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The community ecology of *Aedes* egg hatching: implications for a mosquito invasion

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Abstract. 1. A recently introduced treehole mosquito from Asia, *Aedes albopictus*, is spreading throughout eastern North America, especially in tyre-refuse piles. Previous studies have identified inhibitory effects of larvae on egg hatch as a potential population regulatory mechanism within *Aedes*. Larva–egg interactions may also occur between species. This experiment assesses the ability of larvae of *A.albopictus* and two possible competitors in North America, *A.triseriatus* and *A.aegypti*, to suppress hatching of conspecific and congeneric eggs.

2. We exposed eggs of each species to varying combinations of larval species and density for 24 h and assessed subsequent hatch rates. *Aedes albopictus* eggs exhibited the lowest level of inhibition when exposed to high larval densities; moreover, at the lowest larval density they imposed the most intense interspecific hatch inhibition.

3. Discretionary hatching in response to larval density may influence community composition by promoting the spread of *A.albopictus*, perhaps even leading to its dominance within North American *Aedes* communities.

Key words. *Aedes aegypti*, *Aedes albopictus*, *Aedes triseriatus*, biological invasion, egg hatch inhibition, interspecific competition, mosquito, treehole communities.

Introduction

Aedes albopictus Skuse (Diptera: Culicidae) has recently colonized many southern and midwestern states in the United States (recent reviews: Hawley, 1988; Rai, 1991). Used tyres traded from Japan most likely provided the source of eggs (Hawley *et al.*, 1987), and interstate movement of tyres promoted further dispersal of the population. Because of this new arrival, North American mosquito communities appear to be changing in species composition and dominance structure. The introduced species' generalized oviposition habits (Hawley, 1988; Sota *et al.*, 1992) may have promoted its spread into artificial containers as well as natural treeholes (Foster, 1989). In either case, *A.albopictus* has colonized habitats occupied by potential congeneric competitors. Previous studies have addressed two possible mechanisms that would allow the establishment of *A.albopictus* in the United States: mating interference and larval competition.

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Asymmetric mating interference may explain displacement by *A.albopictus* of other *Aedes* populations in the laboratory (e.g. Gubler, 1970) and in the field (Rozeboom & Bridges, 1972). Aggressive interspecific mating by *A.albopictus* males may partially explain the recent decline in *A.aegypti* L. in Louisiana (Nasci *et al.*, 1989).

Competition with established North American container-breeding mosquitoes, specifically *A.aegypti*, which had prevailed in exposed tyre habitats in the southeast before the introduction, and *A.triseriatus* Say, which dominates eastern treeholes and tyres discarded in forests, will not likely prevent the establishment of *A.albopictus*. Experiments using artificial medium and *A.aegypti* or *A.triseriatus* as a competitor suggest a potential for coexistence between *A.albopictus* and resident species occupying tyre habitats (Black *et al.*, 1989; Ho *et al.*, 1989). Experiments using treehole and tyre fluid and *A.triseriatus* as a competitor support the prediction of stable coexistence between *A.albopictus* and *A.triseriatus* in treeholes, and displacement of *A.triseriatus* from tyre habitats (Livdahl & Willey, 1991).

A third mechanism that could promote the establishment

of *A.albopictus* in the United States, not considered in earlier studies of interspecific interactions within mosquito communities, is the influence of larvae on eggs. Field (Livdahl & Edgerly, 1987) and laboratory (Livdahl *et al.*, 1984) studies on *A.triseriatus* demonstrated that egg hatch declines in response to increases in larval density, thus regulating cohort recruitment. If other *Aedes* display similar behaviour, egg hatch inhibition may affect the spread of *A.albopictus* by altering the rate at which larvae enter the habitat. The process may pre-empt, amplify, or even reverse the effects of mating interference or larval competition, and the interaction has the potential to enable rapid changes in larval species composition.

In *Aedes*, eggs are laid in clusters on the sides of aquatic containers usually above the water line, where embryogenesis proceeds without interruption within a few days after oviposition (Shroyer & Craig, 1980). Following inundation with rainwater, micro-organisms colonize the egg surfaces and the subsequent decline in dissolved oxygen, due to microbial respiration, triggers hatch (Gjullin *et al.*, 1941; Judson, 1960; references in Hawley (1988) for *A. albopictus*). Despite the presence of hatching stimuli, a portion of the eggs often remain dormant until the water level has risen and receded repeatedly, a phenomenon known as installment hatching (e.g. Andreadis, 1990; references in Gillett, 1971). In *A.aegypti* (Gillett *et al.*, 1977) and *A.triseriatus* (Edgerly & Marvier, 1992), larvae impede hatching by grazing micro-organisms from egg surfaces; as larval density increases, more bacteria are consumed and egg hatch rate declines.

Because larval feeding on micro-organisms seems a basic and simple mechanism for hatch inhibition, we anticipated that foraging larvae of other *Aedes* species may impose interspecific, as well as intraspecific, hatch inhibition. We also anticipated that different *Aedes* larval species would exert interchangeable effects on the hatching of different *Aedes* egg species.

In this study we evaluate egg hatch rates of *A.albopictus* and two possible competitors, *A.aegypti* and *A.triseriatus*, as they respond to varying larval densities of each species.

Materials and Methods

We examined responses of eggs of each species exposed to final instar larvae of each species at a range of densities within which intraspecific hatch inhibition occurs for *A.triseriatus* (Livdahl *et al.*, 1984): 0 (control), 4, 12, and 24 larvae per 25 ml vial. We obtained eggs from laboratory populations started from field-collected adults: *Aedes albopictus* and *A.aegypti* from New Orleans in 1989 and 1991, and *A.triseriatus* from central Massachusetts in 1991. Females laid eggs on moist paper, which we stored at $22 \pm 1°C$, ≈70% relative humidity (16L:8D) until needed.

To test hatch responses, we submerged batches of twenty-four eggs in nutrient broth (DISCO brand; 1.0 g/l), a known hatching stimulus (Novak & Shroyer, 1978), and then immediately added larvae. Our design included six replicate vials of each egg species, larva species and density combination, except for those with twenty-four *A.albopictus* larvae, which had five replicates with *A.aegypti* eggs and four replicates each with *A.triseriatus* and *A.albopictus* eggs. After 24 h, we counted the number of hatched and unhatched eggs. For each replicate, we subtracted the number of infertile eggs, determined by dissection following the trial, from the total number of unhatched eggs so that our final analysis included only eggs that could have hatched.

Significant departures from normality due to skewed distributions in opposite directions (Shapiro-Wilk test, SAS Institute Inc., 1991), and significant inequalities of variances (O'Brien's, Brown-Forsyth and Levene's tests, SAS Institute Inc., 1991) necessitated a statistical approach that did not rely on assumptions about distributions. Therefore, we employed nonparametric tests for main effects and interactions among factors in lieu of analysis of variance. We inspected the fraction hatched as the dependent variable as a function of (1) the control groups with no larvae, using a Kruskal-Wallis test (SAS Institute, Inc., 1991) and (2) combinations of egg species, larva species and density. We analysed species differences in control batches separately from those with larvae because egg batches exposed to zero larvae could not be assigned to a larva species treatment. In a manner analogous to a factorial analysis of variance, we used the methods of Wilson (1956) to test for overall effects of experimental factors (egg species, larva species and density), pairwise interactions between factors, and a three-way interaction.

Results

Hatching in the absence of larvae

The species varied in their baseline hatch rates without larvae (Kruskal-Wallis $\chi^2 = 11.8$, $P < 0.01$). The differences occurred between *A.triseriatus* and each of the other two species (Fig. 1); *A.aegypti* and *A.albopictus* hatch rates did not differ significantly (Kruskal-Wallis $\chi^2 = 0.7$, $P > 0.40$).

Hatching in the presence of larvae

The diversity of hatch response patterns resulted from differences in egg sensitivity and larval inhibitory effects (Fig. 1). Egg species, larva species and density, as well as interactions involving egg species and density as well as larva species and density, significantly influenced hatch rates (Table 1). Although each larva species inhibited egg hatch and each egg species exhibited inhibition to some degree relative to control eggs, this experiment revealed species-specificity for responses of each egg species to larvae and for larval inhibitory tendency. For example, unlike eggs of *A.triseriatus* and *A.aegypti*, a relatively large fraction of *A.albopictus* eggs hatched even at high larval densities, regardless of larva species.

We inspected the significant interactions by excluding each egg species and larva species in turn and repeating

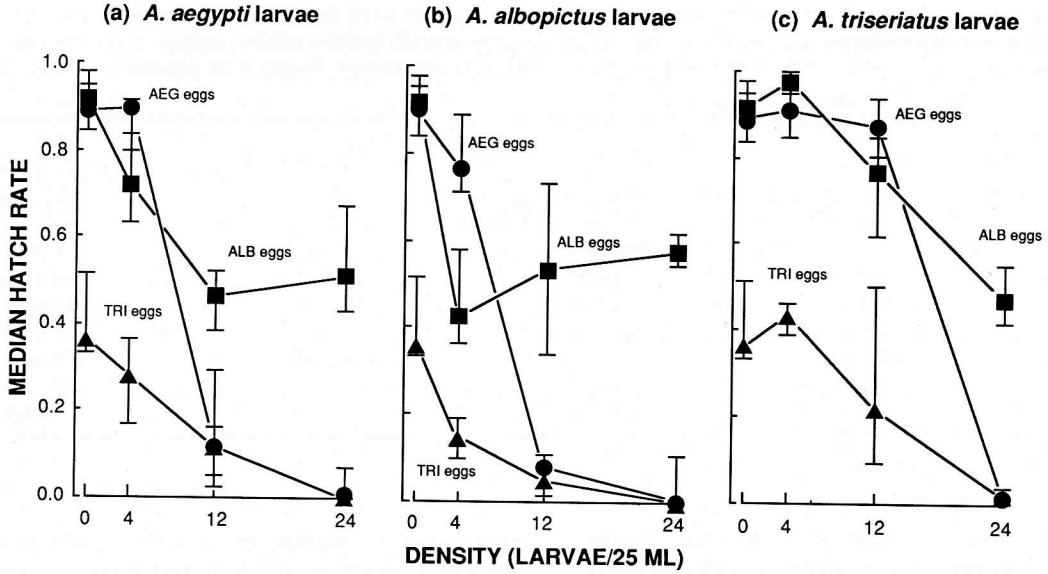

Fig. 1. Larval effects on hatch rates of *Aedes albopictus*, *A.aegypti* and *A.triseriatus*. Each point represents the median proportion hatched for each combination of larval density, larval species [(a) *A.aegypti* larvae, (b) *A.albopictus* larvae, and (c) *A.triseriatus* larvae] and egg species. Egg species are designated as *A.aegypti* (●), *A.albopictus* (■) and *A.triseriatus* (▲). Error bars depict the range between the 25th and 75th percentiles associated with each median.

the analysis on the remaining subsets of data. Our rationale was that a switch from a significant to a nonsignificant interaction would indicate that the excluded data contributed to the significant interaction obtained in the full analysis. This technique identifies the sources of the species-specific responses of eggs to larvae.

When data for eggs of either *A.aegypti* or *A.albopictus* were removed from the analysis, the Egg species × Density (E × N) interaction became nonsignificant, indicating that the pattern of response for *A.triseriatus* does not differ from the response pattern of either of the other species, despite the low overall hatch rate for *A.triseriatus*, and that the response patterns of *A.aegypti* or *A.albopictus* differed due to the more intense inhibition of *A.aegypti* eggs at high densities (Fig. 2a).

The interaction between Larva species and Density (L × N) became nonsignificant when we excluded egg batches exposed to *A.triseriatus* larvae from the analysis (Table 1), but not when data were removed for either of the other larva species. Thus, *A.triseriatus* larvae elicited a differential egg hatch response. *Aedes albopictus* and *A.aegypti* larvae had similar general patterns of density-

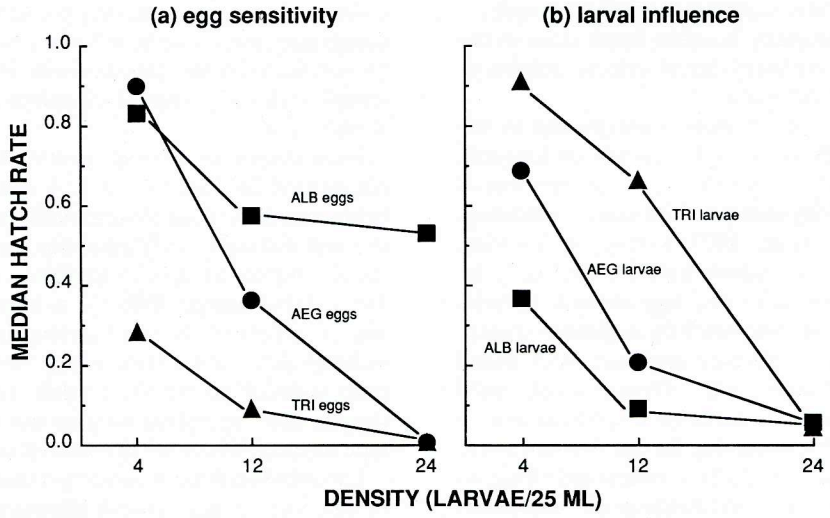

Fig. 2. Egg responses and larval effect for each species of *Aedes* [designated as *A.aegypti* (●), *A.albopictus* (■) and *A.triseriatus* (▲)]. (a) Species comparisons of egg sensitivity. Points represent the median proportion hatched for each egg species at each density level, pooled across all three larval species. (b) Species comparisons of larval influence. Each point represents the median proportion of eggs hatched at each density of larvae, pooled across all three egg species.

Table 1. Chi-squared values from Wilson's (1956) nonparametric analogue of a factorial analysis of variance for overall effects of egg species, larva species and density on hatch of *Aedes* eggs. Results are shown for the full analysis, and for analyses of subsets of the data that produced nonsignificant Larva species × Density and Egg species × Density interactions. Numbers in parentheses denote degrees of freedom.

		Analyses of data subsets, excluding:		
Source	Full analysis	*A.aegypti* eggs	*A.albopictus* eggs	*A.triseriatus* larvae
Egg species (E)	15.4 (2)**	15.4 (1)**	3.7 (1)ns	11.9 (2)**
Larva species (L)	10.9 (2)**	8.5 (2)*	8.7 (2)*	1.4 (1)ns
Density (N)	30.1 (2)**	6.4 (2)*	44.1 (2)**	15.5 (2)**
E × L	1.6 (4)ns	0.6 (4)ns	1.6 (4)ns	1.5 (2)ns
E × N	16.2 (4)**	4.7 (2)ns	4.0 (2)ns	18.0 (4)**
L × N	15.6 (4)**	13.7 (2)**	9.1 (2)*	2.9 (2)ns
E × L × N	7.1 (8)ns	1.3 (4)ns	4.5 (4)ns	2.1 (4)ns

* $P(>\chi^2) < 0.05$; ** $P(>\chi^2) < 0.01$; ns, $P(>\chi^2) < 0.05$.

dependent effects on egg hatch, but *A.triseriatus* larvae were less inhibitory than their congeners at low and intermediate densities (Figs 1 and 2b).

Discussion

Our results defy a simple mathematical description that could incorporate the diverse responses of these species to larval density. Depending on the species combination, response patterns can have concave or convex shape, or a combination of convexity and concavity, with abrupt descents to zero or with asymptotic descents to a minimum greater than zero. In addition, some responses have humps: although the rise in hatch rate for *A.triseriatus* eggs at low *A.triseriatus* density was not significant, three previous studies with *A.triseriatus* (Livdahl *et al.*, 1984; Livdahl & Edgerly, 1987; Edgerly & Marvier, 1992) have also shown either slight or strong stimulatory effects at low density. A model of these responses will likely require a minimum of four parameters: baseline hatch rates in the absence of larvae, stimulatory larval effects, inhibitory effects, and minimal hatch rates.

Although the precise mechanisms contributing to the observed species-specificity are not presently understood, our results point most strongly toward a combination of differential egg sensitivity and larval inhibitory tendency. Previous work (Gillett *et al.*, 1977; Edgerly & Marvier, 1992) suggests that larvae inhibit hatching indirectly by grazing micro-organisms from the egg surface, thereby removing agents that stimulate hatch by depleting oxygen. In *A.triseriatus*, grazing intensity increases with larval density (Edgerly & Marvier, 1992). Oxygen levels could also be influenced directly by cuticular respiration and/or turbulence generated by swimming larvae. Furthermore, egg responsiveness to such factors varies according to larval instar within *A.triseriatus* (Livdahl *et al.*, 1984) and, according to these results, between species as well. These and other factors merit more thorough examination. We hypothesize, based on the diversity of hatch response patterns, that species-specific responses result from differences in larval feeding rates, respiration rates, oxygen thresholds required for hatch, and differential changes in larval grazing behaviour with increased density.

Previous work on *Aedes* eggs also indicates that the timing of hatch varies not only among eggs within the same batch but also among strains within species. For example, clusters of eggs laid by *A.triseriatus* derived from treeholes exhibit highly asynchronous hatch whereas most eggs from a tyre population as well as those from an established laboratory strain generally require only one stimulus (Means *et al.*, 1977). The low hatch rate of *A.triseriatus* control batches in our experiment resembles the response of feral eggs, as found by Means *et al.* Feral and domestic strains of *A.aegypti* (Gillett, 1955) and *A.albopictus* (Mogi, 1982) also differ; a greater proportion of domestic eggs hatch following a single flooding. In our study, control eggs of these two species hatched at high rates, consistent with previous studies of domestic strains. The intensity of egg hatch inhibition by larvae may depend on these strain qualities in ways that are not yet determined. Predictions concerning the outcome of interactions should therefore be restricted to the strains used: in this case, domestic strains of *A.aegypti* and *A.albopictus*, and a feral strain of *A.triseriatus*.

Even though we utilized small vials that may not resemble natural habitats, we believe our results may predict behaviour in the field. Previous field work with *A.triseriatus* showed that egg hatch inhibition as a function of larval density occurs in natural treeholes (Livdahl & Edgerly, 1987). Furthermore, Edgerly & Marvier (1992) triggered similar discretionary egg hatching in a laboratory study utilizing petri dishes with a high surface area to volume ratio, especially compared to vials. Together, these results suggest that egg behaviour may not depend on container type as much as on larval encounters.

Larva-induced hatch inhibition clearly has the potential to influence the spread of *A.albopictus* in North America. The relative effectiveness of *A.albopictus* in inhibiting egg hatch of *A.triseriatus* and *A.aegypti*, combined with their propensity to hatch into occupied habitats, may provide *A.albopictus* with a significant advantage as they encounter

resident populations. This mechanism, when combined with the competitive ability of *A.albopictus* larvae, at least with *A.triseriatus* in tyre-water (Livdahl & Willey, 1991), may help to explain why *A.albopictus* has achieved rapid dominance in domestic container habitats previously occupied by *A.triseriatus* or *A.aegypti* in southern states (Sprenger & Wuithiranyagool, 1986; Nasci *et al.*, 1989; Rai, 1991).

Beyond competitive interactions, the ability of the invader to coexist with, or even dominate, resident species in aquatic containers may depend on their response to other factors influencing community organization, such as cold hardiness (Hawley, 1988), drought (Bradshaw & Holzapfel, 1988), differential response to oviposition cues (e.g. Gubler, 1971) and vulnerability to predators, especially *Toxorhynchites rutilus* (Coq.) in the southeast (Bradshaw & Holzapfel, 1983; Lounibos, 1985; Rai, 1991). Habitat changes can also have dramatic effects on the outcome of biological invasions; rampant urbanization in southeast Asia apparently stimulated an increase in abundance in *A.aegypti* introduced from Africa, at the expense of native *A.albopictus* (see Hawley, 1988, for review).

This experiment indicates that larva-induced hatch inhibition may be widely distributed among *Aedes* species. Over evolutionary time, egg hatch inhibition may represent a compromise between negative consequences of delayed reproduction and potentially more severe effects incurred when a younger cohort hatches and competes with older larvae (Livdahl, 1982; Edgerly & Livdahl, 1992). Such responses of eggs to larvae may, over ecological time, influence the outcome of a mosquito invasion in eastern North America.

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