and J. Turner for technical assistance, O. Sholes, R. Bertin, and two anonymous reviewers for helpful suggestions, J. Freier for *A. albopictus* eggs, and NIH for financial support (R15AI27940 to T.P.L.).

21 January 1991; accepted 30 April 1990

Structural Features That Give Rise to the Unusual Stability of RNA Hairpins Containing GNRA Loops

HANS A. HEUS* AND ARTHUR PARDI†

The most frequently occurring RNA hairpins in 16*S* and 23*S* ribosomal RNA contain a tetranucleotide loop that has a GNRA consensus sequence. The solution structures of the GCAA and GAAA hairpins have been determined by nuclear magnetic resonance spectroscopy. Both loops contain an unusual G-A base pair between the first and last residue in the loop, a hydrogen bond between a G base and a phosphate, extensive base stacking, and a hydrogen bond between a sugar 2′-end OH and a base. These interactions explain the high stability of these hairpins and the sequence requirements for the variant and invariant nucleotides in the GNRA tetranucleotide loop family.

RNA HAIRPINS CONSISTING OF A double-stranded stem and a single-stranded loop are among the most common structural motifs in RNA. RNA hairpin loops have been viewed simply as a means for the RNA strand to fold back on itself, and it was thought that the sequence of the loop might not be very critical. However, recent phylogenetic and thermodynamic studies have revealed strong size and sequence dependencies for hairpin loops in RNA (*1*). In particular, hairpins containing a tetranucleotide loop seem to be very important. Phylogenetic studies show that in ribosomal RNAs (rRNAs) tetranucleotide loops comprise more than 55% of all loops, and more than 50% of all tetranucleotide loops have the consensus sequence GNRA (where N can be any nucleotide and R is either G or A) (*1*). The GNRA hairpin loops also occur with high frequency in catalytic, phage, and eukaryotic signal-recognition particle (SRP) RNAs (*2*). The solution structure of another frequently occurring RNA hairpin, which belongs to the UNCG family of tetranucleotide loops, has been reported (*3*).

RNA hairpins containing the GNRA loop sequence have been shown to be substantially more stable, with melting transition temperatures (T_m) more than 4°C higher than other less frequently occurring sequences (*4*). These thermodynamic and phylogenetic data led to the proposal that GNRA tetranucleotide loops may be evolutionarily selected because of their high stability, which allows them to serve as nucleation sites for proper folding of larger RNAs (*5*). In this report we identify the structural properties that give rise to the sequence requirements and high stability of hairpins containing GNRA loops.

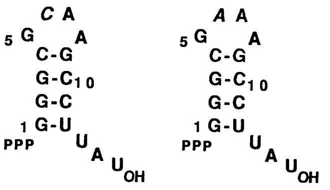

Fig. 1. The full sequences for the GCAA and GAAA RNA hairpins (*17*).

The GCAA and GAAA loops were chosen for these nuclear magnetic resonance (NMR) studies because they are the most frequently occurring members of the GNRA family. The full sequences of the two hairpins are shown in Fig. 1 (*6*). Proton and ^{31}P resonances were assigned by standard two-dimensional (2-D) NMR techniques (*7*). Short (<4.5 Å) ^{1}H-^{1}H distances were identified by 2-D nuclear Overhauser effect (NOE) spectroscopy, and Fig. 2 shows a portion of the spectrum that was used to assign aromatic and sugar C-1′ proton resonances for the GCAA hairpin. Assignment

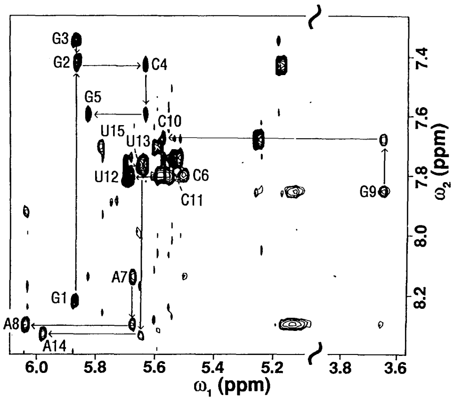

Fig. 2. A portion of the 2-D NOE spectrum of the GCAA hairpin in D_2O showing the aromatic H2/H8/H6 to sugar H1′/H5 region. This spectrum was taken with ~1.9 mM RNA hairpin in 100 mM NaCl, 10 mM sodium phosphate, pD = 6.8, at 25°C. Examples of sequential resonance assignment pathways involving H6/H8(n)-H1′9(n)-H6/H8($n+1$) resonances are illustrated. Note the unusual upfield shift of the G9 H-1′.

Department of Chemistry and Biochemistry, University of Colorado at Boulder, Boulder, CO 80309.

*Present address: Department of Biophysical Chemistry, University of Nijmegen, Tournooiveld, 6525 ED Nijmegan, Netherlands.

†To whom correspondence should be addressed.