Sex-specific reaction norms to intraspecific larval competition in the mosquito *Aedes aegypti*

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Abstract

As the relationship between a given life-history trait and fitness is not necessarily the same for the two sexes, an 'intersexual ontogenetic conflict' may arise. We analysed the phenotypic reaction to intraspecific larval competition of the mosquito, *Aedes aegypti*, asking: (i) Do both sexes pay the cost of competition with the same life-history traits and are they equal competitors? (ii) Is there a specific cost of competition beyond sharing food resources? We found that competition incurs a specific cost that was expressed differently by the two sexes. Indeed, each sex maintained the more important life-history trait(s) for their fitness (developmental time for males and body weight and size for females) at the expense of other traits, thus minimizing the effects of competition on their fitness. The competition exerted by females was estimated as being more intense, probably linked with the greater importance of body size for their fitness.

Introduction

The fitness consequences of variation in a given life-history trait are not necessarily the same for males and females because particular traits may contribute differently towards the reproductive success of each sex. Such differences can arise because sexual selection acts more intensely on the traits of one sex or due to the sexes having different ecological requirements (Lande, 1980; Slatkin, 1984). Either or both of these mechanisms can lead to the evolution and maintenance of sexual dimorphism.

This sexual dimorphism can, however, provoke an 'intersexual ontogenetic conflict' for genes that are expressed, but selected differently, in each sex (Rice & Chippindale, 2001). Evidence for the existence of sexually antagonistic genes, where increasing a genotype's male fitness is at the expense of its female fitness, and vice versa, has been demonstrated in a series of

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experiments involving *Drosophila melanogaster* (Rice, 1992, 1996, 1998; Chippindale *et al.*, 2001; Rice & Chippindale, 2001). Other studies, although less conclusive, support the idea that sexually antagonistic genes may be common. In particular, studies involving dioecious plants have estimated the contribution of various life-history traits towards the reproductive success of males or females and found them to be negatively correlated at the phenotypic (Kohorn, 1994) or genotypic (Meagher, 1992, 1994) level.

What is less known is the extent to which male and female traits can be independently expressed in response to different environmental conditions. In other words: Are reaction norms sex-specific? Do these responses maintain fitness in a sex-specific fashion? From an adaptationist perspective, selection would be expected to favour the ability of a genotype to maintain investment in the traits most closely aligned to the individual's fitness (whether male or female), at the expense of investment into traits less important for fitness.

We studied the phenotypes of male and female *Aedes aegypti* (L.) mosquitoes when subjected to different degrees of intraspecific competition as larvae; a source of stress known to strongly influence various life-history

traits of each sex and their potential fitness (Agnew *et al.*, 2002). Our results are compared with those from other studies also reporting sex-specific reaction norms.

Biological model and specific questions and predictions

Knowledge of the biology and ecology of *Ae. aegypti* allows us to make some specific predictions about the differences in selection pressures exerted on males and females. Larval cohorts within a site are often synchronized, egg hatching being triggered by a single immersion event, and can lead to non-overlapping generations (Christophers, 1960). As in other mosquitoes, females often become refractory to further mating after the first successful copulation (Clements, 1992). These two life-history features are advantageous to early emerging males as they have greater access to virgin females, and so potentially a higher reproductive success. Indeed, in this protandrous species, male developmental time is thus selected to be short. On the contrary, there is a trade-off at the individual level between being a big adult and emerging early. Whereas this trade-off exists both in males and females, the correlation between size and fecundity is stronger in females: van den Heuvel (1963) reported a positive correlation between size (thorax length) and ovariole number in *Ae. aegypti*. Positive correlations between female body size (estimated by wing length) and fitness traits such as blood-feeding success and clutch size have also been reported for *A. cantans* (Renshaw *et al.*, 1994) and other mosquito species. The link between size of a male and its reproductive success is less well established. To the best of our knowledge, there are no data for *Ae. aegypti*. However, it was found to be weak in the pitcher-plant mosquito (Benjamin & Bradshaw, 1994). The trade-off between developmental time and adult size combined with the correlation between adult size and fecundity in females should select for large, slowly developing females, whereas the same trade-off combined with the fitness advantages of emerging early in males should select for small, fast developing males.

Intraspecific competition is a relevant stress for *Ae. aegypti* because they experience strongly contrasting conditions of larval density in the field. Indeed, *Ae. aegypti* larvae grow in natural or artificial containers, such as flower pots, bamboo shoots or water storage jars (Southwood *et al.*, 1972). These sites vary in size and food availability over time. Furthermore, density depends on the volume of these containers at the time of hatching and the number of eggs eclosing. For these reasons, competition conditions are very heterogeneous in this species. In addition, life-history traits of *Ae. aegypti* (Gilpin & McClelland, 1979; Dye, 1982, 1984) and of other mosquitoes (Bradshaw & Holzapfel, 1989; Hard *et al.*, 1989; Agnew *et al.*, 2000) are already known to show strong phenotypic responses to larval competition. In general, when larval competition becomes more intense, developmental time increases and adult body weight and size decreases.

The above-mentioned arguments were examined in a study on the mosquito *Ae. sierrensis* (Kleckner *et al.*, 1995). This species is univoltine and monogamous, which makes the argument for a benefit of greater access to females for early emerging males even stronger. Their study used field data and a simulation model that focused on the influence of temporal heterogeneity in female fecundity on the level of protandry. A positive correlation between larval density and protandry was found and was due to an increase in female developmental time at high larval density, indicating that sexual selection among males is not the only cause of protandry. Constraints imposed on the two sexes by environmental conditions, and particularly by intraspecific competition, should also be considered.

The difference in importance between fitness components for each sex could have another consequence in the framework of intraspecific competition: if the maintenance of different traits in each sex requires different amounts of resources, the prediction can be made of an asymmetry in the intensity of competition either exerted or experienced by each sex. In *Ae. aegypti*, we can predict that developmental time is more important for male fitness, and that body weight and size are more important for female fitness. However, because no data are available for the relative costs, in terms of resource exploitation, of the maintenance of each of these traits, we cannot make any specific prediction for the sex exerting or experiencing the more intense competition in this biological model. We return to this point in the Discussion section.

Our aim in the present study was to characterize the reaction of several life-history traits of *Ae. aegypti* in response to larval competition, in experimental conditions where density was closely controlled. More specifically, we wanted to answer the following questions: (1) Do the sexes respond similarly to competition or do they modify different life-history traits? (2) Are both sexes equal competitors? Moreover, our design allowed us to address a third question, namely whether competition can be simply attributed to a division of resources.

Materials and methods

We used a strain of *Ae. aegypti* collected in Senegal by colleagues at the Pasteur Institut in Dakar that was maintained in the laboratory for 10 generations in outbred conditions (with a minimum of 500 reproductive individuals of each sex).

The data used in this study is from an experiment in which we tested the interaction between intraspecific competition and parasitism by the microsporidia *Vavraia culicis*. Unfortunately, the infection rate was very low so that only the analysis of the effects of intraspecific

competition was possible. Because unexposed and exposed-but-uninfected individuals reacted similarly to competition (test not shown), we combined data from both types of individuals. All the effects reported as statistically significant remain so even if we restrict the analysis to the unexposed individuals.

Different levels of competition were imposed by varying both food regime and larval density. Contrary to most studies (see previously cited), larval density was represented by either one, two or three larvae per standard *Drosophila* vial. This approach of manipulating larval density is not representative of natural conditions, but offers several advantages: first, we had the 'density one' treatments that served as 'without competition' controls. Secondly, we controlled density by only using data of replicates which had conserved their initial density throughout the experiment. Indeed, density will vary both within and between replicates because of mortality. Moreover, it may vary systematically across treatments, if they affect survival differentially, as competition and food regime are likely to do. Our minimalist approach allowed us to circumvent these caveats. Thirdly, the low number of individuals per replicate allowed us to carry out analyses at the within-replicate level: we could use the gender composition of individuals within the vials and have a sufficient number of replicates for each subclass.

Forty-eight-hour-old larvae (post-hatching) were transferred to standard *Drosophila* vials (diameter 25 × 95 mm) containing 5 ml of demineralized water. One, two or three larvae were introduced in each vial. Prior to transfer, larvae were kept in Petri dishes and fed fish food (0.06 mg on day 0 and 0.08 mg on day 1 per larva) (Tetra, Melle, Germany). During development, larvae were daily fed food dissolved in 1 ml of demineralized water. Two food regimes were adopted: 50 and 100% of a standard regime. The 100% food regime consisted of 0.16 mg on day 2, 0.32 mg on day 3 and 0.64 mg per vial from day 4 onwards. In the 50% food regime, these quantities were divided by two. Each treatment (density × food) was replicated between 50 and 79 times. Vials were arranged in racks containing four rows. We distributed the 50% food regime in two rows and the 100% food regime in the other two rows in order to facilitate daily food distribution. Within each food group, density treatments were arranged randomly. We divided the experiment into six blocks in order to reduce the effects of uncontrolled environmental variations. The experiment was carried out in a room maintained at 25 °C and a photoperiod of 12L : 12D.

Vials were examined every 12 h and age at pupation was recorded. Pupae were transferred to individual vials containing 4 ml of demineralized water and the vials were covered with a fine nylon gauze. Adult emergence was noted with a precision of 12 h, the sex of mosquito was recorded and water poured out of the vial to prevent drowning. Water was provided to adults by placing soaked paper on the gauze; this was resoaked every 24 h. Adults were starved to death and their day of death was noted to the closest 24 h. After adults had died they were transferred to 1.7 ml plastic vials and placed in a 65 °C stove for a minimum of 12 h. Their dry weight was recorded to an accuracy of 1 μg (with a Mettler Toledo MX 5 microbalance, Greifensee, Switzerland). One wing was removed from each adult and the distance between the allula notch and the tip of the wing was measured to a precision of 0.03 mm using a dissecting microscope fitted with a graduated eyepiece. Between each step of data collection, dead mosquitoes were conserved at −20 °C.

Statistical analyses were performed with JMP, version 3.2.2 (SAS Institute 1995, Cary, NC, USA). Only replicates where all individuals survived to adulthood were used for the analyses. The size of the data set was thus reduced but ensured that larval mortality did not influence the life-history traits of the remaining individuals.

For each trait, we performed a three-way ANOVA taking larval density, food regime and sex as factors. For these analyses we only used vials in which all the individuals were of the same sex; competitive interactions between sexes were analysed separately. We measured four traits: larval developmental time, adult longevity (day of death − day of pupation), wing length and starved adult dry weight. Data for larval developmental time were transformed to their inverse square in order to have normally distributed residuals. Adult longevity represents the time an individual survived on the reserves it accumulated during its larval life. To avoid pseudoreplication (Hurlbert, 1984), we randomly chose one individual per replicate for density 2 and 3 treatments. Hence, the degrees of freedom in our analyses were based on the number of replicate vials and not on the number of individuals in the data set. Repeated testing (100 random choices and tests were made) found that the results of these analyses were not qualitatively altered by the particular individual selected at random from vials in the density 2 and 3 treatments. The results presented are representative of a typical analysis (that is to say an analysis in which the effects that are significant in most of the tests are significant).

To test whether there is a specific cost to growing with a competitor or if competition simply involved sharing the food resource, we compared two treatments: density 2 and a 100% food regime vs. density 1 and a 50% food regime. Indeed the individuals of these two treatments received the same quantity of food per individual, but they differed by the presence (for density 2) or absence (for density 1) of a competitor. These analyses were carried out on all individuals of these two categories by *t*-tests comparing the mean values of the traits of the two categories for each sex separately.

To see whether the costs of competition are borne equally or differentially by both competitors in the density 2 treatment, and if the costs are expressed in

the same or different traits by the two competitors, it is important to consider the order of emergence in pairs formed by two individuals of the same sex. However, simply comparing the average of first (or second) emerging individuals grown under competition conditions to the average of single grown individuals of the same sex could introduce a statistical artefact. Indeed, if there is variability for developmental rate, the comparison of such averages could reveal a significant effect even in the absence of an effect of competition, because one would be comparing the average of the intrinsically fastest (or slowest) individuals to the average of the entire population. To avoid this artefact, we generated artificial pairs by randomly drawing two individuals without replacement among those of the density 1 treatment. We then determined the order of emergence in the artificial pairs and from real pairs of the density 2 treatments. When paired individuals had the same age at emergence, one of them was randomly ascribed to the first emerging individual. We compared the life-history traits of individuals with the same rank of emergence subjected to different density treatments. After the discrimination of first and second emerging individuals in both density treatments, we compared, by *t*-tests, the values of the traits for individuals that grew alone and individuals that grew with a competitor in each class of order of emergence. This procedure was carried out by a C++ program that repeated the sequence, 'arranging in pairs, determination of the order of emergence, and test of the difference in the value of traits' a given number of times (set to 100 for the results given below). In the 'Results' section, this procedure is mentioned as the 'resampling test', and is quantified by the number of tests with a *P*-value <0.05.

To test whether the costs of competition depended on the sex of the competitor, we made comparisons for the four life-history traits between (1) individuals that grew alone and individuals that grew with a male, and (2) individuals that grew alone and individuals that grew with a female. These comparisons were made for each sex separately on the same data set as the previous analyses. In turn, individuals that grew with a male and individuals that grew with a female were compared within each sex separately to see if there was an asymmetry in competition. This last comparison was made using the programme described in the previous paragraph for the same reasons: males that grew with a female were rearranged by pairs, their order of emergence was determined, and their traits compared with the traits of the males that grew with another male. The matching analysis was carried out for females.

Finally, to complete the investigation of the potential asymmetry in competition between the two sexes, we performed an analysis including only replicates with a larval density of three individuals. We used the number of females in the vial as a new factor (i.e. 0, 1, 2 or 3). We performed two-way ANOVAS for the four traits measured

with the number of females and the quantity of food as factors for each sex separately.

Results

Larval mortality was very low (about 1%) and therefore we did not test for treatment effects on this trait. Before proceeding to the analysis of specific costs of competition and of its sex-specific effects, we first briefly report the global effects of larval competition. The effects reported here have already been identified by previous studies. The purpose of this analysis was to verify whether the results of our minimalist approach are comparable with what one may observe in the field or in experiments with larger population sizes in each density replicate.

Global effects of competition

We detected a significant effect of density, food regime and sex on the four traits we analysed. Overall, males emerged earlier than females ($F_{1,334} = 99.21$, $P < 0.001$), as smaller ($F_{1,274} = 673.38$, $P < 0.001$) and lighter ($F_{1,301} = 269.01$, $P < 0.001$) adults. Males also had a greater adult longevity ($F_{1,334} = 39.16$, $P < 0.001$).

When larval density was increased, larval development was longer ($F_{2,334} = 247.36$, $P < 0.001$), adult longevity reduced ($F_{2,334} = 11.98$, $P < 0.001$) and the adults were both smaller ($F_{2,274} = 82.91$, $P < 0.001$) and lighter ($F_{2,301} = 86.65$, $P < 0.001$). In addition, individuals provided with a 100% food regime during larval development developed faster ($F_{1,334} = 243.59$, $P < 0.001$), had a reduced adult longevity ($F_{1,320} = 14.02$, $P < 0.001$) and were both larger ($F_{1,274} = 47.83$, $P < 0.001$) and heavier ($F_{1,301} = 33.34$, $P < 0.001$). For all the effects described until here, all the 100 tests carried out were significant. All these results are in full agreement with previous reports on the effects that these factors have on the mosquito life-history traits (Gilpin & McClelland, 1979; Bradshaw & Holzapfel, 1989; Hard *et al.*, 1989; Agnew *et al.*, 2000).

Specific cost of competition and sex-specific effects

The different reaction norms of males and females were first revealed by the result that sex interacted with other factors in the analyses of the global effects of competition. This suggests that the two sexes do not react identically to competition. These interactions found: when larval density increased (i) females had a significantly greater increase in developmental time (8.01 days at density 1 to 11.32 at density 3, an increase of 41.3%) than males (7.56 to 9.33, an increase of 23.4%) ($F_{2,334} = 5.62$, $P < 0.01$) (90 significant tests/100), (ii) female longevity did not change, whereas male longevity was decreased by 22% (from 13.5 at density 1 to 10.6 at density 3) ($F_{2,317} = 9.97$, $P < 0.001$) (100 significant tests/100), and (iii) males showed a larger relative decrease (−32.6%) in

starved adult dry weight than females (−26.8%) ($F_{2,284} = 4.60$, $P < 0.05$) (90 significant tests/100).

Secondly, to see if competition had a specific cost and if this cost was expressed differently by males and females, we compared the two categories that received the same amount of food per individual but differed for the presence (for the density 2 and 100% food regime) or absence (for the density 1 and 50% food regime) of a competitor. This analysis revealed a significant effect of competition on the developmental time of females ($F_{1,117} = 9.25$, $P < 0.05$) but not of males ($F_{1,135} = 2.15$, n.s.): females that grew alone developed faster than females that grew with a competitor (Fig. 1a). The trend was the same for males but the range of the difference was smaller and the test was not significant. On the contrary, growing with a competitor made males lighter ($F_{1,127} = 18.78$, $P < 0.001$, Fig. 1b), smaller ($F_{1,121} = 7.45$, $P < 0.05$, Fig. 1c) and short-lived adults ($F_{1,135} = 9.84$, $P < 0.05$, Fig. 1d), whereas it did not significantly affect these traits for females (analyses not shown).

The analyses, taking the order of emergence into account, were performed to find out how the cost of competition is shared between the two competitors. The resampling tests revealed that the effect of competition on the developmental time of females was mostly expressed by the second emerging female which had a significantly longer developmental time (100 significant tests/100), whereas the developmental time of the first emerging female was not significantly different from the density 1 individuals. There were no significant differences in other traits of the first and second females. In contrast, on average, males emerged in the same time whether grown in competition or alone. The first to emerge was lighter (97 significant tests/100), smaller (95 significant tests/100) and lived for less time as an adult (95 significant tests/100) compared with the males who grew alone. The only significant difference for the second emerging males was their lighter weight (98 significant tests/100).

A comparison of females that grew alone with females that grew with another female revealed that growing with a female competitor made their developmental time longer ($F_{1,67} = 17.48$, $P < 0.001$; Fig. 2). The trend was the same for females that grew with a male, but the test was not significant ($F_{1,87} = 3.23$, $P = 0.076$). There were no significant differences in the other life-history traits. The matching comparisons for males revealed that growing with competitors of either sex induced a reduction in adult longevity ($F_{1,85} = 5.76$, $P < 0.05$ with a male competitor; $F_{1,102} = 8.64$, $P < 0.05$ with a female competitor), starved adult dry weight ($F_{1,81} = 14.68$;
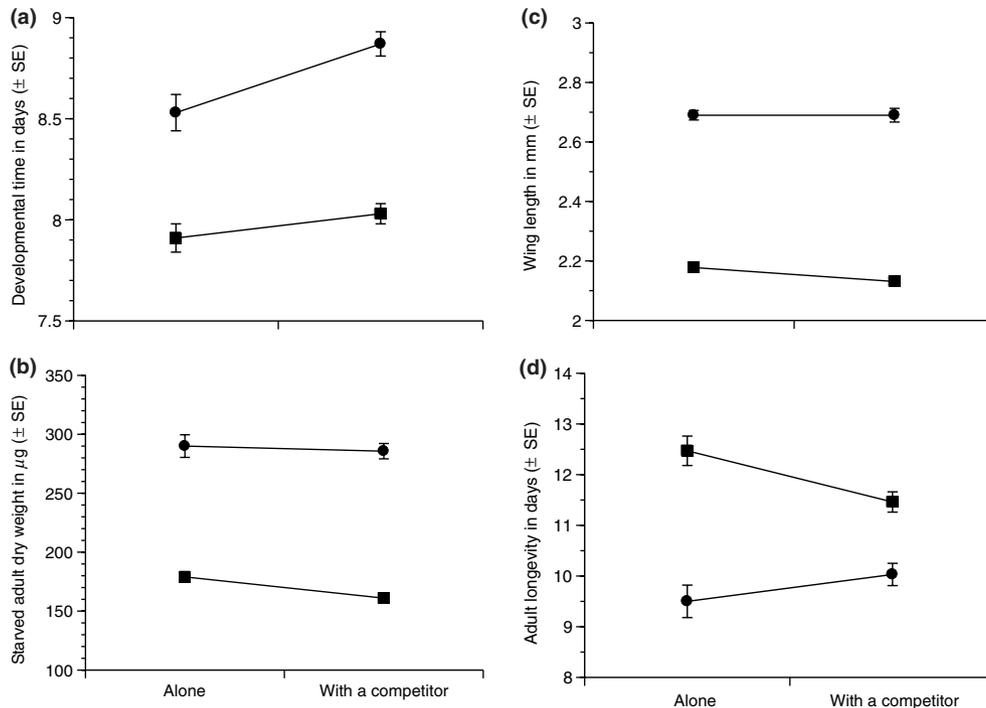

Fig. 1 Effects of the presence of a competitor during larval development for individuals that received the same quantity of food per individual (a) on developmental time (days), (b) on starved adult dry weight (µg), (c) on wing length (mm) and (d) on adult longevity (days). Circles refer to females and squares refer to males.
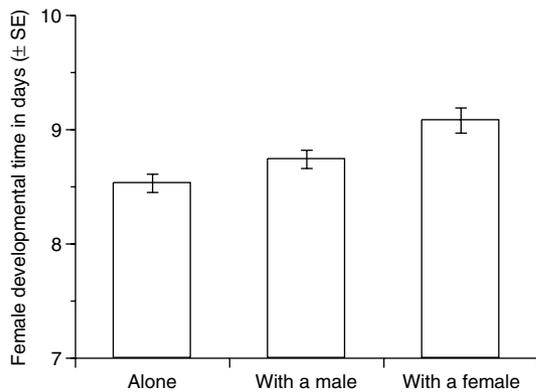
Fig. 2 Developmental time (±SE) of females that grew alone, with a male, or with another female. All the individuals included in this analysis received the same quantity of food.

Fig. 3 Starved adult dry weight (±SE) of males that grew alone, with a male, or with another female. All the individuals included in this analysis received the same quantity of food.

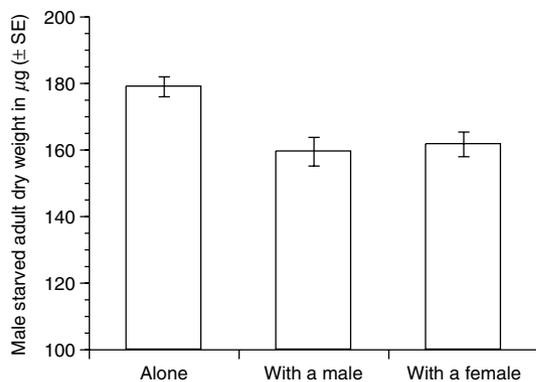$P < 0.001$ with a male competitor; $F_{1,95} = 13.8$, $P < 0.001$ with a female competitor; Fig. 3) and size ($F_{1,75} = 7.28$, $P < 0.05$ with male competitor, $F_{1,87} = 5.01$; $P < 0.05$ with a female competitor). Male developmental time was also longer with a female competitor ($F_{1,102} = 4.5$; $P < 0.05$) whereas there was no difference when the competitor was another male.

The comparison between males that grew with a female and males that grew with another male, performed with resampling tests, revealed no significant differences between these two categories. The matching tests for females showed that the first to emerge of the two females was lighter than the female grown with a male (97 significant tests/100), whereas the second to emerge had a longer developmental time than the female that grew with a male (99 significant tests/100). There were no other significant differences.

With the two-way ANOVA including the number of females and the quantity of food as factors, we investigated the intensity of the competition exerted by each sex.
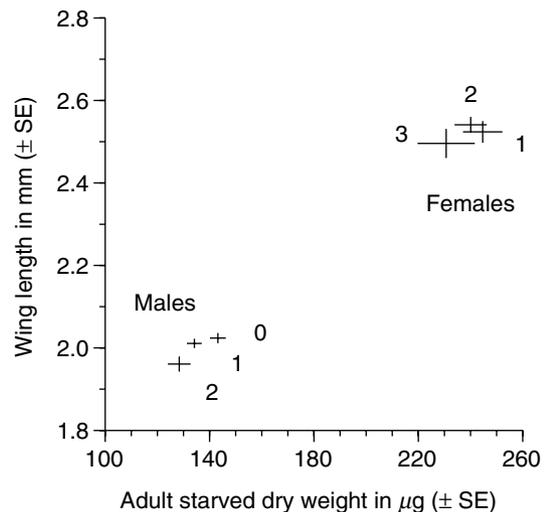

Fig. 4 Effects of number of females in a vial on morphological traits of males and females for treatments with three larvae on wing length (mm) and on starved dry weight (μg). The number next to the point indicates the number of females in the vial.

We found a significant effect of the number of females per vial on male morphological traits. When the number of females increased, males emerged as smaller ($F_{2,120} = 6.74$, $P < 0.05$) and lighter ($F_{2,125} = 6.05$, $P < 0.05$) adults (Fig. 4). Females showed the same trend for body weight but the effect was not significant. The number of females per vial did not have a significant effect on the other traits (developmental time and longevity) of males or females. The effect of food regime was significant in the two sexes and was the same as in the preceding analysis, but the interaction between the food regime and the number of females was not significant.

The main results on specific cost of competition and sex-specific effects are summarized in Table 1.

Table 1 Summary of the effect of larval intraspecific competition on the four male and female life-history traits analysed.

Comparison	Growing with a competitor vs. growing alone		Growing with a male competitor vs. growing alone		Growing with a female competitor vs. growing alone	
	Male	Female	Male	Female	Male	Female
Life-history trait						
Developmental time	0	+++	0	+	++	+++
Adult longevity	– – –	0	– –	0	– – –	0
Starved adult dry weight	– – –	0	– – –	0	– – –	0
Wing length	– –	0	– –	0	– –	0

One symbol: $0.05 < P < 0.1$.
Two symbols: $0.005 < P < 0.05$.
Three symbols: $P < 0.005$.
'+' Indicates an increase in the trait and '−' a decrease.

Discussion

Our results show that there is a specific cost of intraspecific competition in *Ae. aegypti*: when provided with the same amount of food per individual, there were differences in the life-history traits of individuals developing alone or in the presence of a competitor. More interestingly, this cost is expressed through variation of different life-history traits in males and females and this differential expression is in agreement with the expectation that life-history traits differ in importance for male and female fitness. Moreover, females exert a more intense competition than males. These results are discussed in the following paragraphs, but we first discuss the global effects of intraspecific larval competition as they validate our experimental approach.

Global effects of competition

Increasing larval competition resulted in longer juvenile development and smaller adults that starved to death more quickly. These results suggest that competition reduced both growth and resource accumulation rates during the larval stage and increased developmental time. These results follow the general patterns known for mosquitoes. For example, Hard *et al.* (1989) obtained the same type of results with two tree-hole mosquito species, *Ae. geniculatus* and *Ae. triseriatus*, and Bradshaw & Holzapfel (1989) showed that developmental time of *Wyeomyia smithii*, the pitcher plant mosquito, increased when density increased.

We found that males lived longer as adults than females. This result is probably highly dependent on the conditions in which the measure was made: adults were in individual tubes, did not spend a lot of energy in flying, and more importantly had no sexual activity. Figure 1 in Hausermann & Nijhout (1975) suggests that sexual activity reduced male longevity in *Ae. aegypti*. This is a probable explanation for the apparent contradiction between our results and those often observed in population cages where sexual activity is unfettered (Lansdown & Hacker, 1975).

The similarity in the results of our minimalist approach of density and of experiments with a larger number of individuals per replicate indicates that we were able to capture the same effects of competition as in other experimental formats. However, the present experimental design allows us to investigate more competition-specific aspects.

Specific cost of competition

Our data suggest the existence of a cost of competition beyond a limited availability of food. This effect appeared through the comparison of individuals which grew alone at a 50% food regime and individuals which grew with a competitor at a 100% food regime. Although in both cases the quantity of food available per individual was the same, developmental time of females increased in the presence of a competitor (Fig. 1a), whereas males maintained their developmental time but were lighter (Fig. 1b), smaller (Fig. 1c) and survived for less time on their reserves (Fig. 1d; see also Table 1). These effects suggest that competition is a more complex phenomenon than a simple sharing of food resources among the individuals present and implied additional costs.

A first hypothesis to explain this cost is the stress induced by the presence of another individual. Indeed, *D. melanogaster* lines selected at high and low population densities during at least 150 generations in controlled laboratory conditions (Mueller, 1988) revealed that an adaptation to crowded environments is a higher tolerance of nitrogenous wastes (Borash *et al.*, 1998). The cost observed in our experiment could be due to a higher accumulation of nitrogenous wastes at higher densities. We tested this hypothesis in another experiment (S. Bedhomme, unpublished data) and found that 'pollution by conspecifics' was costly but that these costs were not sex-specific. Previous studies (Bar-Zeev, 1957; Moore & Fischer, 1969; Moore & Whithacre, 1972; Dye, 1982, 1984) on the effects of intraspecific competition in *Ae. aegypti* varied in the demonstration of a 'growth retardant' effect depending on the range of density they used and the traits they measured. This 'growth retardant' could be the nitrogenous wastes produced by the competitors. A second hypothesis is that physical contact could induce a stress and cost. Figure 1 in Dye (1984) suggests that physical interference plays a role in larval competition in *Ae. aegypti*. A third hypothesis to explain the specific cost of competition is a faster resource depletion expressed by a higher feeding rate and a reduced energetic efficiency: this phenomenon was reported by Joshi & Mueller (1996) for *Drosophila* lines adapted to crowding.

Involvement of nitrogenous waste accumulation during competition reinforces the idea that competition is not a simple phenomenon whose intensity is solely determined by the number of individuals in the population (Mueller, 1997). For example, environmental conditions affected by density are not constant in time – nitrogenous wastes accumulate and competition for food and space decreases as larvae pupate.

Sex-specific reaction to competition

The significant interactions between sex and density in the global analysis and the sex-specific expression of the cost of competition indicate that the two sexes do not react in the same way to the same environmental conditions. In these two types of analysis, developmental time was more sensitive to environmental conditions for females than for males: the increase in developmental time was larger for females than for males when density increased, and the specific cost of competition was

expressed by a lengthening of developmental time in females, particularly the second emerging female, whereas developmental time of males grown alone or with a competitor was equal. On the contrary, longevity and body weight was more reduced for males than for females as density increased, and the specific cost of competition was expressed by a reduction in weight, wing length and longevity of males, particularly for the first to emerge. Thus, not only did the two sexes pay the specific cost in different proximal currencies (life-history traits), but also, within a given gender, the cost is unequally expressed by the two competitors revealing a hierarchy between the two.

The differential reaction norms of males and females are consistent with the idea that traits involved in the trade-off, at the individual level, between being big adults and emerging early, do not have the same importance for the fitness of the two sexes. The maintenance of developmental time under competitive conditions in males, accompanied by a reduction in morphological traits and accumulated reserves (suggested by reduced adult longevity), is consistent with the fact that developmental time is more important for male fitness than body size because of the advantage of short developmental time in male–male competition for access to mates. On the contrary, the delay in developmental time, but maintenance of size and accumulated reserves under competition conditions for females, can be explained by the correlation between female body size and reproductive success (van den Heuvel, 1963). Our results thus suggest that the cost of competition is expressed through greater phenotypic modifications of the life-history traits that are least important for the fitness of each sex or through the modification of only the 'least important fitness trait', depending on the level of analysis. In this way, individuals of each sex may minimize the cost imposed by an environmental stress or constraint.

Similar results were found in the protandrous butterfly *Lycaena hippothoe sumadiensis* submitted to various temperatures during larval and pupal development: under stressful high temperatures males sacrificed body weight to maintain protandry, whereas females maintained size without altering developmental time (Fischer & Fiedler, 2001).

Such sex-specific reaction norms were also found in dioecious plants submitted to stresses, such as defoliation (Elmqvist & Gardjfell, 1988; Delph, 1990) or density and water stress (Lyons *et al.*, 1994). When defoliated, male *Silene dioica* (Elmqvist & Gardjfell, 1988) have a delayed flowering peak, whereas females flowered at the same time as the control. Females also have a higher regrowth rate after defoliation. In the subdioecious shrub *Hebe subalpina* (Delph, 1990), defoliated males produced fewer fruits but more leaves than females. In *S. dioica*, when density increased, females showed a larger decline in biomass whereas males showed a larger decline in flower

number (Lyons *et al.*, 1994). Although clearly sex-specific, all these reactions are not very easy to interpret because the link between the measured traits and fitness was not always well established. Moreover, the investment in reproductive organs occurs at different times in male and female plants and in the case of defoliation, the choice of the date at which stress was applied should influence the intensity of the cost induced for the two sexes.

Asymmetry between the sexes in the intensity of competition

This specific cost of competition appeared irrespective of the sex of the competitor (Figs 2 and 3, Table 1). However, the lack of a difference in developmental time between a female that grew alone and a female that grew with a male, together with the longer developmental time of the males that grew with a female, compared with males that grew alone, suggest that females exerted a more intense competition. This is further partially confirmed by the cost in developmental time (for the second to emerge) and in body weight (for the first to emerge) for a female that grew with another female compared with a female that grew with a male. The decrease in the size of male morphological traits when the number of females per vial increased to three (Fig. 4) is yet another argument for the more intense competition exerted by females. Why this more intense competition is not expressed in the same sex and on the same traits in the two types of conditions remains unclear. The more intense competition exerted by females suggests that the maintenance of their larger size and weight requires more food and induces a more intense competition for food when the number of females increases. This difference in food requirements and the mechanisms that lead to the differences in size and weight (difference in growth rate and filtration rate) are, in themselves, another index of the difference in importance of the accumulation of reserves for the two sexes.

Concluding remarks

In summary (see Table 1), our study first underlines the fact that the effects of competition cannot be completely predicted just from the amount of available food per competitor, as competition was shown to have a specific cost that may be, at least partially, due to nitrogenous wastes or physical contact. Secondly, we showed that the two sexes do not have a symmetrical role in competition, females being harsher competitors. Thirdly, the two sexes differ in their reactions to competition, managing the trade-off between being big adults and emerging early in ways that reflect the importance of each trait for their fitness. This difference ultimately reflects the fact that at least the life-history traits under consideration are not

under the same selective pressure in males and females. The reactions we observed result not only from phenotypic plasticity, but they may well be also involved in evolutionary processes, for example in adaptation to new environmental conditions such as a higher competition, provided the appropriate genetic variation exists in mosquito populations. The genetic variation is at least present for developmental time, as shown by Koella & Agnew (1999), who successfully selected lines of *Ae. aegypti* for early and late pupation.

The difference in importance of a given trait for the fitness of each sex may create a conflict of interest because traits do not evolve independently for the two sexes (Lande, 1980). This hypothesis was directly tested in *S. latifolia* (Meagher, 1994): two generations of sex-specific selection were imposed on traits having different consequences on male and female fitness. He obtained a reaction both in the sex on which the selection was exerted and in the other sex showing these traits will not evolve independently in each sex. Moreover, in a series of papers, Rice and Chippindale (Rice, 1992, 1996, 1998; Chippindale *et al.*, 2001; Rice & Chippindale, 2001), using very carefully designed experiments, showed that genes increasing fitness in males decreased fitness in females, and vice versa. These trade-offs result from the different contributions made by (at least some) life-history traits to either a male's or a female's fitness. Further developments in this area can be expected as our understanding of the ontogenetic mechanisms that permit genotypes to express sexually dimorphic traits increases (Badayev, 2002).

Acknowledgments

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