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Thibaut Bagni, David Siaussat, Annick Maria, Philippe Couzi, Martine Maïbèche, et al.. A maternal effect influences sensitivity to chlorpyrifos pesticide in the pest moth *Spodoptera littoralis*. *Ecotoxicology and Environmental Safety*, 2020, 204, pp.111052. 10.1016/j.ecoenv.2020.111052 . hal-02969470

HAL Id: hal-02969470

<https://hal.science/hal-02969470>

Submitted on 16 Oct 2020

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A maternal effect influences sensitivity to chlorpyrifos pesticide in the pest moth *Spodoptera littoralis*

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ABSTRACT: Transgenerational effects on sensitivity to pesticides are poorly studied. This study investigated the transgenerational influences of maternal body mass in the major pest moth *Spodoptera littoralis*, with a focus on sensitivity to chlorpyrifos pesticide. In 147 clutches of a laboratory strain of *S. littoralis*, we compared larval mortality between control larvae and larvae treated with chlorpyrifos. Because of the classic positive relationships between offspring size and maternal size and between offspring size and offspring quality, sensitivity to chlorpyrifos was predicted to be lower in larvae of larger mothers. Surprisingly, we found the opposite result, with higher pesticide toxicity in larvae of larger mothers. This result is partly explained by the lack of a relationship between larval mass and larval sensitivity to chlorpyrifos. This means that another offspring characteristic linked to maternal size should have affected larval sensitivity to chlorpyrifos. More generally, knowledge of the effects of the traits and ecological environments of mothers on offspring sensitivity to pesticides remains limited. Ecotoxicologists should pay more attention to such maternal effects on sensitivity to pesticides, both in pests and non-target species.

Keywords: Transgenerational effect, insecticide, crop insect pest, mortality, body mass, offspring size.

1. Introduction

The characteristics, fitness and response to environmental factors of individuals can depend on the environments experienced by their parents. These nongenetic parental effects are referred to as transgenerational effects (Latzel, 2015) and the individual variation caused by transgenerational effects can create lagged responses that are important for population dynamics (Benton et al., 2006). Consequently, transgenerational effects should be considered for the management of pest species and in risk assessment procedures on non-target species (Müller, 2018). Transgenerational mechanisms involve embryo modifications (energetic provisioning, hormones, toxins, offspring size, ...) or epigenetic alterations (DNA methylation and histone modification) (Bernardo, 1996a, 1996b; Latzel, 2015). Whether parental effects may be maternal or paternal, maternal effects are thought to be more common (Falconer and Mackay, 1996), but see Bonduriansky and Head (2007). Indeed, offspring quality often depends on maternal traits and maternal environmental history (Marshall et al., 2010; Mousseau et al., 2009; Mousseau and Fox, 1998), notably in insects (Mousseau and Dingle, 1991; Mousseau and Fox, 1998). This is especially well illustrated by a recent review of animal populations (Moore et al., 2019), which also showed that maternal effects on morphology and life-history traits in juvenile stages are especially strong.

The impact of transgenerational effects on sensitivity to pesticides is poorly studied. Most related studies are on the effects of pesticides on several generations (Brausch and Salice, 2011; Sarkar et al., 2018; Silva et al., 2019). However, studies of other transgenerational effects on pesticide sensitivity are especially scarce (Pieters and Liess, 2006; Tran et al., 2018, 2019). To the best of our knowledge, in insects, this has been investigated only in the vector mosquito *Culex pipiens* (Tran et al., 2018, 2019). The authors highlighted a synergistic effect

between temperature and pesticide exposure in the parental generation that influenced the impact of chlorpyrifos pesticide on offspring survival (Tran et al., 2018). In this context, we aimed to test for a transgenerational effect on pesticide sensitivity in another insect model, the major crop pest insect *Spodoptera littoralis*, a widespread African and Mediterranean moth: its larvae damage 87 plant species of economic importance, such as cotton, maize, and tomato, making this species one of the most destructive agricultural pests. Instead of studying the parental effects of pesticide exposure and temperature, we focused here on the effects of maternal body mass on the sensitivity of offspring to a pesticide. Indeed, maternal body size is often observed to be an influential factor for offspring quality (Marshall et al., 2010).

As in the study on the mosquito *C. pipiens*, we tested the transgenerational effect of chlorpyrifos pesticide. Chlorpyrifos is one of the most commonly used insecticides (Eaton et al., 2008), and it is indeed commonly used against the moth *S. littoralis* (Dewer et al., 2016). This organophosphate insecticide inhibits the enzyme acetylcholinesterase, which hydrolyses the neurotransmitter acetylcholine. Consequently, chlorpyrifos overstimulates the nervous system of insects, leading to their death. In *S. littoralis*, sublethal doses of chlorpyrifos have been also shown to alter metabolic pathways involved in energy production (Dewer et al., 2016). If a transgenerational effect is present as in *C. pipiens*, what effect can be predicted? Classically, offspring size is related positively to maternal body size (Lim et al., 2014). Thus, if offspring size reflects offspring quality as observed in many taxa (Allen et al., 2008; Rollinson and Hutchings, 2013), we expect sensitivity to chlorpyrifos to be lower in larvae of larger mothers. Precisely, in *S. littoralis*, we test whether the body mass of the mother influences the survival rate of her larvae when treated with chlorpyrifos.

2. Materials and methods

2.1. Study system and experiment

A laboratory strain of *S. littoralis* was used, with larvae reared on a semi-artificial diet (Hinks and Byers, 1976) at 23°C, 70% relative humidity, and a 16:8-h light-dark cycle until emergence. We collected and weighed adults one day after their emergence, at 7 days, in 2018 (11 and 19th of June, 2nd and 3rd of July, 13, 14 and 15th of November). The first experimental series in June-July was performed on adults collected in our laboratory rearing where larval density was not strictly controlled. We standardized density at the fourth larval instar visually (but without a precise count of the number of larvae in our basic laboratory routine) with density usually closes to 40-60 larvae in rearing boxes of 22.5 x 14.5 x 7.2 cm. Food was provided *ad libitum* as in all our experimental series. The second experimental series in November was performed on adults reared with strictly controlled larval density, *i.e.* with 1 *versus* 15 *versus* 40 larvae at the fourth larval instar. Except for density, all experimental conditions were the same between the two experimental series. Clutches were obtained from mating of pairs consisting of one female and one male, which were two- and three-day-old adults. Mating was performed in plastic cups (10 cm in diameter) with sugar water (20 g/L) as a food source and a strip of Whatman paper as a laying surface. We collected clutches only 24 hours after mating (at the peak of egg laying) to homogenize the sampling of eggs. We reared clutches for 8-9 days at 29°C, 70% relative humidity, and a 16:8-h light-dark cycle until the fourth larval instar was reached (L4 larvae). For each of the 201 clutches sampled, we counted the number of eggs and quantified their hatching rate. In a subsample of 147 clutches, we selected 30 L4 larvae from each clutch and weighed them before testing their sensitivity to chlorpyrifos (45395, Sigma-Pestanal, France). Although we paid attention to the time spent from the egg stage to minimize age variation when we collected L4 larvae, it cannot be ruled out that an age variation biased our measurements of larval mass. A better control of the age

of larvae could have been achieved with larvae reared individually to determine more precisely their age from their third molt. However, we found that rear larvae individually had a dramatic effect in *S. littoralis* (Supplementary Material, Figure S1). Sensitivity to chlorpyrifos was tested by comparing larvae treated with chlorpyrifos and control larvae, with 15 larvae per clutch and per larval treatment. Chlorpyrifos was diluted in hexane to obtain a concentration of 44 mg/L, and control larvae were treated using hexane. We chose a chlorpyrifos concentration of 44 mg/L, which was revealed to be close to the LD₅₀ in a preliminary test, with a larval mortality of 56% ($N = 195$). Larval mortality was 58% in the present study with this concentration. We performed our treatments by topical applications of 0.5 μ L of the test solutions to the head of larvae using a microapplicator (Hamilton 25 μ L syringe and Hamilton dispenser). The mortality of larvae was checked 72 hours after treatment. We estimated larval survival rates as the proportion of the surviving larvae tested per clutch and per treatment, *i.e.*, from samples of 15 larvae treated with chlorpyrifos and 15 control larvae.

2.2. Statistical analyses

We tested the effect of maternal body mass on clutch quality (number of eggs laid 24 hours after mating and hatching success), offspring quality (larval body mass and survival rate), and offspring sensitivity to chlorpyrifos (survival rate of treated *versus* control larvae). We tested for both direct relationships between maternal body mass and these parameters and indirect effects by testing the relationships between the parameters of clutch and offspring quality. The number of eggs laid 24 hours after mating was analyzed with a regression analysis including maternal body mass and the square of maternal body mass (quadratic term to test for nonlinearity of the relationship) as factors. For hatching success, we performed logistic regressions on the frequency of clutches with high hatching success, namely, clutches with at least 90% hatched eggs. We discretized hatching success into a binomial variable because

most clutches had a high success (mean hatching success of 88.7%, with 81.1% of the 201 clutches sampled having at least 90% hatched eggs). The first logistic regression included the number of eggs laid and its quadratic term as factors, and the second logistic regression included maternal body mass and its quadratic term as factors. Linear and quadratic relationships between larval body mass and maternal body mass, as well as between larval body mass and the number of eggs laid, were tested with regression analyses. Analyses were performed on the average body mass of 30 larvae per sampled clutch. For the analyses of larval survival rate, we performed analyses of covariance that included the pesticide treatment and its interaction with larval body mass or maternal body mass as additional factors. In all analyses, the full models were simplified by backward stepwise removal of terms for which $P > 0.10$. We checked normality of the residuals of full models in parametric analyses. Analyses were performed with JMP software (JMP Pro 15, SAS Institute Inc., USA).

3. Results

3.1. Clutch parameters

We analyzed the influence of female body mass on the number of eggs laid 24 hours after mating and hatching success. We found a quadratic relationship between the number of eggs laid 24 hours after mating and female body mass ($F_{1,198}=13.5$ $P<0.001$). This nonlinear relationship was clarified with partial tests for female body masses lower and higher than 300 mg (see Fig. 1). In females with a body mass lower than 300 mg (*i.e.*, in most (81%) of the females sampled), we found a positive relationship between female body mass and the number of eggs laid ($P<0.001$). In the heaviest females, no significant relationship was found between female body mass and the number of eggs laid ($P=0.749$). From our data of the experimental series that strictly controlled the larval density encountered by females during their ontogeny (with 1 *versus* 15 *versus* 40 larvae), we performed a complementary test on the

effect of larval density on the number of eggs laid by females. The effect of larval density was tested on the residuals of the quadratic relationship with female body mass to avoid a collinearity issue. Body mass of females was indeed dependent on the larval density they encountered during ontogeny (Supplementary Material, Figure S1). In this complementary test, the number of eggs laid was not influenced by larval density ($F_{2,113}=1.3$ $P=0.275$).

In the analysis of hatching success, we found a quadratic relationship between the frequency of clutches with high hatching success and the number of eggs laid ($X^2_1=4.3$ $P=0.038$), and this relationship also fit the percentage of hatched eggs well (Fig. 2). The lowest frequencies of successful clutches and lowest hatching percentages were observed in both the smallest and largest clutches. Despite the relationship between the number of eggs laid and female body mass, hatching success was not significantly related to female body mass ($X^2_1=0.5$ $P=0.494$ for the linear relationship, $X^2_1=0.5$ $P=0.470$ for the quadratic relationship). Moreover, hatching success was not related to the larval density encountered by females during their ontogeny ($X^2_2=3.2$ $P=0.202$: test of the density factor in the statistical model that also included the quadratic effect of the number of eggs laid).

3.2. Offspring traits

We analyzed the maternal influence on larval body mass and larval survival rate. Larval body mass was negatively related to the number of eggs laid (Fig. 3). The larger the number of eggs laid was, the lighter larvae were. This relationship was linear ($F_{1,140}=5.8$ $P=0.017$), not quadratic ($F_{1,139}=0.4$ $P=0.542$). The slope of the relationship did not significantly differ between the two body mass classes of mothers (lighter *versus* heavier than 300 mg as defined in Fig. 1) (Fig. 3; $F_{1,138}=1.6$ $P=0.207$). This relationship coupled with the relationship between the number of eggs laid and female body mass did not result in an overall relationship between larval body mass and maternal body mass ($F_{1,145}=2.6$ $P=0.107$ for the linear

relationship, $F_{1,144}=1.1$ $P=0.295$ for the quadratic relationship). Moreover, larval body mass was not related to the larval density encountered by mothers during their ontogeny ($F_{2,99}=0.5$ $P=0.627$: test of the density factor in the statistical model that also included the linear effect of the number of eggs laid). The larval and maternal masses were also not related in the mothers lighter than 300 mg ($F_{1,109}<0.1$ $P=0.866$) or in the mothers heavier than 300 mg ($F_{1,34}=0.7$ $P=0.414$). However, larval body mass differed between the two body mass classes of mothers. The larvae of females heavier than 300 mg were heavier than those of females lighter than 300 mg ($F_{1,139}=4.8$ $P=0.030$) (Fig. 3).

In the analyses of larval survival rate, we found a positive relationship between larval body mass and survival (Fig. 4; $F_{1,286}=51.2$ $P<0.001$, $F_{1,282}<0.1$ $P=0.883$ for the quadratic relationship). The slope of this relationship did not differ between control larvae and larvae treated with chlorpyrifos ($F_{1,283}=0.4$ $P=0.536$), and we observed only the expected decrease in survival in response to chlorpyrifos ($F_{1,286}=166.7$ $P<0.001$) (Fig. 4). The positive relationship between larval body mass and survival was also similar between the two body mass classes of mothers ($F_{1,284}=0.7$ $P=0.399$), and larval survival did not significantly differ between the two body mass classes of mothers ($F_{1,285}=0.1$ $P=0.700$). In addition, larval survival was not related to the larval density encountered by mothers during their ontogeny ($F_{2,206}=2.4$ $P=0.091$: test of the density factor in the statistical model that also included the effects of larval body mass and chlorpyrifos).

3.3. Offspring sensitivity to chlorpyrifos

Maternal body mass influenced offspring sensitivity to chlorpyrifos for mothers with body masses less than 300 mg (Fig. 5). Indeed, we found an interaction between pesticide treatment and maternal body mass ($F_{1,218}=11.1$ $P=0.001$), with a negative relationship between larval survival and maternal body mass in larvae treated with chlorpyrifos ($F_{1,109}=13.6$ $P<0.001$) but not in control larvae ($F_{1,109}=0.6$ $P=0.446$). Therefore, pesticide toxicity was higher in larvae of larger mothers. The relationship between larval survival and maternal body mass was linear ($F_{1,218}=5.5$ $P=0.019$), not quadratic ($F_{1,217}=0.1$ $P=0.818$). In the class of mothers heavier than 300 mg, we did not find a significant influence of maternal body mass ($F_{1,69}=0.1$ $P=0.732$ for the linear effect, $F_{1,68}=2.8$ $P=0.101$ for the quadratic effect, and $F_{1,67}=1.6$ $P=0.210$ for the interaction with pesticide treatment).

4. Discussion

Mothers often have a strong influence on the energetic provisioning of embryos and offspring size (Bernardo, 1996a, 1996b). Consequently, maternal effects are a great cause of variation in offspring morphology and life history traits, as confirmed in a recent meta-analysis (Moore et al., 2019). This was also observed in our study, with an influence of maternal body mass on larval body mass (difference between the two body mass classes of mothers, Fig. 3) and larval survival (Fig. 5). Specifically, we tested whether the sensitivity of larvae to chlorpyrifos was dependent on maternal body mass. We expected a transgenerational effect as previously observed in *C. pipiens* (Tran et al., 2018). Because offspring size (*sensu lato*, i.e., also mass) is usually related positively to maternal body size (mass) (Lim et al., 2014) and offspring size usually reflects offspring quality (Allen et al., 2008; Rollinson and Hutchings, 2013), sensitivity to chlorpyrifos was expected to be lower in the larvae of heavier mothers. Surprisingly, we found the opposite result.

How can we explain this unexpected result? We must question the hypotheses of relationships between offspring mass and maternal mass and between offspring mass and offspring quality. Our unexpected result cannot be explained by an unexpected negative relationship between offspring mass and offspring quality because we found that larval mass was positively related to larval survival (Fig. 4). Finding the explanation therefore requires searching with respect to the hypothesis that offspring mass is related positively to maternal mass. Indeed, we did not find an overall relationship between offspring and maternal masses or a specific relationship between offspring and maternal mass in mothers lighter than 300 mg (81% of sampled females), in which we observed an effect on offspring sensitivity to chlorpyrifos. We found only that offspring mass differed between the two body mass classes of mothers (lighter *versus* heavier than 300 mg), but this does not explain the result of sensitivity to chlorpyrifos observed in larvae of the mothers lighter than 300 mg (Fig. 5). Therefore, larval mass did not appear to contribute to larval sensitivity to chlorpyrifos. This is also supported by the fact that the slope of relationships between larval mass and survival did not differ between control and treated larvae. The possible age bias in our measurements of larval mass (see Materials and methods) did not seem to be an issue here because larval mass did not contribute to larval sensitivity to chlorpyrifos.

Other offspring characteristics should have been linked to maternal body mass and have contributed to the variation in larval sensitivity to the pesticide. If offspring size is a common target of maternal effects, a range of other offspring characteristics can also be influenced by maternal effects (Bernardo, 1996a; Moore et al., 2019). Indeed, maternal effects can cause offspring to differ in various ways, such as energetic provisioning, proteins, nutrients, hormones, and toxins (Wolf and Wade, 2009). In the mothers lighter than 300 mg, for which we found a maternal effect on sensitivity to chlorpyrifos, we also observed a positive relationship between the number of eggs laid and maternal body mass (Fig. 1). This higher

investment in reproduction could have been performed to the detriment of egg quality because of lower energetic provisioning to embryos (Bernardo, 1996a, 1996b; Mousseau and Dingle, 1991) or any other aspect of offspring (Wolf and Wade, 2009). Indeed, such a reproductive trade-off between fecundity and offspring quality is commonly expected (Smith and Fretwell, 1974; Stearns, 1992). What could be the alternatives to this reproductive trade-off hypothesis linked to a maternal effect on offspring quality? Epigenetic alterations (DNA methylation and histone modification) in offspring related to maternal body mass are also possible (Latzel, 2015; Oppold et al., 2015), and it will be especially interesting to test for them in the future. In contrast, direct genetic covariation between maternal body mass and offspring quality is not a likely alternative because the source of variation in maternal body mass was mainly environmental in our rearing of *S. littoralis*. Indeed, we observed that adult female body mass showed rapid temporal fluctuations (Supplementary Material, Figure S2). A cause of these fluctuations was likely an effect of larval density during ontogeny because adult female body mass was negatively related to larval density (Supplementary Material, Figure S1). Because we provided food *ad libitum* in the rearing boxes to avoid global food limitation, we hypothesize that the limiting effect of larval density was an indirect effect of density on social interactions (chemical or/and behavioral interactions among larvae). Independently of the effect of larval density on adult female body mass, larval density was not found to influence the number of eggs laid by females and hatching success, and to lead to intergenerational effects on larval body mass and survival.

A small portion of ecotoxicological literature is dedicated to the impact of pesticides on several generations (Brausch and Salice, 2011; Sarkar et al., 2018; Silva et al., 2019), but studies on the transgenerational effects of other factors on sensitivity of pesticides are particularly limited. A study on the mosquito *C. pipiens* revealed an effect of the temperature experienced by the parental generation (interacting with parental pesticide exposure) on

offspring sensitivity to chlorpyrifos pesticide (Tran et al., 2018). Low food availability in the parental generation in *Daphnia magna* was found to increase the size of offspring, reduce their age at first reproduction, increase their reproductive output, and decrease their sensitivity to fenvalerate pesticide (Pieters and Liess, 2006). Current knowledge of the effects of maternal traits and ecological environments on offspring sensitivity to pesticides remains limited compared to the literature dedicated to other maternal effects. The global literature on maternal effects expanded greatly over the last three decades and showed that these effects are common and affect a diversity of traits (Moore et al., 2019). Thus, we encourage ecotoxicologists to pay more attention to the impact of maternal effects on sensitivity to pesticides, both in pests and non-target organisms.

CRedit authorship contribution statement

Thibaut Bagni: Conceptualization, Methodology, Writing - original draft, . **David Siaussat:** Funding acquisition, Writing - review & editing. **Annick Maria:** Methodology. **Philippe Couzi:** Methodology. **Martine Maibèche:** Funding acquisition, Writing - review & editing. **Manuel Massot:** Conceptualization, Funding acquisition, Writing - review & editing, Supervision.

Acknowledgements

This research was supported by the Région Ile-de-France (DIM R2DS 2014).

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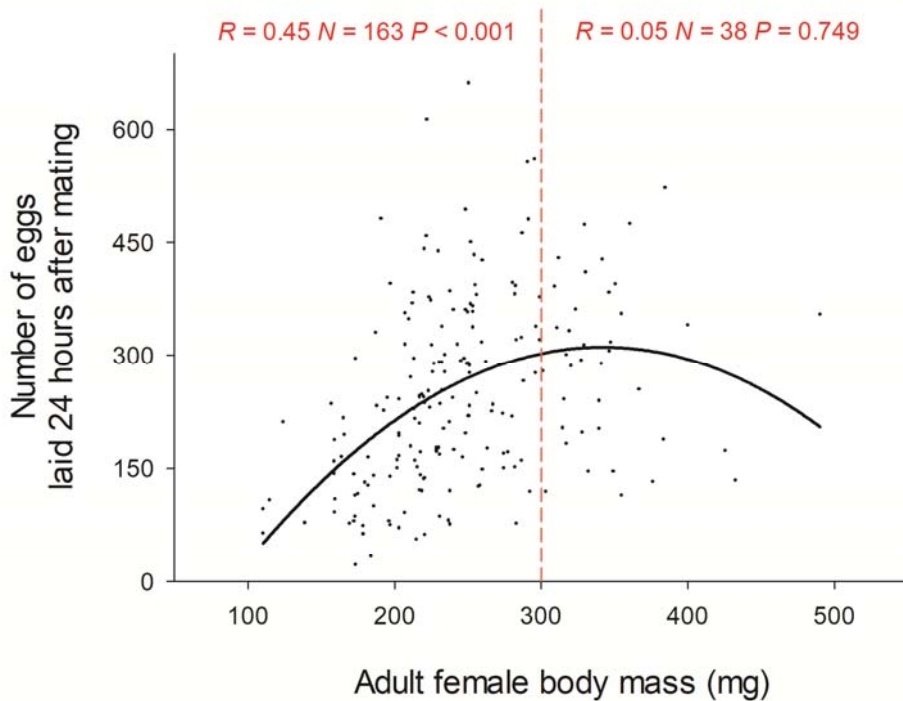


Fig 1. Quadratic relationship between the number of eggs laid 24 hours after mating and female body mass. The results of partial tests of females with body masses lower and higher than 300 mg are reported in red.

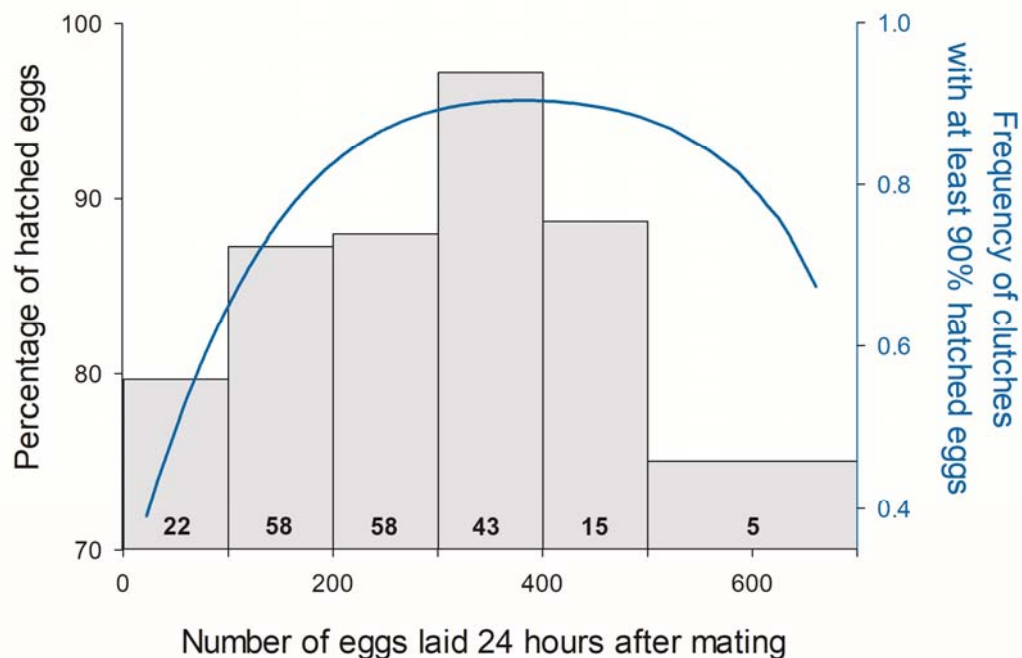


Fig. 2. Relationships between hatching success and the number of eggs laid 24 hours after mating. The histogram shows the percentage of hatched eggs, with the numbers of clutches sampled. The blue curve is the frequency of clutches with a hatching success higher than 90%.

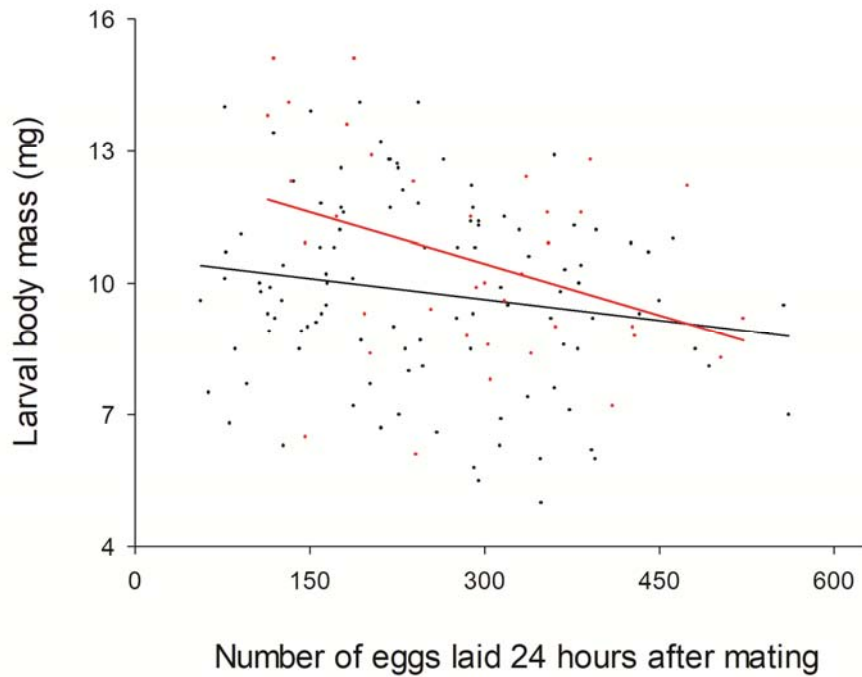


Fig. 3. Relationships between larval body mass and the number of eggs laid 24 hours after mating. The black points and line are for mothers with body masses lower than 300 mg, and the red points and line are for mothers with body masses higher than 300 mg.

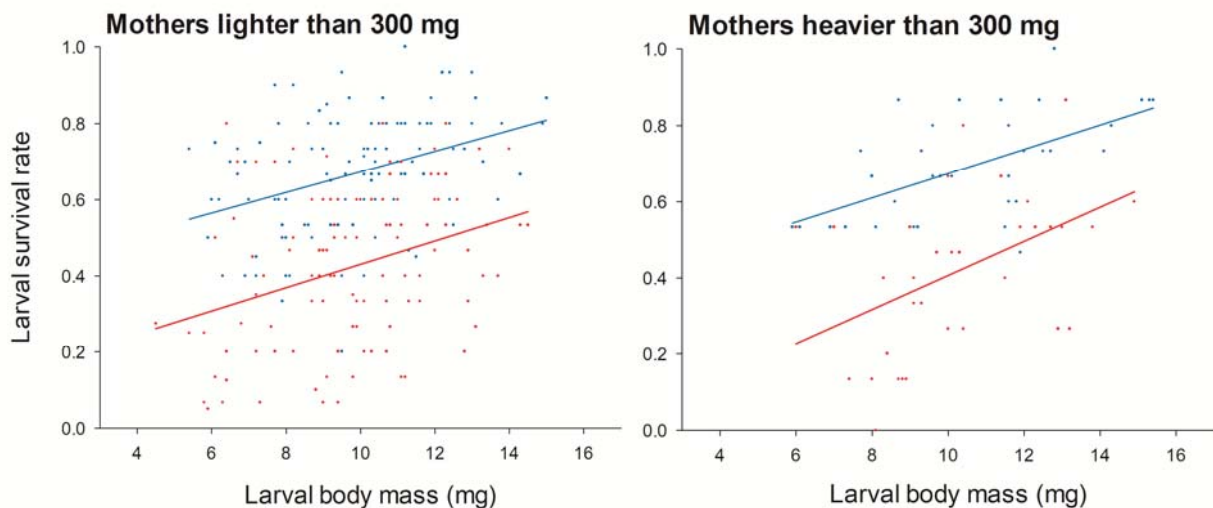


Fig. 4. Relationship between the larval survival rate and larval body mass in larvae of mothers lighter and heavier than 300 mg. Control larvae are in blue, and larvae treated with chlorpyrifos are in red.

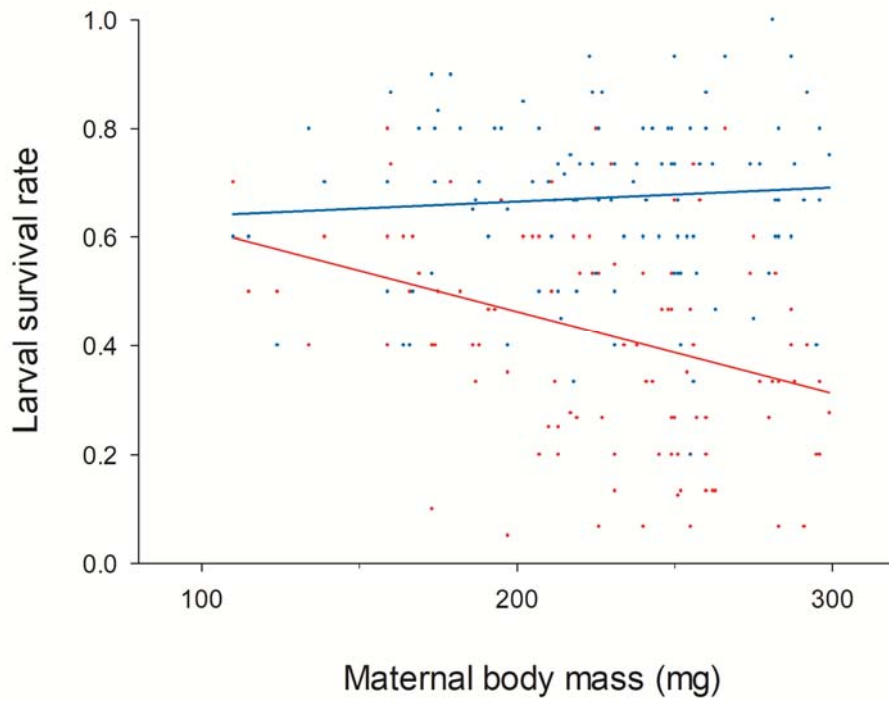


Fig. 5. Relationship between larval survival rate and maternal body mass in mothers with body masses less than 300 mg. Control larvae are in blue, and larvae treated with chlorpyrifos are in red. For mothers with body masses greater than 300 mg ($n = 36$), the mean survival rate \pm s.e.m. was 0.693 ± 0.023 in control larvae and 0.424 ± 0.036 in larvae treated with chlorpyrifos.

APPENDIX A

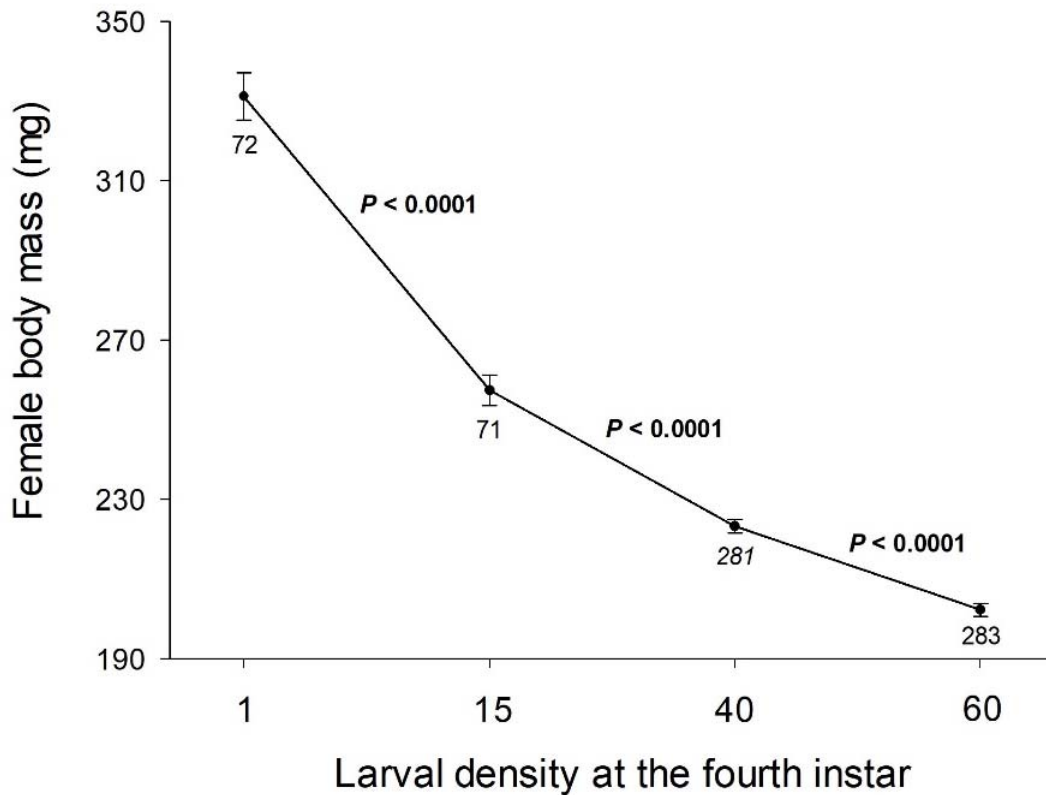


Figure S1. Influence of the larval density during ontogeny on adult female body mass. Larval density was experimentally controlled at the fourth instar (1 vs 15 vs 40 vs 60 larvae per rearing box). We found a negative effect of larval density during ontogeny on female body mass. *P* values of Student's *t* tests are reported in the figure, vertical lines represent s.e.m., and the numbers indicate the sample sizes. The data with the density of 60 larvae did not appear in other analyses of the study because this experimental series was only a complementary test of the larval density effect on female body mass, *i.e.* with no measurements on female reproduction and offspring traits. In this complementary test, the density of 60 larvae was only compared to an experimental group with the density of 40 larvae.

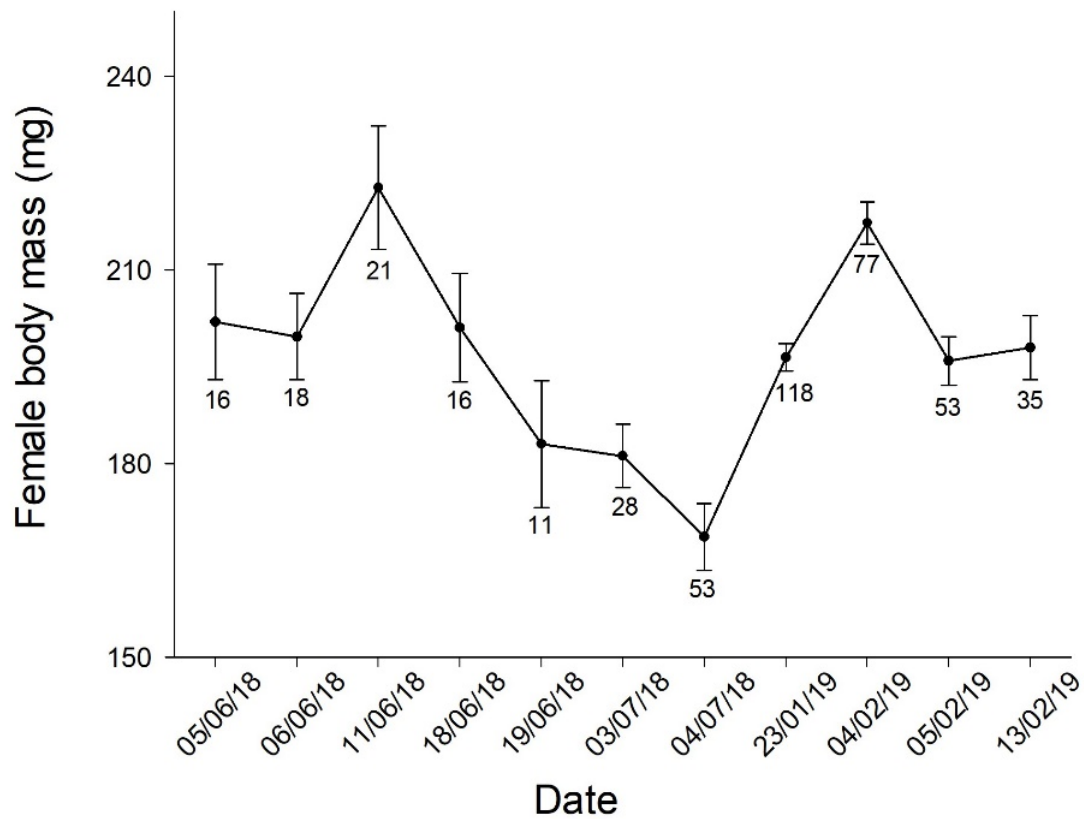


Figure S2. Survey of body mass measured one day after emergence of adult females. Temporal variation was significant ($F_{10,435}=11.0$, $P<0.0001$). Vertical lines represent s.e.m., and the numbers reported in the figure indicate the sample sizes. We increased the sample sizes at the end of the temporal series when it became clear that the body mass of adult females was a key parameter to control in our studies.