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THE NATURE OF EGG HATCHING IN *AEDES TRISERIATUS*: ECOLOGICAL IMPLICATIONS AND EVOLUTIONARY CONSEQUENCES

Todd P. Livdahl and Robert K. Koenekoop

ABSTRACT

Variable delays before egg hatching within *Aedes* populations present ecological problems for larvae that hatch into saturated environments. Using *Aedes triseriatus*, we illustrate the potential magnitude of these ecological problems. Intense competitive effects of large larvae on small ones are shown through manipulation of initial density and concentration of natural fluid in artificial field habitats. The possibility of cannibalism by fourth instars on freshly hatched first instar larvae is established through the disappearance of first instars during 24 h exposures to fourth instar larvae, and a reduced rate of disappearance when a food supply is provided or when the age of the first instars is increased by 24 h. We demonstrate a mechanism by which such ecological problems may be mitigated through an inhibition of egg hatching when large larvae are abundant, and when food is in short supply.

The diversity in hatching time imposes consequences to individual fitness in the form of wide variation in age of reproduction. Sequential application of hatching stimuli in the laboratory reveals more than a 30-fold range of hatching delays. A laboratory comparison of larval fitness components reveals that late hatching groups survive to adulthood at higher rates, develop into adults of both sexes more rapidly, achieve larger adult size, and produce higher fractions of adult females than early hatching groups. Analysis of a composite measure of larval success shows a consistent rise in tolerance to crowding as hatch delay increases. This highly organized covariation among life history traits suggests that hatch delays have evolved in response to a primary level of selection, possibly imposed by frequent or unpredictable drought conditions. The evolution of associations of high larval fitness characteristics with late hatching may have arisen as a secondary, compensating selective process.

INTRODUCTION

An understanding of the interactions that occur within natural populations comprises an important requirement for the prediction of future population size. Hence, the intricacies of such interactions and the selective processes that influence the growth properties of populations merit our continued attention.

Many populations exhibit highly synchronized patterns of birth and development, in which the possible interactions among individuals are limited to a single age group. Organisms that display asynchronous birth and development patterns are subject to a more complex set of potential interactions among individuals.

Interactions within populations of many aedine mosquitoes are confounded by a phenomenon known as "erratic" or "installment" hatching (Breeland & Pickard 1967; Wilson & Horsfall 1970; Gillett 1955a, b, 1972; Gillett et al. 1977), in which only a fraction of eggs of the same age hatches in response to a given stimulus. When subsequent stimuli bring forth succeeding larval cohorts, interactions are possible within cohorts and between cohorts of different developmental stages. In addition, variable hatching delays can influence the degree to which generations overlap. Interactions among different developmental stages can, in turn, influence the extent of hatching delays.

In this paper, we will consider some of the grim circumstances that a larva of Aedes triseriatus (Say) might face if it happens to hatch into an environment that is already occupied by larger larvae. We will suggest that a mechanism exists by which dormant eggs can avoid such situations, which might lead to a very precise method of resource tracking.

We will then show that variation in hatch delays is governed in part by factors that are intrinsic to the eggs themselves, and that this intrinsic variation is considerable. Finally, we will provide evidence that the factors that maintain variation in hatching delays produce a hierarchy of selective pressures which results in superior larval fitness characteristics of late-hatching individuals.

ECOLOGICAL INTERACTIONS: COMPETITION, CANNIBALISM AND RESPONSES OF EGG HATCHING TO LARVAL ABUNDANCE

Competition

The potential for interactions among different hatching groups prompted us to inspect the intraspecific interactions between A. triseriatus larval cohorts of two developmental ages in simulated habitats. These habitats consisted of plastic bottles (300 ml) placed in the forest floor, filled with three concentrations of treehole fluid and stocked with three initial densities of larvae, with an addition of 0 or 20 newly hatched larvae seven days after initiation (Livdahl 1982). The earlier larvae were found to impose a strong adverse effect on the second cohort, which was not reciprocated. On the contrary, the addition of a second cohort reduced the sensitivity of the first cohort's female development time to increasing density and fluid dilution (Fig. 1). In addition, at the intermediate density and low food level (40 larvae/250 ml diluted fluid) the survivorship to adulthood of the earlier larvae was enhanced by the addition of the new cohort (Fig. 2). Facultative cannibalism of the

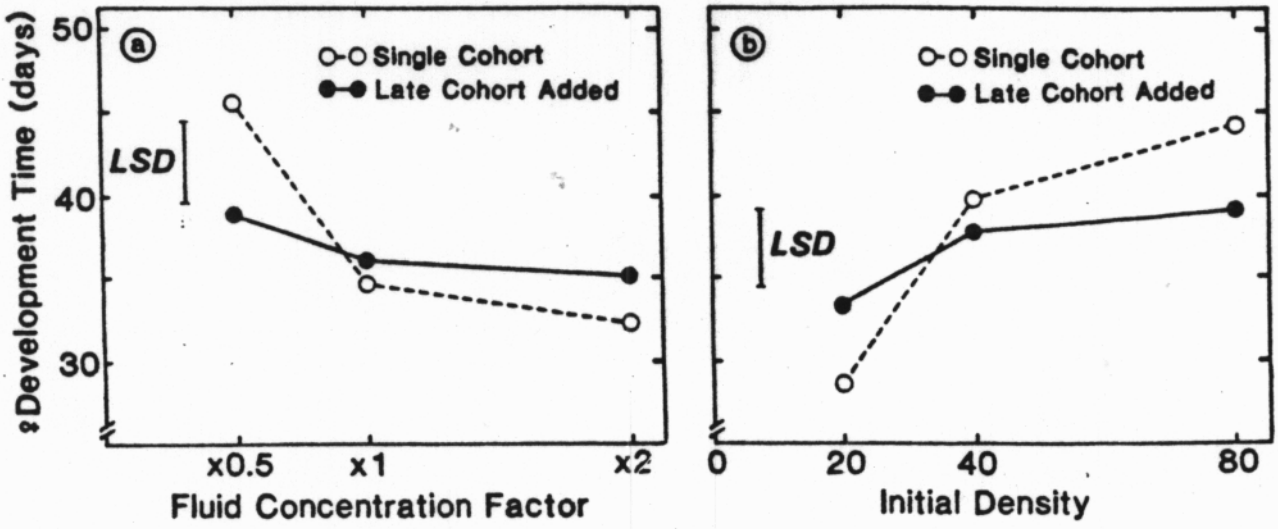

Fig. 1. Effects of two interactions between experimental factors on mean female development time of the first cohort. Females exhibit a diminished sensitivity to dilution of fluid when the second cohort is present (points depict means summed across all density levels). Female developmental response to increased density is moderated by the presence of the second cohort (means summed across all fluid concentrations are shown). Tukey's Least Significant Difference is shown for visual comparison of means.

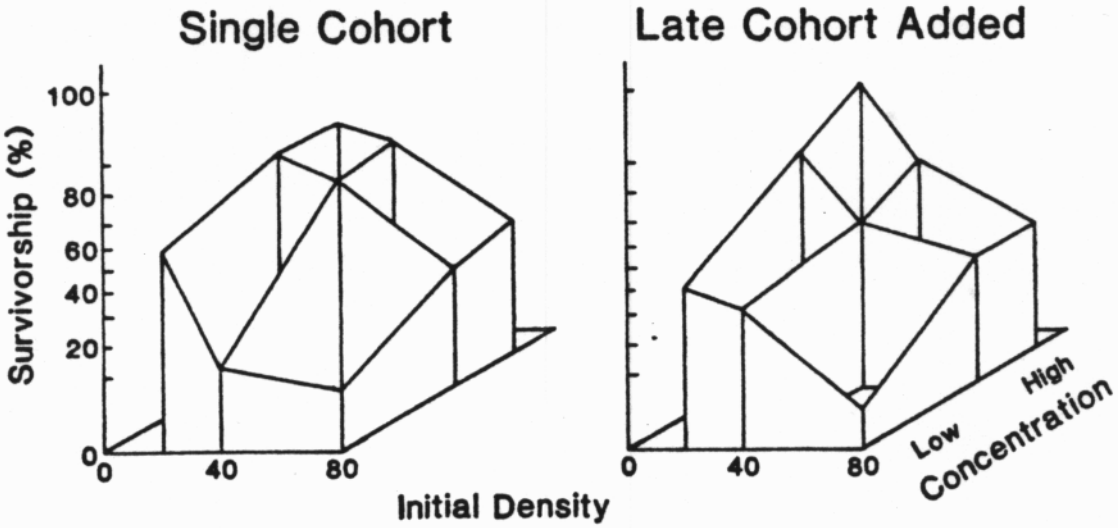

Fig. 2. Survivorship (%) of the first cohort in response to all treatment combinations. The significant third-order interaction arises primarily from the significantly higher survivorship at the low concentration with density 40 for first cohorts grown with the second cohort added, compared to the survivorship in the absence of the second cohort. The vertical scale converts survivorship into the more appropriate angular transformation for proportionate data.

second cohort by the larger larvae was suggested as a possible mechanism for these higher order effects.

It is useful to combine aspects of survival, future fecundity and development time into a composite index of larval success because such variables, when viewed separately, can respond in opposing directions, and may produce conflicting conclusions. This objective can be obtained by numerous possible combinations, but a very informative measure of success is obtained when the variables are incorporated into an estimate of the per capita

rate of population growth:

$$r' = \frac{\ln[(1/N_o)\sum_x A_x f(W_x)]}{D+\sum_x xA_x f(W_x)/\sum_x A_x f(W_x)} \qquad [1]$$

in which N_o denotes the initial number of individuals; A_x is the number of females emerging on day x; W_x is the average female size (measured by either dry mass or wing length in this paper) on the day of emergence, x; D is a time delay between adult emergence and oviposition; and $f(W_x)$ converts female size into a fecundity prediction (Livdahl & Sugihara 1984). In this section, female size is measured by dry weight, and is converted into future female offspring through an empirically obtained linear function, $f(W_x) = 45.85W_x + 7.13$, and D is set at 7 d. An alternative linear function will be used later in the paper, which is based on female wing length: $f(W_x) = 23.17W_x - 51.10$.

The sources of complexity in the responses of first cohort success to combinations of density, food and cohort structure become nonsignificant when the information about success of the first cohort is combined into an estimate of the <u>per capita</u> rate of change, yielding a response to density that contains only the essential linear features of the logistic growth equation (Fig. 3).

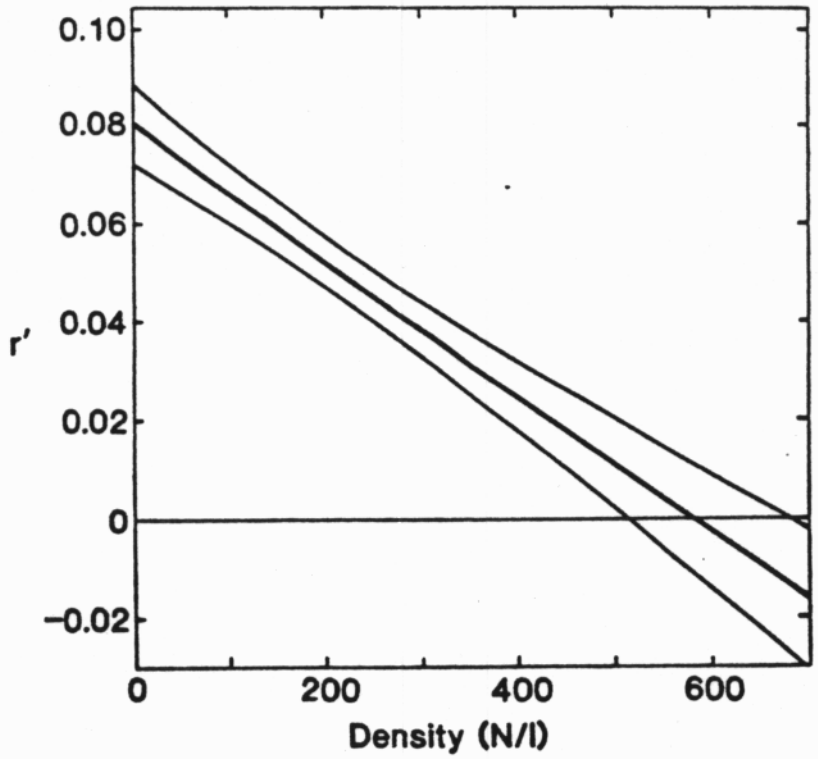

Fig. 3. The least squares line for the composite index of per capita success (r') of the first cohort in response to the number of larvae per liter of treehole fluid (prior to dilution or concentration), and the associated 95% confidence intervals about the predicted r' value.

The estimated per capita rate of change also provides a convenient summary of the hardships suffered by small larvae in the presence of a cohort of larger individuals. Table 1 provides the actual values of r' for each cohort for each bottle in which they were raised together. Without exception, the first cohort has the higher r' value, and the second cohort completely fails to emerge under competitive conditions.

Table 1. Composite indices of per capita larval success (r') of early and late cohorts of A. triseriatus larvae when grown together under different conditions of fluid concentration and first cohort density. Parenthesized numbers denote r' values for the first cohort. Experimental bottles that were colonized by A. barberi are noted with asterisks. In each case, the initial density of the second cohort was 20 per bottle.

First-cohort density	Fluid concentration: Concentrated		Control		Dilute	
20	0.054	(0.129)	0.032	(0.094)	---	(0.082)
	*---	(0.097)	0.023	(0.118)	---	(0.073)
	*---	(0.102)	0.042	(0.108)	---	(0.085)
40	0.028	(0.079)	0.029	(0.087)	---	(0.074)
	0.025	(0.079)	0.016	(0.076)	---	(0.061)
	0.022	(0.101)	---	(0.057)	---	(0.058)
80	*---	(0.051)	---	(0.035)	---	(0.021)
	---	(0.066)	---	(0.046)	---	(-0.008)
	---	(0.062)	---	(0.081)	---	(-0.008)

Despite the simple response of the composite index to combinations of experimental treatments, the presence of moderating second and third order interactive effects of the second cohort on development time and survival of the first cohort presents an interpretive challenge. The following parsimonious explanation of this complexity is consistent with the moderating influence by the second cohort on the first cohort's response to competition as well as the disappearance of the second cohort under competitive conditions.

Facultative cannibalism, which becomes acute when food is scarce and density is high, might allow the first cohort to endure periods of intense competition, thus explaining the moderating influence of the second cohort and the extreme adversity experienced by the second cohort in response to high density of the first group.

Cannibalism

We provide evidence here of the hypothesized facultative cannibalism in A. triseriatus by examining attack rates of fourth instar larvae on first instars in the presence and absence of a food supply. In addition, we report the importance of the developmental age of the first instars to the severity of cannibalism.

This experiment was established according to a factorial design to examine the responses of cannibalism rates by fourth instar larvae to the density of first instar larvae, relative shortage of food, age of the first instar larvae and interactions among these three factors.

Ten days prior to the experiment, larvae were hatched from eggs produced by field-collected females, using the "hatching tube" technique of Novak & Shroyer (1978), and were maintained at low densities with ample food (yeast and ground rat chow) supplied twice weekly. At the time of the experiment these larvae were in their fourth instar.

The first instar larvae were produced through the same hatching technique, which was applied at two separate times prior to the experiment to provide groups of either 0-1.5 or 24-25.5 h of age. Four fourth instars were combined with first instar larvae (first instar densities of 2, 4, 8, 16, 32 and 64 individuals per 35 ml vial were chosen to reflect reasonable field densities). Vials contained deionized water with or without a suspension of brewer's yeast (200 mg/l). Three replicate vials were established for each treatment combination.

The first and fourth instar larvae were kept together for 24 h in an incubator with continuous illumination at 20° C. After this time we removed the fourth instar larvae and counted the remaining first instar larvae.

A control was performed to investigate the mortality rate of first instar larvae without the potential cannibals, by establishing two replicates of all densities of first instars in deionized water or yeast suspension for 24 h.

No mortality was found in the control groups, suggesting that saprophagy cannot account for the disappearance of first instar larvae, and the data shown in Fig. 4 provide clear evidence that cannibalism can occur in A. triseriatus. Under certain circumstances, nearly 50% of the newborn larvae were eaten by this conservative density of fourth instar larvae. The overall mean rate of cannibalism of the entire study is 6.4%.

Contrary to our expectations, the incidence of cannibalism is not significantly affected by the density of the young larvae (Table 2). Within the density range of 2 to 64 larvae per 35 ml, no saturation of cannibalism rates by high density of victims is detectable.

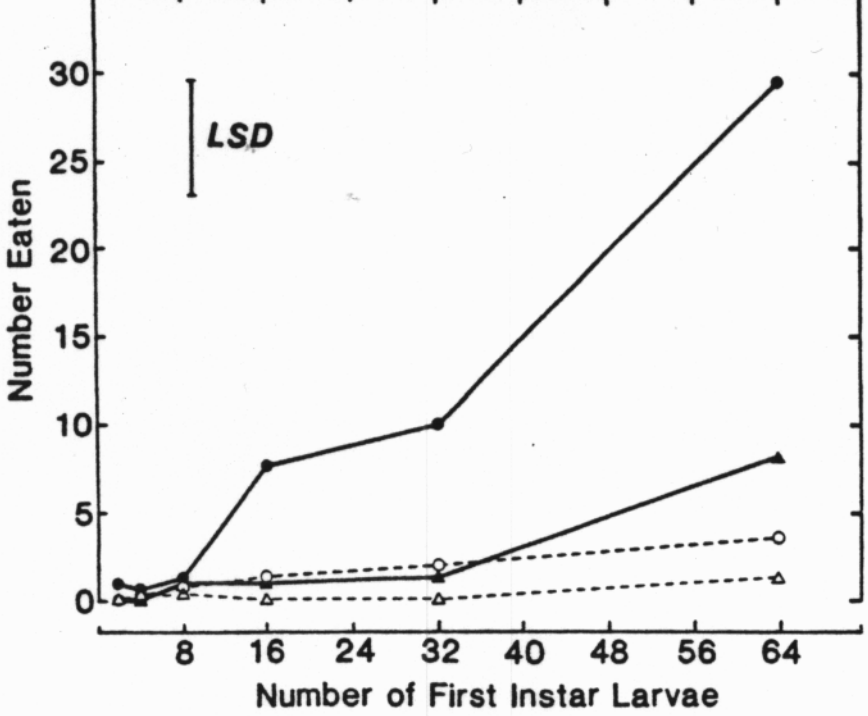

Fig. 4. Functional response of fourth instar larvae to density of first instar larvae. The points represent the means of 3 replicates in each treatment combination. Solid lines indicate no food; dashed lines, food added; circles, 1.5-hour old larvae; triangles, 24-hour old larvae. Tukey's LSD is shown for visual comparison of the cell means.

Table 2. Analysis of variance for the effects of developmental age, food availability, first instar density, and interactions among these factors on the cannibalism rate of fourth instar A. triseriatus larvae. The dependent variable is the number of first instars eaten divided by the initial number of first instars. The angular transformation was applied to reduce the dependence of the variance on the mean in proportionate data.

Source of variation	SS	MS	d.f.	F	P
Age (A)	1.184	1.184	1	19.46	<0.001
Food (F)	1.310	1.310	1	21.52	<0.0001
Density (D)	0.288	0.058	5	0.95	ns
A x F	0.428	0.428	1	7.04	<0.05
A x D	0.256	0.051	5	0.84	ns
F x D	0.382	0.076	5	1.26	ns
A x F x D	0.269	0.053	5	0.88	ns
Error	2.921	0.061	48		

The outcome of cannibalism is significantly altered by the availability of an alternate food source for the fourth instar larvae (Table 2). In the yeast suspension, cannibalism rates are reduced significantly, but are not eliminated (Fig. 5).

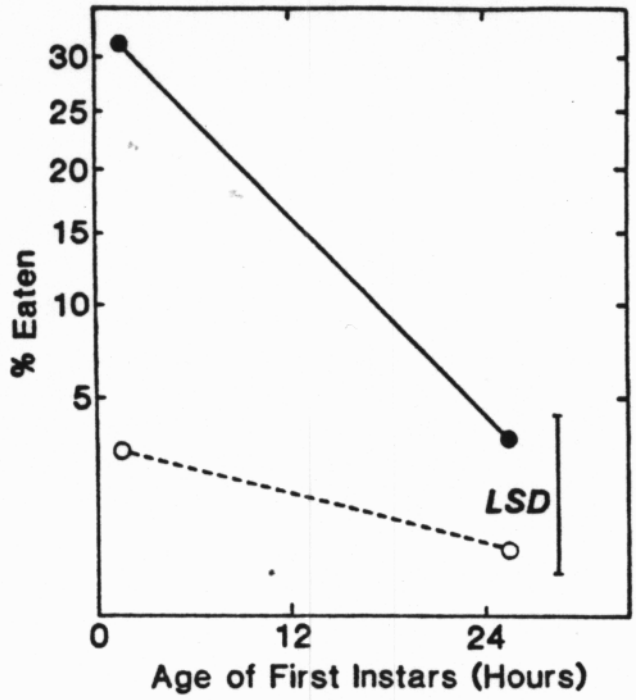

Fig. 5. The effect of the interaction between the age of the first instars and the availability of an alternate food source on the fourth instar attack rate. Solid line, no food; dashed line, food added. The points represent the means summed across all density levels. Tukey's LSD is shown for comparisons of the means. The vertical scale converts attack rate into the more appropriate angular transformation.

The vulnerability of first instars to cannibalism declines dramatically during the first 24 hours of larval life (Table 2). When the first instars are more than 24 hours old, the cannibalism rate is reduced to less than 10%. The significant interaction between time and food arises from the difference in response of the fourth instar larvae to relative food scarcity when the young larvae are a few hours old compared to when they are more than 24 hours old (Fig. 5).

These results provide the first documentation of cannibalism in A. triseriatus. A few studies have reported cannibalism in other mosquito species (Aedes aegypti L. [MacGregor 1915], Eretmapodites spp. [Haddow 1946; Gillett 1972], Anopheles stephensi Liston [Reisen & Emory 1976], Culex tritaeniorhynchus Giles [Mogi 1978b], and Toxorhynchites rutilus Coq. [McCrary 1965; Corbet, this volume]).

The significant interaction between first instar age and food (Table 2) may be interpreted in two ways. The fourth instar larvae might switch from relatively passive filtering to a more active form of hunting behavior under conditions of food scarcity, and the more vulnerable young first instars are rendered particularly susceptible to cannibalism during such periods. Alternatively, the youngest first instars are able to grow out of their vulnerability quite rapidly when food is abundant, thus diminishing the difference between attack rates on old and young first instars. This experiment was not designed to distinguish between the two explanations of this unanticipated

result. By either mechanism, the consequence of the phenomenon is the same: under these conditions, cannibalism in A. triseriatus is a food-dependent, facultative process.

Egg hatching in response to larval abundance

We can now envision at least two reasons for Aedes to avoid hatching into an environment that contains abundant larvae. A density-induced hatching delay would have definite adaptive rewards that could outweigh the cost to fitness of the increase in age at first reproduction. We provide evidence here that density-dependent hatching inhibitions can occur in this species. Facultative cannibalism might have been a strong factor in the evolution of egg hatching inhibition by larvae. An additional benefit to individuals that refrain from hatching into crowded habitats results from the avoidance of severe competitive effects of larger larvae described above.

Two sets of results suggest that a mechanism for judicious density-inhibited hatching may exist in aedine mosquitoes. Carpenter (1983) and Fish & Carpenter (1982) have demonstrated that larvae thrive on the microbial growth that appears on the surfaces of leaves, providing documentation of the ability of larvae to scrape surfaces. Gillett (1959) observed an inhibition of the hatching of "late-laid" eggs by the presence of eggs that had been laid several days earlier, probably induced by the larvae which had already hatched. Gillett et al. (1977) found an association between bacterial abundance on the egg's surface and the tendency to hatch. In addition, they observed a depression of hatching among paired eggs compared to single eggs, supporting the hypothesis that larvae graze bacteria from the walls of eggs around them, removing a source of oxygen depletion, which results in an inhibition of hatching for the eggs that remain.

The importance of the results of Gillett (1959) and Gillett et al. (1977) for A. aegypti may have three limitations. First, in both sets of experiments, the newly-hatched larvae were extremely confined to the immediate environment of the unhatched eggs (volumes of 3 and 1 ml were used by Gillett [1959] and Gillett et al. [1977] respectively). Such confinement resulted in larval densities that far exceed those in natural situations. In addition, the larvae in both studies were given nothing to eat, aside from the microbes found on the walls of eggs. Consequently, the designs of the two experiments may have forced the larvae to exert inhibitory effects. Finally, neither experiment attempted to provide a stimulatory environment for egg hatching (i.e., no deliberate efforts were made to reduce oxygen levels). We established the following experiment to test the hypothesis that larvae inhibit the hatching of eggs despite the presence of an environment conducive to hatching, even when they have an abundant supply of food, and even when they are not confined to the immediate vicinity of the eggs.

We explore these questions further by examining the responses of hatching to the density of each of the four larval instars, in the presence of two hatching media. One medium, nutrient broth

(Horsfall 1956), provides no direct food source for the larvae. A brewer's yeast suspension presents a hatching stimulus while providing a direct source of food. If the larvae elicit an inhibition of hatching through the scraping of eggs, the response to density should be less intense in the yeast suspension, because the larvae will have an alternative to the scraping of eggs for food.

Oviposition by field-collected females occurred in laboratory cages, after which the eggs were stored on moist paper towels in plastic cases under a long (17L:7D) photoperiod for 9 $\pm$ 2 d. Eggs were mixed thoroughly in an effort to randomize our samples of 24 eggs per batch. Larval cultures, reared at low densities with ample food, were established at three periods (3d, 6d and 9d) prior to the experiment to obtain the three largest instars. First instar larvae were obtained by hatching three hours before the experiment.

We selected experimental densities (0, 3, 6, 12, and 24 larvae per 35 ml vial) to reflect a reasonable range of field density situations. Larvae were rinsed in deionized water prior to the setup of the experiment, at which time they were combined with the eggs in the hatching media: an aerated nutrient broth solution (1 g per 1) and a brewer's yeast (200 mg per 1) and sucrose (100 mg per 1) suspension.

We terminated the experiment by removing all eggs from the medium. We then counted the newly hatched larvae, the hatched eggs for each batch as a check on our larval counts, and the viable embryos (criteria of Shroyer & Craig 1980) after bleaching to correct for infertility.

We established five batches with no larvae for each hatching medium, and five replicates for each combination of instar, density and hatching medium treatments.

The effects of larvae on egg hatching permit no easy generalizations based on intuitive expectations, such as an overall negative influence of larvae on hatching rate, or a generally inverse relationship between hatching rate and instar. Instead, the detailed relationship between larvae and egg hatching can only be described with conditional statements due to a complex of interacting factors (Table 3).

The main effects of hatching medium, larval density and instar are confounded by significant interactions among all possible pairs of factors. In addition, each second order effect is complicated by a significant third order effect. In broth, a dichotomy of effects is apparent between density of the two largest and the two smallest instars. Larger larvae provide significant stimulation at low densities, but they become inhibitory to hatching at high densities. The density of smaller instars exerts a relatively consistent positive influence on hatching (Fig. 6a). In the yeast medium, the dichotomy between small and large larvae is disrupted: the stimulatory effects of

Table 3. Analysis of variance for the effects of larval instar, larval density, the nature of the hatching medium, and interactions among those factors on the hatching of A. triseriatus eggs. The dependent variable is the fraction of eggs hatching per vial. The angular transformation was applied to reduce the dependence of the variance on the mean in proportionate data.

Source of variation	SS	MS	d.f.	F	P
Hatching medium (M)	0.491	0.491	1	9.49	<0.01
Instar (I)	1.321	0.440	3	8.53	<0.0001
Density (D)	1.788	0.596	3	11.54	<0.0001
M x I	3.079	1.064	3	19.87	<0.0001
M x D	0.550	0.183	3	3.55	<0.05
I x D	3.363	0.374	9	7.23	<0.0001
M x I x D	1.165	0.129	9	2.51	<0.05
Error	6.613	0.052	128		

low densities of large larvae remain, but inhibition at high densities is ameliorated (most dramatically, the third instars at density 12 switch from inhibition in broth to stimulation in yeast); the positive influence of second instars at the highest density is significantly reduced; and the first instars exhibit inhibitory effects at low and moderate densities (Fig. 6b).

These results demonstrate the existence of at least two larval processes that have antagonistic effects on the hatching behavior of eggs. Respiration by larvae might provide a mechanism for their positive influence on hatching in some conditions, through the removal of dissolved oxygen from the eggs' environment (an effect also observed by Young [1922]). This stimulus can be countered by an inhibitory process which may result from the grazing of bacteria from the eggs' surfaces, thereby removing a source of hatching stimulation. The stimulatory process appears to outweigh the inhibitory process at high densities of small larvae in broth and low densities of large larvae in both media. The inhibitory process surpasses the stimulatory process at high densities of large larvae and, curiously, at moderate densities of first instars in the yeast medium. The production of inhibitory chemicals by larvae has been raised as another inhibitory mechanism (Gillett 1959), but it has yet to receive a direct experimental test (analogous chemical interference phenomena among larvae have been sought with ambiguous results [Dye 1982]). Our results support the hypothesis of grazing effects through the moderating influence of a food supply for the larvae and through the levelling of inhibitory effects at high densities of large larvae in broth. If the eggs are thoroughly scraped by a certain density of larvae, no further inhibition by higher densities would be

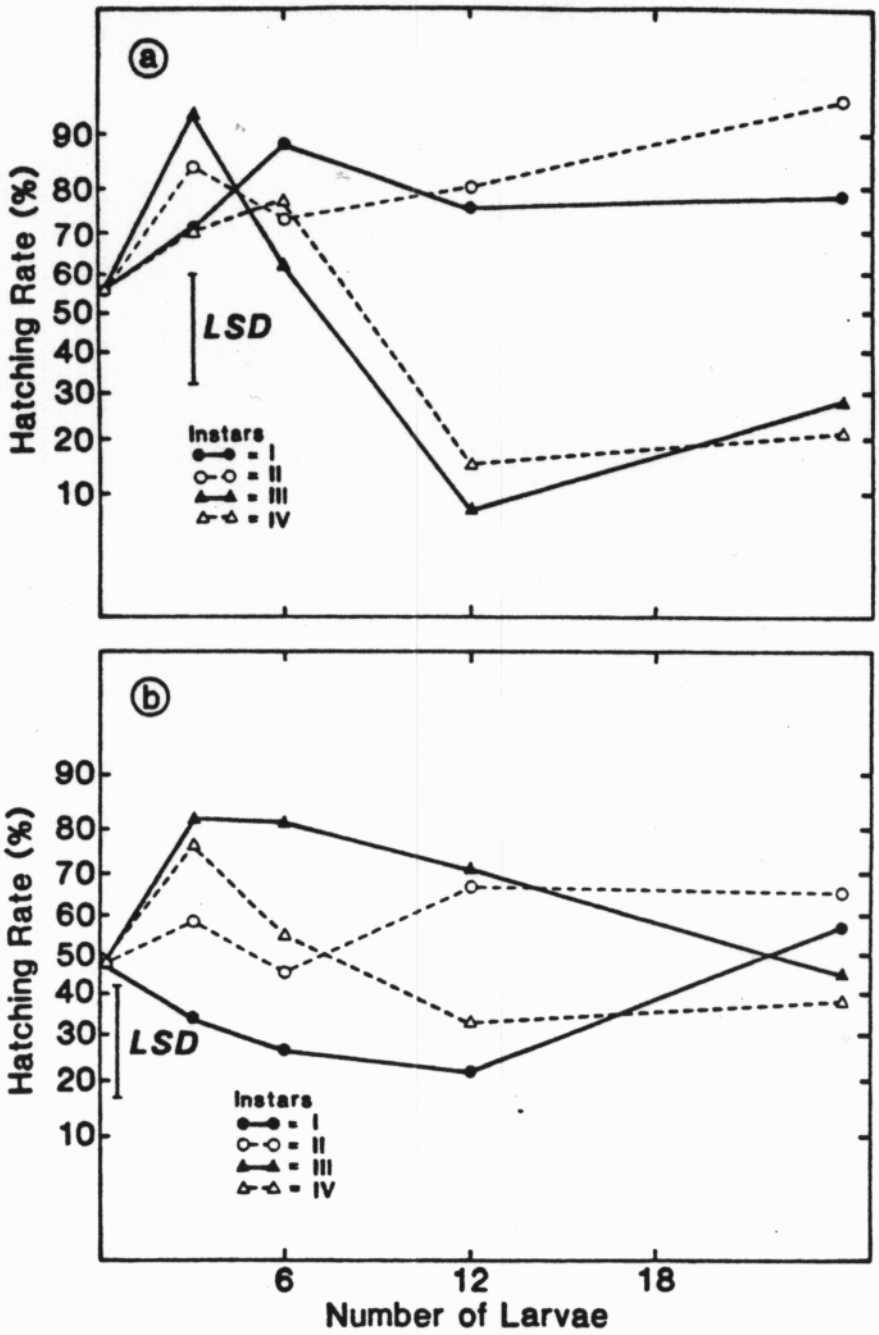

Fig. 6. Responses of egg hatching to densities of each larval instar in the the two media, nutrient broth (a) and yeast suspension (b). Hatching rates are transformed from fractions into radians by the angular transformation. Each point represents the mean of 5 replicate egg batches in each treatment combination. Tukey's LSD is shown for visual comparison of the cell means.

possible. A chemical inhibition mechanism should gain influence consistently as density increases.

The complexity added by the first instars to the interaction is most daunting to explain, and our attempt is presented elsewhere (Livdahl et al. 1984).

The complex of factors that may stimulate or inhibit hatching provides a mechanism for very timely hatching by the embryos, and for precise resource tracking by the population, in contrast to

the findings of Istock et al. (1976b) for Wyeomyia smithii (Coq.) which has no substantially variable delays in hatching time. Inhibition of hatching in the presence of large larvae when food is scarce could allow the embryos to avoid the catastrophic encounters of competition and cannibalism mentioned above.

The cannibalism and hatching inhibition possibilities pose a potential problem for an attempt to draw inferences about density-dependent regulation: unless eggs of Aedes are counted, and the temporal patterns of their hatching behavior are known, two subtle sources of density-dependence could be overlooked completely in a posteriori searches for intraspecific, and possibly interspecific competition (e.g. Chubachi 1979; Southwood et al. 1972; Bradshaw & Holzapfel 1983). The need for experimental field verification of these results is clear.

THE INTRINSIC NATURE OF HATCHING DELAYS AND ASSOCIATED LIFE-HISTORY ORGANIZATION

It should be apparent that a grasp of the extent of variation in the time required for hatching is paramount to the understanding of aedine population dynamics. We have shown above that the timing of hatching behavior can influence the eventual success of larvae, and that the hatching stimulus itself may be subject to larval modification. Additional environmental sources of variation in hatch delay include diversity in critical responses of eggs to diapause inducing or releasing factors, principally temperature and photoperiod (Shroyer & Craig 1980; Sims, this volume); the spatial locations of eggs and the associated likelihood of receiving a suitable hatching stimulus; temporal variation in the suitability of hatching stimuli; and possibly variation in the moisture conditions experienced by the eggs shortly after oviposition.

Novak & Shroyer (1978) have asserted that all variation in hatching time is induced by heterogeneous strengths of stimuli, because they found a technique by which virtually all eggs can be hatched within a few hours. However, some contrary evidence can be found in the literature. Differences in hatching responses to suboptimal conditions have been found between Aedes species (Novak & Shroyer 1978; Lounibos 1981), sympatric ecotypes (Saul et al. 1980), new and old laboratory strains (Schwann & Anderson 1980), geographic strains (Gillett 1955a,b), and strains of A. triseriatus taken from treehole and tire populations (Means et al. 1977). Gillett (1955b) observed intermediate hatching rates in the offspring from crosses of geographically distinct strains. In view of such differences, it seems likely that mean hatching rates of populations in suboptimal conditions may be intrinsic, genetically determined population properties. However, the amount of genetically based variation in hatching time within a population has yet to be determined.

We suggest here that the variation in hatching time within A. triseriatus populations is partly a result of intrinsic variation

among the eggs themselves. This statement rests partly on the substantial variation that we can still observe when we remove some of the obviously environmental sources of variation in hatching time, including diapause inducing factors, spatial and temporal heterogeneity in stimulus strength, and larval effects. Obviously, it is not possible to remove all possible sources of environmental variation, but a successful randomization of unknown or uncontrollable factors should result in a constant probability (h) of egg hatching with each succeeding stimulus, producing a distribution of hatching frequencies in the form of an exponential decay through time:

$$\frac{L_t}{E_o} = (1-h)^t \qquad [2]$$

where E_o denotes the initial number of eggs and L_t is the number of larvae hatching after a certain number of stimuli, t. The hypothesis of purely random environmental factors can be rejected if hatching occurs in clumps around certain stimuli, or if a departure from linearity is observed when the hatching fractions are plotted on a logarithmic scale.

The intrinsic nature of hatching delays would also be supported by the observation of life-history differences among the different hatching cohorts. A convenient array of life-history features that provides information about larval success includes the development time of larvae, the fraction surviving to adulthood, and the size of emergent adults. The potential reproductive rate of a cohort of larvae should also be influenced by changes in the sexual composition of adults. Each of these life history features contributes to the potential for future population growth by a cohort, and it is again convenient to combine these aspects of success into a composite index (eqn. 1).

A comparison of the magnitudes of these properties among different hatching cohorts under a single set of conditions provides only a partial picture of life-history differences, because these properties are subject to change under varying density and food conditions. The sensitivity of these features to crowded conditions can reveal a different aspect of larval fitness, which provides a relative measure of crowding tolerance.

Aedes triseriatus eggs for this study were obtained from females caught during seven biting collections conducted from late June through late September, 1982 in a woodlot in Worcester, Massachusetts. After oviposition, eggs were transferred to damp storage conditions on filter paper squares in batches of 100 eggs per batch. Eggs were stored under constant illumination for 14-24 d to permit the completion of embryonic development, and were immersed for 24 h once per week thereafter, with intervening 6 d periods of damp storage. This process was applied to 108 batches, which entered the experiment in a stepwise fashion as new females were brought into the laboratory. Thus, after all

batches of eggs had been introduced to the study, 10,800 eggs were rinsed into the hatching medium (the yeast and sucrose suspension used in the previous experiment) and manually removed to fresh filter paper every week. The process continued until each batch had experienced 31 hatching stimuli. At the end of this process, the hatching stimulus of Novak & Shroyer (1978) was applied, to see if any viable eggs remained unhatched (only 0.06% of all hatching occurred after the 31st stimulus), and to permit a correction of hatching frequencies for infertility.

Single cohorts of larvae hatching after each stimulus were grown to adulthood in standard petri dishes at various initial densities (1, 2, 3, 4, 5, 10, or 20 larvae per 30 ml water), provided with one of three food levels (equal weights of brewer's yeast and ground laboratory rat chow totalling approximately 14, 7 or 3.5 mg per feeding). Larval densities of less than 5 per dish received the highest food level, densities of 5 per dish received all three food levels, and densities of 10 or 20 per dish received only the lowest food level. Hence, 9 different levels of initial density per unit food were applied to larvae hatching from 32 different hatching groups. We quantify these density per unit food food levels with the following sequence of relative levels: 0.25, 0.5, 0.75, 1.0, 1.25, 2.5, 5, 10 and 20. Larval cultures received fresh food and water on alternating three and four day intervals, and pupae were housed in vials covered with inverted vials to facilitate a daily record of adult emergence, after which one wing of each adult was measured with an ocular micrometer.

Variation of hatch delays

The first set of observations that emerges from these procedures is a view of the substantial variation in the time that the eggs wait before hatching (Fig. 7a). The departure of this distribution from a random expectation is most easily seen by the bumps of hatching activity at specific stimuli when the hatching fractions are plotted on a logarithmic scale, and the obvious departure from a linear relationship (Fig. 7b).

This view of the potential diversity in hatching time prompts our curiosity about the frequency and strength of stimuli in the natural habitat. We wonder how old some of the biting adults really are, and if it is possible for an egg to wait for months or years to hatch in nature. The complications to our view of *Aedes* population dynamics imposed by variable egg dormancy could be considerable.

The hierarchical organization of life-history traits

The comparison of life-history features of different hatching groups was organized into an analysis of covariance design to permit judgments about the relative overall success of the hatching groups and the intensity of their response to the competition treatment.

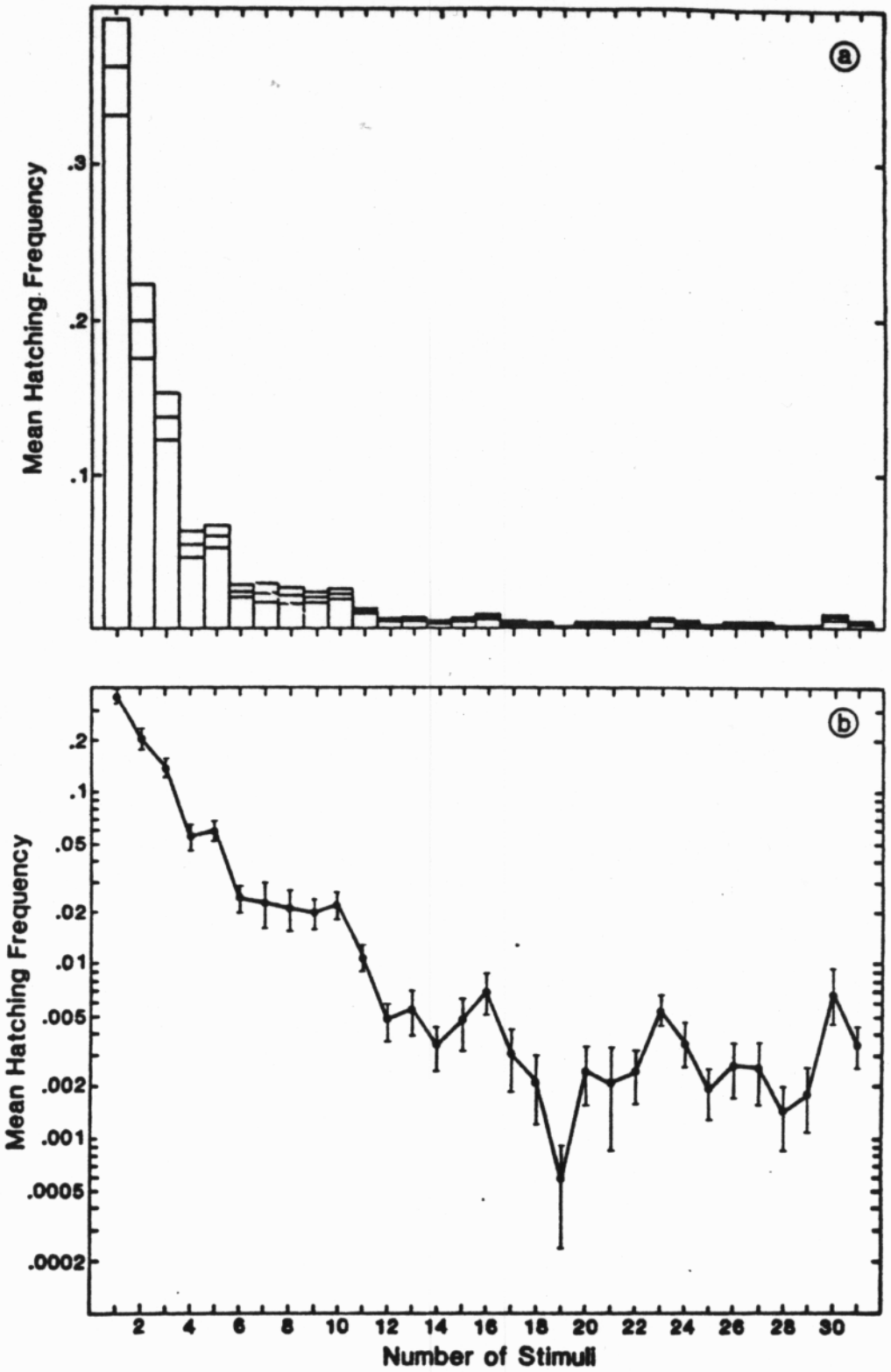

Fig. 7. a) The fraction of initial eggs hatching, after corrections for infertility, of 108 batches of 100 eggs immersed weekly for 31 weeks. Mean hatching fractions of all batches are shown with standard errors above and below each mean. b) The hatching fractions of eggs at successive stimuli plotted on a logarithmic scale. Vertical lines show locations of one standard error above and below each mean, according to the calculations for (a).

Some of the hatching groups produced so few individuals that it was not possible to expose them to all of the density/food levels. Because of the uneven abundance of hatching groups, we have pooled the groups according to octaves of the number of immersions required to hatch, providing five hatching groups among which comparisons are made. The distribution of replicate cultures and a summary of each life-history feature in response to initial density per unit food appears in Table 4.

Table 4. Summaries of life history characteristics of the five hatching groups as they respond to the initial density per unit food, compared by analysis of covariance.

Hatching group	N	Adjusted mean (±SE)	Slope (±SE)	Slope difference	Elevation difference
Survivorship (radians)[1]					
1	166	0.86±0.03	-0.024±0.004	$F_{4,659}$=1.80	$F_{4,663}$=9.80***
2-3	197	0.80±0.03	-0.008±0.004		
4-7	124	0.85±0.04	-0.012±0.006		
8-15	101	1.00±0.04	-0.008±0.008		
16-32	81	1.12±0.05	-0.010±0.014		
Male Development Time (Days)[2]					
1	143	22.98±0.24	0.439±0.034	$F_{4,493}$=1.84	$F_{4,497}$=7.46***
2-3	152	23.46±0.23	0.404±0.036		
4-7	90	22.91±0.30	0.469±0.045		
8-15	67	21.46±0.35	0.290±0.034		
16-32	51	21.72±0.41	0.402±0.076		
Female Development Time (Days)[2]					
1	137	26.09±0.25	0.590±0.033	$F_{4,536}$=2.12	$F_{4,540}$=14.79***
2-3	160	26.85±0.23	0.640±0.036		
4-7	106	26.41±0.28	0.686±0.051		
8-15	85	24.68±0.31	0.547±0.034		
16-32	58	23.91±0.39	0.480±0.075		
Fraction Male (radians)[1]					
1	156	0.81±0.04	0.003±0.005	$F_{4,603}$=0.84	$F_{4,607}$=5.74***
2-3	180	0.73±0.03	-0.001±0.005		
4-7	113	0.60±0.04	0.004±0.006		
8-15	93	0.58±0.05	0.008±0.008		
16-32	71	0.69±0.05	-0.001±0.015		
Female Wing Length (mm)[2]					
1	137	3.60±0.02	-0.015±0.002	$F_{4,535}$=1.53	$F_{4,539}$=3.59**
2-3	160	3.56±0.01	-0.017±0.002		
4-7	106	3.56±0.02	-0.013±0.002		
8-15	85	3.63±0.02	-0.009±0.003		
16-32	58	3.63±0.02	-0.009±0.007		

[1]Transformation $y' = \arcsin(y^{0.5})$ employed for proportionate data.

[2]Mean culture values are used to preserve the independence of observations.

*P<0.05 **P<0.01 ***P<0.001

The later hatching groups exhibit the highest overall larval survivorship. Analysis of covariance reveals no significant differences among slopes of regressions, although each group

responds in a significant negative manner to resource shortages. Elevation differences among the five hatching groups are highly significant.

Females that hatch late develop to adulthood more rapidly than do the earlier hatching groups. The same statement applies to males. Neither sex shows a difference among the slopes of regression relationships, but our analyses reveal highly significant differences among the elevations of those regressions.

Females of the later hatching groups achieve a larger size as adults, as indicated by wing length. Again, there are no significant slope differences among hatching groups, but the elevations of the groups exhibit highly significant differences.

An analysis of covariance reveals no significant differences among the slopes of regressions for the response of the fraction of male adults to density per unit food. In fact, no significant response occurs, even when all hatching groups are pooled together. However, significant differences among the overall adjusted mean male fractions are present. The significant differences arise from the tendency for the later hatching groups to produce lower proportions of emergent adult males. The nonsignificant response of the fraction male to crowding or food shortage suggests that the sex ratio differences are not a result of differential survival of the two sexes for any of the groups.

Based on what has been shown above for separate features of larval success, it is not surprising that an index that combines those features displays significant differences among hatching groups in the overall magnitude of r', computed according to eqn. 1. The composite measure of success increases with hatching delay (Fig. 8a). In this case, however, the slopes of the regression lines depict a decreasing sensitivity to competition as hatch delay increases (Fig. 8b), a result that analyses of separate aspects of success failed to detect. This finding demonstrates that the late hatchers possess features that contribute not only to larval success at low densities, but that the difference in success actually becomes amplified at high densities. This index reveals that the late hatching groups should be superior competitors in saturated environments.

DISCUSSION

This set of life history differences among hatching groups bolsters the hypothesis that hatch delays result in part from genetic differences among hatching groups. If that hypothesis survives the direct examinations of inheritance which are now in progress, these life-history data will provide a very clear example of coadaptation of life-history traits into what could quite properly be called a "life-history strategy."

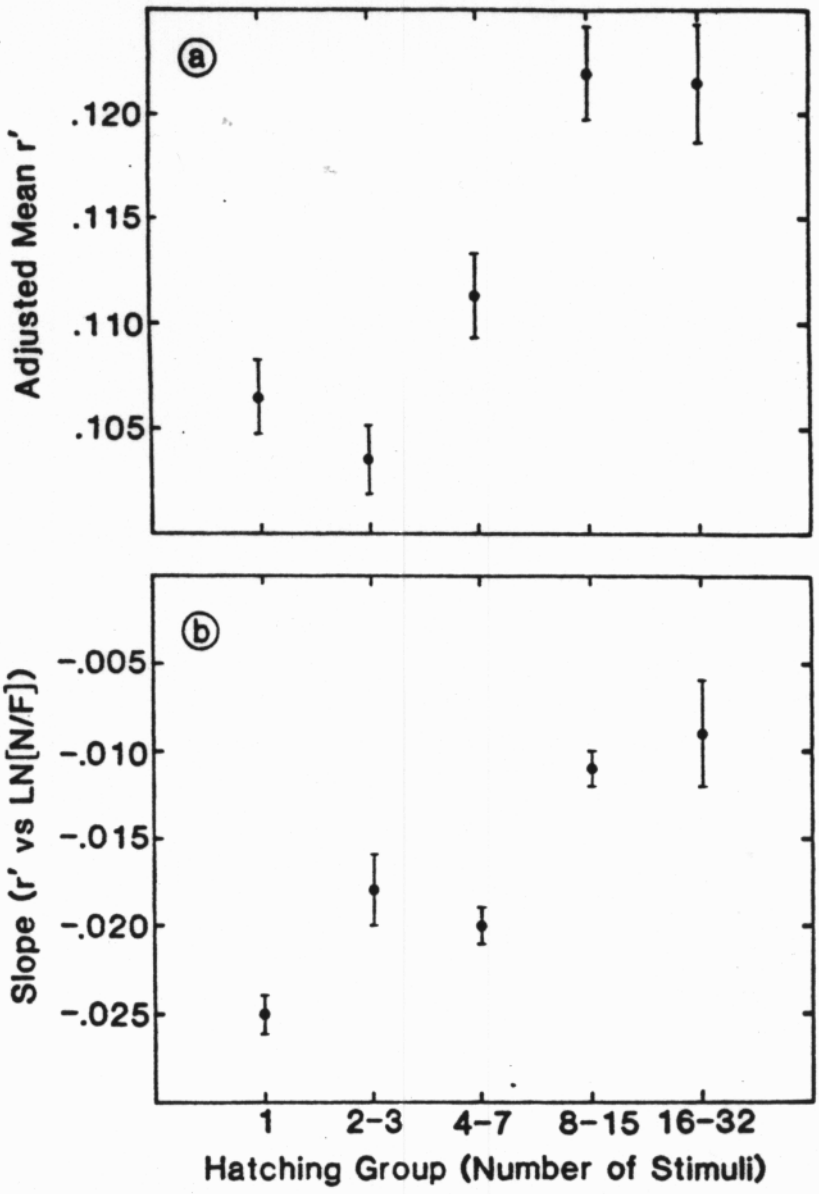

Fig. 8. A summary of the hierarchical organization of larval fitness according to hatching time: a) composite measure of success (r') of laboratory cohorts hatching after various numbers of stimuli, pooled into five groups as shown on the abscissa. Adjusted means (± S.E.) have been corrected by the pooled regression relationship between r' and ln(initial density/ unit food). Differences among adjusted mean r' values are detectable through analysis of covariance ($F_{4,539}$ = 15.20, P<0.001). b) The sensitivity of the five hatching groups to crowding and resource shortage, depicted as the slopes (± S.E.) of the regression relationships between r' and ln (initial density/unit food). Slope differences are highly significant ($F_{4,535}$ = 14.34, P<0.001).

"Covariation of life-history traits within a population has central importance to the theory of life-history evolution, which often depends on the existence of closely coadapted traits (Stearns 1976, 1977). Although such covariation has been seen through comparisons of separate populations (e.g., Livdahl 1984; Dingle et al. 1982; Giesel et al. 1982), theoretical treatments frequently require that separate traits be closely associated within populations (e.g., MacArthur & Wilson 1967; Roughgarden 1971; Charlesworth 1971; Gadgil & Bossert 1970; Maynard Smith 1976). Such reliance is frequently implicit in references to

the evolution of suites of characteristics or "strategies". In addition, variation in the expression of the coadapted genes must appear for life-history tactics to respond to selection.

Using the possibility of a genetic basis for hatching delays as a working hypothesis, we suggest that life-history evolution is a hierarchical process, and that intense selection on one major trait can drive selection of other traits as secondary events.

Selection resulting in variable life cycle delays can occur in populations that inhabit temporary or unpredictable habitats if the individuals that delay their life cycle can endure or avoid a catastrophe (Cohen 1968; Den Boer 1968; Livdahl 1979a; Jain 1982). Environmental uncertainty has been posited as an explanation for the installment hatching phenomenon of Aedes mosquitoes (Novak & Shroyer 1978; Gillett et al. 1977; Gillett 1955a,b). One consequence of these variable life-cycle delays is that a fraction of the dormant individuals will be present and viable after a catastrophe has passed, while it is possible that all of the individuals that had no delay could be lost.

Another possible consequence of these variable delays has received less attention: during favorable environmental periods, while the population is growing, there should be intense selection against individuals that delay their life cycles. If the catastrophes occur with enough frequency to prevent the elimination of the delayed genotypes, but are interrupted by long periods of population growth, we can expect this primary level of selection to produce features among the late hatchers that compensate, in part, for their long generation time. The result of this hierarchy of selection pressures could be an organized, coadapted set of life-history variates within a population.

The work described here underscores some fundamental weaknesses in our understanding of Aedes population dynamics. Until we know how long the eggs are capable of waiting to hatch, what influences the larvae have on the hatching behavior of embryos and on their subsequent survival, and the mortality schedule for eggs that remain dormant, we may have great difficulties in predicting future adult abundance. The importance of our laboratory work will lie more in the promotion of field experiments to test the importance of cannibalism, larval inhibition or stimulation of hatching, and variability of hatch delays than in the mere demonstration of the possibility of such phenomena in artificial settings.

Our understanding of processes that influence the evolution of life histories could be greatly enriched by an awareness of the central problems in this field among mosquito ecologists, who may already have answers to questions that transcend taxonomic boundaries. A reciprocal benefit lies in store for mosquito ecology, which may have a greater practical need to understand the evolution of population growth properties than any other guild of ecologists.