

## Reproductive allocation in the western tree-hole mosquito, *Aedes sierrensis*

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We examined the effects of geographic population, larval food, density, and temperature on reproductive allocation between egg number and size in 3 populations of the western tree-hole mosquito, *Aedes sierrensis*. Egg number did not differ among populations but increased allometrically with pupal weight. Egg number also varied with the interactions between food, density, and temperature independently of their effects on pupal weight. Egg size was unaffected by larval environmental factors or by pupal weight but was larger in the two more northern populations. Inter-population larval interactions (competitive and facilitative) depended largely but not exclusively on egg-size differences; facilitative interactions outnumbered competitive interactions. Early reproduction entailed no detectable costs in survivorship or in future reproduction. A negative correlation between egg number and size observed among species of mosquitoes disappeared in pooled populations of *A. sierrensis* and re-emerged within one population but not the other two. Thus, genetic variation exists in *A. sierrensis* both in egg size and in a trade-off between egg number and egg size.

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Reproductive investment is the proportion of total resources committed to reproduction. Reproductive allocation is then the distribution of these resources among the components of reproduction. The premise that variation in the apportionment of resources to reproductive effort affects an organism's fitness has been a cornerstone of life-history theory since its conception (Fisher 1930, Cole 1954, Williams 1966). Among the most prominent predictions are that current reproductive effort has a cost to future reproduction or survivorship (Williams 1966, Schaffer 1974, Charlesworth 1980). Less well understood are the fitness consequences of different patterns of allocation to the various components of current reproductive effort. Central to the trade-offs between these components is the relationship between reproductive effort and allocation (Wilbur 1977). Once a female has invested resources in reproduction, these resources must be packaged. Females

cannot produce an infinite number of optimally sized propagules. Under a fixed reproductive effort, increased propagule number results in reduced propagule size and, usually, offspring fitness (Salisbury 1942, Svårdson 1949, Lack 1954, Baker 1972, Wilbur 1977, but see Janzen 1969, Capinera 1979).

Explicit models of the trade-off between propagule number and size, under the premise that natural selection acts on both total reproductive effort and on investment in individual propagules, have been developed by Smith and Fretwell (1974), Brockelman (1975), Parker and Macnair (1978), Parker and Begon (1986), Temme (1986), Lloyd (1987), McGinley et al. (1987), and Winkler and Wallin (1987). Numerous studies on diverse taxonomic groups claim to have detected this trade-off (e.g., Olsson 1960, Perrins 1965, Primack 1978, Montague et al. 1981, Allan 1984, Hawley 1985a, Galen and Weger 1986), but many have not (e.g.,

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Parker 1970, Wilson and Fudge 1984, Congdon and Gibbons 1985, Kaplan 1987, Michaels et al. 1988, Schnebel and Grossfield 1988). Important problems may confound interpretation of these results. A trade-off between propagule number and size may not be observed for several reasons, including environmental covariance between the traits, unknown correlations with other traits, and fluctuating selection or selection on correlated traits (Clutton-Brock and Harvey 1984, van Noordwijk and de Jong 1986). The relationship between reproductive components may also be taxon-dependent, where high correlations often appear only among higher taxa (Lande 1979, Pagel and Harvey 1988). But comparisons of reproductive components among more taxonomically distant groups may be weak tests of the theory because environmental constraints vary among different species and allometric relationships may change.

The analytical technique used to detect negative correlations between fitness correlates must take into account allometry and, as far as possible, minimize trait variation explained by other factors. Linear regression (LR) is typically used to detect negative correlations. However, the true slope of a relationship for mutually variable traits may be estimated better by geometric mean regression (GMR, Ricker 1984) if the correlation between traits is not too low and if independent variability in one trait from systematic factors can be discounted. Moreover, GMR is more appropriate if description of a central trend, rather than a predictive equation, is desired. Unlike LR, GMR does not assume that all individual variability lies in the dependent variable (Ricker 1984). We use both techniques to analyze factors affecting reproductive components.

In this paper, we describe patterns of reproductive allocation in female western tree-hole mosquitoes, *Aedes sierrensis* Ludlow, from three populations distributed across 11° of latitude in western North America. We describe the effects of geography and larval environment on variation in egg number and size. By measuring several fitness correlates, we demonstrate that variation in egg size affects subsequent intraspecific interactions. Finally, we show that variation in egg number and size does not necessarily result from a trade-off between these traits, that differences between populations in this relationship are genetically based, and that the nature of this relationship changes with the taxonomic scale of the comparison.

## Natural history of *Aedes sierrensis*

*Aedes sierrensis* occurs in western North America from southern California (33°N) to British Columbia (55°N), most commonly west of the continental divide (Darsie and Ward 1981). *Aedes sierrensis* is exclusively a tree-hole inhabitant in its immature stages. Adult females

are iteroparous but require a blood meal to produce eggs in each ovarian cycle. Hawley (1985a), who found a negative interspecific correlation between weight-specific egg number and size among 9 species of mosquitoes from 3 genera, showed that compared to most other mosquitoes for which data are available, *A. sierrensis* females have high weight-specific fecundity and lay small eggs (Hawley 1985a). Eggs are laid in deciduous tree holes from May to September, even when these habitats are frequently dry (Hawley 1985b, c). The desiccation-resistant eggs are capable of an aestival diapause that enables them to remain dormant during the warm, dry summers typical of this region (Jordan 1980a). Hatching occurs from September to May after tree holes fill with water. Short days induce and maintain larval diapause (Jordan and Bradshaw 1978, Jordan 1980b). Larvae resume development during the spring.

Larval survivorship of *A. sierrensis* in nature is density-independent, but size of males and females at metamorphosis (pupal weight) is inversely correlated with larval density (Hawley 1985b). Survivorship and fecundity of adult females increase with pupal weight, so that females within a population realize a 30-fold variation in gross lifetime fecundity (Hawley 1985b, c). Large males can fly at lower temperatures than smaller males and thus have greater accessibility to females during cool weather; consequently, male fitness may increase with pupal weight as well (Hawley 1985b, c). Thus, in this mosquito, density-dependent size at metamorphosis is "capable of regulating the population" (Hawley 1985c). Clearly, factors in the larval environment that affect size at metamorphosis can indirectly influence adult reproduction, but it is not known whether these factors also affect reproduction independently of their effects on metamorphic size.

## Methods

### Collection and culture of populations

Collections of *A. sierrensis* were made during 1984–86 either as eggs laid on hardwood slats previously inserted in tree holes or as larvae siphoned directly from water-filled holes. Collections were made from 3 localities: San Diego, California (33°N), Oakland, California (38°N), and Eugene, Oregon (44°N), USA. Mosquitoes from all localities were raised for at least 2 but no more than 5 generations in the laboratory to eliminate residual field effects. *Aedes sierrensis* readily mate in small cages without recourse to induced copulation. To minimize inbreeding and drift, the minimum founding population sizes during this period exceeded 1000 individuals, and founding adults were selected haphazardly from those ecdysing each day. Eggs were then chilled at 4–5°C in a refrigerator for at least 90 d to terminate embryonic diapause and increase the rate and synchrony of hatching.

After four subsequent long (L:D > 16:8) days at room temperature (23–25°C), the eggs were flooded with water containing rotten guinea-pig chow to stimulate hatching. Larvae in each population were reared to pupation in plastic 150 × 25 mm petri dishes filled with 100 ml distilled water, at a density of 50–60 larvae per dish. Larval mosquitoes were fed every 1–3 d with 5–10 ml suspension of ground, sifted guinea pig chow and freeze-dried brine shrimp in a 2:1 ratio; several kg of this diet were prepared in 1983 and have since served as a standard diet for *A. sierrensis*. Pupae were removed every 1–3 d and placed in plastic dishes filled with 50–75 ml of distilled water. Adults were allowed to ecdyse and mass swarm in humidified screen cages (55 × 27 × 60 cm) and had constant access to fresh pesticide-free raisins. Anesthetized rats were offered twice weekly as blood-meal sources. Within screen cages, females laid eggs inside of glass jars painted flat black externally and lined with moist paper toweling. After being exposed to long-day photoperiod (L:D > 16:8) and room temperature (23–25°C) for 15 d to permit embryonation, the eggs were transferred on the moistened paper toweling to sealed plastic petri dishes and chilled at 4–5°C for at least 90 d before the onset of experiments.

## Experimental procedures

### *Variation in egg number and size*

We raised mosquitoes from the 3 populations concurrently under controlled conditions, after first removing residual field effects, to determine the genetic basis for variation among populations in fecundity and egg size. Upon hatching, experimental *A. sierrensis* larvae from the 3 populations were raised in 100 × 25 mm plastic petri dishes filled with 75 ml distilled water. To determine the effects of latitude (°N = L), temperature (°C = T), larval density (larvae/dish = D), and food level (g dry laboratory diet/dish = F) on egg number and size, larvae from each of the 3 populations were placed on a photoperiod of L:D = 16:8 and reared at 1 of 5 temperatures, 1 of 5 densities, and 1 of 5 food levels. Our intent was to spread treatments out in the design space with maximum efficiency for use in response surface analysis (e.g., Hard et al. 1989). Combined with a composite design (Box et al. 1978), this approach permitted the efficient estimation of linear, quadratic, and first-order interaction effects with a fraction of the treatments required for a full-factorial design. The drawback of the composite design is the lumping of higher-order interactions into the model's error term.

We first constructed a 2-level factorial design to estimate linear and first-order interaction effects involving L (33 and 44°N), T (13 and 21°C), D (15 and 25 larvae/dish), and F (0.10 and 0.20 g/dish). These levels were based on pilot data and were selected to maximize both the range of environmental conditions experienced by larvae and their survival to pupation. We then created a

composite design by supplementing these 16 treatments with a central treatment (L = 38°N, T = 17°C, D = 20 larvae/dish, F = 0.15 g/dish) and eight paired axial treatments, where the axial levels of T, D, and F were equal to the central level  $\pm \sqrt{2}$  times the difference between the central level and either corresponding factorial level (T = 11.3, 22.7°C, D = 13, 27 larvae/dish, F = 0.08, 0.22 g/dish). For L, 33 and 44°N were substituted for "axial" levels. The addition of these 9 treatments allowed the estimation of quadratic effects (which require at least 3 levels), and with the exception of L, rendered the factor levels orthogonal. The 25 unique experimental treatments were each replicated 3 times, for a total of 75 trials – 6 less than in the corresponding 3-level factorial design without any replication.

Female pupae from each treatment replicate were weighed live to the nearest 0.1 mg and their development times recorded every 1–3 d. The replicates were terminated after 275 d, when 5 larvae (0.3%) remained alive. Pupal weight was used as an index of adult size because the two traits are tightly correlated in *A. sierrensis* ( $r^2 = 0.95$ , Hawley 1985b). To determine the effects of environmental conditions experienced by the larvae on subsequent egg number and size, the smallest, largest, and median female pupae (by weight) were selected from each treatment replicate and allowed to ecdyse and mate with 1–3 males in a one-quart (0.95 l) glass jar filled with 60 ml distilled water and covered with a screen lid. Jars were kept in a humid chamber at  $23 \pm 1^\circ\text{C}$  and L:D = 16:8 for the duration of the experiment. Adults had continuous access to fresh raisins. A 5 × 10 cm piece of moist paper toweling that adhered to the jar wall provided an oviposition substrate. Jars were checked for eggs and dead females 3 times per week, and an anesthetized rat was provided every 2–4 d as a blood-meal source. The experiment was terminated when all females had died.

Eggs laid in each female's complete batches were counted and, except for smaller batches ( $n < 20$ ), 20 eggs were sampled haphazardly from each batch and each egg's length and maximum width measured with an ocular micrometer and dissecting microscope at 60× magnification. Egg shape was assumed to be a prolate spheroid and egg volumes were estimated to the nearest  $1 \times 10^{-4} \text{ mm}^3$  (Hawley 1985a). Seventy-four females produced complete initial egg batches; of these, 8 (16.2%) laid a second batch. Two females laid 3 complete batches. Egg batches were considered complete if a female that had previously laid eggs had taken a subsequent blood meal and had not oviposited for at least a week thereafter.

In mosquitoes, the larval environment can affect size at metamorphosis and subsequent reproductive potential. To determine whether conditions during larval development affect fitness of female *A. sierrensis* independent of their effects on size at metamorphosis (pupal weight), pupal weight was factored out and residual variation was then tested for significant correlations.

Table 1. Interpretation of possible means, standard deviations ( $s$ ), and skewnesses ( $g_1$ ) observed when comparing cohorts hatching from a mixture of different egg-size classes (mixed) with the average of cohorts from each egg-size class reared separately (avg separate). nsd = not significantly different from; PW = pupal weight; DT = development time. Interpretations follow from Wilbur and Collins (1973), Wilbur (1976), Kaplan (1980), Travis (1984), and Begon (1984).

Parameter	Result	Interpretation
Mean	Mixed nsd avg separate	Larvae from different egg sizes interact additively
	Mixed PW > avg separate and/or mixed DT < avg separate	Larvae from different egg sizes interact facilitatively or possess exclusive resources
	Mixed PW < avg separate and/or mixed DT > avg separate	Larvae from different egg sizes mutually interfere with one another
$s$ or $g_1$	Mixed nsd avg separate	No directional interaction is taking place
	Mixed < avg separate	Facilitation and/or exclusive resources exist
	Mixed > avg separate	Some form of directional competition is taking place

We first performed a log-log regression of the number of eggs in the first batch (hereafter referred to as egg number), mean egg volume in the first batch (egg size), adult longevity (d from pupal eclosion to death), and batch volume (measured as egg no.  $\times$  mean egg size) on pupal weight. We then assessed the significance of a correlation by testing for deviation of the slope ( $v$ ) of the central trend line (CTL, Ricker 1984) from zero, and assessed the significance of an allometric relationship by testing for deviation of  $v$  from 1.0. Tests used the 95% confidence limits of  $v$  calculated by the formula of Jolicoeur and Mosimann (1968). Then, (1) if  $v$  did not differ from zero, we concluded that the dependent variable was not significantly correlated with pupal weight and we subsequently regressed the dependent variable directly on conditions of the larval environment (see (4) below). (2) If  $v$  differed from zero but not 1.0, we concluded that the dependent variable was significantly correlated with pupal weight and that the relationship was isometric; consequently, we performed GMR of the dependent variable on pupal weight and then regressed the deviation from the resulting CTL on conditions of the larval environment (see (4)). (3) If  $v$  differed from both zero and 1.0, we concluded that the dependent variable was significantly correlated with pupal weight and that the relationship was allometric; consequently, we performed log-log GMR of the dependent variable on pupal weight and then regressed the deviations from the CTL on conditions of the larval environment. (4) Finally, the appropriate variable from (1-3) directly above was subjected to LR on all linear, quadratic, and first-order interaction terms involving latitude (L), temperature (T), food level (F), and larval density (D): L, L<sup>2</sup>, T, T<sup>2</sup>, F, F<sup>2</sup>, D, D<sup>2</sup>, L $\times$ T, L $\times$ F, L $\times$ D, T $\times$ F, T $\times$ D, and F $\times$ D. In this way, the effects of

these factors on each fitness correlate, independently of their effects on pupal weight, were measured.

Variation in egg size within and among females and within and among populations was analyzed with nested analysis of variance (ANOVA).

#### *Egg size, viability, and fitness correlates*

Egg viability within populations was defined as the proportion of eggs that hatched or were embryonated. Viability was measured in a sample of 460 small ( $\leq 8.5 \times 10^{-3}$  mm<sup>3</sup>) and 460 large ( $\geq 11.5 \times 10^{-3}$  mm<sup>3</sup>) eggs from the Eugene (44°N) population. Embryonation was determined by flooding unhatched eggs with 5.25% sodium hypochlorite solution for 30-40 min and examining the cleared chorions for segmented embryos. Differences were tested with the G-test of independence using Williams' correction (Sokal and Rohlf 1981).

Fitness correlates were determined by rearing larvae hatching from large and small eggs either separately or together in a basic de Wit replacement series (de Wit 1960, Begon and Mortimer 1986). Because there was no reliable method for distinguishing individuals that hatched from eggs of different sizes, indirect methods were used to infer the consequences of egg size on subsequent development time (recorded daily) and pupal weight. Inferences were based on comparisons of the mean, standard deviation, and skewness in each mixed cohort with the average of the comparable parameters in the two cohorts reared separately (Table 1).

Larvae from the Eugene (44°N) population were used to determine the consequences of egg size on fitness correlates within a population. The experimental design incorporated 3 replicates each of 3 combinations of larvae from small or large eggs: 100% large, large:small = 1:1, and 100% small. For each treatment replicate, 24 freshly hatched larvae were placed in a 100  $\times$  25 mm

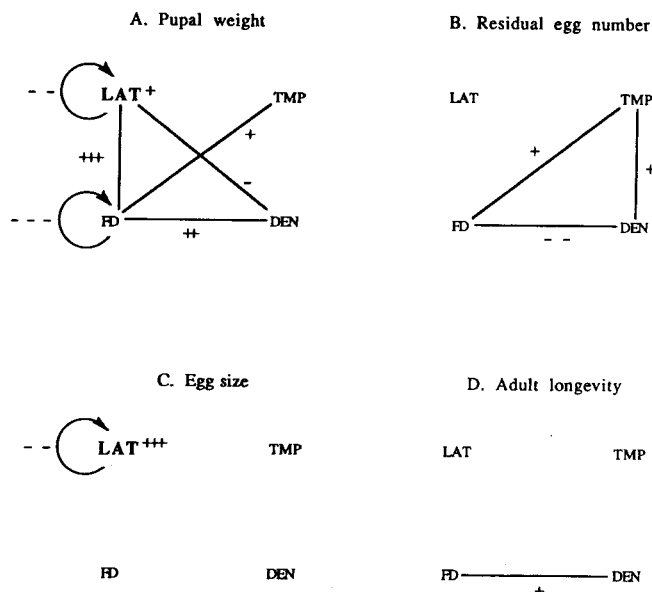


Fig. 1. Summary of regression analyses of variation in (A) pupal weight, (B) egg number laid in the first batch, (C) mean size in the first batch, and (D) adult female longevity in three populations of *Aedes sierrensis*. Egg size and female longevity did not vary with pupal weight; egg number depended on pupal weight and is expressed as residuals from its geometric mean regression on pupal weight (log-log). Factors tested are latitude (LAT), temperature (TMP), food level (FD), and larval density (DEN). Bold factors indicate significant main effects, straight lines connecting factors denote significant interactions between factors, and circular arrows indicate significant quadratic effects. Sign and level of significance are denoted by the number of plus or minus marks: 1 mark,  $P < 0.05$ ; 2 marks,  $P < 0.01$ ; 3 marks,  $P < 0.001$ .

plastic petri dish, to which was added 75 ml distilled water and 0.10 g dry laboratory diet. All experimental trials were run concurrently at  $21 \pm 1^\circ\text{C}$  and a L:D = 16:8 photoperiod. The experiment was terminated after 52 d, when 4 larvae remained alive. Separate cohorts hatching from large or small eggs were paired at random by turning cards. Variation in cohort survivorship (survival from hatch to pupation) was tested with one-way ANOVA after arcsine transformation. The mean, standard deviation, and skewness of male and female pupal weight and development time were averaged for each pre-determined pair of separate cohorts and then compared with one-way ANOVA to the values for the mixed cohorts.

Larvae from the Eugene ( $44^\circ\text{N}$ ) and San Diego ( $33^\circ\text{N}$ ) populations were raised together in 2 de Wit replacement series to determine the consequences of egg size on fitness correlates between populations. First, larvae hatching from unselected eggs from Eugene (large eggs) and San Diego (small eggs) were reared alone or together (Eugene:San Diego = 1:1) as above in 9 replicates each to determine the consequences of egg-size variation between the populations. Second, larvae hatching from large ( $\geq 9.2 \times 10^{-3} \text{ mm}^3$ ) San Diego eggs and small ( $\leq 10.0 \times 10^{-3} \text{ mm}^3$ ) Eugene

eggs were reared alone or together (Eugene:San Diego = 1:1) as above in 6 replicates each to control for differences between populations that were unrelated to egg size. This procedure rendered mean egg sizes in the two selected populations equivalent. Analyses and interpretation of mean, standard deviation, and skewness of pupal weight and development time were made as in Table 1.

#### Allocation of reproductive investment

To assess whether a negative correlation exists between egg number and size, the relationship between these traits was determined for the 3 populations of *A. sierrensis* by performing GMR of deviation from mean log egg size on residuals from GMR of log egg number on log pupal weight. To test for the effect of reproductive investment on the probability of laying a second egg batch (i.e., probability of being iteroparous), a one-way ANOVA of batch volume in the first egg batch in females that laid one vs two egg batches was performed. Putative costs in adult longevity due to early reproductive effort were assessed by regressing the deviations from mean adult longevity on the residuals from GMR of log egg number in the first batch on log pupal weight. To determine whether females incur physiological costs in future reproduction through early reproductive investment, the relationship between weight-specific early and late reproductive investment was examined by regressing the residuals from GMR of log egg number in the second batch on log pupal weight on residuals from GMR of log egg number in the first batch on log pupal weight.

## Results

### Egg number

The combined LR of pupal weight on latitude, larval density, food level, and temperature and their quadratic and interaction effects was highly significant ( $R^2 = 0.63$ ,  $F_{14,334} = 39.68$ ,  $P < 0.001$ ) (Fig. 1A). Pupal weight was positively correlated with the linear effect of latitude ( $t = 2.49$ ,  $P < 0.05$ ), negatively correlated with the quadratic effects of latitude ( $t = -2.71$ ,  $P < 0.01$ ) and quadratic effects of food level ( $t = -6.11$ ,  $P < 0.001$ ) and with the interaction between latitude and density ( $t = -2.12$ ,  $P < 0.05$ ), and positively correlated with the interactions between latitude and food level ( $t = 3.32$ ,  $P < 0.001$ ), density and food level ( $t = 2.78$ ,  $P < 0.01$ ), and food level and temperature ( $t = 2.55$ ,  $P < 0.05$ ). These interactions show that the decline in pupal weight with density was less at higher latitudes and that the contribution of food level to pupal weight increased with temperature or larval density. The effect of food level was nonlinear, indicating that increased food yields diminishing returns in pupal weight. Thus, females in different populations differed in pupal weight,

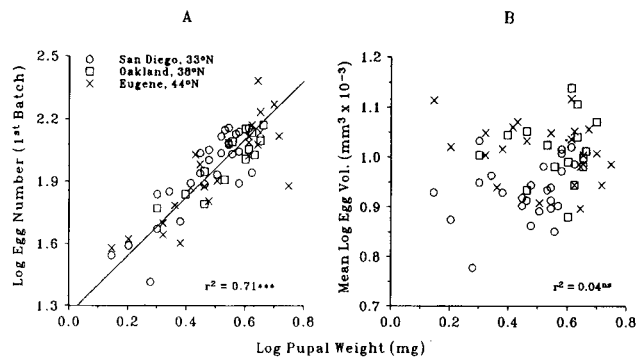


Fig. 2. Relationships of log pupal weight to (A) log egg number and (B) mean log egg size oviposited in the first batch in 3 populations of *Aedes sierrensis*. The central trend line relating log egg number to log pupal weight in (A) indicates allometry (lower confidence limit of  $v$ ,  $v_L = 1.16$ ). \*\*\*  $P < 0.001$ , ns  $P > 0.05$ .

and each aspect of the larval environment had a significant direct or indirect effect on pupal weight.

The number of eggs produced by a female in her first batch depended on her pupal weight (Fig. 2A). The pooled GMR of log egg number on log pupal weight was significantly allometric ( $v = 1.41$ ,  $P < 0.001$ ). The slope of the CTL differed significantly among populations ( $P < 0.001$ , method of Jolicoeur and Mosimann 1968); the slope of the CTL in log-log GMR was significantly greater than 1.0 among females from Eugene (lower limit of  $v$ ,  $v_L = 1.08$ ,  $P < 0.05$ ) and San Diego ( $v_L = 1.09$ ,  $P < 0.01$ ) but not Oakland ( $v_L = 0.92$ ,  $P > 0.05$ ). Regardless of differences in slope, GMR revealed a significant correlation between pupal weight and fecundity in all populations.

To examine the effects of the larval environment on fecundity independently of its direct effects on pupal weight, the analyses below consider residuals from the CTL of log egg number on log pupal weight. The combined LR of these residuals on latitude, larval density, food level, and temperature and their quadratic and interaction effects was significant ( $R^2 = 0.44$ ,  $F_{14,49} = 2.69$ ,  $P < 0.01$ ) (Fig. 1B). There were significant interaction effects between larval density and temperature (positive:  $t = 4.28$ ,  $P < 0.001$ ), larval density and food level (negative:  $t = -2.77$ ,  $P < 0.01$ ), and food level

Table 2. Nested ANOVA of egg size ( $\times 10^{-3}$  mm<sup>3</sup>) in the first ovarian cycle for three populations of *Aedes sierrensis*. Satterthwaite's approximation was used to calculate a synthetic mean square among females (within localities) to test the effect of locality on egg size (Sokal and Rohlf 1981). \*\*\* =  $P < 0.001$ .

Source	df	SS	MS	F'	Expected MS
Among localities	2	1615.38	807.69	21.06*** <sup>a</sup>	807.70
Among females, within localities	98 <sup>a</sup>	3524.88	38.35	10.42***	35.25
Within females	1690	5715.75	3.38		3.38
Total	1972	10856.01			

<sup>a</sup> Values calculated from Satterthwaite's approximation.

Table 3. Viability and hatchability of large and small *Aedes sierrensis* eggs from the Eugene, OR (44°N) population.

Egg size	Initial no. eggs	Viability (%)	Hatching success (%)
$\leq 8.5 \times 10^{-3}$ mm <sup>3</sup>	460	100.0	70.3
$\geq 11.5 \times 10^{-3}$ mm <sup>3</sup>	460	99.8	74.2

and temperature (positive:  $t = 2.63$ ,  $P < 0.05$ ). These interactions show that larval density effects were more acute at higher temperature or lower food levels and that increased larval food made a greater contribution to subsequent adult egg production at higher than lower temperature. Thus, interactions between larval density, food level, and temperature transcended metamorphosis to modify the weight-specific number of eggs laid by adult females in their first batch but the extent of these modifications did not vary among populations.

### Egg size

Mean egg size per female in the first batch was not significantly correlated with pupal weight (Fig. 2B). The combined LR of egg size on latitude, larval density, food level, and temperature and their quadratic and interaction effects was highly significant ( $R^2 = 0.59$ ,  $F_{14,79} = 3.01$ ,  $P < 0.001$ ). Egg size did not vary with larval density, food level, or temperature (Fig. 1C) but did increase with latitude ( $t = 3.35$ ,  $P < 0.001$ ). There were no significant quadratic or interaction effects of or among larval density, food level, and temperature but the effect of latitude was significantly non-linear (Fig. 1C;  $t = -3.27$ ,  $P < 0.01$ ). Thus, egg size showed evidence of divergence among populations that was unaffected by the larval environment of their parents.

Nested ANOVA of egg size showed that considerable differences exist among females within and among localities (Table 2). The estimated variance components indicate that approximately 20% of the overall variation in egg size was due to differences among localities, an additional 28% to differences among females within localities, and the remaining 52% to differences among eggs within females.

Table 4. Summary of ANOVAs of effects of egg-size distribution on larval competition and subsequent mean, standard deviation (s), and skewness ( $g_1$ ) of fitness correlates in *Aedes sierrensis*. Each entry in the table shows the mean value of larvae hatching from mixed (1:1) egg sizes minus the mean value of larvae reared separately ( $\pm 1$  SE) after hatching from each egg-size category (see text). Survivorship = arcsin (no. pupating/no. in original cohort); PW = pupal weight; DT = development time. A) Competition of larvae of large vs small eggs from Eugene (EU). B) Competition of larvae from Eugene (EU) vs San Diego (SD) eggs unselected for size. C) Competition of larvae from EU vs SD eggs of equivalent size. Degrees of freedom for the ANOVAs were 1 and 4 in A, 1 and 17 in B, and 1 and 12 in C. Not significant except \* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ . c = competition, f = facilitation (Table 1).

Parameter	Male		Female	
	PW (mg)	DT (d)	PW (mg)	DT (d)
A. Large vs small EU; difference in survivorship = $0.07 \pm 0.33$				
mean	$-0.03 \pm 0.02$	$0.13 \pm 0.57$	$0.06 \pm 0.12$	$4.75 \pm 1.96^*c$
s	$0.15 \pm 0.07$	$2.19 \pm 1.08$	$-0.29 \pm 0.07^*f$	$0.60 \pm 0.44$
$g_1$	$0.10 \pm 0.35$	$2.15 \pm 0.88^*c$	$-1.53 \pm 0.93$	$-1.43 \pm 0.27^*f$
B. Unselected EU (larger mean size) vs unselected SD (smaller mean size); difference in survivorship = $0.04 \pm 0.05$				
mean	$0.04 \pm 0.06$	$-2.84 \pm 0.47^{***}f$	$-0.04 \pm 0.11$	$-1.78 \pm 1.35$
s	$0.10 \pm 0.03^{**}c$	$-2.58 \pm 0.80^*f$	$0.02 \pm 0.09$	$1.88 \pm 1.71$
$g_1$	$0.61 \pm 0.17^{**}c$	$-1.20 \pm 0.41^*f$	$-0.17 \pm 0.37$	$0.61 \pm 0.45$
C. Small EU vs large SD (equivalent mean size); difference in survivorship = $0.16 \pm 0.09$				
mean	$-0.06 \pm 0.13$	$-6.12 \pm 3.33^*f$	$-0.15 \pm 0.28$	$-5.34 \pm 4.71$
s	$0.03 \pm 0.03$	$-1.44 \pm 0.73$	$0.16 \pm 0.10$	$1.47 \pm 1.25$
$g_1$	$0.63 \pm 0.46$	$-1.02 \pm 0.63$	$0.46 \pm 0.33$	$0.24 \pm 0.25$

Eggs laid by San Diego (mean  $\pm 2$  SE =  $8.69 \pm 0.36$  mm<sup>3</sup>  $\times 10^{-3}$ ) females were smaller than eggs laid by either Oakland (mean  $\pm 2$  SE =  $10.68 \pm 0.73$  mm<sup>3</sup>  $\times 10^{-3}$ ) or Eugene (mean  $\pm 2$  SE =  $10.16 \pm 0.47$  mm<sup>3</sup>  $\times 10^{-3}$ ) females ( $P < 0.01$ , GT2-method, Sokal and Rohlf 1981); eggs of the two more northern populations did not differ in size (Fig. 2B).

### Egg size, viability, and fitness correlates

*Aedes sierrensis* eggs showed no size-dependent differences in viability or hatchability (Table 3). For both small ( $\leq 8.5 \times 10^{-3}$  mm<sup>3</sup>) and large ( $\geq 11.5 \times 10^{-3}$  mm<sup>3</sup>) eggs, viability was  $>99\%$ . Hatching successes of small and large eggs after one flooding with the same hatching stimulus did not differ significantly ( $G = 1.60$ , d.f. = 1,  $P > 0.05$ ). In the de Wit replacement series forcing encounters of larvae from large and small EU eggs, an ANOVA showed no significant variation in survivorship to pupation between mixed and the mean of separate cohort (Table 4,  $P > 0.75$ ). The encounters had no effect on means or standard deviations of male pupal weight or development time relative to the average of the separate cohorts (Table 4A). The encounters also had no effect on mean female pupal weight or on the standard deviation of female development time, but they resulted in an increase in mean female development time and a decrease in the standard deviation of female pupal weight. The encounters produced an increase in the skewness of male development time and a decrease in the skewness of female development time,

but did not affect skewness of either male or female pupal weight. Of 4 significant interactions, 2 were competitive and 2 were facilitative.

Encounters between Eugene and San Diego larvae from eggs unselected for size did not affect cohort survivorship ( $P > 0.25$ ) or means or standard deviations of female pupal weight or development time (Table 4B). The encounters had no effect on mean male pupal weight, but the standard deviation of male pupal weight increased relative to the average of the separate cohorts. Both the mean and standard deviation of male development time declined in these treatments. The encounters increased skewness in male pupal weight but decreased that in male development time; skewness in female pupal weight and development time was not affected. Of 5 significant interactions, 2 were competitive and 3 were facilitative.

When encounters were controlled for differences between Eugene and San Diego *A. sierrensis* in egg size, cohort survivorship did not differ ( $P > 0.05$ ). In these encounters (Table 4C), only mean male development time differed between treatment means, declining relative to the average of the separate cohorts. Encounters involving larvae from these treatments produced no detectable changes in standard deviation or skewness in any fitness correlates. The only significant interaction was facilitative.

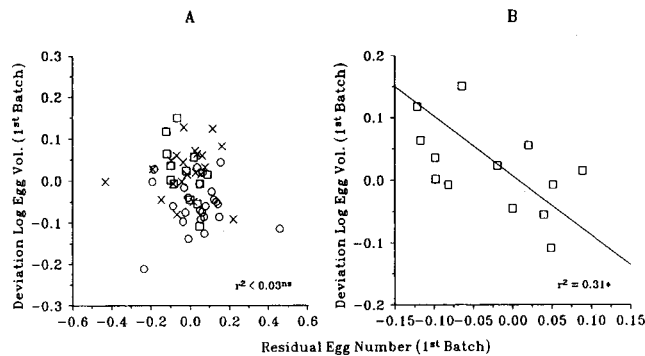


Fig. 3. Relationship between egg number and size in first-batch eggs in *Aedes sierrensis*, expressed as the correlation between deviation from mean log egg size and residuals from geometric mean regression of log egg number on log pupal weight. (A) Comparison of Eugene, OR, Oakland, CA, and San Diego, CA populations. Symbols as in Fig. 2A. (B) The relationship for the Oakland population, showing the central trend line (upper confidence limit of  $v$ ,  $v_U = -0.56$ ).  $^{ns} P > 0.05$ ,  $^* P = 0.05$ .

### Allocation of reproductive investment

When populations were pooled, GMR of deviation from mean log egg size and residuals from GMR of log egg number on log pupal weight revealed no significant covariation of egg size with weight-specific fecundity (Fig. 3A). When each population was examined separately, the Oakland population, but not the other two, showed a significant (negative) correlation between these variables (Fig. 3B).

Female batch volume in the first batch (egg no.  $\times$  mean egg size) increased allometrically with pupal weight (log-log GMR:  $v_L = 1.16$ ,  $P < 0.001$ ). However, this correlation ( $r^2 = 0.67$ ) did not differ detectably from that between log egg number and log pupal weight ( $r^2 = 0.68$ ). Unlike egg number, variation in egg size therefore contributed little to variation in female batch volume.

Adult longevity of females did not vary significantly with pupal weight ( $r^2 = 0.01$ ,  $P > 0.05$ ). The combined LR of adult longevity on latitude, larval density, food level, temperature, and their quadratic and interactive effects was highly significant ( $R^2 = 0.53$ ,  $F_{14,87} = 2.46$ ,  $P < 0.01$ ), but only the positive interaction between larval density and food level had a detectable effect on adult longevity ( $t = 2.42$ ,  $P < 0.05$ ) (Fig. 1D). Independent of pupal weight, adult females lived longer if they had been reared at higher food and density.

Deviation from mean log female longevity (Fig. 4) was not significantly correlated with the residuals from GMR of log egg number in the first batch on log pupal weight. Batch volume in the first batch did not differ among females that laid one vs two egg batches (ANOVA:  $F_{1,55} = 0.83$ ,  $P > 0.25$ ). The mean residual from GMR of log egg number in the first batch on log pupal weight did not differ between the 57 females that produced only one batch of eggs (mean  $\pm 2$  SE =  $-0.01$

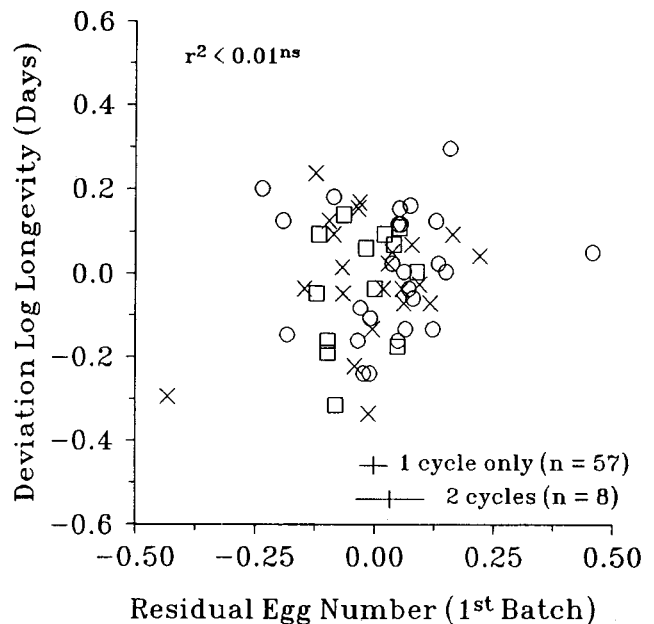


Fig. 4. Relationship of reproductive investment to adult longevity in female *Aedes sierrensis*, expressed as the correlation between deviation from mean log longevity and residuals from geometric mean regression of log egg number in the first batch on log pupal weight. Symbols as in Fig. 2A. Lines below the plot show means  $\pm 2$  SE for females laying one and two egg batches.  $^{ns} P > 0.05$ .

$\pm 0.03$ ) and the 8 that produced 2 batches (mean  $\pm 2$  SE =  $0.03 \pm 0.07$ ). Neither mean residual differed from zero ( $P > 0.05$ ), and the direction of the nonsignificant trend was toward an increased probability of a second batch among females with a high weight-specific fecundity in the first batch. The log number of eggs laid in the second batch was positively correlated with log pupal weight (Fig. 5A). There was a positive correlation ( $P < 0.05$ ) between the residuals from GMR of log egg number in the second batch on log pupal weight and the corresponding residuals for eggs in the first batch (Fig.

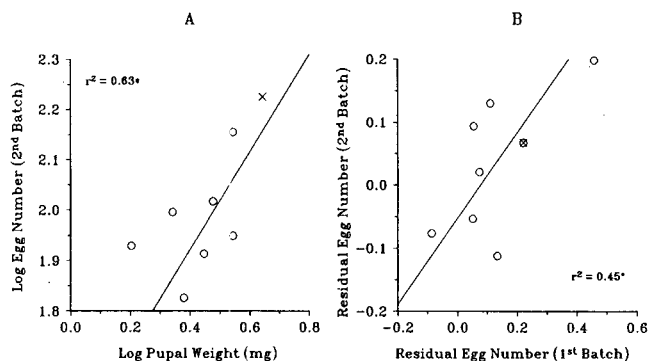


Fig. 5. Temporal allocation of egg number in *Aedes sierrensis*. (A) Log number of eggs laid in the second batch as a function of log pupal weight. (B) Allocation of eggs laid to first and second batches (residuals from geometric mean regressions of log egg number in each batch on log pupal weight). Central trend lines are fitted. Symbols as in Fig. 2A.  $^* P < 0.05$ .



5B). Thus, there was no evidence for physiological costs of early reproductive investment, either in adult longevity or in later reproductive investment.

## Discussion

Environmental conditions experienced by larval *Aedes sierrensis* combine in a complex manner to affect pupal weight (Fig. 1A) and, subsequently, both weight-dependent (Figs 2, 5A) and weight-independent (Fig. 1B) number of eggs laid. The interactions between food, density, and latitude affecting pupal weight (Fig. 1A) mean that each population of *A. sierrensis* views the combination of food and density as a different environmental mosaic. As in at least one other mosquito (*Wyeomyia smithii*, Bradshaw and Holzapfel 1992), the relationship between egg number and pupal weight is strongly allometric; larger females in both species lay disproportionately more eggs per unit weight than smaller females. Because mean egg size does not vary significantly with the previous larval environment (Fig. 1C) or with pupal weight (Fig. 2B), reproductive investment in *A. sierrensis* may be equated with weight-specific production of eggs. We failed to detect any negative effect of reproductive investment during the first ovarian cycle on either adult longevity (Fig. 4) or reproductive investment during the second ovarian cycle (Fig. 5B). Most females in this laboratory study survived to complete only a single ovarian cycle; in nature, survival of adult females per ovarian cycle is 50% or less (Hawley 1985b). The low probability of successfully laying more than one egg batch suggests to us that second and third egg batches may be relicts. Egg number laid in the first batch is the major determinant of fitness within natural or laboratory populations of *A. sierrensis*.

About 71% of the variation in the number of eggs laid in the first batch is accounted for by pupal weight (Fig. 2A). Of the residual 29%, 62% is accounted for by factors in the larval environment that are independent of pupal weight (Fig. 1B). Thus, the larval environment determines roughly 71% of the variation in adult female reproductive success indirectly through its effect on pupal weight, and an additional 18% (62% of 29%) directly through its effects that transcend metamorphosis. Pupal weight is therefore the major but not exclusive determinant of reproductive success in female *A. sierrensis*.

The larval environment of *A. sierrensis* may have several direct effects on fitness characters. Broadie and Bradshaw (1991) found that *A. sierrensis* larvae of the same size but reared at different densities consume food at different rates. When transferred to a uniform density and food level, larvae reared at higher densities consumed more food than larvae of the same weight but reared at lower densities. Thus, the larval environment

may directly modify competitive ability. In the present case, well-fed larvae may achieve greater fitness than poorly fed individuals of the same size by sequestering more resources as larvae and subsequently diverting them to egg production, rather than using them directly for somatic growth. We identified 7 pairs of pupae with equivalent weight ( $\pm 0.2$  mg) that had developed at different levels of per-capita food. The resulting females did not differ in egg number ( $t = -0.24$ , d.f. = 6,  $P > 0.25$ ) but females that had experienced greater food as larvae laid smaller eggs ( $t = -2.24$ , d.f. = 6,  $P < 0.05$ ), although batch volume was unaffected ( $t = -0.82$ , d.f. = 6,  $P > 0.25$ ). These results suggest that in addition to its greater evolutionary plasticity, egg size may show greater developmental plasticity than weight-specific egg number within populations. Egg size may be subject to greater revision during both the ontogeny and phylogeny of *A. sierrensis* than is weight-specific egg number. The greater plasticity of egg size relative to weight-specific egg number may be due in part to the observation that yolk is added simultaneously, rather than sequentially, to individual ova (Clements 1963).

Although reduced fecundity is a result of metamorphosis at low pupal weight in *A. sierrensis*, there is no evidence that small females incur additional costs related to egg size (Fig. 2B). Variation in egg size among females within populations is only about 55% of that within females (Table 2); by contrast, most egg-size variation (measured as egg length) in the congeneric mosquito *A. aegypti* occurs among females and is about 100 times that within females (Steinwascher 1984). However, egg size in *A. aegypti* is sensitive to female size and blood-meal size and varies considerably among full sibships (Steinwascher 1984). These observations together with the variation in *A. sierrensis* egg size given in Table 2 suggest a stronger genetic component of variation in egg size in *A. aegypti* than *A. sierrensis*.

Egg number in *A. sierrensis* increases much more rapidly with pupal weight ( $33.5 \pm 3.6$  eggs/mg) than that in *A. aegypti* ( $9.9 \pm 1.4$  ovarioles/mg; Steinwascher 1984). Hawley (1985a) suggested that the high fecundity and small egg size of *A. sierrensis* relative to other mosquitoes, including *A. aegypti*, is an adaptation to an unpredictable larval environment that allows females to spread their reproductive investment, perhaps even for a single ovarian cycle, among several habitats. Small egg size in *A. sierrensis* may therefore have evolved in response to selection for high fecundity. However, variation in egg size within populations may still have fitness consequences. Steinwascher (1984) demonstrated that female *A. aegypti* hatching from larger eggs developed faster and metamorphosed at higher weights than females from smaller eggs. Within the Eugene population, egg size did not affect viability or hatching success (Table 3) but encounters of larvae hatching from eggs of different sizes resulted in both facilitative and competitive interactions (Table 4A). Encounters of larvae hatching from eggs of different populations (Eu-

gene and San Diego) also resulted in both facilitative and competitive interactions (Table 4B) but 4 of the 5 interactions became non-significant when the eggs from the two populations were first matched in mean size (Table 4C). In this case, the effects of larval encounter on male development time suggest facilitative interactions between the larvae of the two populations. Thus, the Eugene and San Diego populations have diverged in their larval interactions but this divergence depends largely (although not entirely) on population differences in egg size.

We detected evidence for a trade-off between egg number and size in *A. sierrensis* in one population but not the other two. In this study, these populations were cultured concurrently under closely controlled conditions in an experiment designed to detect genetic differences among them. Since the populations differ with respect to this phenotypic correlation, the presence or absence of the correlation itself has a genetic basis. Consequently, significant genotype  $\times$  environment interaction exists among populations that is not revealed when the populations are pooled and that may or may not reflect genetic correlation or genotype  $\times$  environment interaction within populations. The egg number-egg size relationship in mosquitoes can now be examined at three taxonomic levels: among species, among populations, and within populations. The correlation between log egg size and log weight-specific egg number is strongly negative among species (Hawley 1985a:  $r^2 = 0.95$ ,  $P < 0.001$ ). In *A. sierrensis*, the correlation between deviation from mean log egg size and the residual from GMR of log egg number on log pupal weight disappears when populations are pooled (Fig. 3A) and re-emerges within only one of the three populations (Fig. 3B). Thus, the nature of the relationship between mosquito egg number and size changes with the taxonomic scale of the comparison. These results support the contentions of Reznick (1985) and Schnebel and Grossfield (1988) that interspecific comparisons are unreliable indicators of reproductive allocation patterns within populations. Even broad intraspecific comparisons may be too coarse to predict relationships within populations (Fig. 3A, B).

Comparisons among individuals within populations are more likely to reflect accurately the underlying genetic correlations, if the environmental correlations among traits are small (van Noordwijk and de Jong 1986, Chaverud 1988). In this study, environmental factors that had strong effects on pupal weight and, consequently, egg number (Fig. 1A, B) had no detectable effect on egg size (Fig. 1C), indicating that environmental correlations between them are indeed small. In the absence of known genetic correlations, phenotypic correlations within populations obtained under experimental conditions where environmental variation can largely be controlled are likely to represent better these genetic correlations than are phenotypic correlations obtained under less controlled laboratory conditions,

from field observations, or from comparisons among more distantly related taxonomic groups. Our results suggest that independent variation in egg size and number is not greatly constrained physiologically or developmentally; consequently, the covariation we have observed in these traits is likely to have a substantial genetic basis. It would be interesting to learn if the fitness consequences of egg-size variation were greater in Oakland *A. sierrensis*, where significant covariation in egg size and number exists.

We conclude that both phenotypic and genetic tests of reproductive allocation should begin with comparisons within populations but, in order to minimize the confounding effects of environmental covariance among traits on the determination of their evolutionary relationships, tests should assess the effects of many environmental variables, and the interactions between them, on these traits. In this paper, use of this approach has enabled us to demonstrate that genetic variation exists both for egg size and for the allocation of reproductive investment between egg size and egg number in *Aedes sierrensis*.

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