

# Effects of Density and Larval Competition on Selected Life History Traits of *Culex pipiens quinquefasciatus* (Diptera: Culicidae)

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**ABSTRACT** The effects of larval densities of one to four individuals in standard *Drosophila*-vials (diameter 25 by 95 mm) on the age at pupation, starved dry weight, and wing length of *Culex pipiens quinquefasciatus* Say were studied. This approach required relatively few larvae per replicate and included a control treatment, where individual larvae developed in the absence of competition. This design also tested for competitive interactions between male and female larvae. Mosquitoes pupated later, and emerged with lighter starved dry adult weight and shorter wings as larval density increased. The size of adult female mosquitoes, particularly their starved dry weight, was sensitive to larval density and also was influenced by the presence or absence of competition with another female larva. In contrast, the life history traits of males did not vary as a function of competition with female larva. Female larvae were also more likely to die in the highest density treatment. This design confirmed previous results and offered a potentially useful experimental approach to investigate the effects of density-dependent competition among mosquito larvae.

**KEY WORDS** *Culex pipiens quinquefasciatus*, density, life history traits, body size

DENSITY-DEPENDENT COMPETITION AMONG larvae is an important factor regulating the growth of mosquito populations. For example, an increase from 300 to 2,400 *Culex tarsalis* Coquillett larvae competing for the same ration of food resulted in delayed median ages at pupation (from day 13 to 17), reduced larval-to-adult survival (47–3%), and a sex ratio at emergence biased toward males (51–68%) (Reisen et al. 1984). These parameters greatly reduced estimates of the net reproductive rate ( $R_0$ ) and intrinsic rate of increase ( $r_m$ ). A similar pattern has been reported for several other species of mosquitoes when competitive interactions among larvae are increased (e.g., Siddiqui et al. 1976, Gilpin and McClelland 1979, Lyimo et al. 1992).

The current study investigated the role of density-dependent competition among the larvae of *Culex pipiens quinquefasciatus* Say. In contrast to previous studies where density treatments involved up to several hundred larvae, densities in our experiment were restricted to one to four larvae per replicate. This approach offered three main advantages: (1) There was a control treatment where individual larvae developed in the absence of competition with other larvae. (2) The very nature of density-dependent interactions among larvae means that individuals within a particular replicate cannot be regarded as statistically independent and mean values per replicate should be used (Hurlbert 1984). The low number of

individuals per replicate in our design provided an efficient manner to increase the number of replicates per experiment. (3) Our design allowed competitive interactions within and between males and females to be compared. Previous studies indicated that females were more susceptible to the effects of density-dependent competition than males (e.g., Reisen et al. 1984, Hard et al. 1989). However, if a treatment contained several individuals of both sexes, it was difficult to determine if the observed effects were caused by competitive interactions within or between the sexes. We compared replicates where a single female larva only experienced male competitors against those where two females were present, and replicates where males experienced no female competition against those where a female larva was present.

## Materials and Methods

**Mosquito Strain and Experimental Protocol.** We used the S-lab strain of *Cx. pipiens quinquefasciatus* maintained at the University of Montpellier II, France. This strain has been maintained in continuous laboratory culture since being collected in the San Joaquin Valley of California in 1950 (Georghiou et al. 1966) and has been used extensively as a control strain in studies on the evolution of insecticide resistance (Raymond et al. 1986).

Within 6–12 h of egg hatch, one, two, three, or four larvae were transferred to standard *Drosophila* vials (diameter 25 by 95 mm) containing 5 ml of deionized water. Twenty replicate vials were used at each den-

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sity; therefore, the experiment included 80 vials containing 200 larvae. Vials were maintained in a room at 28°C, >70% RH, and local photoperiod of ≈16:8 [L:D] h. The amount of finely ground fish food (Tetra MicroFood, Melle, Germany) added to each vial was as follows: day 0, 0.06; day 1, 0.08; day 2, 0.16; day 3, 0.32; day 4, 0.64, and day 5 onward, 1.28 mg.

Vials were examined every 24 h for pupation. Day of pupation was recorded. The pupa was transferred to a separate vial containing 5 ml of deionized water, and the vial covered with fine nylon gauze. The day of adult emergence and the mosquito's sex was recorded and water poured out of the vial to prevent drowning. No sugar water was provided to the adults, but they had access to water from a soaked ball of cotton placed on the vial's nylon gauze.

After the adults died they were transferred to numbered 1.5 ml plastic vials and placed in an 80°C oven for a minimum of 12 h. The starved dry weight of adults was recorded to an accuracy of 1 µg. One wing was removed from each adult and the distance between the alula notch and the tip of the wing (excluding fringe scales) was measured using a dissecting microscope fitted with a graduated eyepiece.

**Statistical Analyses.** A two-tailed log likelihood ratio test determined whether the proportion of replicates in which all individuals survived to adulthood varied among density treatments. The square root of these proportions was arcsine transformed before analysis. Subsequently, a two-tailed Dunnett test examined the proportion of replicates with 100% survival at densities of two, three, or four larvae per vial against that at the control density of one larva per vial (Zar 1996).

Mosquito age at pupation, wing length, and starved dry weight were only analyzed for replicates where all individuals survived to adulthood. This reduced the size of the data set, but avoided underestimating the effects of density on life history traits caused by larval mortality.

In cases where a replicate yielded both male and female data, we avoided pseudoreplication (Hurlbert 1984) by either ignoring data from one sex, or analyzing both sexes separately. Equally, if a replicate yielded data on more than one individual per sex, mean trait values were analyzed. Hence, the degrees of freedom in our analyses were based on the number of replicate vials and not the number of individuals in the data set.

The life history traits of males and females were tested to see if they were influenced by larval density and by competition between male or female larvae. There were not enough data to perform a full three-way analysis of variance (ANOVA), so each sex was analyzed separately. For females, at densities of two, three, and four larvae per vial, we compared replicates where only one female was present against those where two females were present. To maintain this balance across densities, replicates were excluded if they contained three or more females. Similarly for males, we compared replicates where no females were present against those where one female was present (i.e., replicates were excluded if they contained two or

**Table 1.** ANOVA of the effects of larval density and sex on the life history traits of adult mosquitoes

	Source	Sums of squares	df	F
Age at Pupation	Density	12.71	3	13.57***
	Sex	7.54	1	24.17***
	Interaction	0.13	3	0.14
	Error	12.17	39	
Starved Dry Weight	Density	143,449	3	13.89***
	Sex	30,263	1	8.79**
	Interaction	52,394	3	5.07**
	Error	134,297	39	
Wing Length	Density	0.62	3	16.59***
	Sex	1.29	1	103.72***
	Interaction	0.14	3	3.76*
	Error	0.48	39	

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

more females). Data from the density of one larva per vial were not included in these analyses because individuals did not compete with either the same or opposite sex larvae.

All tests were performed by the statistical package JMP, version 3.1.6 (SAS Institute 1994).

## Results and Discussion

The proportion of replicates in which all individuals survived to adulthood varied significantly among density treatments (log likelihood ratio G-test,  $\chi^2 = 19.62$ ,  $P < 0.01$ ). At density one, 16/20 replicates yielded 100% survival, whereas at densities of two, three and four larvae per vial the proportions were, 19/20, 13/20, and 7/20, respectively. Only the proportion of replicates with 100% survival in the latter treatment was significantly lower than when larvae were reared in isolation (two-tailed Dunnett test,  $q' = 3.04$ ,  $df = 4$ ,  $P < 0.01$ ).

There was a total of 21 females and 37 males (median number emerging from each vial, one and two, respectively) emerging from vials originally containing four larvae. There were two plausible explanations: (1) The mortality of male and female larvae was the same, but the original sex ratio was biased in favor of males, or (2) the original sex ratio of males and females was equal, but larval mortality was biased toward females. Unfortunately, the current data could not be used to distinguish between these possibilities. However, previous observations give us no reason to suspect the sex ratio differed from 1:1 (Agnew et al. 1999). Therefore, we attributed this sex ratio distortion to the differential mortality of female larvae.

Life history traits of both male and female mosquitoes were influenced by larval density. Each sex responded similarly to increasing larval density by pupating progressively later (Table 1; Fig. 1). In general, males emerged faster than females in matching density treatments, but the two sexes overlapped considerably across density treatments (Fig. 1).

As larval density increased, the starved dry weight of adult mosquitoes decreased (Table 1; Fig. 1). This was particularly evident for females who were considerably heavier than males when reared in isolation,

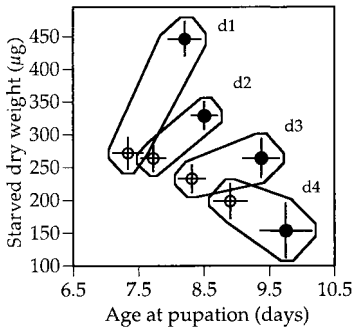


Fig. 1. Age at pupation and starved dry adult weight of mosquitoes reared at densities of 1-4 larvae per vial (d1-d4, respectively). Polygons enclose data (mean ± SE) from males (open symbols) and females (closed symbols) reared at the same density.

but whose weight converged with that of males when four larvae were reared together (Fig. 1).

An interaction between sex and density also was found for adult wing length (Table 1; Fig. 2). The wings of both sexes became shorter as density increased, but more so for the females; between being reared in isolation and at a density of four larvae per vial, there was a 17% decrease in female wing length, but only an 8% decrease in male wing length. Unlike the pattern of starved dry weight, the difference between the sexes remained pronounced with the length of the shortest female wings being longer than the longest male wings (Fig. 2).

The starved dry weight of females was more sensitive to the effects of larval density than was wing length. We assumed that starved dry weight was a fixed trait (after metabolic reserves have been used up), as was wing length. Wing length will be the more conservative of these two traits, because the fresh weight supported during flight needs to allow for a female's weight at emergence (dry weight + reserves), any additional weight gained because of nectar feeding, and a substantial increase in weight caused by blood feeding.

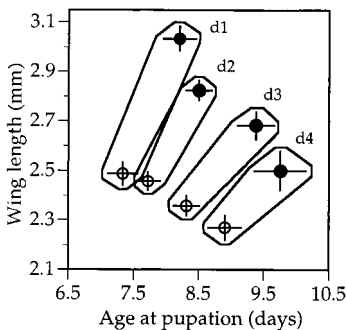


Fig. 2. Age at pupation and wing lengths of mosquitoes reared at densities of 1-4 larvae per vial (d1-d4, respectively). Polygons enclose data (means ± SE) from males (open symbols) and females (closed symbols) reared at the same density.

Table 2. ANOVA of the effects of larval density and sex of larval competitor on the life history traits of female mosquitoes

	Source	Sums of squares	df	F
Age at Pupation	Density	3.60	2	11.41***
	Sex of competitor	0.64	1	4.07 <sup>a</sup>
	Interaction	0.75	2	2.38
	Error	2.3	15	
Starved Dry Weight	Density	112,536	2	15.59**
	Sex of competitor	21,435	1	5.94*
	Interaction	6,706	2	0.93
	Error	54,126		
	Density	0.32	2	15.403***
Wing Length	Sex of competitor	0.04	1	3.41†
	Interaction	0.01	2	0.38
	Error	0.16	15	

<sup>a</sup> 0.1 > P > 0.05. \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

There seemed to be an asymmetry in the competitive interactions between male and female larvae. At densities of two, three, or four larvae per vial, the dry

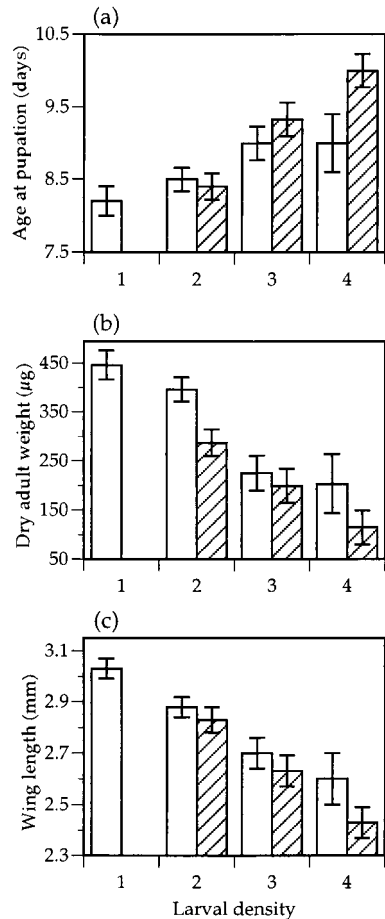


Fig. 3. Life history traits of female mosquitoes at different larval densities and from replicates where only one female was present (open columns) or two females were present (hatched columns). (a) Age at pupation. (b) Starved dry adult weight. (c) Wing length. Each column represents mean value of trait (±SE).

**Table 3.** ANOVA of the effects of larval density and sex of larval competitor on the life history traits of male mosquitoes

	Source	Sums of squares	df	F
Age at Pupation	Density	1.36	2	2.89 <sup>a</sup>
	Sex of competitor	0.30	1	1.25
	Interaction	0.48	2	1.01
	Error	3.78	16	
Starved Dry Weight	Density	18,223	2	8.46**
	Sex of competitor	6	1	0.01
	Interaction	3,667	2	1.70
	Error	17,235	16	
Wing Length	Density	0.08	2	6.74**
	Sex of competitor	0.01	1	0.02
	Interaction	0.01	2	0.65
	Error	0.09	16	

<sup>a</sup> 0.1 > P > 0.05. \*, P < 0.05; \*\*, P < 0.01.

starved weight of female mosquitoes from replicates containing a single female larva was significantly heavier than in replicates where two female larvae were present (Table 2; Fig. 3b). No such pattern was found for males reared in the presence or absence of a female competitor (Table 3). Similar, but weaker trends were detected for the two other life history traits measured; i.e., females tended to pupate later (Fig. 3a) and have shorter wings (Fig. 3c) when in competition with another female larva (Table 2). Analogous trends were not observed for males (Table 3).

The greater increase in the size of adult females as larval density decreased is to be expected; whereas both sexes live longer if larger (e.g., Reisen et al. 1984), only females increase their reproductive capacity by being larger (Benjamin and Bradshaw 1984, Clements 1992). Therefore, as larval growth conditions improve, females have a greater incentive than males to become large adults (see also, Bradshaw et al. 1997).

Within individual vials, growth conditions probably deteriorated as larvae became larger and competitive interference increased, therefore reducing the reproductive benefits gained from continued larval growth. Our results indicate that this was the case when two female larvae were in competition with each other. However, the lack of effect of female competition on male life history traits indicates that males exceeded the developmental threshold favorable for pupation before competition arising specifically from females deteriorated their growth environment. This indicated that the greater susceptibility of females to density-dependent competition was caused predominately by interactions among female larvae rather than with males.

To summarize, the conditions used to test the effects of larval density in our study produced results similar to those using much larger sample sizes. As more larvae competed for the same amount of resources, pupation was delayed and adults emerged with a lighter starved dry weight and shorter wings. The effects of density were more pronounced on the size of adult female mosquitoes, particularly their starved dry weight. Our results also indicate that competitive interactions among female larvae were more intense than among males, or between the sexes. Fi-

nally, at the highest density examined, there was proportionally more larval mortality than when larvae were reared in isolation, and this mortality was differentially biased toward females.

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### References Cited

- Agnew, P., S. Bedhomme, C. Haussy, and Y. Michalakis. 1999. Age and size at maturity of the mosquito *Culex pipiens* infected by the microsporidian parasite *Vavraia culicis*. Proc. R. Soc. Lond. B 266: 947-952.
- Benjamin, S. N., and W. E. Bradshaw. 1984. Effects of flight activity and body size on male reproductive success in the pitcher-plant mosquito, *Wyeomyia smithii* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 87: 331-336.
- Bradshaw, W. E., C. M. Holzapfel, C. A. Kleckner, and J. J. Hard. 1997. Heritability of development time and protandry in the pitcher-plant mosquito, *Wyeomyia smithii*. Ecology 78: 969-976.
- Clements, A. N. 1992. The biology of mosquitoes: development, nutrition and reproduction. Chapman & Hall, London.
- Georghiou, G. P., R. L. Metcalf, and F. E. Gidden. 1966. Carbamate-resistance in mosquitoes: selection of *Culex pipiens fatigans* Wiedemann (= *C. quinquefasciatus* Say) for resistance to Baygon. Bull. WHO 35: 691-708.
- Gilpin, M. E., and G.A.H. McClelland. 1979. Systems analysis of the yellow fever mosquito *Aedes aegypti*. Fortschr. Zool. 25: 355-388.
- Hard, J. J., W. E. Bradshaw, and D. J. Malarkey. 1989. Resource- and density-dependent development in tree-hole mosquitoes. Oikos 54: 137-144.
- Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187-211.
- Lyimo, E. O., J. Takken, and J. C. Koella. 1992. Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae*. Entomol. Exp. Appl. 63: 265-271.
- Raymond, M., D. Fournier, J. M. Bride, A. Cuany, J. Bergé, M. Magnin, and N. Pasteur. 1986. Identification of resistance mechanisms in *Culex pipiens* (Diptera: Culicidae) from southern France: insensitive acetylcholinesterase and detoxifying oxidases. J. Econ. Entomol. 79: 1452-1458.
- Reisen, W. K., M. M. Milby, and M. E. Bock. 1984. The effects of immature stress on selected events in the life history of *Culex tarsalis*. Mosq. News 44: 385-395.
- SAS Institute. 1994. User's manual, version 3.14. SAS Institute, Cary, NC.
- Siddiqui, T. F., Y. Aslam, and W. K. Reisen. 1976. The effects of larval density on selected immature and adult attributes in *Culex tritaeniorhynchus* Giles. Trop. Med. 18: 195-202.
- Zar, J. H. 1996. Biostatistical analysis, 3rd ed. Prentice-Hall, Englewood Cliffs, NJ.

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