

A minimalist approach to the effects of density-dependent competition on insect life-history traits

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Abstract. 1. Due to its effects on the phenotypic and genotypic expression of life-history traits, density-dependent competition is an important factor regulating the growth of populations. Specifically for insects, density-dependent competition among juveniles is often associated with increased juvenile mortality, delayed maturity, and reduced adult size.

2. The aim of the work reported here was to test whether the established phenotypic effects of density-dependent competition on life-history traits could be reproduced in an experimental design requiring a minimal number of individuals. Larvae of the mosquito *Aedes aegypti* were reared at densities of one, two, or three individuals per standard *Drosophila* vial and in six different conditions of larval food availability. This design required relatively few individuals per independent replicate and included a control treatment where individuals reared at a density of one larva per vial experienced no density-dependent interactions with other larvae.

3. Increased larval densities or reduced food availability led to increased larval mortality, delayed pupation, and the emergence of smaller adults that starved to death in a shorter time (indicating emergence with fewer nutritional reserves).

4. Female mosquitoes were relatively larger than males (as measured by wing length) but males tended to survive for longer. These differences increased as larval food availability increased, indicating the relative importance of these two traits for the fitness of each sex. The role of nutritional reserves for the reproductive success of males was highlighted in particular.

5. This minimalist approach may provide a useful model for investigating the effects of density-dependent competition on insect life-history traits.

Key words. *Aedes aegypti*, density, life-history traits, mosquito.

Introduction

Since Nicholson's seminal experimental work on captive populations of the Australian sheep-blowfly *Lucilia cuprina* (Nicholson, 1957), a particular form of competition that has been studied intensively is that of intraspecific density-dependent competition (Mueller, 1997). The aim of

Nicholson's work was to gain a clearer understanding of the mechanisms that allow animal populations to adapt to the conditions they experience. In particular, the advantages of working in an ecologically simplified laboratory environment were advocated so as to permit individual factors to be manipulated and their influence to be identified without being obscured by uncontrolled or unknown factors.

Nicholson's results showed that manipulating the amount of food available to either larvae or adults of *L. cuprina* could produce replicable patterns of larval mortality and adult fertility that governed the observed population dynamics. Further evidence was cited to show that such manipulations could lead to evolutionary change such that populations exposed regularly to food limitation

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required less protein to produce eggs than the original populations (Nicholson, 1957).

The theory of density-dependent natural selection was developed further in the 1960s, notably by MacArthur (1962) and MacArthur and Wilson (1967), to suggest that many aspects of an organism's life history could be explained by the population densities experienced by their ancestors (Mueller, 1997). Subsequent controlled laboratory experiments over many generations, mainly with *Drosophila* spp., have verified these predictions by showing that density-dependent selection leads to genetic divergence among populations for various life-history traits (e.g. Mueller *et al.*, 1991; Joshi & Mueller, 1996; Roper *et al.*, 1996; Sokolowski *et al.*, 1997).

Most studies involving the manipulation of density involve single generations and reveal how different phenotypes are likely to be exposed to selection from this type of competition. Many of these studies have involved insects whose offspring develop in discretely defined patches where density-dependent competition for resources is likely to be encountered. The diversity of these experiments includes manipulating densities of coleopteran eggs per seed or grain (Credland *et al.*, 1986), the number of bark beetles allowed to attack a host tree (Reeve *et al.*, 1998), altering how many parasitoid larvae develop in the same host (Dijkstra, 1986), or the number of lepidopteran larvae per Petri dish (Leonard, 1968).

In each experiment, the increase in density-dependent competition for a limiting resource affected at least one of the traits of juvenile mortality, delayed maturity, smaller adult size, or fertility. These responses are also in general accord with predictions from life-history theory of how individual genotypes should alter their phenotype to maximise their fitness in response to deteriorating conditions of juvenile growth (e.g. Stearns & Koella, 1986; Berrigan & Koella, 1994).

A feature common to almost all experiments involving manipulations of density is that they compare various treatments ranging from low to high densities of individuals. What defines a low number of individuals is usually arbitrary, and high-density treatments may be up to several orders of magnitude greater (e.g. Reisen *et al.*, 1984). In the work reported here, Nicholson's approach to simplifying potential confounding factors was taken to its lower bounds by studying the effects of density-dependent competition in treatments containing one, two, or three individuals. This approach had the particular advantage of a control treatment in which individuals developed in the complete absence of any density-dependent interactions with other individuals. A second advantage of the low number of individuals per treatment was that the number of treatments and statistically independent replicates could be increased with relative efficiency.

The organism in this experiment was the yellow fever mosquito *Aedes aegypti* (L.). This species is particularly well adapted to laboratory conditions as its larvae usually develop in containers of a natural or artificial origin (Christophers, 1960). Furthermore, due to the interest in

the ability of density-dependent processes to curb the population growth of mosquitoes (Washburn, 1995), the results from this experiment could be compared with those of previous field and laboratory studies in more realistic environmental conditions and involving a range of mosquito species. Despite their differences, these studies generally found that an increase in larval density resulted in increased larval mortality, delayed pupation, and the emergence of smaller, less fecund adults (e.g. Bar-Zeev, 1957; Wada, 1965; Southwood *et al.*, 1972; Siddiqui *et al.*, 1976; Reisen *et al.*, 1984; Bradshaw & Holzapfel, 1989; Hard *et al.*, 1989; Lyimo *et al.*, 1992).

The results of this experiment are also discussed in relation to the relative investment into adult size or nutritional reserves that males and females made as larval food availability increased, and how these two traits contribute towards the fitness of each sex.

Materials and methods

The strain of *Ae. aegypti* used was isolated in Senegal by colleagues at the Institut Pasteur and has been maintained in the laboratory for ≈ 10 generations with a minimum population size estimated conservatively to be 500 breeding females.

Eggs from the base colony were hatched synchronously by exposure to a partial vacuum and, within 6 h of hatching, larvae were transferred to standard *Drosophila* vials (diameter 20 mm \times 95 mm) containing 5 ml of deionised water. Larvae were reared at a density of one, two, or three individuals per vial and provided with 1, 2, 3, 4, 5, or 6 mg of powdered fish food (Tetramin MicroFood, Melle, Germany). These 18 treatments were repeated 12 times, with replicates being arranged physically in a block design in a room maintained at a constant temperature of 27 °C and 50–70% relative humidity.

Vials were monitored daily for pupation. When individuals pupated, they were transferred to another vial containing 5 ml of distilled water and the vial was covered with a fine nylon gauze. After emergence, the water within the vial was poured out. No food was provided to the adult mosquitoes but they had access to water from soaked sheets of paper placed over the vials; these sheets were resoaked daily. This condition effectively forced individuals to survive by metabolising nutritional reserves accumulated during larval life; death ensues when at least one essential resource has been expended (Briegel, 1990). Adult mortality was recorded daily. Subsequently one wing was removed from each individual and measured to the nearest 0.03 mm using a dissecting microscope fitted with a graduated eyepiece.

Statistical analysis

The treatment effects of larval density and larval food availability were classified as nominal and continuous

effects respectively in all analyses. When included in an analysis, sex was classified as a nominal effect.

Due to the low numbers of individuals per replicate, larval mortality was analysed as a binary variable at the level of individual replicates: either all individuals within a replicate survived to adulthood or they did not. The log-likelihood that all individuals within a replicate would survive to adulthood was tested using a nominal logistic regression.

To determine the effects of treatment conditions and sex on the traits of age at pupation, wing length, and adult longevity (day of death – day of pupation), a three-way ANOVA was used with the parameter replicate classed as a random block effect.

The nature of density-dependent interactions means that individuals from within a replicate cannot be considered independent. To avoid pseudoreplication (Hurlbert, 1984), each replicate made only a single contribution to each analysis. This was achieved by eliminating the contribution of either males or females randomly when a replicate yielded data from both sexes, and by taking the mean value of the sex analysed when data were available from more than one individual of that sex. The results of additional analyses (not shown) where the opposite sex was eliminated were not qualitatively different from those presented.

Three replicates were eliminated from the data set before analyses were carried out as they were found to contain an additional larva to that prescribed by their density treatment.

Results

All individuals from within a replicate survived to adulthood in 113 of the 213 replicates (53%) analysed. At the lowest food treatment of 1 mg, only three individuals (each from the density 1 treatment) survived to adulthood, making survival with this amount of resources essentially independent of density conditions (likelihood-ratio test, $\chi^2 = 4.635$, $P = 0.099$). Due to the low number of individuals surviving to adulthood in the 1-mg treatment, it was excluded from subsequent analyses. In the remaining treatments, there were trends for increasing survival to adulthood as food availability increased and larval density decreased (Table 1, Fig. 1).

Of the 262 individuals that pupated, 163 (62%) did so on the sixth day of the experiment. The remaining individuals mainly pupated on days 5 and 7; males accounted for the bulk of pupation on day 5, whereas females dominated

Table 1. Log likelihood-ratio test on the probability of all individuals within a replicate surviving to adulthood.

Source	d.f.	χ^2	<i>P</i>
Density	2	11.872	0.003
Food	1	11.528	<0.001
Interaction	2	1.214	0.545

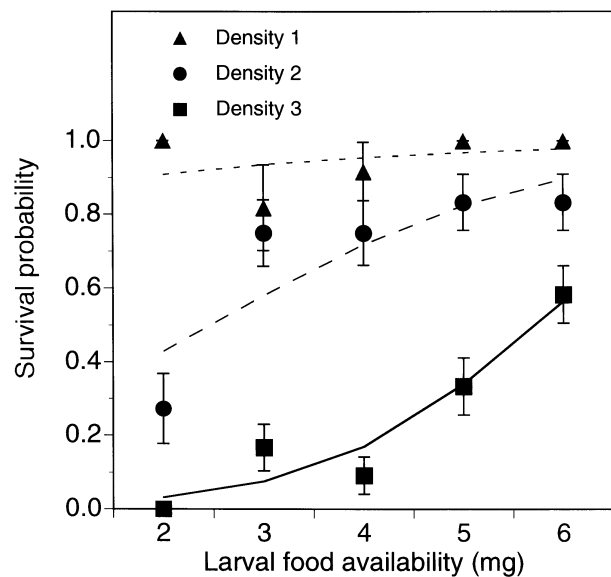


Fig. 1. Effects of larval food availability on the probability that all individuals within a treatment will survive to adulthood. Each point represents the mean (\pm SE) of each density treatment. The lines represent the logistic regressions for densities of one (---), two (-.-), and three (—) larvae per replicate.

those pupating on day 7. Consequently, the overall age structure, with respect to pupation, was weak, however an interaction was detected between larval density and food availability for mean age at pupation (Table 1). For both sexes, individuals reared in isolation pupated earlier than those reared with other larvae when food availability was low, but their respective ages at pupation converged on day 6 as larval food availability increased (Fig. 2a,b). Care should be taken when interpreting these results as these differences were small relative to the 12-h margin of error associated with the 24-h sampling protocol.

Measurements of wing length were less affected by sampling error. For both sexes, wing length increased with food availability but females remained larger than males, such that even the largest males were of an equivalent size to the smallest females (Table 2, Fig. 2c,d). Also, for both sexes,

Table 2. ANOVA for the mean age at pupation of mosquitoes as a function of their sex and larval treatment conditions.

Source	d.f.	Sums of squares	<i>F</i>	<i>P</i>
Block	11	3.450	1.182	0.305
Density	2	3.745	7.053	0.001
Food	1	0.098	0.371	0.544
Sex	1	1.900	7.157	0.008
Density \times food	2	1.973	3.717	0.027
Density \times sex	2	0.365	0.688	0.505
Food \times sex	1	0.422	1.591	0.209
Density \times food \times sex	2	0.156	0.293	0.746
Error	137	36.369		

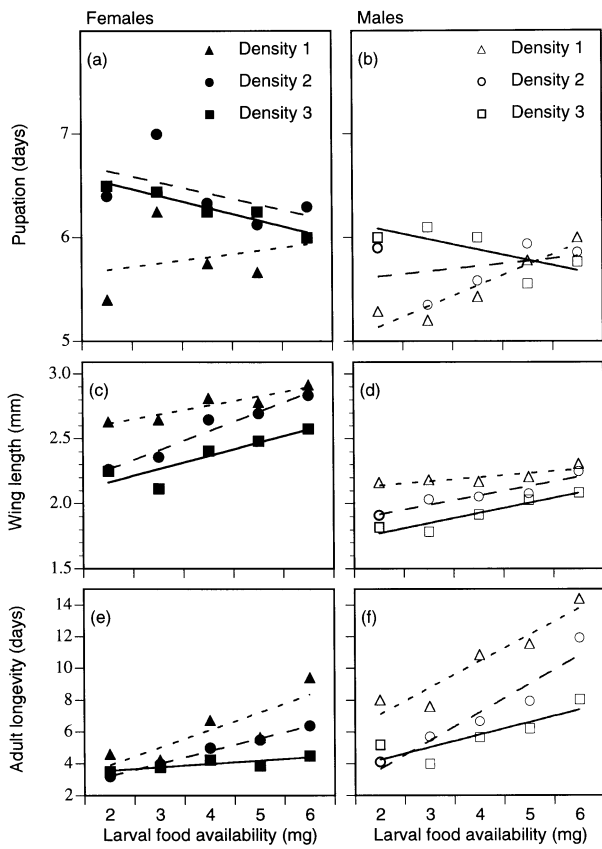


Fig. 2. Linear regressions of the life-history traits of female and male mosquitoes as a function of larval food availability: (a,b) age at pupation, (c,d) adult wing length, (e,f) adult longevity. In each case, points represent the mean (\pm SE) of each treatment. The lines represent the regressions for densities of one (---), two (—), and three (—) larvae per replicate.

the increase in wing length with food availability was more pronounced in the treatments where larvae developed in the presence of other larvae than for those developing in isolation (Table 3). The interaction between food and sex (Table 3) showed that the increase in size with food was stronger for females.

Table 3. ANOVA for mean wing length (mm) of adult mosquitoes as a function of their sex and larval treatment conditions.

Source	d.f.	Sums of squares	F	P
Block	11	0.278	1.148	0.329
Density	2	0.718	16.299	< 0.001
Food	1	1.837	83.372	< 0.001
Sex	1	0.215	9.737	0.002
Density \times food	2	0.202	4.595	0.012
Density \times sex	2	0.039	0.889	0.414
Food \times sex	1	0.181	8.222	0.005
Density \times food \times sex	2	0.029	0.663	0.517
Error	137	3.018		

Table 4. ANOVA for mean adult longevity of mosquitoes as a function of their sex and larval treatment conditions.

Source	d.f.	Sums of squares	F	P
Block	11	49.090	1.225	0.277
Density	2	11.995	1.646	0.197
Food	1	277.315	76.092	< 0.001
Sex	1	0.007	0.002	0.965
Density \times food	2	31.906	4.377	0.014
Density \times sex	2	13.583	1.864	0.159
Food \times sex	1	28.381	7.788	0.006
Density \times food \times sex	2	3.544	0.486	0.616
Error	137	499.294		

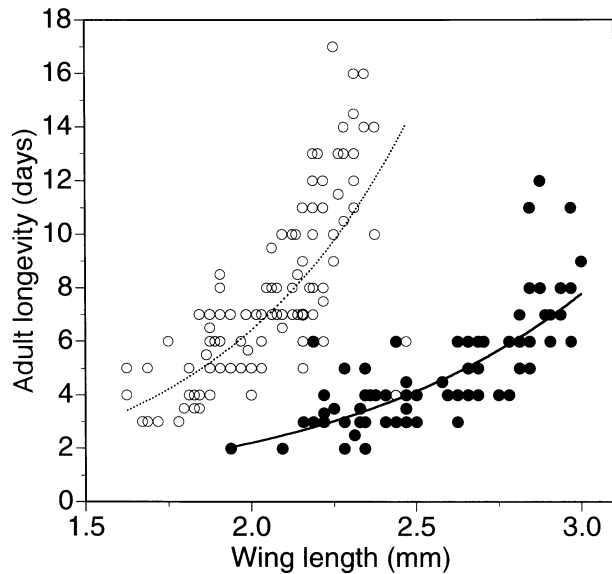


Fig. 3. Exponential relationships between mean adult longevity and mean wing length of male (○) and female (●) mosquitoes per replicate.

The distribution of adult longevity was sufficiently broad for the sampling protocol to detect meaningful differences among treatments. For both sexes, adult longevity increased with food availability, with males generally surviving for longer than females (Table 4, Fig. 2e,f). Also, for both sexes, the increase in longevity with food availability was more pronounced as larval density decreased (Table 4). The interaction between food and sex (Table 4) showed that the increase in longevity with food was stronger for males.

The overall exponential relationships between mean adult longevity and mean wing length for male and female mosquitoes are illustrated in Fig. 3.

Discussion

Density-dependent interactions among mosquito larvae were manipulated by altering the number of larvae per

replicate and the initial amount of food available to them. Each of the life-history traits measured in this experiment was influenced by at least one of these treatments. Furthermore, the basic pattern of results as larval density increased or food availability decreased (reduced survival to adulthood, delayed pupation, and the emergence of smaller adults) is qualitatively similar to those in the experiments cited above concerning mosquitoes, where greater numbers of larvae interacted in physically larger environments. This indicates that the low number of individuals per replicate and the physical environment used in this experiment provide a suitable and efficient design for studying the effects of density-dependent interactions among *Ae. aegypti* larvae. A related protocol using similarly low numbers of individuals per replicate has also been applied successfully to *Culex pipiens quinquefasciatus* (Agnew *et al.*, 2000), suggesting that this approach may be generally applicable to mosquitoes and to other insects that inhabit environments manipulated easily in laboratory conditions.

As the total food provided to larvae was present at the beginning of the experiment, their initial growth was unlikely to have been limited by food availability. A lack of physical space was also unlikely to have constrained initial growth as three first-instar larvae could feed comfortably at the water's surface without coming into physical contact with each other (P. Agnew, pers. obs.). The influence of these density-dependent factors is likely to have increased as food (or the bacteria growing on it) was consumed and as larvae grew in size. *In vitro* studies with *Ae. aegypti* have indicated that most of the energy assimilated during early larval development is converted into structural growth (Gilpin & McClelland, 1979). It appears that there are developmental thresholds that must be satisfied before the sequence of events leading to pupation can be initiated. The detailed nature of these thresholds is unclear but they appear to be related to a combination of an individual's age, size, sex, and nutritional status (Clements, 1992). Whatever these requirements, research has found that an increasing proportion of resources is allocated to energetic reserves in the form of lipids as pupation is approached, particularly when food is not limiting (Gilpin & McClelland, 1979; Briegel, 1990; Timmermann & Briegel, 1999). Some of these resources will contribute towards the energetic demands of metamorphosis, the surplus being available to maintain the adult until it finds further nutrition or for allocation towards its reproductive activity.

Apart from the 1-mg food treatment in which almost no individuals survived to adulthood, survival to the adult stage was influenced by the food resources available and the number of individuals in competition for them (Table 1, Fig. 1). As more individuals competed for fewer food resources, there was an increasing probability that at least one individual per replicate would be displaced in competition and die while still a larva. These individuals presumably failed to acquire sufficient resources for the requirements of pupation before the remaining food had been depleted to a lower limit that was unable to sustain further growth. The condition of having all food resources

available at the beginning of the experiment probably favoured those individuals with a relatively fast growth rate and may help to explain the limited distribution in ages at pupation. Had food been provided on a daily basis, slower-growing individuals would have continued to have access to nutritional resources after other competitors had pupated and hence they would have been able to pupate at a later date (Agnew *et al.*, 2000). This also suggests that the manner in which food is presented to larvae may provide a useful experimental means of increasing or decreasing the age structure in a cohort of adults.

For the larvae reared at a density of one individual per vial, an increase in food availability resulted in an increase of adult size and longevity (Tables 3 and 4), suggesting that both traits are correlated positively with an individual's fitness (van Noordwijk & de Jong, 1986). The generally larger size of females (Fig. 2c,d) and the greater average longevity of males (indicating a greater investment into nutritional reserves) probably reflect the relative importance of these traits to each sex's fitness. The relatively greater increase in longevity rather than size as food availability increased indicated that investment into size was more important when larval food resources were limiting.

The effects of increasing larval density were most apparent in conditions of low food availability. Males and females reared in competition with other larvae were smaller than those reared in isolation (Fig. 2c,d). Males also showed reduced longevity when in competition with others for limited food (Fig. 2e). As food availability increased in these density treatments, there was an overall trend for a relatively greater increase in size rather than reserves (Tables 3 and 4). This conservative bias towards investing in size may be explained partially by noting that this trait is fixed at emergence, whereas nutritional reserves can be supplemented in adult life.

Larval density was not the only factor to interact with food availability in determining an adult's size or longevity. The effect of food availability on these traits also differed for males and females (Tables 3 and 4). Females invested relatively more than males into increased adult size as food availability increased (Fig. 2c,d) whereas the opposite was true for investment into nutritional reserves, the increase in male longevity with food availability being greater than for the females (Fig. 2e,f). These differences between the sexes probably reflect the relative contribution that each trait makes towards male or female fitness.

As for many species of mosquito, the fecundity of female *Ae. aegypti* is correlated closely with adult size (Christophers, 1960), giving a direct link between this trait and fitness. There is also a direct link between female fitness and the amount of nutritional reserves carried over from the larval stage in species of mosquito with obligate or facultative autogeny: these reserves enable a female to mature a first clutch of eggs without needing to take the risk of being killed while blood-feeding. The facultative expression of autogeny, however, depends on larval growth conditions and the ability of female larvae to accumulate sufficient reserves for the first gonotrophic cycle (Clements, 1992).

Autogeny has been reported in some populations of *Ae. aegypti* (Trpis, 1977) but seems to be rare and has not been observed in the strain used in this study (P. Agnew, pers. obs.). Emerging with abundant nutritional reserves does, however, increase the efficiency with which female *Ae. aegypti* convert a bloodmeal into egg production (Briegel, 1990) and therefore is likely to be correlated positively with fitness in this species, though less directly than for the relationship between female size and fitness.

The relationship between reproductive success and the size of male mosquitoes is less well known than for females but is thought to be weaker (Benjamin & Bradshaw, 1984). A more important parameter for reproductive success may be a male's nutritional condition. Comparative data from other male Diptera show that the ability of male Mediterranean fruit flies *Ceratitis capitata* to engage in lekking behaviour is determined by their nutritional condition (Yuval *et al.*, 1998; Kaspi *et al.*, 2000), and the duration of swarming behaviour by males of the midge *Chironomus plumosus* is reduced when they have to rely on stored reserves rather than a recently acquired source of food (Neems *et al.*, 1998). It is also known that the courtship and mating behaviour of male *Drosophila melanogaster* is physiologically demanding, reducing a male's longevity (Partridge & Farquhar, 1981) and immuno-competence (McKean & Nunney, 2001). The exponential increase in longevity with wing length shown by both sexes, but more so by males (Fig. 3), resembles closely the relationships between wing length and lipid reserves of teneral male and female *Ae. aegypti* (Briegel, 1990; Timmermann & Briegel, 1999). As mosquitoes metabolise lipids mainly when at rest, the patterns of longevity observed in this experiment may reflect the amount of lipids accumulated during larval life. The relative increase in male longevity with increased larval food availability and in reduced conditions of larval competition suggests that the accumulation of lipid resources is more closely related to the reproductive success of male *Ae. aegypti* than is their adult size.

To summarise, a minimalist approach to studying the effects of density-dependent interactions among mosquito larvae in laboratory conditions was adopted. The overall results for the effects of density were comparable with those from field and laboratory experiments involving mosquitoes and a diversity of other insects, suggesting the general utility of this experimental approach. The design also had the advantages of a control treatment, where density-dependent interactions were absent, and an efficient means to increase the number of independent replicates and/or treatments per experiment.

The results were also discussed in terms of how larval density and food availability influenced the body size and nutritional reserves of male and female mosquitoes and the relative importance of these traits for the fitness of each sex.

The basic approach and experimental design are adaptable to meet the requirements of other organisms that are reared easily in laboratory conditions and to test hypotheses related to the effects of density-dependent competition on life-history traits.

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