

Pollution by conspecifics as a component of intraspecific competition among *Aedes aegypti* larvae

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Abstract. 1. The role of pollution by conspecifics in the costs associated with larval intraspecific competition was investigated for *Aedes aegypti* (Diptera: Culicidae).

2. The growth of larval *A. aegypti* mosquitoes reared in clean water and water in which another larva had previously grown was compared; this procedure eliminates interactions through food consumption between competitors and allows the effects of other processes to be expressed.

3. A cost of growing in polluted water was found: this cost was expressed as an increase in developmental time and a reduction of adult longevity when starved, starved adult dry weight, and wing length.

4. Contrary to previously reported results of an experiment allowing for competition for food, these costs were not expressed in a sex-specific manner and were independent of the sex of the polluter.

5. It was thus demonstrated that competition arises from both resource depletion and other effects that result in deterioration of the environment, with chemical pollution of the environment being the most likely cause.

Key words. *Aedes aegypti*, chemical interference, intraspecific competition, mosquito, pollution by conspecifics.

Introduction

When population density increases, resulting environmental modifications create detrimental effects on individuals, and intraspecific competition becomes intense. Environmental conditions, particularly if they are experienced during juvenile development, are likely to have an important influence on life-history traits and reproductive success of the adult stage. The most commonly mentioned and most obvious environmental modification caused by an increase in population density is the reduction of per capita food availability that influences traits such as growth, resource accumulation, body size, and fecundity (Prout & McChesney, 1985 and references therein).

In a previous study, the effects of larval intraspecific competition on life-history traits of the mosquito *Aedes aegypti* (L.) were investigated, and it was shown that when density increases, developmental time increases and adult longevity, size, and weight decrease (Bedhomme *et al.*, 2003). By comparing two treatments in which the larval density was different but the quantity of food per larva was the same, evidence was obtained that the costs of an increase of population density are not completely due to the reduction of per capita food availability. There are at least three other factors linked with density that could be involved in the competition phenomenon and partly explain the costs of competition expressed through modifications of life-history traits. First, as density increases the frequency of physical contact among individuals increases and could be a source of stress as suggested for *Ochlerotatus cantans* (formerly *Aedes cantans*) larvae in Renshaw *et al.* (1993). Second, a mechanism of resource appropriation could appear in reaction to higher levels of intraspecific competition. *Resource appropriation* means that at high density,

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individuals have a higher feeding rate and reduced energetic efficiency; this was observed in *Drosophila melanogaster* lines reared for more than 100 generations at high densities (Joshi & Mueller, 1996). In this case, *resource appropriation* was genetically determined but in principle it can also appear by phenotypic plasticity. Third, an increase in density could result in an accumulation of toxic chemical products to higher concentrations in a given environment. These products could be either allelopathic substances synthesised in reaction to the increase of competition intensity or waste produced by the individuals (particularly nitrogenous wastes). The existence of allelopathic substances has been mainly studied in plants (e.g. Ohno *et al.*, 2001; Kato-Noguchi, 2003) and in bacteria (e.g. Riley & Gordon, 1999). In plants, these substances seem to have a role exclusively in interspecific competition, whereas bacteriocins, highly specific toxins acting against individuals of other strains of the same species, are a component of intraspecific competition. It is believed that there is no example of an allelopathic substance identified in animals. On the other hand, the role of the toxic effects of nitrogenous wastes in intraspecific competition has been demonstrated for *D. melanogaster* (Borash *et al.*, 1998): ammonia and urea accumulated to higher concentrations in the environment when larval density was higher and populations adapted to higher densities had a higher tolerance to ammonia and urea.

The goal of the present study was to go one step further in understanding one of these potential mechanisms explaining the specific costs of larval intraspecific competition in *A. aegypti*: the accumulation of chemical products in the environment. The first question is thus: do accumulated chemical substances play a role in the negative effects of intraspecific competition?

The two following questions were also asked: (1) Are these potential effects expressed identically in males and females? (2) Do male and female *polluters* generate the same cost? It was decided to include these sex-specific components of competition in the design of the experiment because previous studies on the effects of larval density on life-history traits of *A. aegypti* (Gilpin & McClelland, 1979; Agnew *et al.*, 2000; Bédhomme *et al.*, 2003) and other mosquitoes, *Wyeomyia smithii* (Bradshaw & Holzapfel, 1989), *Aedes triseriatus*, and *Aedes geniculatus* (Hard *et al.*, 1989), have mentioned sex-specific reactions to competition. A difference in the intensity of competition exerted by the two sexes was also reported in Agnew *et al.* (2000) and Bédhomme *et al.* (2003).

Materials and methods

Larvae from a strain of *A. aegypti*, collected in Senegal by workers at the Pasteur Institute in Dakar were used. This strain has been maintained in the laboratory for 10 generations in outbred conditions (with a minimum number of 500 reproductive individuals of each sex). The experiment was conducted in a room maintained at 25 °C and a photoperiod of LD 12:12 h.

The experiment was divided into two steps. In the first step, larvae, the *polluters*, were reared in vials for a measured period of time. In the second step, other larvae, the *polluted*, were grown in the water in which the polluters had developed. This protocol maintains the pollution by the conspecifics and eliminates other components of intraspecific competition: competition for food resources, physical contact, food appropriation.

In the first step of the experiment, 400 recently hatched larvae (<6 h) were each put into their own individual *Drosophila* vial (diam. 25 mm × 95 mm) containing 5 ml of softened water. Larvae were given food daily: 0.06 mg of Tetramin (fish food) on day 0, 0.08 mg on day 1, 0.08 mg on day 2, 0.16 mg on day 3, and 0.32 mg per vial from day 4 onwards. Food was suspended in 1 ml of softened water. When a larva became a pupa, the date was noted (to the nearest 12 h) and the vial was covered by a fine nylon gauze. When the adult emerged, the sex of the individual was noted and the adult was killed. Polluted water for which the sex of the polluter and the duration of pollution were known was thus obtained. The duration of pollution was noted as *A. aegypti* females typically have a longer developmental time than males and this factor was controlled to see if the potential difference of the effects of pollution by males and females came from this difference in developmental time. Six categories of pollution were defined: polluted by a male or by a female for 7.0, 7.5, or 8.0 days respectively. Water was pooled in each of these categories. Only water from vials in which the individual survived to the adult stage were used. The food regime was such that only negligible amounts of food may have remained in the water at the end of this first step of the experiment. Indeed, it is known from a previous experiment (Bédhomme *et al.*, 2003) that larvae given larger amounts of food than in the present experiment develop faster than individuals of this experiment, thus suggesting that the regime used here represents conditions of food limitation. The only suspended food likely to have been unconsumed was that administered on the day the larva pupated. It is possible that some nutrients were dissolved in water and could have been beneficial to microbial growth. Effects due to any residual food or microorganisms in polluted treatments are likely to work against the hypothesis of there being costs of developing in a polluted environment. Such food, however, would have had little time to deteriorate and could have made a positive contribution towards larval growth in the second step of the experiment. The procedure is thus conservative.

In the second part of the experiment, vials were prepared with 5 ml of water, either from a polluted source or with softened water. One hundred and twenty vials of softened water were used as a control. The number of vials in each category (determined by the sex of the polluter and duration of the pollution) are given in Table 1. A recently hatched larva (about 5 h old) was added to each vial. There was a total of 389 larvae. Vials were positioned randomly in 10 racks. The same food regime as above was administered to these larvae and the food was distributed daily and diluted in 1 ml of softened water. Vials were examined every 12 h

Table 1. Number of *Aedes aegypti* larvae in each polluted category for the second step of the experiment (growth in polluted or control water).

Duration of pollution (days)	Sex of the polluter		Total
	Males	Females	
7.0	89	17	106
7.5	49	40	89
8.0	19	55	74
Total	157	112	269

and age at pupation was recorded. Pupae were transferred to vials containing 5 ml of softened water and the vials were covered with a fine nylon gauze. Adult emergence was noted to the nearest 12 h, the mosquito's sex was recorded, and water was poured out of the vial to prevent drowning. Adults were starved to death and their day of death was noted to the nearest 24 h. After adults had died they were transferred to 1.7 ml plastic vials and placed in a 65 °C oven for a minimum of 12 h. Their dry weight was recorded to an accuracy of 1 µg (with a Mettler Toledo MX 5 microbalance). 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 (SAS, 1995). MANOVA was used to assess the joint response to treatment effects of larval developmental time, adult longevity (day of death - day of pupation), wing length, and starved adult dry weight. Adult longevity represents the time during which the individual survived on the reserves it accumulated during its larval life. *Rack* was introduced as a random factor to take micro-environmental variability into account. In this analysis, all the individuals submitted to a polluted environment were pooled in a single class and the factors of the analysis were the sex of the polluted individuals and the environment of growth (polluted vs. control). This was to test for the global effects of pollution and if these effects differentially affected trait expression of males and females. A MANOVA was then performed on a data file including only the individuals exposed to pollution. The factors considered were the sex of the individual, the sex of the polluter, and the duration of the pollution. This last factor was introduced to test whether the potential differences induced by the sex of the competitor were due to differences in the duration of pollution (longer for females than for males). When MANOVAs revealed significant effects, separate ANOVAs were performed on each life-history trait including the same factors.

Results

Larval mortality was low (14/388 individuals died before adulthood) and so a treatment effect on this trait was not tested for.

In the first analysis, all the individuals exposed to pollution were pooled in a single category and compared with control individuals. The aim was to explore the effects of conspecific secreted substances on an individual's life-history traits and if this varied according to the individual's sex. The MANOVA revealed that an individual's sex and the treatment of pollution had a significant effect (Table 2). The ANOVAs for each trait supported the established differences between males and females: males developed faster ($F_{1,305} = 218.2$, $P < 0.001$) and emerged as smaller ($F_{1,305} = 1377.6$, $P < 0.001$) and lighter adults ($F_{1,305} = 599.8$, $P < 0.001$). Males also lived longer as adults ($F_{1,305} = 319.9$, $P < 0.001$). Pollution increased developmental time ($F_{1,305} = 69.2$, $P < 0.001$; Fig. 1a) and decreased adult longevity ($F_{1,305} = 21.9$, $P < 0.001$; Fig. 1b), starved dry weight ($F_{1,305} = 45.1$, $P < 0.001$; Fig. 1c), and wing length ($F_{1,305} = 31.3$, $P < 0.001$; Fig. 1d). The interaction between sex and pollution was significant for longevity only: the reduction of longevity induced by pollution was more important for males than for females ($F_{1,305} = 9.99$, $P = 0.002$; Fig. 1b).

The second analysis with an individual's sex, sex of the polluter, and the duration of the pollution as factors was performed to test whether the sex of the polluter had an effect on the life-history traits of the individuals exposed to their pollution. The effect of an individual's sex was significant and the effects of the Sex factor were the same as in the first analysis (Table 3). Neither the sex of the polluter nor the duration of the pollution had a significant effect.

Discussion

Does environmental degradation play a role in intraspecific competition?

Growing in water in which another individual had already grown was costly for *A. aegypti* since it had negative fitness effects: it increased developmental time and reduced an adult's longevity, wing length, and starved dry weight. Pollution of the environment by conspecific individuals is thus responsible for a part of the negative effects of intraspecific competition. Indeed the amount of toxic chemical products that will accumulate in a given environment is proportional to larval density and thus imposes an additional cost to that due to competition for nutritional resources.

Table 2. MANOVA of the effects of sex and pollution by conspecifics on developmental time, adult longevity, starved adult dry weight, and wing length of *Aedes aegypti*. Test are based on Wilk's lambda.

Factor	d.f.	F ratio	P
Rack	36	2.4	<0.0001
Sex	4	682.8	<0.0001
Pollution	4	25.1	<0.0001
Sex × Pollution	4	4.5	0.0015

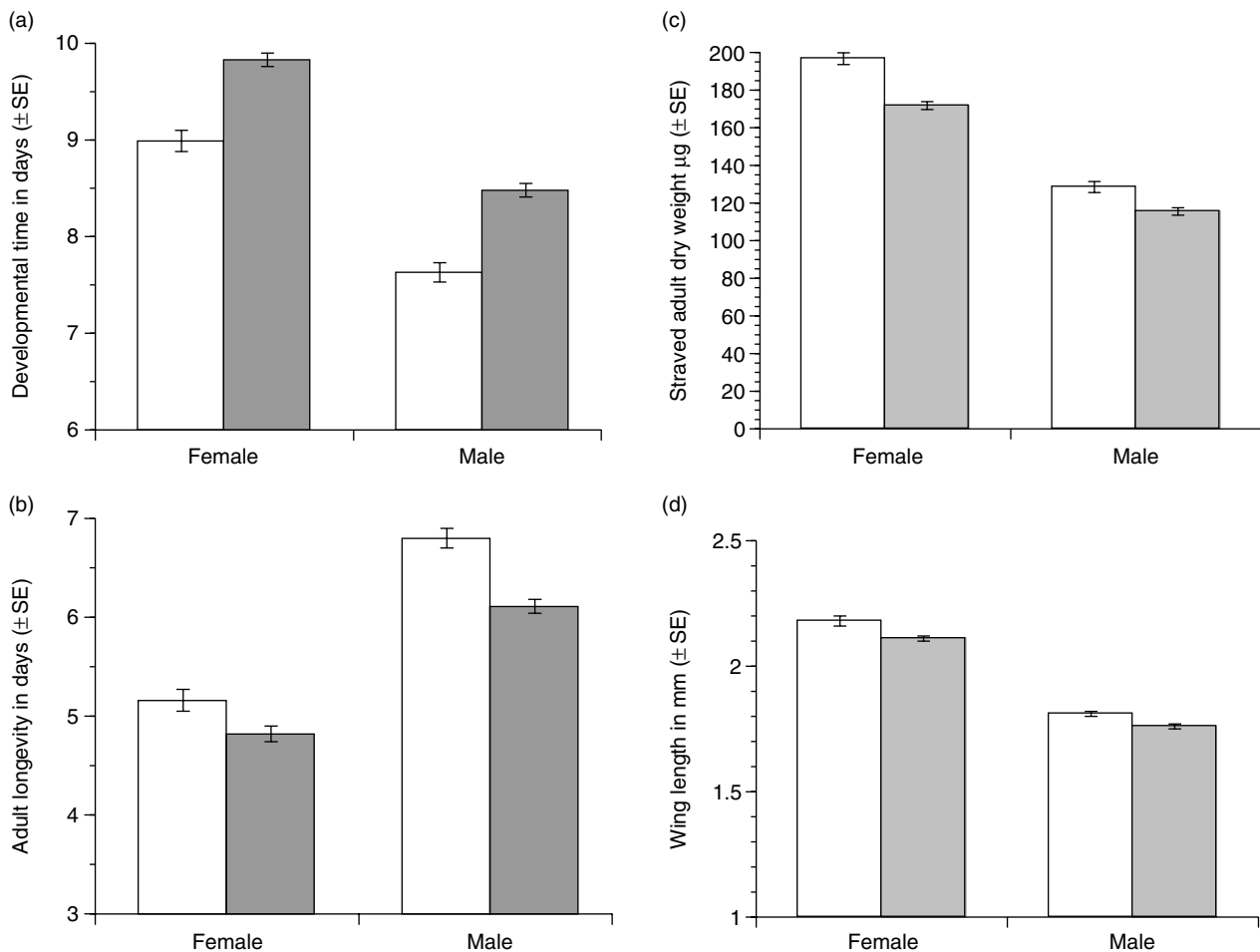


Fig. 1. Effects of pollution by conspecifics on life-history traits of *Aedes aegypti*. Larvae were reared in water in which another larva had grown previously and clean demineralised water as a control. (a) Effects on developmental time, (b) effects on adult longevity, (c) effects on adult starved dry weight, and (d) effects on wing length. White bars refer to control individuals and grey bars to polluted individuals. Error bars represent standard errors.

Previous studies investigating the role of density in intraspecific competition and population regulation in *A. aegypti*, and particularly the role of chemical interference, did not all reach the same conclusions. Some of them identified a specific effect of density (Wada, 1965) or the existence of a *growth retardant* produced by growing larvae (Moore & Fischer, 1969; Ikeshoji & Mulla, 1970; Moore & Whithacre, 1972; Dye, 1984), whereas others concluded that competition does not include factors other than food shortage (Bar-Zeev, 1957; Dye, 1982). As mentioned by Dye (1982), this divergence among the conclusions may be due to several factors: first, different lines of *A. aegypti* may not be equally sensitive to chemical interference. This sensitivity could particularly be linked with the history of the line: lines reared in laboratory conditions at very high densities for many generations could have lost their sensitivity to chemical substances due to the intense selection pressures exerted at high densities. Due to this, working on mechanisms and effects of competition on

laboratory lines, as done in Dye (1982), may be misleading. The present study used a strain removed from field conditions since a relatively small number of generations (10), under conditions minimising the effects of toxic wastes accumulation (water changed every 2–3 days). Second, in some of these studies the effects of chemical and physical interference are combined or confounded, which is not the case for the present study. Moreover, in the experimental design used, the *polluter* individuals were alone, so that the retardant substances are not secreted in response to competition but are normally excreted products. Finally, some of the analyses in the above studies were limited to the effects on the larval period of growing in water where other larvae have grown: they did not include adult life-history traits and thus may have underestimated the potential effects of pollution and its role in intraspecific competition.

A good candidate for the chemical products accumulated in the environment during larval growth are nitrogenous wastes. Mosquito larvae are thought to mainly excrete

Table 3. MANOVA of the effects of sex, conspecific polluter's sex, and duration of pollution on developmental time, adult longevity, starved adult dry weight, and wing length of *Aedes aegypti*. Test are based on Wilk's lambda.

Factor	d.f.	F ratio	P
Rack	36	747.5	0.0155
Sex	4	393.9	<0.0001
Polluter's sex	4	0.1	0.9949
Pollution duration	8	1.4	0.1786
Sex × Polluter's sex	4	0.2	0.9332
Sex × Pollution duration	8	1.3	0.2378
Polluter's sex × Pollution duration	8	1.8	0.0809
Sex × Polluter's sex × Pollution duration	8	1.2	0.2803

ammonia as do many aquatic organisms (Wright, 1995). Enzyme activity studies (von Dungern & Briegel, 2001) demonstrated that they also synthesise urea and uric acid from the second larval stage but third and fourth instar larvae and pupae do not excrete uric acid. Excreting nitrogen in the form of ammonia requires 10 times more water than excreting urea and the conversion of ammonia to urea is energy demanding (Wright, 1995). Moreover, ammonia is toxic at lower concentrations than urea. It modifies pH regulation and is neurotoxic. Linked with these constraints, the excretion of ammonia is commonly found in aquatic organisms that do not face the problem of desiccation and can excrete ammonia continuously (ammonotelic organisms). The implication of ammonium in density-dependent regulation has been suggested in the tree-hole mosquito *Aedes triseriatus*. Indeed, Carpenter (1982) demonstrated that the addition of ammonia to artificial microcosms had negative effects on numerous life-history traits including survival and adult mass. Subsequently, Walker *et al.* (1991) showed that ammonia accumulated in tree-hole occupied by *Ae. triseriatus* larvae. These two results indicate that increases in ammonia concentration linked with the presence of competing larvae will have negative effects contributing to the costs of intraspecific competition. In a more recent study, Sunahara and Mogi (2002) reared cohorts of *Aedes albopictus* and *Tripteroides bambusa*, two other tree-hole mosquito species, in microcosms where another cohort of larvae of one of the species had already grown. A negative impact of the growth of a previous cohort was shown on survival rate. One of the hypotheses to explain these negative effects was the accumulation of nitrogenous wastes. This hypothesis was confirmed by the reduction of the amplitude of the negative effects when polluted water was replaced by fresh water.

Although nitrogenous wastes seem reasonable candidates for explaining the observed results, it can not be excluded that the negative effects of growing in the water in which another larva has grown are due to a modification of the microbial community by the presence of the first larvae. These modifications could either lead to an increase in the population size of a detrimental microorganism [as shown for interspecific competition in Mokany & Shine (2003)] or

a change in the microbial community rendering the food less edible or less nutritious.

Are there sex-specific effects in this component of intraspecific competition?

In this experiment, the interaction between an individual's sex and pollution was not significant for developmental time, body weight, and size. It was significant for longevity, with males being more affected by pollution. The cost induced by pollution are thus mainly expressed similarly by males and females. The absence of sex specificity is probably linked to the toxicity of any accumulated substances affecting general physiology of the organism.

The measured traits of individuals that grew in water polluted by males and females were not significantly different. Duration of pollution was included in the analysis to control for the difference of developmental time of males and females that could be an explanation for the observation that females have a stronger competitive effect than males (Bedhomme *et al.*, 2003). Specific effects of duration of pollution were not interesting in themselves. The absence of significant differences in traits of individuals growing in environments polluted by males or females indicated that the cost imposed by pollution due to the competitor is not dependent on its sex.

*Comparison of this experiment and the competition experiment (Bedhomme *et al.*, 2003).*

In the previous study on *A. aegypti* reaction norms to intraspecific competition among larvae (Bedhomme *et al.*, 2003), a specific cost of competition was established by the comparison of two categories of individuals that received the same quantity of food per individual but differed by the presence or absence of a competitor. This comparison showed that competition cannot be reduced to simply a sharing of the food resources available. The cost was not expressed by the same life-history traits of the two sexes. The present experiment was conducted in conditions as similar as possible (food, volume, temperature) to those of the competition experiment in order to directly compare life-history traits differences in the two cases. More specifically, the goal was to test whether pollution by conspecifics could be an explanation to the specific cost of competition identified here. For each trait and sex, the difference between the mean for *control* and for *treatment* individuals and its standard deviation were calculated for the pollution and the competition experiment. *Control* refers to individuals grown in clear water in the pollution experiment and to individuals grown alone with a 50% food regime in the competition experiment. *Treatment* refers to individuals grown in polluted water in the pollution experiment and to individuals grown with another larva and a 100% food regime in the competition experiment. These differences therefore represent the mean effects of pollution

Table 4. Means and differences of means of the four measured life-history traits in the pollution and in the competition experiments. The differences of the means are compared by *t*-tests. In the pollution experiment, 'Cont.' are individuals that grew in unpolluted water and 'Treat.' are individuals that grew in water polluted by conspecifics. In the competition experiment (Bedhomme *et al.*, 2003), 'Cont.' are individuals that grew alone and 'Treat.' are individuals that grew with a competitor. Values in parentheses are \pm SE.

	Pollution experiment			Competition experiment			<i>t</i> -test (1 d.f.)	<i>P</i> -value
	Cont. mean	Treat. mean	Difference of the means	Cont. mean	Treat. mean	Difference of the means		
Male developmental time (days)	7.611 (0.101)	8.471 (0.066)	0.861 (0.120)	7.896 (0.077)	7.978 (0.054)	0.082 (0.094)	68.13	<0.001
Male adult longevity (days)	6.736 (0.093)	6.056 (0.061)	-0.680 (0.111)	12.481 (0.263)	11.458 (0.185)	-1.024 (0.320)	13.19	<0.001
Male starved adult dry weight (μ g)	127.62 (2.63)	114.77 (1.73)	-12.85 (3.13)	178.38 (5.18)	160.63 (3.85)	-17.75 (6.43)	8.49	<0.001
Male wing length (mm)	1.800 (0.011)	1.748 (0.007)	-0.052 (0.013)	2.176 (0.015)	2.132 (0.011)	-0.044 (0.019)	4.27	<0.001
Female developmental time (days)	9.080 (0.114)	9.789 (0.074)	0.709 (0.136)	8.536 (0.094)	8.821 (0.059)	0.285 (0.110)	28.85	<0.001
Female adult longevity (days)	4.969 (0.104)	4.773 (0.068)	-0.196 (0.125)	9.216 (0.321)	9.669 (0.202)	0.452 (0.377)	19.07	<0.001
Female starved adult dry weight (μ g)	190.86 (2.95)	168.45 (21.92)	-22.41 (3.52)	279.35 (6.53)	280.74 (4.07)	1.392 (7.651)	31.69	<0.001
Female wing length (mm)	2.175 (0.013)	2.116 (0.008)	-0.059 (0.015)	2.669 (0.019)	2.675 (0.012)	0.006 (0.023)	26.14	<0.001

and competition in the two experiments. A *t*-test was used to show whether these mean effects were significantly different. The results are given in Table 4. This method makes the hypothesis that there is no significant interaction between the block effect (due to the fact that the two experiments were not performed at the same time) and the treatment effect (pollution or competition).

Absolute values for each trait of the control treatment differ for the two experiments, despite efforts to reproduce the same environmental conditions. A possible explanation is that the two experiments were performed at different times in different rooms, with differences in the way temperature and hygrometry are regulated.

The qualitative variation of life-history traits due to pollution was similar to that due to larval competition but the treatment effects are significantly different for each trait in each sex and greater in the pollution experiment than in the competition experiment. The specific cost of intraspecific competition is thus partly explained by the accumulation of substances secreted by the larvae. However, a significant difference in both sexes for each of the traits analysed was found in the pollution experiment, whereas the specific cost of competition was expressed only by developmental time in females and longevity, weight, and size in males. This suggests that both sexes are equally sensitive to environmental pollution. The sex-specific reaction to competition observed by Bedhomme *et al.* (2003) thus reflects other components of intraspecific competition. Moreover as the pollution by males and females did not induce different reactions of life-history traits, the asymmetry in the intensity of the competition exerted by males and females was not due to excreted substances. The origin of this asymmetry thus lies in

another component of intraspecific competition. Finally, the greater amplitude of induced differences in life-history traits in the pollution experiment is probably due to larvae in this experiment having been in contact with secreted substances during their entire larval life, whereas those in the competition experiment accumulated as the larvae grew and polluted their environment.

To summarise, it has been shown that growing in water in which another larva had already grown had negative effects on developmental time, adult longevity, and body weight and size. Secreted substances or other environmental modifications are thus involved in intraspecific competition. Nevertheless, this component of intraspecific competition does not induce a differential expression of the costs of competition between the two sexes. In a more general context, the role of environmental modifications and particularly of substances secreted by conspecifics in intraspecific competition confirmed two ideas pointed out by Mueller (1997): first, intraspecific competition cannot be reduced to a simple partitioning of the food resources available. Second, the environmental changes induced by high population density are not constant in time since secreted substances probably accumulate during larval life and participate in a progressive deterioration of the environment under crowded conditions.

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