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Costs of reproduction and geographic variation in the reproductive tactics of the mosquito *Aedes triseriatus*

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Abstract The eastern treehole mosquito, *Aedes triseriatus* (Say), was used in two experiments testing assumptions and predictions of life history theory. Experiment 1 employed direct, phenotypic manipulations of reproductive investment (blood meal mass) to test for a physiological cost of reproduction expressed as reduced longevity with increasing reproductive investment. As predicted, *A. triseriatus* from Vero Beach, Fla., showed costs increasing with investment in reproduction, but mosquitoes from Ashland, Va., showed the opposite response. This difference in apparent costs of reproduction may influence patterns of reproductive investment documented in the second experiment. To test the hypothesis that different predator-induced expected mortality rates among juveniles result in different patterns of reproductive allocation, we compared the allocation tactics of *A. triseriatus* from eight geographic populations, four sympatric with and four allopatric to the predatory mosquito, *Toxorhynchites rutilus* (Coq). Path analysis identified a phenotypic trade-off between offspring size and number, supporting this fundamental assumption of life history theory. Analysis of covariance revealed significant variation among populations in replete blood meal mass, reproductive output per unit blood meal mass (both measures of reproductive investment), mean egg mass, and egg number, although intrapopulation variation greatly exceeded interpopulation variation for all traits. Despite this variation among populations, there were no consistent or significant dif-

ferences between populations sympatric vs. allopatric to the predator *T. rutilus*. This experiment therefore fails to support the hypothesis that greater mortality of juveniles should be associated with greater offspring size, lower offspring number, and lower reproductive investment. Our results are interesting, however, because they suggest that reproductive investment, reproductive output, and the trade-off between offspring size and number may evolve independently in this system.

Key words Life history evolution · Treehole mosquito · Mortality rate · Predation · Manipulation of reproductive investment

Introduction

Life history theory seeks to explain an organism's reproductive tactics under a given set of conditions (Roff 1992; Stearns 1992). Trade-offs within the life history are assumed to result from allocation of a fixed resource budget among growth, maintenance, and reproduction. Trade-offs may be manifested as costs of reproduction, such as decreases in parental longevity or ability to reproduce later in life associated with increases in early reproductive investment (Gadgil and Bossert 1970; Bell 1980; Partridge and Harvey 1988). Another trade-off occurs within the reproductive budget itself: greater investment per offspring is predicted to reduce fecundity. Given these trade-offs, natural selection is assumed to favor trait combinations that optimize fitness, so that individuals in discrete populations may become locally adapted via evolution of age-specific reproductive investment (Law 1979; Michod 1979; Bell 1980), or how that investment is allocated among offspring (Gadgil and Bossert 1970; Smith and Fretwell 1974; McGinley et al. 1987; Morris 1987; Winkler and Wallin 1987). In this paper we describe experiments identifying physiological costs of reproduction and documenting differentiation in the life history tactics of the eastern treehole mosquito, *Aedes triseriatus* (Say).

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Most empirical studies of life histories employ phenotypic correlations or experimental manipulations to assess reproductive costs (see Partridge and Sibly 1991; Reznick 1992a). The interpretation of such experiments as tests of evolutionary theory is debated (Partridge 1992; Reznick 1992a, 1992b) because these approaches cannot identify the genetic (evolutionarily important) basis of reproductive trade-offs. Ultimately, the evolutionary consequences of such trade-offs or correlations among reproductive traits will be determined largely by the proximate physiological or developmental relationships that cause the correlation; a strong genetic correlation maybe unbreakable and profoundly constrain evolutionary possibilities, or the correlation may be evolutionarily malleable, depending on the physiological or developmental basis of the correlation (Nijhout and Emlen 1998; WA Frankino, JB Wolf, AF Agrawal, ED Brodie III, AJ Moore, unpublished data). Phenotypic manipulations have merit because they can identify the presence of phenotypic trade-offs and proximate costs (Maynard Smith 1991; Partridge 1992; Reznick 1992a; Sinervo and DeNardo 1996) and they have the potential to reveal physiological or developmental relationships which may suggest underlying, genetically based trade-offs of evolutionary importance (Sinervo and DeNardo 1996). Furthermore, such manipulations have the advantage of allowing experimental increase in the variation among individuals for the trait in question (Sinervo and DeNardo 1996). We use such a manipulative approach to investigate proximate costs of reproduction and geographic variation in reproductive tactics in *A. triseriatus*.

In Experiment 1, we manipulate reproductive investment of individual female mosquitoes from two populations to determine if there is a physiological cost associated with reproduction (Gadgil and Bossert 1970; Bell 1980). In the second experiment, we compare reproductive allocation patterns of *A. triseriatus* from eight distinct geographic populations and test for life history differentiation among populations. We test the hypothesis that population co-occurrence with a predator of *A. triseriatus* larvae is associated with lower overall reproductive investment (Law 1979; Michod 1979; Bell 1980) and greater offspring size and lower offspring number (Morris 1987).

Life history predictions for *A. triseriatus*

A. triseriatus larvae develop through four larval instars and pupate in water-filled treeholes or artificial containers. In the southern portion of its range, immature *A. triseriatus* may suffer intense predation by the larvae of another mosquito, *Toxorhynchites rutilus* (Coq.). When present, *T. rutilus* larvae prey on all *A. triseriatus* larval instars, and can dramatically reduce the numbers of *A. triseriatus* that survive to eclosion (Darsie and Ward 1981; Bradshaw and Holzapfel 1983, 1985, 1988; Lounibos et al. 1993, 1997). Predation by *T. rutilus* is size

selective, with early instars preying on early-instar larvae and late instars preying on late-instar larvae and pupae (Steffan and Evenhuis 1981; Bradshaw and Holzapfel 1983; Lounibos et al. 1993, 1997; Juliano 1996). In the northern portion of *A. triseriatus* range, *T. rutilus* is absent, and no major predators replace *T. rutilus* in these northern habitats (Bradshaw and Holzapfel 1985; Nannini and Juliano 1998). Hence, predation by *T. rutilus* larvae in the south is an important source of *A. triseriatus* larval mortality that is absent in the north. Thus, *A. triseriatus* sympatric with *T. rutilus* are expected to have a considerably greater average mortality rate than are *A. triseriatus* allopatric to this predator (see also Juliano and Reminger 1992; Juliano et al. 1993; Juliano 1996; Hechtel and Juliano 1997).

Greater juvenile mortality is predicted to select for lower reproductive investment (Law 1979; Michod 1979) because increased juvenile mortality lowers the average contribution of each offspring to adult fitness, and therefore enhances the value of adult survivorship (Stearns 1992). Thus, this prediction assumes survival costs of reproduction. Lower reproductive investment could be manifested in two, non-exclusive ways in blood-feeding mosquitoes. First, females from populations subjected to greater predation during the larval stages may take smaller replete blood meals relative to body mass. This prediction assumes that the amount of blood ingested is a phenotypic expression of reproductive investment, representing a 'decision' concerning allocation of time and energy among life history components, and that there is some cost (e.g., time, energy expenditure) of taking a blood meal. We predict that *A. triseriatus* from populations co-occurring with *T. rutilus* will take smaller blood meals than will *A. triseriatus* that do not co-occur with this predator. Second, females from populations subjected to greater predation on the larval stages may express lower reproductive investment by producing a smaller mass of eggs (i.e., the product of individual egg mass and egg number) relative to body mass and blood meal mass. This prediction assumes that the female allocates relatively less host blood protein to egg production and that some component of the blood meal (e.g., carbohydrate) is allocated to somatic function. We predict that *A. triseriatus* from populations co-occurring with *T. rutilus* will produce smaller masses of eggs after controlling for both body size and blood meal mass. These two predictions about the relationship of reproductive allotment to co-occurrence with this predator are not mutually exclusive.

Greater mortality of juveniles is also predicted to favor production of higher-investment (larger) offspring (Morris 1987) because it is assumed that greater offspring size increases the probability of surviving to adulthood, either by shortening the time to adulthood or by reducing vulnerability to predators (Stearns 1992). We therefore predict that *A. triseriatus* from populations co-occurring with *T. rutilus* will produce fewer, larger eggs than will *A. triseriatus* from populations that do not occur with this predator.

Our qualitative predictions should be true regardless of the specific rates of mortality of juveniles in sympatric and allopatric populations, as long as immatures sympatric with the predator have appreciably greater expected mortalities than do those allopatric to the predator (Morris 1987). Testing these predictions requires determining whether multiple populations sympatric with and allopatric to this predator differ consistently in the manner predicted. A similar approach, treating populations as replicates and testing for significant differences between regions, has been used productively in studying the evolution of the response of other mosquitoes to geographical variation in selection acting on immatures (e.g., Bradshaw and Holzapfel 1989). Our tests assume that natural mortality rates of adults in nature are similar for sympatric and allopatric populations. Though mortality rates of adults have been examined in a few northern (allopatric) populations (e.g., Walker et al. 1987), we know of no comparable data for southern (sympatric) populations.

Consistent differences in reproductive tactics between regions of sympatry with and allopatry to *T. rutilus* would suggest that selection imposed by this predator is the dominant cause of evolution shaping these life history traits. Absence of the predicted differences in these reproductive tactics between regions would suggest that other sources of natural selection or genetic or other constraints are more important influences on the evolution of these life history traits in *A. triseriatus*, or that predation has selected for differences in other traits, such as age and size at adulthood or behavior (Roff 1992; Stearns 1992; Abrams and Rowe 1996). Other studies of geographic variation in *A. triseriatus* larvae have found significant variation among populations, but no consistent differences between populations sympatric with and allopatric to this predator behavior (Juliano et al. 1993), survival in the presence of the predator (Juliano 1996), and age and size at adulthood (Hechtel and Juliano 1997). Thus, testing for differences in reproductive tactics is a logical next step in testing for evolutionary effects of this predator on its prey.

Materials and methods

A. triseriatus as a model system

A. triseriatus is well suited to tests of life history predictions concerning reproductive tactics for several reasons. First, an essential, limiting resource necessary for reproduction can be quantified and manipulated. Protein for oogenesis is obtained entirely from host blood (Cochrane 1972; Jalil 1974; Mather and Defoliart 1983; Briegel 1985). Reproductive output appears to be limited primarily by availability of ten essential amino acids provided in host blood (Clements 1992) and nitrogen from digested protein not used for oogenesis is excreted (Briegel 1985). Hence, females can control their reproductive investment directly by altering the volume of host blood they acquire. Investigators can exploit *A. triseriatus* dependence on host blood, manipulating blood volume ingested to alter an individual's reproductive investment.

Second, maternal energy reserves derived from carbohydrate feeding, or from synthesis of lipids and glycogen from carbon ob-

tained in excess, non-essential amino acids in blood may be mobilized to meet the energetic demands of oogenesis (Briegel 1990; Clements 1992). These energy reserves may be used either as an energy source for non-reproductive activities (e.g., flight) or for synthesis of yolk proteins for reproduction (Clements 1992). Females can live through multiple oviposition cycles, averaging as little as 11 days in nature (Walker et al. 1987), and realized longevity of adult females in nature can be as high as 39 days (Walker et al. 1987). These data produce a daily survival rate of 0.92 (Walker et al. 1987), implying that approximately 16% of females survive long enough to take multiple blood meals. Thus, trade-offs in energy allocation to reproduction versus somatic function are possible and would constitute costs associated with reproductive investment.

Third, *A. triseriatus* varies geographically for a variety of characters (e.g., Livdahl 1979; Miller et al. 1982; Sims 1985; Juliano 1989; Juliano and Reminger 1992; Juliano et al. 1993). This geographic differentiation indicates that populations are sufficiently isolated to allow local adaptation if appropriate selection pressures exist.

Collection and maintenance of mosquitoes

A. triseriatus larvae and pupae were collected from eight geographic populations in the eastern United States. At four of these sites, the predator *T. rutilus* is absent or only sporadically present and rare (Juliano et al. 1993; Juliano 1996; Hechtel and Juliano 1997). These sites allopatric to the predator are: Fermilab (F), near Batavia, Ill.; Normal (N), Ill.; Worcester (W), Mass., and Romeo (R), Mich. At the other four sites, the predator is very abundant and commonly co-occurs with *A. triseriatus* in the larval environment. These sites sympatric to the predator include: Ashland (A), Va.; Olive Branch (O), Ill.; the Duke Research Forest, Durham (D), N.C., and Vero Beach (V), Fla. Mosquitoes from each population were propagated as free-mating colonies in 0.6-m³ cages. Colony and experimental adults were maintained at 17:7 L:D at 22°C in experiment 1, and at 17:7 L:D and 24 ± 2°C in experiment 2. All adults had continuous access to ~10% sucrose solution. Anesthetized mice served as blood sources for colony and experimental mosquitoes (for methods see Juliano 1989; Juliano et al. 1993).

Experiment 1: proximate costs of reproductive investment

We manipulated initial reproductive investment to determine whether individual mosquitoes showed a physiological cost of reproduction in the form of reduced longevity with increasing reproductive investment. F₁ progeny from the Ashland, Va., and Vero Beach, Fla., populations were hatched synchronously (Novak and Shroyer 1978). Larvae hatching within 24 h were placed into 250-ml beakers (five larvae/200 ml water) with 20.0 mg bovine liver powder. Pupae were isolated and newly emerged adults were handled within 18 h of eclosion. Newly emerged males were placed in 0.3-m³ cages by population. Newly emerged females were captured in a plastic capsule, weighed to the nearest 0.1 µg, released into individual 475-ml cardboard containers with screen lids, and assigned randomly to a treatment. All adults had continuous access to ~10% sucrose solution. We manipulated initial reproductive investment by allowing females to blood feed to one of three levels: (1) low, one-third repletion; (2) medium, one-half repletion, and (3) high, near repletion.

Blood meal mass was determined as the difference between pre- and post-meal weights. Six days after eclosion, females were weighed (0.1 µg) and then provided with hosts. The host was removed when females had reached the appropriate relative meal size. Within 45 min of taking a blood meal, the female was reweighed. Within 5 h of taking a blood meal, the female was lightly anesthetized with ether and force-mated (Munstermann and Wasmuth 1985) with a male from the same population.

Each female was provided with an oviposition cup (50-ml black plastic beaker containing ~30 ml water and lined cup with a paper

towel) 2 days after taking a blood meal. Oviposition cups containing eggs were replaced daily. When the first oviposition cycle ended (no oviposition for 9 consecutive days), mosquitoes were offered a second host. Procedures for the first and second gonotrophic cycles were the same except that during the second cycle, females were allowed to blood feed to repletion and were not remated. Because only 18 females survived to oviposit their second clutch, reproductive output in the second gonotrophic cycle was not analyzed.

The prediction that increased early reproductive investment negatively affects longevity was tested by ANOVA with log of days from eclosion to death as the dependent variable, and population and treatment as main effects. We chose not to analyze survival time as a function of absolute meal size because blood meal mass was manipulated relative to body size; consequently, the absolute mass of a 'low' blood meal for a large mosquito may have exceeded that of a 'medium' blood meal for a small mosquito. We used ANCOVA with log body mass of females as the covariate to test whether size, or its interactions, affected longevity. We found no significant effects of female size (or its interactions), hence only the results of ANOVA for treatment and population effects are reported.

Experiment 2: geographic variation in reproductive tactics

We measured the reproductive allocation of F_1 females to test for consistent differences among populations in reproductive tactics. Eggs from each colony were hatched synchronously (Novak and Shroyer 1978) and larvae were placed in 250-ml beakers (five larvae/200 ml water). To produce a range of adult sizes, one of five food levels (5.0, 10.0, 20.0, 40.0, or 80.0 mg bovine liver powder) was added to each beaker. Pupae and newly emerged adults were handled as in experiment 1, except that females were not assigned to treatments.

Beginning 5 days after eclosion, each female was weighed (0.1 μg) and offered a host every other day until a blood meal was taken. All females were allowed to blood feed to repletion and then force mated (Munstermann and Wasmuth 1985). Determination of female fresh mass and blood meal mass, and forced matings were all done as in experiment 1. Two days after taking a blood meal, an oviposition cup was placed in the container with each female. Oviposition cups were checked and eggs handled as in experiment 1. At death, females were preserved in 70% ethanol. Female reproductive tracts were dissected, and eggs (stage III or later; Bertram 1962) remaining in the body were counted.

Total reproductive output was calculated as the product of a female's mean egg mass and fecundity. For each female, we calculated mean dry egg mass (eggs dried for 48 h at 60°C and weighed individually to 0.1 μg) from 12 eggs which were sampled randomly throughout the oviposition period. To eliminate potentially misleading effects of adult longevity under laboratory conditions, individual fecundities were calculated as eggs oviposited + eggs retained at death. Our statistical conclusions were the same whether we analyzed eggs laid, eggs laid plus mature eggs (stage V) in the ovaries, or all eggs at stage III or greater.

The prediction that mosquitoes from populations sympatric with and allopatric to *T. rutilus* differ in reproductive investment was tested by analysis of covariance (ANCOVA). For the first measure of reproductive investment, log blood meal mass, we tested for effects of region (allopatry/sympatry re. the predator) and population within region as main effects with log of female fresh mass at eclosion as the covariate. For the second measure of reproductive investment, log reproductive output, we tested for effects of region and population within region as main effects with log of female fresh mass at eclosion and log blood meal mass as covariates. The prediction that mosquitoes from populations sympatric and allopatric to *T. rutilus* differ in their reproductive tactics (i.e., how reproductive investment is divided among propagules) was also tested by ANCOVA, testing for effects of region and population within region on log of fecundity and log of mean egg mass, again with both log female fresh mass and log blood meal

mass as covariates. For all ANCOVAs, preliminary tests indicated no significant difference among slopes for the treatment groups in relation to the covariates and residual analysis indicated linearity for all log-log regressions. All tests for differences between regions of allopatry to and sympatry with the predator were made using population within region as the error term. Although populations were not truly chosen at random, we wish to draw inferences about all populations from these two regions. We therefore assume that our populations within regions are representative samples of all populations in that region and therefore conduct these tests as though populations within regions were randomly chosen. When the region effect was not significant it was dropped and variation among all populations was analyzed. Variance components were estimated to determine the relative proportions of random variation associated with populations and individuals within populations. Multiple comparisons were used to determine specific differences among populations for all reproductive parameters.

The possible presence of clinal variation in reproductive tactics was tested by regressing the adjusted population mean for each variable against latitude, longitude, and altitude of the collection sites. In our view, only discrete differences in reproductive tactics between regions of allopatry and sympatry would support our hypotheses; clines in reproductive tactics would suggest that other factors affect the evolution of reproductive tactics. Hence, testing for two different patterns in reproductive tactics among populations allows us to disentangle the effects of predation by *T. rutilus* from other geographic or environmental parameters.

Path analysis (Li 1975; Mitchell 1993) was used to determine the direction and strength of direct and indirect effects of female fresh mass on blood meal mass, and their effects on egg number and mean egg mass. Path analysis allows the partitioning of correlations or covariances among variables into direct, indirect, spurious, and unanalyzed components (see Li 1975; Mitchell 1993; and Results section for definitions and explanations of these components), and is increasingly applied to the study of life histories (e.g., King 1994; Sinervo and DeNardo 1996). To remove population effects, path analyses were performed on residuals from one-way ANOVAs with population as the independent variable. Path coefficients were obtained by multiple regression of each endogenous variable on all exogenous variables hypothesized to have direct effects (Li 1975). Alternative path diagrams were tested by comparing coefficients of non-determination from reduced models to those of the full model. This method is preferred over significance testing of individual path coefficients because it considers the indirect effects of path deletions (Pedhazur 1982; Kingsolver and Schemske 1991; Mitchell 1993). Total-effect coefficients (the sum of the direct and indirect effects of one variable on another) were then calculated for each of the endogenous variables in the path diagram.

Results

Experiment 1: proximate costs of reproductive investment

There was no significant difference between populations in manipulated blood meal mass ($F_{1,34} = 0.19$, $P = 0.6697$); however, there were differences among treatments ($F_{2,34} = 11.60$, $P = 0.0002$; means \pm SE: low = 1.41 ± 0.33 mg; medium = 2.14 ± 0.30 mg; high = 3.56 ± 0.30 mg). Together, these results show that the treatment successfully established the same range of reproductive investment between populations. All females that took a blood meal oviposited, indicating that even our smallest blood meals were sufficient for oogenesis.

Although there was no significant effect of population ($F_{1,29} = 2.76$, $P = 0.1074$) or treatment ($F_{2,29} = 0.54$, $P = 0.5884$) on longevity, there was a significant pop-

ulation by treatment interaction ($F_{2,29} = 3.90$, $P = 0.0317$), indicating that the effect of initial investment in reproduction on longevity depends on the geographic origin of the mosquito (Fig. 1).

Experiment 2: geographic variation in reproductive tactics

Reproductive investment

Replete blood meal mass did not differ between regions of allopatry to and sympatry with *T. rutilus*; however, there was significant variation in blood meal mass among populations (Table 1). Female fresh mass positively affected blood meal mass (Table 1). These results indicate that although blood meal mass increases with female fresh mass consistently across all populations,

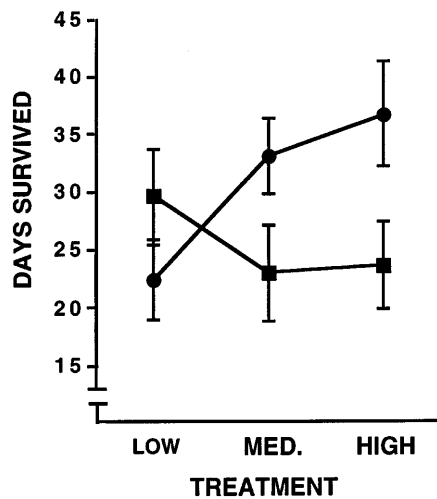


Fig. 1 Treatment means (\pm SE) for longevity for each population in experiment 1 (circles Ashland, Va., squares Vero Beach, Fla.)

Table 1 ANCOVA on reproductive investment (blood meal mass and total reproductive output) in experiment 2. Tests for differences between regions (*Region*) are made using mean square for population within region [*Population(Region)*] as the denominator. The *Region* and *Population(Region)* effect partition the *Population* effect into components due to allopatry to vs. sympatry with *Toxorhynchites rutilus*, and a component of variation within regions, respectively

Effect	Blood meal mass			Reproductive output		
	df	F	P	df	F	P
Population	7	12.70	0.0001	7	5.61	0.0001
Region	1	2.84	0.1428	1	0.66	0.4485
Population(Region)	6	9.08	0.0001	6	6.21	0.0001
Female fresh mass	1	162.19	0.0001	1	30.71	0.0001
Blood meal mass				1	14.33	0.0002
Error	239			112		
		$r^2 = 0.520$			$r^2 = 0.662$	

females of a given size from distinct populations differ in their replete blood meal size (Fig. 2A). The proportion of total random variation due to population (27.9%) was only about one-third of that due to individuals within populations (72.3%).

Our other measure of reproductive investment, total reproductive output (the product of mean egg mass and fecundity) did not differ between regions of allopatry to and sympatry with the predator, but did vary significantly among populations (Table 1, Fig. 2B). Female fresh mass and blood meal mass both positively affected reproductive output (Table 1). These results indicate that for a given blood meal mass, larger females have greater reproductive output than smaller females and that for a constant female size, females taking larger blood meals have greater reproductive output than females taking smaller blood meals. Reproductive output for a given blood meal size and given female size is also

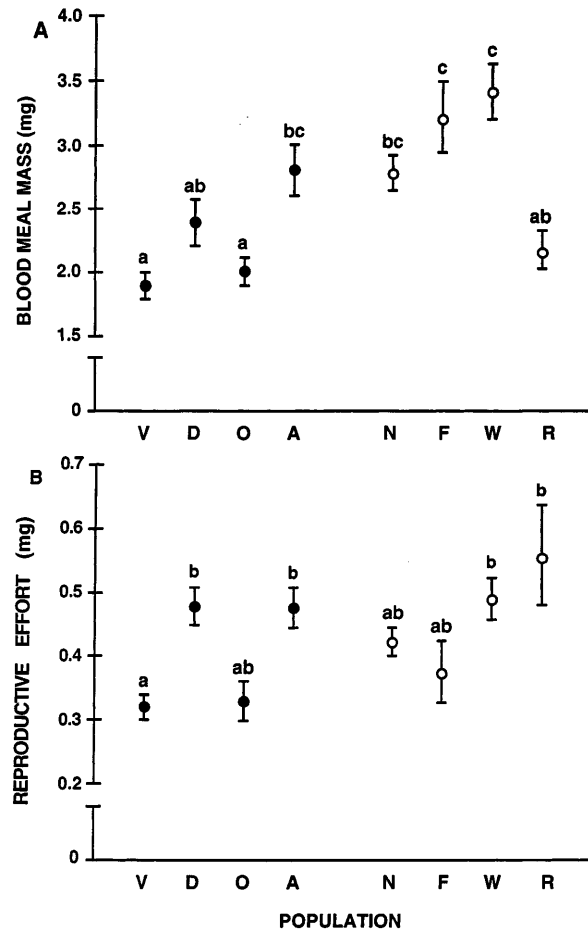


Fig. 2 Back-transformed adjusted population means (\pm SE) for replete blood meal mass (A) and reproductive output (B) in experiment 2. Means have been adjusted for the covariates used in each analysis (see Materials and methods). Open and closed circles indicate populations allopatric to and sympatric with *Toxorhynchites rutilus*, respectively. Population abbreviations are given in Materials and methods. Means associated with the same letter are not significantly different (Bonferroni experimentwise $\alpha = 0.05$)

dependent on the female's geographic origin. The proportion of total random variation due to population is small (15.8%) compared to that due to individuals within populations (84.2%).

Egg number, egg size, and clines

Fecundity did not differ significantly between regions of allopatry to and sympatry with the predator; however, there was significant variation among populations (Table 2). After Bonferroni correction (Neter and Wasserman 1974) for the number of comparisons ($\alpha_e = 0.05$), no statistically significant pairwise differences among population-adjusted means could be identified (Fig. 3A). Female fresh mass and blood meal mass both affected fecundity positively (Table 2). These results indicate that for a given volume of blood, larger females produced more eggs than smaller females, and for a given female size, mosquitoes taking larger blood meals produced more eggs than females taking smaller blood meals. The proportion of total random variation due to population was small (5.9%) compared to that due to individuals within populations (94.1%).

Mean egg mass did not differ between regions of allopatry to and sympatry with the predator; however, there was significant variation among populations (Table 2, Fig. 2B). Female fresh mass did not significantly affect mean egg mass (Table 2). The proportion of total random variation accounted for by population was small (11.4%) compared to that accounted for by individuals within populations (33.2%) or eggs within individuals (55.4%).

Only adjusted mean egg mass varied in a clinal fashion, apparently increasing with latitude ($F_{1,7} = 23.47, P = 0.0029$; Fig. 4). However, this relationship was not significant when mosquitoes from Vero Beach were excluded from the analysis ($F_{1,6} = 2.90, P = 0.1491$).

Table 2 ANCOVA on number of eggs produced (fecundity) and mean egg mass in experiment 2. Tests for differences between regions (*Region*) are made using mean square for population within region [*Population (Region)*] as the denominator. The Region and Population (Region) effect partition the *Population* effect into components due to allopatry to vs. sympatry with *T. rutilus*, and a component of variation within regions, respectively

Effect	Number of eggs			Mean egg mass		
	df	F	P	df	F	P
Population	7	2.27	0.0311	7	7.92	0.0001
Region	1	0.24	0.6434	1	1.44	0.2747
Population(Region)	6	2.32	0.0356	6	6.51	0.0001
Female fresh mass	1	49.29	0.0001	1	3.12	0.0795
Blood meal mass	1	45.63	0.0001			
Error	166			112		
		$r^2 = 0.636$			$r^2 = 0.308$	

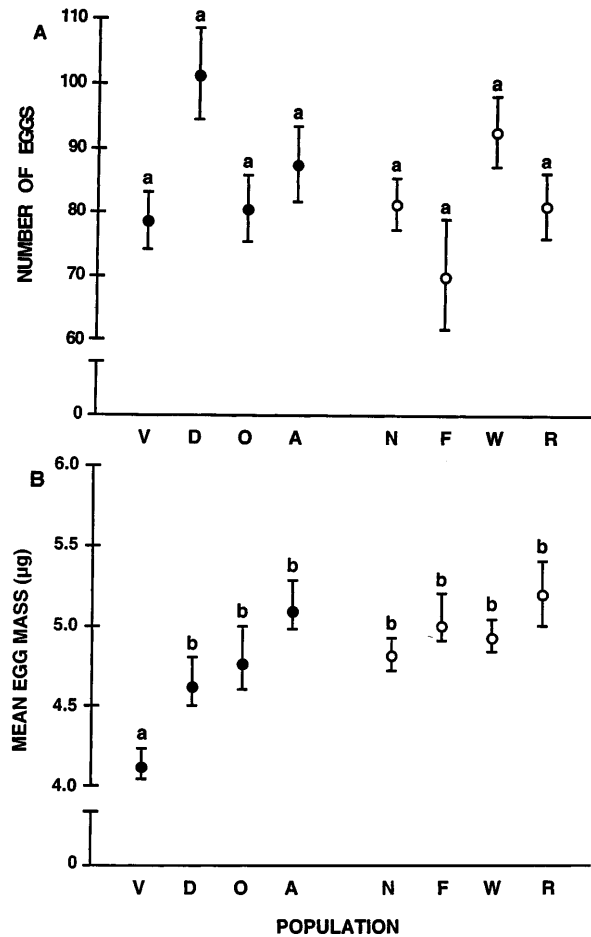


Fig. 3 Back-transformed adjusted population means (\pm SE) for number of eggs produced (A) and mean egg mass (B) in experiment 2. Means have been adjusted for the covariates used in each analysis (see Materials and methods). *Open and closed circles* indicate populations allopatric and sympatric to *T. rutilus*, respectively. *Population abbreviations* are given in Materials and methods. Means associated with the same letter are not significantly different (Bonferroni experimentwise $\alpha = 0.05$)

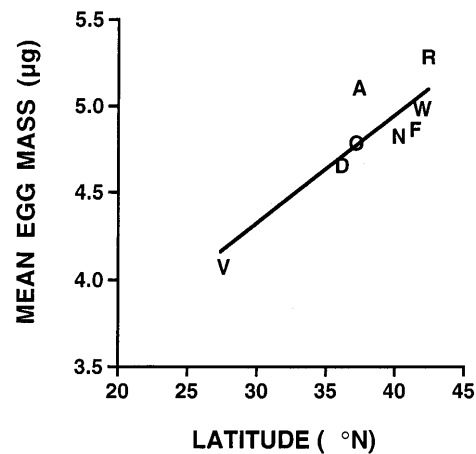


Fig. 4 Relationship between adjusted population means for mean egg mass and latitude of population origin. *Population abbreviations* are given in Materials and methods

Trade-off between offspring size and number

Only 122 mosquitoes could be used in the path analysis due to missing values. The path diagram best accounting for the variance among the variables was the full model (Fig. 5). All other reduced path diagrams (alternative hypotheses of causal connections among variables) yielded significant lack of fit. The full path model accounted for 43% of the variation in residual blood meal mass, 4% of the variation in residual egg mass, and 40% of the variation in residual egg number (i.e., residuals after the effects of population are removed). In Fig. 5, arrows indicate postulated causation, and path coefficients (direct-effect coefficients) show the direction and magnitude (in standard deviation units) of the direct effect of one variable on another.

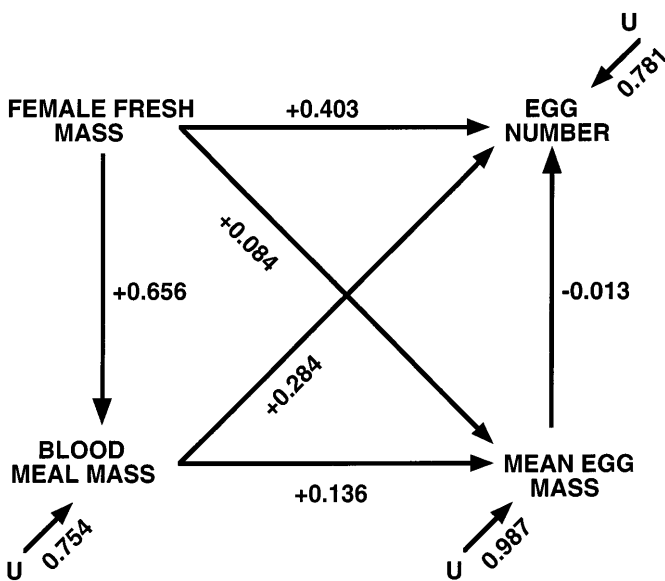


Fig. 5 Path diagram of hypothesized causal relationships among female fresh mass, blood meal mass, mean egg mass, and egg number for *Aedes triseriatus*. Coefficients of non-determination (residual variation from partial regressions) are designated as unexplained (U). See text for interpretation

Indirect effects of one variable on another (Table 3) can be calculated by multiplying the coefficients of paths connecting one variable through intervening variables. The total effect of one variable on another (Table 3, total-effect coefficient) is the sum of its direct and indirect effects. Spurious effects (Table 3) are produced by the relationship of two variables to other causal variables. Raw correlations (Table 3) between variables can be reconstructed by summing the products of all connecting paths between the two variables (equivalent to summing direct-, indirect-, and spurious-effect coefficients).

Path coefficients in Fig. 5 reveal the large, positive effects of female size on egg number both directly and indirectly through blood meal mass. The indirect effect of female size on egg size (through blood meal mass) is as large as the direct effect (Table 3). Blood meal mass also positively affects both egg number and egg mass, independent of the effect of female fresh mass (Fig. 5, Table 3). Egg mass has a negative direct effect on the number of eggs produced, indicating a trade-off between offspring size and number. Although this direct effect has a small coefficient (Fig. 5), any model omitting this path showed a significant reduction in goodness of fit. Comparison of the raw correlations with effect coefficients (Table 3) illustrates the advantage of path analysis. Both analyses yield similar relationships among female fresh mass, blood meal mass, and mean egg mass; however, path analysis revealed that nearly one-half of the observed raw correlation between blood meal mass and egg number is a spurious effect due to a common relationship to female fresh mass (Table 3). More dramatically, the *direction* of the relationship between egg mass and egg number differs between the two analyses (compare direct effect and correlation coefficients, Table 3). Simple correlations failed to detect the trade-off between offspring size and number because both variables share strong positive correlations with common causes (female fresh mass and blood meal mass). Strong positive effects of female size and blood meal mass produce a large spurious effect (Table 3) that masks the relatively small, but significant, negative relationship between offspring size and number.

Table 3 Coefficients from path analysis of the variables in Fig. 5. Total-effect coefficient = direct-effect coefficient + indirect-effect coefficient. Pearson correlation coefficient = total-effect coefficient + spurious-effect coefficient (– indicates effect coefficient that is undefined in the path model given in Fig. 5)

Causal variable	Direct-effect coefficient	Indirect-effect coefficient	Total-effect coefficient	Spurious-effect coefficient	Pearson correlation coefficient
Effects on blood meal mass					
Female fresh mass	+0.656	–	+0.656	–	+0.656
Effects on mean egg mass					
Female fresh mass	+0.084	+0.089	+0.174	–	+0.174
Blood meal mass	+0.136	–	+0.136	+0.055	+0.191
Effects on egg number					
Female fresh mass	+0.403	+0.184	+0.587	–	+0.587
Blood meal mass	+0.284	–0.002	+0.282	+0.264	+0.546
Mean egg mass	–0.013	–	–0.013	+0.124	+0.111

Discussion

Even though we have detected no consistent differences in life history traits of *A. triseriatus* between the regions of allopatry to and sympatry with *T. rutilus*, there are interesting relationships among populations in the reproductive parameters measured. Because all mosquitoes were raised under the same laboratory conditions, the observed differences in life history traits probably reflect genetic differences among populations in allocation of reproductive investment, and in the consequences of that allocation. For example, experiment 1 detected a cost of reproduction, specifically reduced longevity, with increased early reproductive investment for mosquitoes from Vero Beach, Fla., but not for those from Ashland, Va. This difference in the relationship of longevity and investment is interesting because these populations differ in natural levels of reproductive investment (blood meal mass or total reproductive output, Fig. 1). If mosquitoes from Vero Beach are more sensitive to the physiological stress of reproduction, then they may reduce this cost by lowering reproductive investment.

Furthermore, the second experiment revealed that, within the region of sympatry with the predator, mosquitoes from Vero Beach have the smallest investment in reproduction whereas those from Ashland have the greatest. Blood meal mass showed the greatest variation among populations, and mean egg mass the least (Figs. 1, 2). Mosquitoes from Fermilab were the least efficient in egg production, taking large blood meals, producing the fewest eggs, and having relatively low total reproductive outputs. In contrast, mosquitoes from Durham were the most efficient by the same measures. Relative to mosquitoes from other populations, those from Vero Beach took small blood meals, produced small eggs, and had low reproductive outputs. However, none of the predicted differences between regions of allopatry to and sympatry with the predator are present, and we therefore conclude that mortality due to predation by *T. rutilus* has not been the primary selective factor influencing these life history traits of *A. triseriatus*. Only mean egg mass exhibited a well-defined geographic pattern (Fig. 4), and this apparent cline is heavily influenced by the population from Vero Beach, Fla., which may be genetically or phylogenetically distinct from other *A. triseriatus* (Zavortink 1972; Munstermann 1985).

While our phenotypic manipulation cannot reveal genetically based trade-offs or costs associated with reproduction (Reznick 1992a), the differences in expression of physiologically based costs in our common garden experiment suggest that there may be genetic differences influencing how such costs are expressed. That we detected significant differentiation among populations in life history traits supports the notion that these reproductive parameters can diverge evolutionarily.

The refuted prediction of lower reproductive investment under conditions of relatively greater mortality of juveniles (i.e., in sympatry) depends on an assumed

trade-off between reproduction and parental growth or maintenance (Law 1979; Michod 1979). Because adult size is fixed at metamorphosis and host blood protein is used only for egg production (Briegel 1985), the assumed trade-off between reproduction and maintenance may be reduced in anautogenous mosquitoes, although there is likely to be some trade-off between maternal energy reserves allocated to protein metabolism for reproduction (Briegel 1990) versus that allocated toward somatic maintenance. The reduced trade-off may nonetheless enhance the relative importance of other selective factors as determinants of reproductive investment. Relatively low variation in reproductive output among these populations is interesting because females' investment in reproduction and allocation of reproductive resources among offspring varied among populations, suggesting that reproductive investment, reproductive output, and the trade-off between offspring size and number may evolve independently. This is best illustrated by comparing the similar reproductive outputs of mosquitoes from Fermilab and Durham. Females from Fermilab took larger blood meals and produced fewer, larger eggs, whereas those from Durham took smaller blood meals and produced more numerous, smaller eggs.

The absence of significant differences between regions may be related to high intrapopulation variation in aspects of the larval environment. The life historical shifts predicted under different levels of juvenile mortality assume constant levels of predator-induced mortality within a population. Although *A. triseriatus* commonly co-occurs with *T. rutilus* in the south, contact may not be predictable at a small spatial scale (Juliano 1989). *T. rutilus* immatures are more frequent in larger, more permanent water-filled containers, whereas *A. triseriatus* immatures exploit a range of container sizes that vary in their degree of permanence (Bradshaw and Holzapfel 1983, 1985, 1988). In addition, Lounibos et al. (1997) showed that in Florida, the presence and absence of *T. rutilus* can vary in time, and it can become locally extinct for relatively long periods. This temporal variation also seems likely to occur at other sympatric sites. Such variable predation may result in spatially or temporally variable selection on *A. triseriatus* by *T. rutilus*. Also, treeholes themselves vary in their effects on larval success (Léonard and Juliano 1995), due to variation in volume, per capita food availability, water chemistry, and density of predators and competitors (Bradshaw and Holzapfel 1983, 1988; Kitching 1987). All these factors combine to make the juvenile environment highly variable on a local scale. Such ecological variability may maintain high intrapopulation variation in life history traits and retard consistent differentiation between sympatric and allopatric regions (Roff 1992).

Lack of sympatric-allopatric differentiation may also result if different selective agents favor similar phenotypes. For example, consider selection acting on egg size. In the north, larvae are likely to experience a short developmental season (Juliano 1989) and develop under conditions of density dependence (Bradshaw and

Holzapel 1983, 1985), both of which theoretically favor greater propagule size (Parker and Begon 1986; Hard and Bradshaw 1993). Density dependence occurs in the south, but when developing in a treehole with *T. rutilus*, *A. triseriatus* larvae may be freed from density dependence via mortality due to predation (Chambers 1985; Lounibos et al. 1993), which is also predicted to select for greater propagule size (Michod 1979; Morris 1987). Thus, similar optimal offspring sizes may result from two different sources of selection.

Although there are numerous differences among populations, the geographic pattern in reproductive traits is not clearly related to presence/absence of *T. rutilus*. This same pattern of population differentiation without a consistent sympatric-allopatric dichotomy is present for *A. triseriatus* behavior (Juliano et al. 1993), size at and time to pupation (Hechtel and Juliano 1997), and vulnerability to predation (Juliano 1996). It appears that we must look to other ecological or genetic factors, particularly those specific to particular populations, to understand geographic variation in reproductive tactics in *A. triseriatus*. In other studies of life history variation in container mosquitoes, similar results showing variation among populations but no consistent differences between regions have been obtained (Bradshaw and Holzapel 1989). The similarity of these results for two container-dwelling species suggests that conditions in container habitats vary considerably among populations, and that there is little consistent difference between major geographic regions (Bradshaw and Holzapel 1989). Local differences in costs of reproduction, suggested by our first experiment, local patterns of predation and density dependence, and local variation in physical factors may all contribute to interpopulation differentiation in reproductive tactics.

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References

- Abrams PA, Rowe L (1996) The effects of predation on the age and size of maturity of prey. *Evolution* 50:1052–1061
- Bell G (1980) The costs of reproduction and their consequences. *Am Nat* 116:45–76
- Bertram TS (1962) Annex on the ovary and ovarioles of mosquitoes. In: Detinova TS (ed) *Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria* World Health Organization, Geneva
- Bradshaw WE, Holzapel CM (1983) Predator-mediated non-equilibrium coexistence of tree-hole mosquitoes in southeastern North America. *Oecologia* 57:239–256
- Bradshaw WE, Holzapel CM (1985) The distribution and abundance of treehole mosquitoes in eastern North America: perspectives from Florida. In: Lounibos LP, Rey JR, Frank JH (eds) *Ecology of mosquitoes: proceedings of a workshop*. Florida Medical Entomological Laboratory, Vero Beach, pp 3–24
- Bradshaw WE, Holzapel CM (1988) Drought and the organization of tree-hole communities. *Oecologia* 74:507–514
- Bradshaw WE, Holzapel CM (1989) Life-historical consequences of density-dependent selection in the pitcher-pant mosquito *Wyeomyia smithii*. *Am Nat* 133:869–887
- Briegel H (1985) Mosquito reproduction: incomplete utilization of the blood meal protein for oogenesis. *J Insect Physiol* 31: 15–21
- Briegel H (1990) Metabolic relationship between female body size, reserves, and fecundity in *Aedes aegypti*. *J Insect Physiol* 36:165–172
- Chambers RC (1985) Competition and predation among larvae of three species of treehole breeding mosquitoes. In: Lounibos LP, Rey JR, Frank JH (eds) *Ecology of mosquitoes: proceedings of a workshop*. Florida Medical Entomological Laboratory, Vero Beach, pp 25–54
- Clements AN (1992) *The biology of mosquitoes, vol 1. Development, nutrition, reproduction*. Chapman & Hall, London
- Cochrane A (1972) Body weight and blood meal weight as factors affecting egg production of the tree-hole mosquito, *Aedes triseriatus* (Say). *Proc NJ Mosquito Extermination Assoc* 59:65–78
- Darsie RF, Ward RA (1981) Identification and geographical distribution of the mosquitoes of North America, north of Mexico. *Mosquito Syst Suppl* 1:1–313
- Gadgil M, Bossert WH (1970) Life historical consequences of natural selection. *Am Nat* 104:1–24
- Hard JJ, Bradshaw WE (1993) Reproductive allocation in the western tree-hole mosquito, *Aedes sierrensis*. *Oikos* 66:55–65
- Hechtel LJ, Juliano SA (1997) Effects of a predator on prey metamorphosis: plastic responses by prey or selective mortality? *Ecology* 78:838–851
- Jalil M (1974) Observations on the fecundity of *Aedes triseriatus* (Diptera: Culicidae). *Entomol Exp Appl* 17:223–233
- Juliano SA (1989) Geographic variation in vulnerability to predation and starvation in larval treehole mosquitoes. *Oikos* 56:99–108
- Juliano SA (1996) Geographic variation in *Aedes triseriatus* (Diptera: Culicidae): temperature-dependent effects of a predator on survival of larvae. *Environ Entomol* 25:624–631
- Juliano SA, Reminger L (1992) The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences. *Oikos* 63:465–476
- Juliano SA, Hechtel LJ, Waters JR (1993) Behavior and risk of predation in larval treehole mosquitoes: effects of hunger and population history of predation. *Oikos* 28:229–241
- King RB (1994) Determinants of offspring number and size in the brown snake, *Storeria dekai*. *J Herpetol* 27:175–185
- Kingsolver JG, Schemske DW (1991) Path analysis of selection. *Trends Ecol Evol* 6:276–280
- Kitching RL (1987) Spatial and temporal variation in food webs in water-filled treeholes. *Oikos* 48:280–288
- Law R (1979) Optimal life histories under age-specific predation. *Am Nat* 114:399–417
- Léonard PM, Juliano SA (1995) Effect of leaf litter and density on fitness of *Aedes triseriatus*. *Ecol Entomol* 20:125–136
- Li CC (1975) *Path analysis – a primer*. Boxwood, Pacific Grove

- Livdahl TP (1979) Evolution of handling time: the functional response of a predator to the density of sympatric and allopatric strains of prey. *Evolution* 33:765–768
- Lounibos LP, Nishimura N, Escher RL (1993) Fitness of a treehole mosquito: influences of food type and predation. *Oikos* 66:114–118
- Lounibos LP, Escher RL, Nishimura N, Juliano SA (1997) Long-term dynamics of a predator used for biological control and decoupling from mosquito prey in a subtropical treehole ecosystem. *Oecologia* 111:189–200
- Mather TN, DeFoliart GR (1983) Effect of host blood on the gonotrophic cycle of *Aedes triseriatus*. *Am J Trop Med Hygiene* 32:189–193
- Maynard Smith J (1991) The evolution of reproductive strategies: a commentary. In: Harvey PH, Partridge L, Southwood TRE (eds) *The evolution of reproductive strategies*. Royal Society, London, pp 103–104
- McGinley MA, Temme DH, Geber MA (1987) Parental investment in offspring in variable environments: theoretical and empirical considerations. *Am Nat* 130:370–398
- Michod RE (1979) Evolution of life histories in response to age-specific mortality factors. *Am Nat* 113:531–550
- Miller BR, Beaty BJ, Lorenz LH (1982) La Crosse virus filial infection rates in geographic strains of *Aedes triseriatus* (Diptera: Culicidae). *J Med Entomol* 19:213–214
- Mitchell RJ (1993) Path analysis: pollination. In: Scheiner SM, Gurevitch J (eds) *Design and analysis of ecological experiments*. Chapman & Hall, New York, pp 211–232
- Morris DW (1987) Optimal allocation of parental investment. *Oikos* 49:332–339
- Munstermann LE (1985) Geographic patterns in genetic differentiation in the treehole mosquito *Aedes triseriatus*. In: Lounibos LP, Rey JR, Frank JH (eds) *Ecology of mosquitoes: proceedings of a workshop*. Florida Medical Entomological Laboratory, Vero Beach, pp 327–343
- Munstermann LE, Wasmuth LM (1985) *Aedes triseriatus*. In: Singh P, Moore RF (eds) *Handbook of insect rearing*, vol 2. Elsevier, Amsterdam, pp 15–24
- Nannini MA, Juliano SA (1998) Effects of the facultative predator *Anopheles barberi* on population performance of its prey *Aedes triseriatus* (Diptera: Culicidae). *Ann Entomol Soc Am* 91:33–42
- Neter J, Wasserman W (1974) *Applied linear statistical models, regression, analysis of variance, and experimental designs*. Irwin, Homewood
- Nijhout HF, Emlen DJ (1998) Competition among body parts in the development and evolution of insect morphology. *Proc Natl Acad Sci USA* 95:3685–3689
- Novak RJ, Shroyer DA (1978) Eggs of *Aedes triseriatus* and *A. hendersoni*: a method to stimulate optimal hatch. *Mosquito News* 38:515–521
- Parker GA, Begon M (1986) Optimal egg size and clutch size: effects of environment and maternal phenotype. *Am Nat* 128:573–592
- Partridge L (1992) Measuring reproductive costs. *Trends Ecol Evol* 7:99–100
- Partridge L, Harvey PH (1988) The ecological context of life history evolution. *Science* 241:1449–1455
- Partridge L, Sibly R (1991) Constraints in the evolution of life histories. In: Harvey PH, Partridge L, Southwood TRE (eds) *The evolution of reproductive strategies*. Royal Society, London, pp 3–13
- Pedhazur EJ (1982) *multiple regression in behavioral research explanation and prediction*, 2nd edn. Holt Rinehart Winston, New York, pp 577–682
- Reznick D (1992a) Measuring the costs of reproduction. *Trends Ecol Evol* 7:42–45
- Reznick D (1992b) Measuring reproductive costs: response to Partridge. *Trends Ecol Evol* 7:134
- Roff DA (1992) *The evolution of life histories: theory and analysis*. Routledge, Chapman & Hall, New York
- Sims S (1985) Embryonic and larval diapause in *Aedes triseriatus*: phenotypic correlation and ecological consequences of the induction response. In: Lounibos LP, Rey JR, Frank JH (eds) *Ecology of mosquitoes: proceedings of a workshop*. Florida Medical Entomological Laboratory, Vero Beach, pp 359–369
- Sinervo B, DeNardo DF (1996) Costs of reproduction in the wild: path analysis of natural selection and experimental tests of causation. *Evolution* 50:1299–1313
- Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. *Am Nat* 108:499–506
- Stearns SC (1992) *The evolution of life histories*. Oxford University Press, New York
- Steffan WA, Evenhuis NL (1981) Biology of *Toxorhynchites*. *Annu Rev Ecol Syst* 26:159–181
- Walker ED, Copeland RS, Paulson SL, Munstermann LE (1987) Adult survivorship, population density, and body size in sympatric populations of *Aedes triseriatus* and *Aedes hendersoni* (Diptera: Culicidae). *J Med Entomol* 24:485–493
- Winkler DW, Wallin K (1987) Offspring size and number: a life history model linking effort per offspring and total effort. *Am Nat* 129:708–720
- Zavortink TJ (1972) Mosquito studies (Diptera: Culicidae). XVII. The New World species formerly placed in *Aedes* (Finlaya). *Contrib Am Entomol Inst* 8:1–205