Evolutionary consequences of pesticide exposure include transgenerational plasticity and potential terminal investment transgenerational effects

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Transgenerational plasticity, the influence of the environment experienced by parents on the phenotype and fitness of subsequent generations, is being increasingly recognized. Human-altered environments, such as those resulting from the increasing use of pesticides, may be major drivers of such cross-generational influences, which in turn may have profound evolutionary and ecological repercussions. Most of these consequences are, however, unknown. Whether transgenerational plasticity elicited by pesticide exposure is common, and the consequences of its potential carryover effects on fitness and population dynamics, remains to be determined. Here, we investigate whether exposure of parents to a common pesticide elicits intra-, inter-, and transgenerational responses (in F0, F1, and F2 generations) in life history (fecundity, longevity, and lifetime reproductive success), in an insect model system, the seed beetle Callosobruchus maculatus. We also assessed sex specificity of the effects. We found sex-specific and hormetic intergenerational and transgenerational effects on longevity and lifetime reproductive success, manifested both in the form of maternal and paternal effects. In addition, the transgenerational effects via mothers detected in this study are consistent with a new concept: terminal investment transgenerational effects. Such effects could underlie cross-generational responses to environmental perturbation. Our results indicate that pesticide exposure leads to unanticipated effects on population dynamics and have far-reaching ecological and evolutionary implications.

KEY WORDS: Callosobruchus maculatus, epigenetics, nongenetic inheritance, pesticide-induced transgenerational effects, sex-specific transgenerational effects, terminal investment transgenerational effects.

Environmental conditions are constantly changing, sometimes in a highly predictable way, as in seasonal variation, tidal cycles, or daily photoperiod variation, and sometimes in a much less predictable way, as when prey encounter predators or hosts are exposed to pathogens. When environmental changes occur, populations require substantial standing genetic variation to persist (Matuszewski et al. 2015; Lai et al. 2019). However, adaptation under rapid environmental change can also occur without apparent genetic differentiation through adaptive plasticity and/or nongenetic inheritance of adaptive phenotypes (Pigliucci 2005; Suzuki and Nijhout 2008; Gomez-Mestre and Jovani 2013; Lind et al. 2015; Bonduriansky and Day 2020). Organisms may respond to global anthropogenic disturbances like rises in temperature, pesticide pollution, or irruption of invasive predators, with plastic changes in phenology, morphology, or physiology, rather than on differential survival and selection on genetic polymorphisms (Przybylo et al. 2000; Charmantier et al. 2008; Hua et al. 2015; Sgrò et al. 2016).
In addition to intragenerational plasticity, organisms can also experience transgenerational plasticity such that the interaction between parents and their environment can lead to phenotypic modifications in the offspring and even in subsequent generations through nongenetic inheritance (Salinas et al. 2013; Bonduriansky and Day 2020). The mechanisms underlying nongenetic inheritance are complex and linked to epigenetics (Jablonska and Raz 2009; Herman and Sultan 2016; Lindeman et al. 2019; Bonduriansky and Day 2020) and parental effects (Mousseau and Fox 1998a; Räsänen and Kruuk 2007; Curley et al. 2011; Crean and Bonduriansky 2014). Parental effects refer to the influence of parents on the phenotype of their offspring exerted by means other than allelic transmission (Shama and Wegner 2014; Garcia-Gonzalez and Dowling 2015; Brevik et al. 2018; Lymbery et al. 2020). These effects may be determined by behavior, condition, or the environment (including the social environment) experienced by parents. Maternal effects have been extensively studied and are well known to be prominent in shaping offspring features and adaptive evolution (Mousseau and Fox 1998b; Räsänen and Kruuk 2007). Seemingly simple maternal investment decisions have profound implications for the offspring’s phenotype, as it is the case with oviposition site choices and provisioning in dung beetles, where these decisions determine key offspring sexual traits (Moczek and Emlen 1999; Hunt and Simmons 2000).

In birds, for instance, testosterone deposition in the yolk is a common mechanism for females to modulate offspring’s growth and immune status (Schwabl 1993; Gil et al. 1999; Mazuc et al. 2003). Paternal effects have received comparatively less attention, probably because males were supposed to contribute little else than genetic material to the next generation, except in species with direct paternal care. However, the paternal role in nongenetic inheritance has lately gotten renewed attention (García-González and Simmons 2007; Lane et al. 2014; Simmons and Lovegrove 2019). Paternal contributions to offspring phenotypes are known to be transmitted via the nonsperm fraction of the ejaculate, or through epigenetic programming via DNA methylation, histone modifications, and noncoding sperm RNAs (Rodgers et al. 2015; Klosin et al. 2017; Conine et al. 2018; Evans et al. 2019).

Recent studies demonstrate that chemical substances introduced into the environment by human activity, such as toxicants and endocrine disruptors, can be major drivers of phenotypic changes, and not only in the individuals directly being exposed to these substances but also in their progeny (Räsänen and Kruuk 2007; Curley et al. 2011; Soubry et al. 2014; Evans et al. 2019). Human-altered activities and environments may thus have profound evolutionary and ecological consequences via unanticipated transgenerational effects on subsequent generations. Most of these consequences are, however, unknown. A greater focus on whether toxicants, pollutants, and anthropic activities in general play a role in transgenerational plasticity is, therefore, critically needed (Donelan et al. 2020). Pesticides and insecticides impose a sudden harsh environmental change to insects and other organisms, representing a major challenge to them (Zhang 2018). The way these toxicants are applied in the field is often recurrent in time and persistent in the environment. The role of these compounds driving the evolution of insecticide resistance due to selection is well known (Jacomb et al. 2016). More recently, however, it has been noted that pesticides may drive the evolution of resistance via epigenetic mechanisms (Brevik et al. 2018). Regardless of whether the use of pesticides boosts the evolution of resistance, recent evidence indicates that pesticides can elicit intergenerational effects when sublethal concentrations are applied. Margus et al. (2019) found higher survival for offspring of female Colorado potato beetles exposed to insecticides compared to offspring from control mothers. It remains to be determined whether these effects are common, whether they underlie transgenerational plasticity, what their underlying mechanisms are, and what consequences they have, not only for individual fitness, but also for population dynamics and pest control.

Here, we test whether exposure to sublethal doses of a commonly used pesticide elicits intra-, inter-, and transgenerational responses (parental, offspring, and grandoffspring responses) in an insect model system, the seed beetle Callosobruchus maculatus (Coleoptera, Chrysomelidae, Bruchinae), which is a pest of stored legumes (Southgate 1979; Utida 1981; Fox 1993a). This species is distributed worldwide and multiple measures to control their populations have been designed, often involving pesticides (Sanon et al. 2018). We experimentally exposed males and females to sublethal concentrations of Deltamethrin, a common pyrethroid pesticide broadly used for insect pest control that interferes with the normal nerve signals by affecting the calcium and sodium ion channels (Deng et al. 2021). Insecticides and pesticides are known to elicit hormesis, that is, biphasic dose-dependent effects such that a low dose can be stimulatory and a higher dose can be inhibitory, or vice versa (Guedes and Cutler 2014; Tang et al. 2019), and a pattern characteristic of many endocrine disruptors is that they produce nonlinear dose-dependent relationships (resulting in, for instance, greater mortality at lower doses than at higher doses) (Vom Saal 2007; Larderie et al. 2015). To assess hormetic effects in our system, we used two different concentrations of the pesticide.

Sexual dimorphism, sex-specific selection, and sex-specific responses are common features of sexually reproducing species. Nevertheless, little research has been done to assess sex-specific transgenerational effects, to test for interactions between the sex of progenitors exposed to environmental cues and the sex of their offspring, or to investigate causal relationships between sexual conflict and sex-specific transgenerational plasticity (Bell and Hellmann 2019; Burke et al. 2020; Hellmann et al. 2020). Here, we examine the existence of sex-specific transgenerational
effects, both attending to the sex of the parent being exposed to the environmental stressor and the sex of the offspring and grand-offspring potentially being affected via nongenetic inheritance. Our main aims were thus to (i) test for transgenerational effects resulting from exposure of the parental generation to the toxicant; (ii) assess the relative importance of paternal and maternal effects in driving phenotypic and life-history modifications in their offspring; and (iii) test whether transgenerational effects differ in magnitude and persistence according to the descendant’s sex. To this end, after parental exposure to the pesticide, we measured three key life-history traits (longevity, fecundity, and lifetime reproductive success [LRS]), across three generations, without further contact with the pesticide beyond F0 exposure.

Stress exposure in the parental generation could simply constitute a carryover effect that would drag the development, growth, or reproduction of the subsequent generation. Alternatively, costly stress exposure could trigger an additional effort in resource allocation to the current reproductive event at the expense of future reproduction and survival, a phenomenon called terminal investment (Williams 1966; Clutton-Brock 1984; Velando et al. 2006; Creighton et al. 2009; Froy et al. 2013). For instance, Bowers et al.’s (2012, 2015) studies on the house wren found that females whose immune system was challenged increased pre- and postnatal maternal investment to offspring, leading to positive effects in the F1 generation. However, “terminal investment transgenerational effects,” that is, effects of terminal investment at generation F0 permeating through multiple successive generations, have not yet received attention. In this study, we introduce the idea that inter- and transgenerational terminal investments may play a role in adaptive or nonadaptive responses to environmental disturbances. We found evidence for remarkable intergenerational and transgenerational effects of pesticide exposure on offspring fitness. We also found evidence for a potential terminal maternal investment effect triggered by pesticide exposure impacting offspring life histories.

Materials and Methods

Callosobruchus maculatus is a seed beetle that infests dry legumes and causes damage in grain stores worldwide, having an important impact on the economy (Prevett 1961; Javaid and Poswal 1995). It has a short generation time (3–4 weeks) and high fecundity, each female being able to lay around 80 eggs during her life span (Fox 1993a; Canal et al. 2021). This species constitutes a relevant and tractable laboratory model system for studying transgenerational effects. Beetles can be raised in high numbers and measures of LRS can be obtained for several generations over the course of a few months. Adults search for mates, mate, and reproduce using metabolic water and the resources ac-
quired during larval development (i.e., they are capital breeders) (Fox et al. 2003). The fact that adult beetles do not eat or drink (they cannot feed on the seeds, only the larvae feed on the inside of seeds) eliminates sources of variation that may obscure the assessment of paternal effects. Furthermore, the laboratory conditions under which the animals are maintained mimic those in grain storages to which the species has adapted for thousands of years (e.g., Berger et al. 2014).

We established the stock population used for this study at Doñana Biological Station, CSIC (Seville, Spain) in 2013 using several hundreds of founders sourced from the South Indian population (Fox et al. 2003; Bilde et al. 2008; Berg and Maklakov 2012). The population has been maintained in high numbers (Zajitschek et al. 2018), and with nonoverlapping generations since then in climate chambers at a constant temperature of 29°C, with 40% humidity and a 12:12 L:D photoperiod using mung beans (Vigna radiata) as host. Females of this polygamous species lay eggs on the surface of dry beans. In our population, females typically lay a single egg per bean when mung beans are used. After hatching, the larva burrows into the bean, where it feeds until it reaches the pupal stage. Once the adult stage is reached, beetles drill a hole out of the bean, and they are ready for reproduction soon after emergence.

EXPERIMENTAL SETUP

We individually exposed male and female beetles from the stock to either water (controls) or sublethal concentrations of pesticide. We applied the experimental treatments only to the parental generation, and recorded longevity, fecundity, LRS, and body size (assessed as dry body weight; see below) of individuals up to the second generation (F2), after mating the focal parent to standardized mates. Sample size doubled with each subsequent generation as we randomly selected one son and one daughter from each pair of parents to start each subsequent generation to test for sex-related differences in transgenerational effects (Fig. 1). The total number of individuals scored was expected to be 1050 (75 F0 mothers, 75 F0 fathers, 150 F1 daughters, 150 F1 sons, 300 F2 granddaughters, 300 F2 grandsons), but due to some individuals escaping and accidental mishaps, the final sample sizes were slightly lower and varied for the different traits (sample size for each analysis is indicated in the tables in the Results section).

We placed inoculated beans individually in Eppendorf tubes to ensure virginity of the emerging adult beetles and to keep track of individual age. Bean isolation was done 11 days after female oviposition, to minimize potential effects of mechanical manipulation on freshly laid eggs or early instar larvae inside the beans. The age of individuals at the time of exposure to the experimental treatments (toxicant/control) ranged between 1 and 4 days. We exposed each individual beetle to either one of two
nonlethal concentrations of the pesticide (1 g/L and 2 g/L COMBO Deltamethrin, 2.5% w/v, Sarabia) or to carbon-filtered dechlorinated tap water (control group) inside a flow cabinet at room temperature for 24 h. We previously conducted pilot tests to identify nonlethal concentrations of the pesticide for this species (see Fig. S1). We aimed to expose parental beetles to sublethal concentrations so as not to confound potential transgenerational effects (influences on offspring’s phenotype via nongenetic inheritance) with genetic changes due to differential survival, that is, selection. Each beetle was randomly allocated to a single treatment (control, low pesticide, high pesticide). We exposed 25 F0 individuals from each sex to each treatment, for a total of 150 initial individuals (25 individuals × 2 sexes × 3 treatments; Fig. 1).

To expose each parental beetle to its corresponding treatment, we impregnated the ends of cotton swabs with 30 µL of either pesticide or water and placed these in 2-mL Eppendorf tubes that we kept open. We then introduced the target beetle in another tube with the bottom cut out and fit this tube into the one containing the impregnated swab, placing a mesh in between both tubes. This setup allowed exposure of beetles to airborne pesticide but prevented direct contact with it. After the exposure phase, focal beetles were individually placed into 26-mL plastic vials with pinholes in the cap to allow airflow, and ad libitum beans to be used as oviposition substrate. Each individual shared the vial with a tester nonexposed mate (male or female). These tester individuals were sourced from a standardized heterozygote tester line that was generated by crossing two near-isogenic lines generated after 17 generations following a brother-sister mating protocol. The use of these genetically homogeneous tester individuals minimizes sampling variance arising from random sampling of mates (see for rationale and application Garcia-Gonzalez and Evans 2011; Garcia-Gonzalez and Dowling 2015; Travers et al. 2015), and also ensures that transgenerational effects would be only minimally obscured by genetic or nongenetic variation introduced by individuals used as mates (Garcia-Gonzalez and Dowling 2015). We allowed beetles to mate and lay eggs in the vial for 48 h. Afterward, we extracted males from the vials and individually kept them in Eppendorf tubes, to track their longevity.

We then transferred mated females (focal females mated to tester males, or tester females mated to focal males) to a second 26-mL container with ad libitum beans where they laid eggs for the remainder of their life span. We counted the lifetime number of eggs (fecundity) and number of adult offspring (LRS) produced by each female. LRS is a good estimate of fitness, as it measures the lifetime production of sexually mature progeny. We did not expose the two subsequent generations (F1 and F2) to the pesticide, but kept housing and mating conditions identical to those of the parental generation. To obtain virgin F1 individuals, we waited until day 11 after oviposition by parental females (see above) and individually isolated beans from the mating vials in Eppendorf tubes, as described above. We followed the same procedure in the F2 generation.

We tracked longevity by checking the survival of isolated individuals daily. We determined fecundity by carefully inspecting all beans and counting the total number of eggs laid by each female, both in the mating vial and the subsequent oviposition vial (only for F0 and F1 generations). We measured LRS as number of adults produced by each female during her lifetime. To obtain these data, we froze vials on day 28 since the last possible day of oviposition (in the second oviposition vial, the day the female was found dead), and later counted the number of adult individuals present in the vial. This procedure ensures that our LRS measurement excludes individuals from a subsequent generation (i.e., produced by crossings between siblings), because
we take into account female egg laying span and the timing of egg-to-adult transition. Reproducing females only live a few days (15 days maximum; mean longevity ± SE = 8.38 ± 0.069; 99% of females lived less than 13 days) and their fecundity is maximal the first few days after mating (Zajitschek et al. 2018), hardly laying any eggs after the first week postmating (unless they are housed with males and remate during this period). Moreover, new adults take about 23 days to emerge from newly laid eggs under our experimental conditions and virtually all adults emerge from the beans within 28 days (Rodriguez-Exposito 2018; Rodriguez-Exposito and Garcia-Gonzalez 2021). We therefore preserved the vials after all adults have emerged, but before any of their subsequent offspring could have emerged. LRS has two components. First, an intragenerational component, because a female’s total number of adult offspring produced over its life span is largely determined by fecundity and effects of mothers on egg-to-adult viability (including maternal effects such as egg provisioning). Second, an intergenerational component, because egg-to-adult viability is expected to be largely dependent on properties of the individual offspring. This consideration also affects the interpretation of changes in LRS in the F1 and F2 generations discussed below, and generally, it implies that our conclusions on the extent of cross-generational influences of pesticide exposure are conservative.

We measured the dry weight of focal beetles with a Sartorius Cubis MSA6.6S microbalance (readability 0.001 mg; Sartorius, Göttingen, Germany). Beetles were previously frozen at −20°C (after death, to be able to track longevity), and afterward thawed and dried to constant weight on an oven at 40°C for ~1 week, hence removing variation in body weight due to variation in time elapsed between death and freezing.

**STATISTICAL ANALYSES**

We used linear models (LMs; F0 and F1 generations) and general linear mixed models (GLMMs; F2 generation) to test whether experimental treatments applied to parental individuals affected not only their phenotype and life-history traits, but also those of subsequent generations. Our response variables were longevity, fecundity, and LRS, all of which were normally or approximately normally distributed. Models included the experimental treatment as a fixed factor. Longevity was included as covariate in models testing effects on fecundity and LRS. Body size of the focal individual was included as covariate for longevity of those focal individuals, whereas body size of the female (focal or tester) was included as covariate for fecundity and LRS. Individuals’ age at mating (1–4 days old) was also entered as a covariate. Longevity was also analyzed by means of survival analysis using Cox models, and Cox mixed models for the F2 generation. Results from these proportional hazards models are consistent with those from the LMs and the GLMMs (Table S4).

To ease the interpretation of results and also because in this species males and females have distinct life-histories (Hallsson and Björklund 2012), we conducted the analyses separately according to sex of the focal individuals and by the sex of the parent/grandparent (for F1 and F2, respectively) initially exposed to treatment. We fitted LMs to the data from the F0 and F1 generations using the function “lme” in R (R Core Team 2016). Since we only took one male and one female per cross in each generation, and because we conducted the analyses separately by sex, F1 data analyses did not require inclusion of a random effect to correct for parental ID. For the F2 generation, we fitted GLMMs using the “lmer” function in the “lme4” package (Bates et al. 2014) including grandparent ID as a random factor. P-values were calculated on maximum likelihood models, whereas restricted maximum likelihood (REML) models were run to obtain parameter estimates. The package pbkrtest (Halekoh and Hojsgaard 2014) was used to calculate the degrees of freedom for the estimates in the case of GLMMs. A few instances of F0 LRS data <10 were excluded from the analyses a priori because such low fertility data are likely outliers due to intrinsic individual health problems or infertility problems (Rodriguez-Exposito 2018). The few cases of LRS <10 were distributed across groups, including the controls, which supports the notion that these cases are unrelated to the treatment (number of cases in the female-treated dataset: two in the control group, one in the low group, two in the high group; number of cases in the male-treated dataset: one in the control group, two in the low group). Due to the large number of P-values estimated throughout our study, we controlled the false discovery rate using the p.adjust function in R applying the Benjamini-Hochberg method (Benjamini and Hochberg 1995). We corrected P-values within each generation and show adjusted P values in the Results section and the tables.

We also calculated cross-generational multiplicative fitness for females and males exposed to treatment and we did so both via daughters and via sons (Fig. S2). In these calculations, a 1:1 sex ratio was assumed. Multiplicative fitness via daughters for each F0 individual was calculated by multiplying its LRS (halved, as half of the number of adults produced would be daughters, assuming equal sex ratio) by the LRS of his/her daughters (F1), and by the LRS of his/her grandoffspring (F2) produced via daughters. We followed a similar procedure to calculate multiplicative fitness via sons. Due to the multiplicative nature of these calculations, sample sizes in these analyses are reduced compared to the analyses above because missing values for any family in any given generation implies a missing value for the net fitness of a particular lineage across the three generations (see final sample sizes for the lineages in the Results section). As before, F0 LRS data <10 were excluded a priori from the analyses. Effect sizes (Cohen’s d, i.e., the mean difference
Table 1. Results from linear models testing the effects of pesticide exposure on intragenerational responses in females and males. Significant adjusted P-values following Benjamini-Hochberg procedure are bolded. Final sample sizes in the analyses: F0 exposed females (70), F0 exposed males (72).

| Effect                      | Sum of squares (SS) | d.f. | P-value | β (SE) d.f. = 65 |
|-----------------------------|---------------------|------|---------|------------------|
| **Female longevity**        |                     |      |         |                  |
| Intercept                   | 9.168 (1.329)       |      | 0.000   |                  |
| Treatment                   | 1.585 2             |      | 0.639   | 0.253 (0.268)    |
|                              | 3.181 1             |      | 0.000   | 0.214 (0.489)    |
| Female’s body size          | 8.671 1             |      | 0.002   | 2.759 (0.825)    |
| Female’s age at mating      | 49.921 1            |      | <0.001  | 0.308 (0.068)    |
| Residuals                   | 53.285 65           |      |         |                  |
| **Male longevity**          |                     |      |         |                  |
| Intercept                   | 6.412 (2.216)       |      | 0.000   |                  |
| Treatment                   | 4.778 2             |      | 0.407   | 0.378 (0.498)    |
|                              | 19.384 1            |      | 0.000   | 0.275 (0.549)    |
| Male’s body size            | 3.181 1             |      | 0.000   | 0.214 (0.489)    |
| Male’s age at mating        | 19.384 1            |      | 0.000   | 0.275 (0.549)    |
| Residuals                   | 167.605 64          |      |         |                  |
| **Female fecundity**        |                     |      |         |                  |
| Intercept                   | 69.575 (22.528)     |      | 0.000   |                  |
| Treatment                   | 589.8 2             |      | 0.300   | 0.087 (3.470)    |
|                              | 199.9 1             |      | 0.306   | 0.050 (3.457)    |
| Female’s body size          | 199.9 1             |      | 0.230   | 0.067 (8.254)    |
| Female’s age at mating      | 0.9 1               |      | 0.006   | 0.307 (2.422)    |
| Female’s longevity          | 17.2 1              |      | 0.000   | 0.282 (2.422)    |
| Residuals                   | 8704.9 64           |      |         |                  |
| **Male’s mate fecundity**   |                     |      |         |                  |
| Intercept                   | 46.312 (13.639)     |      | 0.000   |                  |
| Treatment                   | 40.0 2              |      | 0.819   | 2.195 (2.996)    |
|                              | 1559.0 1            |      | 0.001   | 0.282 (2.422)    |
| Tester’s female’s body size | 14.6 1              |      | 0.147   | 0.307 (2.422)    |
| Tester’s female’s age at mating | 156.2 |      |         |                  |
| Female’s longevity          | 2956.9 1            |      | <0.001  | 0.000 (2.996)    |
| Residuals                   | 6484.1 65           |      |         |                  |

(Continued)
### Table 1. (Continued).

| Effect                        | Female lifetime reproductive success | Male’s mate lifetime reproductive success |
|-------------------------------|------------------------------------|------------------------------------------|
|                               | Sum of squares (SS) | d.f. | Female’s current body size (d.f. = 64) | Sum of squares (SS) | d.f. | Male’s current body size (d.f. = 65) |
| Intercept                     | 49.265 (19.469)     |  | 23.642 (12.125)                       | 23.642 (12.125)     |
| Treatment                     | 533.0               | 2   | 3  | 0.221 | 0.080 | 0.257 | 6.831 (2.998)                       | 2.868 (2.988) |
| 1 g/L                         | −6.831              | 1   | 0.926 | 0.340 | 0.623 | 6.864 (7.133)                       | 2g/L          |
| 2 g/L                         | −2.868              |     |     |       |       |                                   | −1.643 (2.663) | −1.628 (2.605) |
| Female’s age at mating        | 37.2                | 1   | 0.366 | 0.547 | 0.803 | −1.266 (2.093)                      | 441.1         |
| 1 g/L                         | −1.266              | 1   |     |       |       |                                   | −3.917 (1.656) |
| Female’s longevity            | 0.8                 | 1   | 0.008 | 0.929 | 0.937 | −0.124 (1.381)                      | 1508.5        |
| 1 g/L                         | −0.124              | 1   |     |       |       |                                   | 19.134 <0.001 | 0.142 | 4.054 (0.927) |
| Residuals                     | 6501.1              | 64  |     |       |       |                                   | 5124.5        | 65  |
Results

INTRAGENERATIONAL EFFECTS
Pesticide exposure had a marginal effect on the LRS of F0 females, though this effect did not pass correction for false discovery rate (Table 1). Notwithstanding, given that crucial interpretations in our study hinge on the existence of costs of pesticide exposure for treated individuals, we calculated effect sizes for the difference in multiplicative fitness between groups were calculated using the package `compute.es` (Re 2013). Cohen’s $d$ for intragenerational effects of the pesticide on female fitness (see below) was calculated from raw data using `compute.es`, but also using marginal means from the model, with the package `emmeans` (Lenth et al. 2018).

For simplicity, we do not state or discuss specific influences of covariables on the response variables. Their effects are nonetheless reflected on the tables. Notably, transgenerational effects were detected even after controlling for these influences. Graphical representation of data for response variables that were not affected by treatment can be found in Figures S3–S7. Tukey post hoc tests for all models can be found in Table S1.

INTERGENERATIONAL AND TRANSGENERATIONAL EFFECTS

F1: Offspring whose mothers were exposed to treatment
Fecundity and LRS of daughters whose mothers were exposed to pesticide were significantly increased compared to those whose mothers were not (Table 2). Fecundity of daughters whose mother was exposed to 2 g/L of pesticide was 12.45% higher (Fig. 3a), and their LRS 26.95% higher (Fig. 3b) compared to daughters from the control group (Table S1).

The fecundity and LRS of tester females mated to sons of exposed mothers were not affected by treatment. Nevertheless, sons from mothers exposed to 2 g/L pesticide showed a significant 19.9% increase in their longevity compared to sons from control mothers (Tables 2 and S1; Fig. 3c).

F1: Offspring whose fathers were exposed to treatment
We found no effect of male exposure to pesticide on their offspring’s life-history traits (Table S2).

F2: Grandoffspring whose grandmothers were exposed to treatment
Longevity of granddaughters (Fig. 4a) but not of grandsons (Fig. 4b) was significantly and negatively affected by pesticide exposure of their grandmothers (Tables 3 and S4).

F2: Grandoffspring whose grandfathers were exposed to treatment
Longevity of grandoffspring, both granddaughters (Fig. 5a) and...
Table 2. Results from linear models testing the effects of pesticide exposure on intergenerational responses in daughters and sons whose mother was exposed to treatment, either pesticide (1 g/L or 2 g/L) or water (control). Significant adjusted $P$-values following Benjamini-Hochberg procedure are bolded. Final sample sizes in the analyses: F1 daughters’ longevity (74), fecundity (73), and LRS (66); F1 sons’ longevity (68), sons’ mate fecundity (68), and LRS (56).

F1 whose mother was exposed

| Effect                  | Sum of squares (SS) | d.f. | $F$   | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. = 68 |
|-------------------------|---------------------|------|-------|-----------|--------------|----------------------|
| Intercept               | 7.950 (1.276)       | 2    | 0.414 | 0.663     | 0.741        | 2 g/L                |
| Treatment               | 1.091 (0.335)       | 2    | 0.030 | 0.057     | -0.823 (0.561)| 2 g/L                |
| Daughter’s body size    | 2.835 (0.335)       | 1    | 2.150 | 0.147     | -0.823 (0.561)| 2 g/L                |
| Daughter’s age at mating| 28.597 (0.059)      | 1    | 21.689 |<0.001    | 0.742 (0.159)| 2 g/L                |
| Residuals               | 89.658 (0.059)      | 68   |       |           |              |                      |

Daughters’ fecundity

| Effect                  | Sum of squares (SS) | d.f. | $F$   | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. = 68 |
|-------------------------|---------------------|------|-------|-----------|--------------|----------------------|
| Intercept               | 101.013 (1.718)     | 2    | 5.586 | 0.006     | 0.027        | 2 g/L                |
| Treatment               | 1807.9 (3.733)      | 2    | 5.586 | 0.006     | 0.027        | 2 g/L                |
| Daughter’s body size    | 494.3 (3.733)       | 1    | 3.054 | 0.085     | -11.031 (6.312)| 2 g/L                |
| Daughter’s age at mating| 4907.4 (2.028)      | 1    | 30.322 |<0.001   | -11.166 (6.312)| 2 g/L                |
| Daughter’s longevity    | 849.9 (1.344)       | 1    | 5.252 | 0.025     | 3.079 (1.344)| 2 g/L                |
| Residuals               | 10,843.4 (1.718)    | 67   |       |           |              |                      |

Sons’ longevity

| Effect                  | Sum of squares (SS) | d.f. | $F$   | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. = 61 |
|-------------------------|---------------------|------|-------|-----------|--------------|----------------------|
| Intercept               | 9.784 (2.513)       | 2    | 5.729 | 0.005     | 0.027        | 2 g/L                |
| Treatment               | 43.933 (2.513)      | 2    | 5.729 | 0.005     | 0.027        | 2 g/L                |
| Son’s body size         | 0.339 (0.619)       | 1    | 0.088 | 0.767     | 0.823        | 2 g/L                |
| Son’s age at mating     | 2.514 (0.619)       | 1    | 0.656 | 0.421     | 0.639        | 2 g/L                |
| Residuals               | 233,904 (0.619)     | 61   |       |           |              |                      |

Daughters’ mates fecundity

| Effect                  | Sum of squares (SS) | d.f. | $F$   | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. = 61 |
|-------------------------|---------------------|------|-------|-----------|--------------|----------------------|
| Intercept               | 87.858 (20.031)     | 2    | 1.449 | 0.243     | 0.465        | 2 g/L                |
| Treatment               | 594.8 (3.703)       | 2    | 1.449 | 0.243     | 0.465        | 2 g/L                |
| Tester female’s body size| 6.4 (4.407)        | 1    | 0.301 | 0.860     | 0.873        | 2 g/L                |
| Tester female’s age at mating| 255.0 (4.407)    | 1    | 1.242 | 0.270     | 0.494        | 2 g/L                |
| Tester female’s longevity| 48.7 (4.407)      | 1    | 0.237 | 0.628     | 0.741        | 2 g/L                |
| Residuals               | 12,524.0 (4.407)    | 61   |       |           |              |                      |

(Continued)
### Table 2. (Continued).

| Effect                        | Sum of squares (SS) | d.f. | $F$   | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. = 60 |
|-------------------------------|---------------------|------|-------|-----------|--------------|-------------------------|
| **Daughters’ lifetime reproductive success** |                     |      |       |           |              |                         |
| Intercept                     | 53.378 (14.476)     | 1    |       | 0.015     |              |                          |
| Treatment                     | 1509.3              | 2    | 7.563 | 0.001     | 0.015        |                          |
| Daughter’s body size          | 28.4                | 1    | 0.285 | 0.596     | 0.741        |                          |
| Daughter’s age at mating      | 4737.9              | 1    | 47.482| <0.001    | <0.001       |                          |
| Daughter’s longevity          | 919.2               | 1    | 9.212 | 0.004     | 0.022        |                          |
| Residuals                    | 5987                | 60   |       |           |              |                          |
| **Sons’ mates lifetime reproductive success** |                     |      |       |           |              |                         |
| Intercept                     | 81.0                | 2    | 0.425 | 0.656     | 0.741        |                          |
| Treatment                     | 810                 | 2    | 0.425 | 0.656     | 0.741        |                          |
| Tester female’s body size    | 58.8                | 1    | 0.617 | 0.436     | 0.640        |                          |
| Tester female’s age at mating| 88.7                | 1    | 0.931 | 0.340     | 0.533        |                          |
| Tester female’s longevity     | 2.5                 | 1    | 0.026 | 0.873     | 0.873        |                          |
| Residuals                    | 4671.1              | 49   |       |           |              |                          |
Table 3. Results from linear mixed models (GLMM), including grandparent ID as a random factor, testing the effects of pesticide exposure on transgenerational responses in granddaughters and grandsons whose grandmother was exposed to treatment, either pesticide (1 g/L or 2 g/L) or water (control). Significant adjusted $P$-values following Benjamini-Hochberg procedure are bolded. Final sample sizes in the analyses: F2 granddaughters’ longevity (127), and LRS (127); F2 grandsons’ longevity (129), grandsons’ mate fecundity LRS (125).

| Effect                      | $\chi^2$ (Wald) | d.f. | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. | $\beta$ (SE) d.f. |
|-----------------------------|-----------------|------|-----------|--------------|-------------------|-------------------|
| Intercept                   | 9.498           | 2    | 0.009     | **0.015**    | 8.016 (0.785)     | 118.144           |
| Treatment                   | 5.528           | 2    | 0.063     | 0.084        | 4.426 (1.348)     | 119.535           |
| Granddaughters’ body size  | 7.292           | 1    | 0.007     | **0.013**    | -0.884 (0.327)    |                   |
| Granddaughters’ age at mating | 245.862        | 1    | <0.001    | **<0.001**   | 0.922 (0.059)     |                   |
| Grandsons’ body size       | 6.028           | 1    | 0.014     | **0.021**    | 1.712 (0.697)     |                   |
| Grandsons’ age at mating   | 46.022          | 1    | <0.001    | **<0.001**   | 0.644 (0.095)     |                   |

| Effect                      | $\chi^2$ (Wald) | d.f. | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. | $\beta$ (SE) d.f. |
|-----------------------------|-----------------|------|-----------|--------------|-------------------|-------------------|
| Intercept                   | 52.017 (11.835) | 2    | 0.423     | 0.447        | 5.2017 (11.835)   |                   |
| Treatment                   | 2.208           | 2    | 0.332     | 0.389        | 2.208 (2.308)     |                   |
| Granddaughters’ body size  | 3.701           | 1    | 0.054     | 0.076        | -7.224 (3.755)    |                   |
| Tester female’s body size  | 7.930           | 1    | 0.005     | **0.010**    | -9.292 (3.300)    |                   |
| Granddaughters’ age at mating | 19.082         | 1    | <0.001    | **0.019**    | -4.982 (1.141)    |                   |
| Tester female’s age at mating | 21.902         | 1    | <0.001    | **0.010**    | -7.204 (1.539)    |                   |
| Granddaughters’ longevity  | 8.024           | 1    | 0.005     | **0.010**    | 2.841 (1.003)     |                   |
| Tester female’s longevity  | 13.398          | 1    | 0.0003    | **0.001**    | 4.137 (1.130)     |                   |
grandsons (Fig. 5b), was significantly and positively affected by pesticide exposure of their grandfather, such that the longevity of grandoffspring increased when their grandfather was exposed to pesticide treatment (Tables 4 and S1).

MULTIPlicative FITNESS

For females exposed to the different treatments, we found a significant difference in the multiplicative fitness, via daughters, between the low and high pesticide exposure groups. Females exposed to lower pesticide concentration had lower cross-generational fitness than females exposed to the higher pesticide concentration (Fig. 6a; effect size $d$ [95% CI] = $-0.78 [-1.49, -0.07]$). As for the multiplicative fitness of females via sons, a large decrease in fitness in the low concentration exposure compared to the control group cannot be ruled out based on the effect size’s CIs (Fig. 6b; $d = 0.61 [-0.08, 1.31]$). As for males exposed, differences in multiplicative fitness via daughters between the high pesticide dose (lower cross-generational fitness) and the control group (greater fitness) cannot be dismissed attending to the effect size’s CIs (Fig. 6c; $d$ [95% CI] = $0.59 [-0.03, 1.21]$). No significant differences were detectable in multiplicative fitness via sons for lineages generated from males exposed (Fig. 6d).

Discussion

Cross-generational effects elicited by pesticide exposure may, at the very least, affect the population dynamics of pest species,
Table 4. Results from linear mixed models (GLMM) testing the effects of pesticide exposure on transgenerational responses in granddaughters and grandsons whose grandfather was exposed to treatment, either pesticide (1 g/L or 2 g/L) or water (control). Grandparent ID was included as a random factor. Significant adjusted $P$-values following Benjamini-Hochberg procedure are bolded. Final sample sizes in the analyses: F2 granddaughters’ longevity (134), and LRS (132); F2 grandsons’ longevity (134), grandsons’ mate fecundity LRS (132).

| Effect                        | $\chi^2$ (Wald) | d.f. | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. = 126.277 |
|-------------------------------|-----------------|------|------------|--------------|-----------------------------|
| Intercept                     |                 |      |            |              |                             |
| Treatment                     | 11.048          | 2    | 0.004      | **0.010**    | 7.616 (0.941)              |
|                               | 2g/L 0.637      |      |            |              |                             |
| Granddaughters’ body size     | 6.254           | 1    | 0.012      | **0.019**    | −0.953 (0.381)             |
| Granddaughters’ age at mating | 189.273         | 1    | $<$0.001   | **$<$0.001** | 0.826 (0.060)              |
| Granddaughters’ lifetime reproductive success | | | | | |
| Intercept                     |                 |      |            |              |                             |
| Treatment                     | 5.262           | 2    | 0.072      | 0.092        | 5.270 (2.305)              |
|                               | 2g/L −2.260 (2.257) |  |       | | |
| Granddaughters’ body size     | 0.883           | 1    | 0.347      | 0.389        | −4.059 (4.320)             |
| Granddaughters’ age at mating | 42.996          | 1    | $<$0.001   | **0.031**    | −6.665 (1.017)             |
| Granddaughters’ longevity     | 21.611          | 1    | $<$0.001   | **0.010**    | 4.288 (0.922)              |

| Effect                        | $\chi^2$ (Wald) | d.f. | $P$-value | $P$ adjusted | $\beta$ (SE) d.f. = 117.957 |
|-------------------------------|-----------------|------|------------|--------------|-----------------------------|
| Intercept                     |                 |      |            |              |                             |
| Treatment                     | 10.356          | 2    | 0.006      | **0.011**    | 4.936 (1.415)              |
|                               | 2g/L 1.120 (0.349) |  |       | | |
| Grandsons’ body size          | 0.620           | 1    | 0.431      | 0.447        | 0.592 (0.751)              |
| Grandsons’ age at mating      | 51.503          | 1    | $<$0.001   | **$<$0.001** | 0.795 (0.111)              |
| Grandsons’ lifetime reproductive success | | | | | |
| Intercept                     |                 |      |            |              |                             |
| Treatment                     | 0.477           | 2    | 0.788      | 0.788        | 0.478 (2.204)              |
|                               | 2g/L −1.561 (2.306) |  |       | | |
| Tester female’s body size     | 0.911           | 1    | 0.340      | 0.389        | −3.654 (3.829)             |
| Tester female’s age at mating | 42.401          | 1    | $<$0.001   | **$<$0.001** | −7.715 (1.185)             |
| Tester female’s longevity     | 21.376          | 1    | $<$0.001   | **0.010**    | 4.254 (0.920)              |

Transgenerational Effects of Pesticide Exposure

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Figure 5. Longevity of F2 grandoffspring whose grandfathers were exposed to either pesticide (1 g/L or 2 g/L) or water (control). The upper graph in each panel shows a boxplot indicating the median (black line) and 25% and 75% quartiles (box) for each treatment. The bottom chart shows the effect size (mean difference) of the pesticide treatments levels compared to the control (zero mean). (a) Longevity of granddaughters. (b) Longevity of grandsons.

Figure 6. Multiplicative fitness (across three generations) via daughters or sons for females and males exposed. Boxplots indicate the median (black line) and 25% and 75% quartiles (box) for each treatment. Numbers above the boxes indicate the sample sizes in terms of number of lineages. (a) Multiplicative fitness via daughters for females exposed. (b) Multiplicative fitness via sons for females exposed. (c) Multiplicative fitness via daughters for males exposed. (d) Multiplicative fitness via sons for males exposed. Asterisks indicate large effect sizes that cannot be dismissed according to its confidence limits (see the MULTIPLICATIVE FITNESS section in the Results).

with marked ecological and economic consequences (Margus et al. 2019). Nevertheless, research on cross-generational effects of pesticides is very limited (Brevik et al. 2018), and studies typically focus on intragenerational and intergenerational rather than transgenerational effects (e.g., Margus et al. 2019). Whether, and how, pesticide exposure leads to transgenerational effects remains poorly understood. Differentiating intergenerational (affecting the next generation) and transgenerational
effects (affecting >1 generations) is important not only from the perspective of understanding longer term impacts (e.g., on population dynamics), but also because the relative importance of genetic versus epigenetic mechanisms of inheritance likely differs between these two types of effects.

Here, using the seed beetle *C. maculatus*, we found transgenerational effects caused by exposure to sublethal concentrations of pesticide. Females from the F0 generation exposed to pesticide did not experience sizeable changes in their life-history traits compared to control females after controlling for false discovery rate. However, evidence from effect size analyses indicated that exposed females may incur a cost in the form of lower LRS relative to nonexposed females. Striking effects emerged when looking at the consequences of pesticide exposure in future generations. For exposed F0 females, the longevity of their sons was extended, and the fecundity and LRS of their daughters increased, compared to control females. We also observed transgenerational effects of female exposure in the F2 generation, in the form of decreased granddaughters’ longevity. In comparison, males exposed to pesticide in F0 did not lead to intragenerational or intergenerational effects, but they also resulted in transgenerational effects (F2) in the form of an increased longevity of their grandsons and granddaughters. Inter- and transgenerational effects were thus sex specific as the resulting phenotype/life history of the offspring differed depending on whether the father or the mother got exposed. Notably, although most studies documenting sex-specific cross-generational plasticity focus on intergenerational plasticity (e.g., see Garcia-Gonzalez and Dowling 2015; Hellmann et al. 2020; Moschilla et al. 2022), our results extend these findings to transgenerational scenarios.

Sex-specific transgenerational effects may imply that the mechanisms behind the transmission of environmental information could differ between sexes, via epigenetics, genomic imprinting, or genomic regulation (Bonduriansky and Day 2009). For instance, mothers have the possibility to make allocation decisions to manipulate the amount of hormones present in their eggs (Wilson and McNabb 1997; Gil et al. 1999), and fathers have the ability to determine offspring’s phenotype or offspring’s fate via the nonperm fraction of the ejaculate (García-González and Simmons 2007; Immler 2018; Simmons and Lovegrove 2019) or, generally, through ejaculate-mediated paternal effects (Evans et al. 2019). Moreover, both maternal and paternal effects could be interacting in shaping the phenotype of individuals across subsequent generations (Bromfield et al. 2014; Crean and Bonduriansky 2014; Evans et al. 2019). Pesticide resistance can be a mechanism linked to sex (Argentine et al. 1995), suggesting that mechanisms underlying the transgenerational effects of pesticides such as epigenetics and gene regulation (Oppold and Müller 2017) could also be different depending on sex. The study species is characterized by marked sexual dimorphism, which could be associated to differential sensitivity to stress (Blanckenhorn 2005). Furthermore, hormetic effects (i.e., dose-dependent responses to toxicants where low and high concentrations have markedly distinct effects) can take place in a sex-specific manner, as in *Drosophila melanogaster* exposed to mild heat stress (Hercus et al. 2003). Sexually dimorphic patterns in transgenerational plasticity can be linked to sexual conflict and sexually antagonistic selection. For instance, the contrasting effects of maternal and paternal environmental influences on offspring phenotype, or the contrasting responses of offspring to such influences depending on offspring’s sex, may be favored by selection to resolve intralocus sexual conflict (Burke et al. 2020). However, despite the growing interest in the study of sex specificity underlying transgenerational plasticity (Pembrey et al. 2006; Uller 2008; Burke et al. 2020; Hellmann et al. 2020), the causes and consequences of sex-specific transgenerational effects are largely unknown. *Callosobruchus maculatus* is a system characterized by intense sexual conflict adaptations (Zuk et al. 2014; Dougherty et al. 2017; Martinossi-Allibert et al. 2019; Sayadi et al. 2019; Rodriguez-Exposito and Garcia-Gonzalez 2021). Future research into the interplay between sexual conflict evolution and the evolution of sex-specific transgenerational effects using this species is bound to generate important insights.

Sublethal pesticide exposure may induce hormetic effects in organisms by inducing increased enzyme production, regulated by epigenetic mechanisms, responsible for detoxification processes (Guedes and Cutler 2014). In our experiment, we observed hormetic cross-generational effects in the F1 generation on sons and daughters, in the form of increased longevity and LRS, respectively, only manifested when the pesticide dose was higher (2 g/L). Detoxification mechanisms can lead to energy-conflicting trade-offs between different life-history traits that can even spill over across generations (Brevik et al. 2018; Tang et al. 2019), and hormetic effects can be inherited via parental effects through epigenetic modifications (Kishimoto et al. 2017). Our results support the notion that intergenerational and transgenerational effects of pesticide exposure may be dependent upon the concentration of the toxicant and the sex of the parent exposed (Tang et al. 2019; Hellmann et al. 2020).

Our results support Margus et al.’s (2019) findings in another pest insect that stressful exposure to insecticide may lead to maternal intergenerational effects that could facilitate population growth. Our results show positive maternal intergenerational effects from pesticide exposure, and extend these findings by showing that pesticide use can backfire through transgenerational (i.e., reaching the grandoffspring generation), and not just intergenerational, effects. We found that cross-generational multiplicative fitness (and hence population sizes and population growths) are consistent with the patterns in transgenerational plasticity.
dynamics) is impacted by transgenerational effects of pesticide exposure in such a way that high exposure may actually trigger population increases in future generations. These results on multiplicative fitness should be taken with caution because the estimations of LRS for the different lineages (the families originated by the experimental F0 females or F0 males) are based on the LRS of a limited number of individuals per lineage. Nevertheless, the large effect sizes for some of the differences in multiplicative fitness between groups (values of \( d \) around 0.8 are considered large; see Nakagawa and Cuthill 2007) should not be taken lightly. These effects suggest that pesticide exposure can have profound and unexpected effects on population dynamics. Some of these effects implicate hormesis, such that F2 population sizes may be actually larger rather than smaller, when high dosage of pesticide is used. Thus, our results advocate for extreme caution when pesticides are used, because they may, through transgenerational effects, have the opposed effect to that intended.

Interestingly, the inter- and transgenerational maternal effects detected in this study are consistent with a phenomenon that could be described by a new concept: terminal investment transgenerational effects. Intergenerational effects in the context of terminal investment are known (e.g., Bowers et al. 2012, 2015). However, to the best of our knowledge, not much attention has been paid to the possibility that such terminal investment could be manifested through transgenerational parental effects. Terminal investment transgenerational effects are theoretically plausible and fit with the formal terminal investment hypothesis of life-history theory, even though they have not been contemplated so far. We observed a decrease in the number of offspring of female beetles exposed to pesticide, but we found that the daughters of females exposed to the pesticide experienced an increase in total fitness, in the form of higher fecundity and LRS (Tables 2 and S3). Moreover, sons from pesticide-exposed mothers showed increased longevity (Tables 2 and S3), which would likely translate into greater LRS under natural conditions where males are not constrained to mating with a single female, as in our experiments, but rather have access to multiple partners. To the extent that LRS has an intergenerational component due to egg-to-adult viability variation (see Methods), our data demonstrating a fitness increase in F1 are therefore consistent with the existence of terminal investment impacting successive (i.e., more than one, from F0 to F2) generations. We are, however, cautious with this claim as the evidence for a cost to exposed mothers was limited to an analysis of effect sizes. Nevertheless, the importance and implications of these effects in the face of environmental disturbances are so large that they should not be dismissed lightly in transgenerational studies. Future investigations on these effects, and the underlying mechanisms and trade-offs involved, as well as their evolutionary implications, are warranted. In particular, in our study system, resource allocation to eggs by females is known to have important consequences for offspring performance (Fox 1993b, 1994; Yanagi and Miyatake 2002), so females exposed to the highest pesticide concentration in our experiment could have invested terminally via resource allocation to eggs. Further research will be required to reveal the mechanisms by which individuals can induce such a shift in their offspring’s life history, and why the sexes are affected differently.

Conclusions

Our results show intergenerational and transgenerational effects triggered by sublethal concentrations of pesticide exposure in seed beetles. These effects on the life-history traits of both the offspring and grandoffspring generations demonstrate that there is transmission of environmental information linked to pesticide exposure to subsequent generations. A next step would be to study the mechanisms underlying these phenomena and whether they constitute adaptive (e.g., anticipatory) or nonadaptive parental effects (Uller et al. 2013; Sánchez-Tójar et al. 2020; Zhang et al. 2020). The evolutionary implications of our results are significant. Considering the current extended use of pesticides and herbicides, and the fact that these chemicals are periodically applied and some of them are quite persistent in ecosystems, transgenerational plasticity might be an important mechanism allowing adaptation to toxic environments. Our results imply that organisms are able to respond to anthropogenic toxicants via non-genetic inheritance, and that these responses permeate through generations with complex consequences for individual and population fitness. Moreover, this transgenerational plasticity implies complex and unforeseen evolutionary consequences, depending on the underlying genetic correlations between the parental and the offspring traits affected (Evans et al. 2019). Furthermore, our results have direct far-reaching economic implications because of the ramifications for pest control and agroecosystems management. Among these, increased knowledge of mechanisms underlying transgenerational responses to pesticides might be key for developing methods to circumvent the evolution of pesticide resistance in nature. This underscores the potential of applying evolutionary knowledge to problems of social and economic relevance. Another key avenue for future research is to investigate the potential synergies (or lack thereof) between transgenerational plasticity elicited by pesticide exposure (non-genetic inheritance) and the evolution of resistance (genetic inheritance and selection).

Transgenerational effects found in this experiment differ depending on the sex of the parent that was exposed to pesticide.
treatment and the sex of the offspring. Moreover, the dosage of pesticide used has been shown to determine different phenotypic responses in males, females, and their offspring depending on their own sex and whose parent was exposed to the toxicant. We also uncovered potential, thus far ignored, terminal investment transgenerational effects, which may play an important role in life-history evolution and may be common in explaining transgenerational responses to environmental change.

AUTHOR CONTRIBUTIONS

VCS, IGM and FGG conceived the study and designed the experiments. VCS conducted the experiment, obtained the data and analyzed it with assistance from FGG and IGM. VCS wrote a first draft of the MS, to which then FGG and IGM contributed. FGG and IGM secured the funding for the research.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

All the data used in this study are deposited and publicly available in Dryad (https://doi.org/10.5061/dryad.tdz08kg2s).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Longevity of males (green, n = 75) and females (purple, n = 75) exposed to different concentrations of pesticide from 0 g/L to 1 g/L, assessed in a pilot test.

**Figure S2.** The calculation of multiplicative fitness across the three generations (F0, F1, and F2) was done via daughters (red) or via sons (blue).

**Figure S3.** Results from males exposed to pesticide or control treatment.

**Figure S4.** Results from offspring whose mothers were exposed to pesticide or control treatment.

**Figure S5.** Results from offspring whose fathers were exposed to pesticide or control treatment.

**Figure S6.** Longevity of sons whose fathers were exposed to either pesticide (1 g/L or 2 g/L) or water (control).

**Figure S7.** Results from grandoffspring whose grandmother or grandfather were exposed to pesticide or control treatment.

**Table S1.** Tukey post-hoc results. Bold font indicates p < 0.05. Marginal p values < 0.07 are underlined.

**Table S2.** Results from linear models testing the effects of pesticide exposure on intergenerational responses in daughters and sons whose father was exposed to treatment, either pesticide (1 g/L or 2 g/L) or water (control).

**Table S3.** Summary table with the main results found in our experiment. Long and Fec stand for longevity and fecundity, respectively.

**Table S4.** Results from longevity Cox models and Cox mixed models for F2 generation (hazard models) run using the packages `survival` (Therneau, 2022) and `coxme` (Therneau and Grambsch, 2000), respectively.