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Prolonged diapause has sex-specific fertility and fitness costs

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Abstract

Diapause in seasonal environments allows insects to survive adverse seasons. However, individuals can sometimes enter a prolonged diapause for more than a year, and also skip favourable seasons, which can bring additional costs through e.g. loss of metabolic resources. At the same time, prolonged diapause can be beneficial if it allows individuals to have a risk-spreading strategy to skip potentially suboptimal breeding seasons. We studied if prolonged diapause (2-year diapause) negatively affects the fertility and fitness of female and male Colorado potato beetles (*Leptinotarsa decemlineata*) compared to control (1-year diapause) beetles. We also tested the parental effects on the subsequent chemical stress tolerance of their offspring. We found that prolonged diapause carried fertility costs only for females who were less fertile than the control females. However, no differences in fertility were observed in males. Furthermore, prolonged diapause in females resulted in offspring with lower larvae-to-adult survival even though these offspring had accelerated development times. In contrast, paternal diapause duration had no effects on their offspring larvae-to-adult survival, but prolonged diapause males sired offspring with slower development times than control males. Perhaps to compensate the costs related to prolonged diapause both older parents produced or sired offspring with higher body mass than control parents. Despite the differences in emergence mass, parental diapause duration did not affect offspring insecticide stress tolerance. The difference between females and males most likely results from the observed differences in prolonged diapause females' capacity to fight against cellular oxidative damage which was poorer compared to the control females. Even though prolonged diapause allows individuals to have a risk-spreading strategy it carries sex-specific fertility and fitness costs indicating that selection could favour this in males but not in females.

Keywords Diapause · Extended diapause · Fertility · Fitness · Insecticide · Invasive species · Prolonged diapause · Stress tolerance

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Introduction

Diapause is an adaptation to cope with unfavourable conditions. Diapause, in insects, is a programmed arrest of growth, development, or reproduction, coupled with physiological changes to ensure the survival (Danks 1987; Tatar and Yin 2001). It can last one or more years depending on species and environmental conditions during the pre-diapause and different diapause stages (Tauber et al. 1986; Danks 2006). Prolonged diapause in many insect populations occurs when individuals stay in diapause for 12, or more months and/or miss one or more breeding seasons. In extreme cases, diapause can surpass a decade. It is common among insects and up to 68% of individuals depending on the species can extend diapause for two or more years and most commonly for 2 years (Ushatinskaya 1984; Hanski 1988; Danks 1992; Menu and Debouzie 1993). Several studies have shown that prolonging diapause allows species to respond to the unpredictability of their habitat and thus increase the population stability (Menu and Debouzie 1993; Salman et al. 2016; Higaki 2016). Different diapause duration within a population could be viewed as a diversified bet-hedging or risk-spreading strategy (Menu and Debouzie 1993; Menu et al. 2000), which allows species to decrease the risk of reproductive failure during the unfavourable season. In addition, it could be a dispersal strategy as it has been shown to increase invasion speed in the stochastic environment (Mahdjoub and Menu 2008). In pest species variation in diapause duration could increase their resistance to crop rotation (Tauber and Tauber 2002). All these could be important for the invasive pest species in the colonization phase, where species are more likely to be exposed to environmental conditions they are poorly adapted to.

Prolonged diapause can also be costly for individuals, which can be seen as increased mortality (Sims 1983; Salman et al. 2019), and/or reduced performance (Matsuo 2006). Decreased survival could be due to longer exposure to chronic mortality factors, such as natural enemies and or due to depletion of energy reserves. Surviving long periods without eating is a challenge thus dormant insects have to build up energy reserves such as fat, glycogen and proteins (Arrese and Soulages 2010; Hahn and Denlinger 2011). As a result, diapausing individuals contain ca. 20–30% fat when compared to non-diapausing beetles that contain only 5–10% of fat (Danks 1987; Lehmann et al. 2012). Larger individuals commonly have greater energy reserves and thus have higher survival during diapause (Piiroinen et al. 2011) as well as higher post-diapause fertility (Hahn and Denlinger 2007). Thus, larger individuals have advantages for longer diapause (Matsuo 2006). On the other hand, longer diapause could also result in individuals with lower fat reserves and body weight (Ellers and Van Alphen 2002). Surprisingly, the fat reserves in the Colorado potato beetle are used so economically that they do not differ much between the winter diapause and prolonged diapause and are sufficient for reproduction after three winters (Ushatinskaya 1966). Prolonged diapause may also bring additional age effects, because there are many genes that are shared between diapause, ageing and longevity (Kučerová et al. 2016). Age effects could partly explain the often-observed increased mortality during prolonged diapause but this has not been studied.

Diapause duration does not only affect survival but is also correlated with other traits such as fertility and reproduction, moreover, it could also have transgenerational effects and affect the quality of their offspring (Chang et al. 1996; Wang et al. 2006). Many studies have been comparing non-diapausing individuals to diapausing individuals and have found that diapause has positive or no effect on female fecundity (Peferoen et al. 1981; Jansson et al. 1989). However, prolonged diapause leads to reduced dry

weight, fat reserves, egg production and size (Kroon and Veenendaal 1998; Ellers and Van Alphen 2002). Less known is how prolonged diapause affects fertility and offspring production in males.

Besides quantity, individuals that enter prolonged diapause could also affect their offspring quality. For example, diapause has been associated with increased immune responses and stress resistance to extreme environmental conditions (Hahn and Denlinger 2007; Hand et al. 2016; Kučerová et al. 2016). During winter diapause, insects are exposed to low temperatures, while in prolonged diapause, insects also need protection against high summer temperatures and low humidity (Ushatinskaya 1966). A substantial proportion of genes affected by diapause is shared with the ones revealed in studies of aging and extended lifespan such as detoxification, stress-response pathways (i.e. stress resistance, metabolic resistance and genetic instability) as well as immune responses (Kučerová et al. 2016). As a contrast, if the increased lifespan is related to ageing, then prolonged diapause could also have a negative effect on offspring stress tolerance. Ageing has been associated with weakened stress tolerance, due to an increased homeostatic imbalance, which increases the risk of diseases associated with age (De Loof 2011). For example, it has been demonstrated that older wild *Anopheles gambiae* mosquitoes are more susceptible to permethrin insecticide exposure than younger mosquitoes (Chouaibou et al. 2012).

Here we examine the effects of prolonged diapause on the fertility (a number of eggs laid, hatching rate and the number of batches produced or sired) of both females and males and on the chemical stress tolerance of their offspring (i.e. survival) as well as development time and body mass. We used the Colorado potato beetle (*Leptinotarsa decemlineata* Say) as a study species due to its long-lasting diapause period (Tauber and Tauber 2002). We first investigated whether prolonged diapause reduces fertility. Previous studies that have compared non-diapausing adults to diapausing adults found positive or no effect on the total fecundity (Peferoen et al. 1981; Jansson et al. 1989). We hypothesize that if prolonged diapause brings additional costs it could also lead to reduced fertility. Second, we assessed whether the cost of prolonged diapause is higher in females than males. We expect the costs of prolonged diapause on fertility to be higher in females than males because the costs of producing sperm are lower than producing eggs. Third, we asked does the parental prolonged diapause increase the stress tolerance (i.e. survival) and/or affect the life-history traits (i.e. development time and adult body mass) of their offspring. Longevity in many species has been associated with increased stress tolerance because similar genetic mechanisms are suggested to be involved in both longevity and stress tolerance. Similarly, by skipping a breeding season, individuals can be exposed to unfavourable environmental conditions. Thus, if similar genetic or epigenetic mechanisms are involved in longevity and chemical stress tolerance we would expect increased stress tolerance in the offspring. On the other hand, physiological and reproductive performance decline with prolonged diapause could also result in lower offspring quality and thus decreased stress tolerance.

Materials and methods

Study species

The Colorado potato beetle (*Leptinotarsa decemlineata* Say; hereafter CPB) is an excellent species to study the effects of prolonged diapause because of its long-lasting diapause (Peferoen et al. 1981), which can last up to 9 years (Tauber and Tauber 2002). The beetle

has a facultative diapause during their adult stage, which is induced by a short photoperiod and decreased temperature but also changes in food quality and decreases in the availability of food (de Wilde and Hsiao 1981; de Kort 1990; Yocum et al. 2011). Up to 25% of the overwintering individuals can prolong their diapause for the second year (Alyokhin et al. 2013). This species breaks its diapause when temperatures rise above 10 °C (de Kort 1990). The beetle is mainly univoltine in the northern latitudes, but in case of multivoltine populations, there is always a proportion of first-generation adults entering diapause (Tauber et al. 1988; Tauber and Tauber 2002). The fact that adults from different generations form the next summer population can contribute to the maintenance of the genetic variability (Tauber et al. 1988).

Laboratory conditions

The Colorado potato beetles used in the current study originate from a laboratory colony established in 2010 from individuals collected from the fields in Vermont (USA; 44° 43' N, 73° 20' E). Field collected beetles were mated in the laboratory and the next generation was overwintered individually in 100 ml jars (containing 60 ml of peat and a thin layer of plasticine to retain the moisture) in controlled climate cabinets (Type B3100; WeissTechnic, Reiskirchen-Lindenstruth, Germany) at 5 °C, dark. Thereafter, in each spring adult beetles were mated at 23 °C under a long day photoperiod of 18 h light and 6 h dark to induce reproduction (de Kort 1990; Lehmann et al. 2012). Pairs were kept on taped Petri dishes (Ø 9 cm). Eggs were collected from these matings and hatching was recorded daily. Hatched larvae were grown in small family groups in plastic containers (0.5 l) and fed daily with fresh leaves and stems of potato (*Solanum tuberosum* L. var. Challenger). Newly emerged adults were grown under short day photoperiod of 12 h light and 12 h dark and were allowed to gather reserves to enter diapause (de Kort 1990; Lehmann et al. 2012). Beetles generally burrow for diapause 10–15 days after adult eclosion (Piiroinen et al. 2011). Overwintering was initiated by gradually decreasing temperatures from 23 to 20 °C, 15 °C, 10 °C, and finally at 5 °C, with two-week intervals. The summer conditions were initiated similarly by gradually increasing the temperatures. The '2-year-diapause' beetles did not break their diapause at 15 °C. Thus, they were kept at 15 °C for the summer, after which they were moved to 10 °C, and overwintered at 5 °C for the second winter together with the '1-year-diapause' beetles. In total, the 2-year-diapause beetles stayed in diapause for 22–23 months and the 1-year-diapause beetles for 9–10 months. For long it was believed that diapausing beetles do not usually survive for more than 1 year under laboratory conditions (Peferoen et al. 1981).

Before the actual experiment, we tested the physiological conditions from another set of individuals not used in the experiment. For that, we compared the oxidative status biomarkers between the 2-year-diapause females and males and the 1-year-diapause young females and males. Oxidative status biomarkers: catalase (CAT), glutathione peroxidase (GPx), the ratio of reduced to oxidized glutathione (GSH/GSSG), glutathione-S-transferase (GST), superoxide dismutase (SOD), total glutathione (tGSH), and glutathione reductase (GR) were measured according to Margus et al. (2019) and Rainio et al. (2019). Lipid hydroperoxides was measured according to Vuori et al. (2015) with modifications by Rainio et al. (2019) (Suppl. mat.). Prolonged diapause in females leads to higher principal component (GSH/GSSG↓, GST↑) scores suggesting that prolonged diapause leads to decreases GSH/GSSG and increases GST activities (Suppl. mat.; S1). We identified a similar trend in males ($p=0.094$; Supp. Table S1), meaning that the diapause duration

decreases GSH/GSSG activity and increases GST activity. However, our dataset was likely too small to detect significant differences in males. GSH is the major intracellular non-protein defence against free radical (Collatz and Sohal 1986; Krishnan et al. 2009), thus decreased GSH/GSSG ratio could mean decreased defence against free radicals. This could lead to increased cellular oxidative stress and DNA damage (Asensi et al. 1999). Increased GST activity has been associated with higher insecticide resistance (Enayati et al. 2005).

Experimental setup

To investigate the effect of prolonged diapause on females, fourteen 1-year-diapause females (6th generation, born 2014) and fourteen 2-year-diapause females (5th generation, born 2013) were paired with 1-year-diapause males (6th generation) in summer 2015. Before mating the females with males, we measured the body mass of all the females. We assessed that the 2-year-diapause females had similar body mass as the 1-year-diapause females (independent sample *t* test: $t_{1,26} = 0.004$, $p = 0.997$). Each male was mated with one female from both diapause groups to rule out the paternal effects from the maternal effects. Males were randomly swapped between the females in every third day. The observed mating success was 100%. However, egg-laying success was higher for 1-year-diapause females (= 100%) and lower for 2-year-diapause females (= 86%).

Paternal diapause experiment was conducted similarly as the maternal age experiment. Males from both diapause groups 1-year-diapause (6th generation, born 2014) and 2-year-diapause (5th generation, born 2013) males were mated with two 1-year-diapause females, to ensure mating success. In total 12 males from both diapause groups were each mated with two 1-year-diapause females. Before mating the males with the female, males we measured the body mass of all the males. We found that the 2-year-diapause males had similar body mass as the 1-year-diapause males (independent sample *t*-test: $t_{1,22} = 1.7$; $p = 0.102$). Males were swapped randomly between females in every third day. Both mating and egg-laying success were 100% in both male diapause groups, suggesting the males from both diapause groups were equally successful in mating with the females and that all the females laid eggs.

Mated beetles from both maternal and paternal experiment were fed daily with leaves and stems of fresh potato. Eggs were collected, counted and checked for hatching daily. Total hatching was estimated from a number of larvae that had hatched by the second day (i.e. based on 1-day-old larvae). Thereafter, hatched larvae were fed with fresh potato leaves daily, until the second instar.

Insecticide exposure experiment

To test whether parental diapause duration affects stress tolerance we exposed their offspring to insecticide. Second instar larvae (4–6 days old; based on the size of the head capsule 1.03 ± 0.09 mm) (Boiteau and Le Blanc 1992) were divided from each family into two different treatment groups: insecticide and control. In the insecticide stress exposure group, 3 μ l (123 μ g/ml) of azinphos-methyl (AZ, PESTANAL, Sigma-Aldrich, USA) was applied topically to the dorsal abdominal segment. In the control group, 3 μ l of acetone was used instead. Two hours after the insecticide treatment the survival was recorded, and a fresh potato leaflet was supplied to the viable larvae. After 24 h larvae were transferred into the new Petri dishes and fed daily. At the end of the fourth instar, larvae were transferred into plastic boxes containing soil to allow them to pupate.

Soil boxes were checked daily for adult emergence. Newly emerged adults were sexed (female/male) and weighted (mg; Mettler AM100, Toledo). Survival after 24 h (dead/alive), larvae-to-adult survival (dead/alive), adult emergence body mass (mg) were recorded and development time (days; from egg hatching date until adult emergence date) was calculated.

Statistical analysis

Statistical analyses were computed with IBM SPSS Statistics 22 (New York, USA) and both maternal and paternal diapause duration effects were analysed similarly. Principal component analyses (PCA) were performed to investigate the diapause duration effects on female and male fertility (i.e. total number of egg batches laid, total number of eggs laid and total number of eggs hatched within 10 days since mating). We used PCA to reduce the number of dimensions for further analysis. PCA sampling adequacy was tested with Kaiser–Meyer–Olkin test with Promax rotation (KMO=0.642, Approx $\chi^2=81.8$, $df=3$, $p<0.001$). The PCA for female fertility resulted in a principal component (PC1; total number of eggs \uparrow , total number of batches \uparrow , and total number of hatching larvae \uparrow within the 10 days since mating) explaining 83% of the variance (Eigenvalue 2.5). This PC was used in the further analysis with ANOVA, where we investigated the main effects of maternal diapause duration on the reproduction. Maternal diapause duration was set as a fixed factor and male ID as a random factor. The PCA sampling adequacy for male fertility was also tested with KMO-test with a Promax rotation (KMO=0.611, Approx. $\chi^2=76.8$, $df=3$, $p<0.001$). The PCA for male fertility resulted in a PC (PC1; total number of batches \uparrow , total number of eggs \uparrow , total number of hatched larvae \uparrow within 10 days since mating) explaining 78% of the variance (Eigenvalue 2.3). These PC values were used in further analysis with ANOVA, where we investigated the effects of paternal diapause duration on reproduction. Paternal diapause duration was set as a fixed factor.

Larval survival after 24 h of the insecticide exposure and larvae-to-adult survival were analysed with generalized linear models using GLM binomial distribution with a logit link function. The number of surviving individuals was set as dependent variable and the number of treated individuals as a number of events. Maternal or paternal diapause duration and insecticide exposure treatment (control/insecticide stress) were entered as fixed factors. Interactions were included.

Development time (days; from egg hatching date until adult emergence date) and emergence body mass (mg) of the offspring were analysed with ANOVA. Maternal or parental diapause duration and insecticide exposure were entered as fixed factors. Non-significant interactions were omitted from the final models. Offspring sex was included as a fixed factor in development time analysis. However, the effects of parental diapause duration on body mass were analysed separately for female and male offspring. Normality was checked with Kolmogorov–Smirnov and Shapiro–Wilk test and homogeneity with Levene’s test to meet the assumptions of ANOVA.

We excluded 4 individuals when calculating development times in the paternal experiment due to missing data. We excluded six individuals out of 613 (0.98%) from the maternal diapause experiment (2-year-diapause:1-year-diapause = 3:3), and eleven individuals out of 1129 (0.97%) from paternal diapause experiment (2-year-diapause:1-year-diapause = 5:6) from the body mass analysis due to forewing deformations.

Results

Does prolonged diapause affect fertility in females and males?

The PC1 (total number of eggs \uparrow , a total number of batches \uparrow , a total number of hatched larvae \uparrow within 10 days since mating) of female fertility was affected by the maternal diapause duration ($F_{1,14}=6.9$, $p=0.021$) but not by the paternal ID ($F_{1,14}=1.2$, $p=0.362$). Females that had diapaused for 1 year had higher fertility (mean PC1 = 0.55 ± 0.23 s.e.) than females that had prolonged their diapause for two years (mean PC1 = -0.32 ± 0.23 s.e.) (Fig. 1a–c). The PC1 (number of eggs \uparrow , number of batches \uparrow , number of hatched larvae \uparrow within 10 days since mating) of male fertility was not affected by the diapause duration ($F_{1,46}=0.04$, $p=0.846$) (Fig. 2a–c).

Does parental prolonged diapause affect offspring survival?

Offspring survival after 24 h of the insecticide treatment was decreased by the insecticide exposure but was not affected by the maternal diapause duration, or their interaction (Tables 1, 2). This suggests that the maternal diapause duration does not increase or decrease the sensitivity to insecticide exposure. It can be seen from Table 2, that the survival after 24 h was the highest among the offspring in the control treatment groups and lowest among the offspring in the insecticide stress group. Maternal diapause duration affected larvae-to-adult survival, suggesting that 2-year-diapause mothers produced poorer offspring (Tables 1, 2). As shown in Table 2, larvae descending from the 2-year-diapause mothers were 6% less likely to reach the adult stage when compared to larvae descending from the 1-year-diapause mothers. Insecticide exposure had no effect on the overall larvae-to-adult survival (Table 1).

Offspring survival after 24 h of the insecticide treatment was decreased by the insecticide exposure but was not affected by the paternal diapause duration or their interaction (Tables 1, 2). Survival after 24 h was the highest among the offspring from the

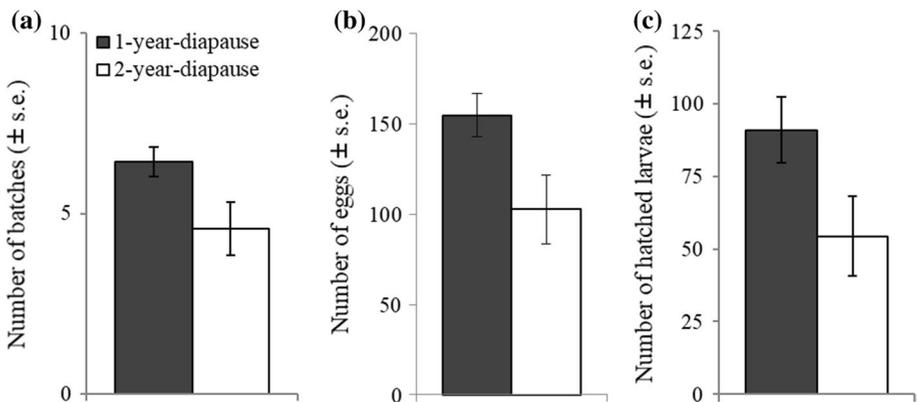


Fig. 1 Maternal diapause duration (2-year-diapause/1-year-diapause) effects on fertility **a** number of egg batches (\pm s.e.), **b** number of eggs (\pm s.e.), and **c** number of hatched larvae (\pm s.e.) within the 10 days since mating

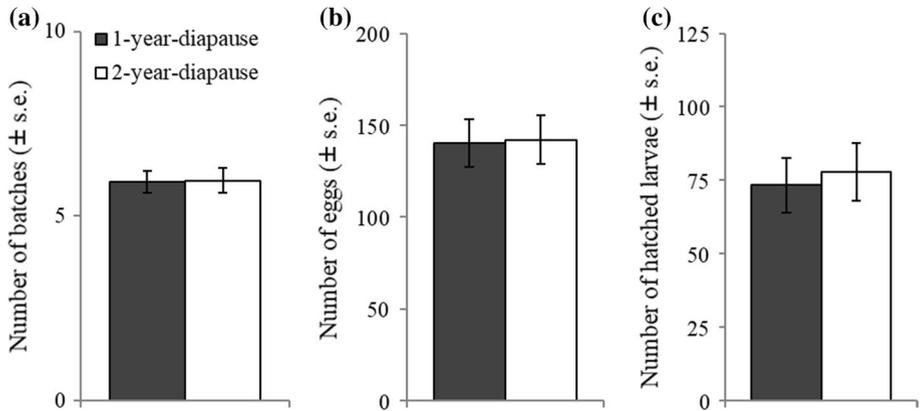


Fig. 2 Paternal diapause duration (2-year-diapause/1-year-diapause) effects on fertility **a** number of egg batches (\pm s.e.), **b** number of eggs (\pm s.e.), and **c** number of hatched larvae (\pm s.e.) within the 10 days since mating

Table 1 Binary logistic regression analysis for larval 24 h and larvae-to-adult survival in relation to maternal or paternal diapause duration and insecticide stress treatment. Significant results are marked in bold

	Survival	Source	Wald Chi square	df	p
Maternal	After 24 h	(Intercept)	149.1	1	<0.001
		Diapause duration	1.3	1	0.254
		Insecticide stress	25.9	1	<0.001
		Diapause duration \times insecticide stress	0.2	1	0.689
	Larvae-to adult	(Intercept)	7.3	1	0.007
		Diapause duration	4.3	1	0.037
		Insecticide stress	0.6	1	0.434
		Diapause duration \times insecticide stress	0.5	1	0.464
Paternal	After 24 h	(Intercept)	199.2	1	<0.001
		Diapause duration	1.3	1	0.250
		Insecticide stress	31.9	1	<0.001
		Diapause duration \times insecticide stress	1.6	1	0.201
	Larvae-to-adult	(Intercept)	<0.01	1	0.953
		Diapause duration	0.7	1	0.394
		Insecticide stress	7.9	1	0.005
		Diapause duration \times insecticide stress	2.1	1	0.151

Significant results are marked in bold

control group and lowest in the insecticide exposure group (Table 2). Larvae-to-adult survival was not affected by the paternal diapause duration but by the insecticide

Table 2 Estimated marginal means for the maternal and paternal diapause duration (1-year-diapause/2-year-diapause) and insecticide treatment (insecticide stress/control) on the survival after 24 h of the insecticide exposure and larvae-to-adult survival

		Survival	1-year-diapause		2-year-diapause	
			Insecticide stress (% ± s.e)	Control (% ± s.e)	Insecticide stress (% ± s.e)	Control (% ± s.e)
Maternal	N		371	372	286	284
	After 24 h		92 (± 1.4)	100 (± 0.3)	88 (± 1.9)	99 (± 0.5)
	Larvae-to-adult		49 (± 2.6)	49 (± 2.6)	41 (± 2.9)	45 (± 3.0)
Paternal	N		553	561	566	565
	After 24 h		92 (± 1.2)	100 (± 0.2)	92 (± 1.1)	99 (± 0.4)
	Larvae-to-adult		46 (± 2.1)	55 (± 2.1)	48 (± 2.1)	51 (± 2.1)

exposure (Table 2). As shown in Table 2, larvae descending from the control group were more likely to reach the adult stage when compared to larvae from the insecticide exposure group.

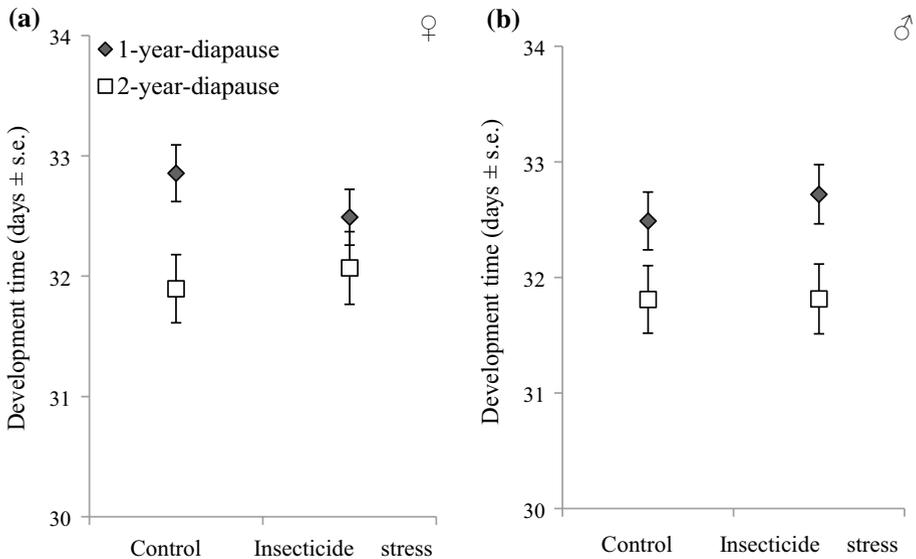


Fig. 3 Maternal diapause duration (2-year-diapause/1-year-diapause) and treatment (insecticide stress/control) effects on development time (days ± s.e.) on **a** female and **b** male offspring. Open squares represent progeny descending from 2-year-diapause mothers and filled diamonds from 1-year-diapause mothers

Does maternal prolonged diapause affect offspring life-history traits?

Offspring descended from the 2-year-diapause mothers had shorter development times than larvae descended from 1-year-diapause mothers ($F_{3,603} = 11.8$; $p = 0.001$; Fig. 3a, b). Insecticide exposure ($F_{3,603} = 0.004$; $p = 0.952$) or offspring sex ($F_{3,603} = 0.31$; $p = 0.581$) had no effect on the development time. The difference in the development time between the two maternal diapause groups was 0.6 days (Fig. 3a, b).

Maternal diapause duration had a significant effect on both female (ANOVA; $F_{2,316} = 11.9$; $p = 0.001$) and male ($F_{2,285} = 12.5$; $p < 0.001$) offspring emergence body mass (Fig. 4a, b). As shown in Fig. 4, two-year-diapause mothers produced on average 6% (5.7 mg) bigger female and 7% (5.2 mg) bigger male offspring when compared to 1-year-diapause mothers. Insecticide exposure had no effect on the emergence body mass of females ($F_{2,316} = 0.02$; $p = 0.884$) or males ($F_{2,285} = 0.64$; $p = 0.423$).

Does paternal prolonged diapause affect offspring life-history traits?

In contrast to maternal prolonged diapause effects, 1-year-diapause fathers sired offspring with shorter development times when compared to offspring from 2-year-diapause fathers ($F_{3,1120} = 4.5$, $p = 0.034$; Fig. 5a, b). The difference in the development time between the two paternal diapause duration groups was 0.3 days and is shown in Fig. 5. Insecticide exposure ($F_{3,1120} = 0.046$, $p = 0.831$) had no effect on the development time but males developed faster than females ($F_{3,1120} = 4.8$, $p = 0.029$).

Two-year-diapause fathers sired both female (ANOVA; $F_{2,545} = 4.4$, $p = 0.035$) and male ($F_{2,564} = 7.9$, $p = 0.005$) offspring with higher emergence body mass (Fig. 6a, b). Insecticide exposure had no effect on the emergence body mass in females ($F_{2,545} = 1.5$,

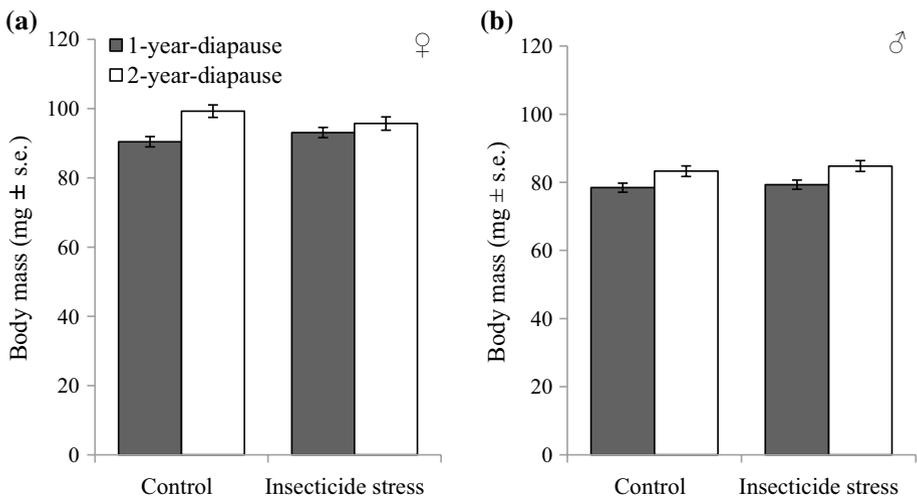


Fig. 4 Maternal diapause duration (2-year-diapause/1-year-diapause) and treatment (insecticide stress/control) effects on emergence body mass (mg \pm s.e.) on **a** female and **b** male offspring. White bars represent the emerged adult body mass of progeny descending from 2-year-diapause mothers and dark bars from 1-year-diapause mothers

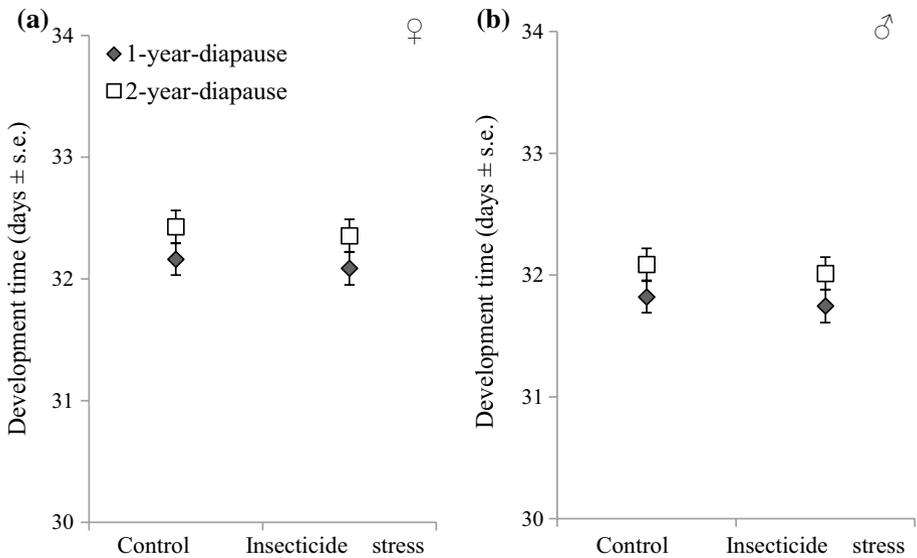


Fig. 5 Paternal diapause duration (2-year-diapause/1-year-diapause) and treatment (insecticide stress/control) effects on development time (days ± s.e.) on **a** female and **b** male offspring. Open squares represent progeny descending from 2-year-diapause fathers and filled diamonds from 1-year-diapause fathers

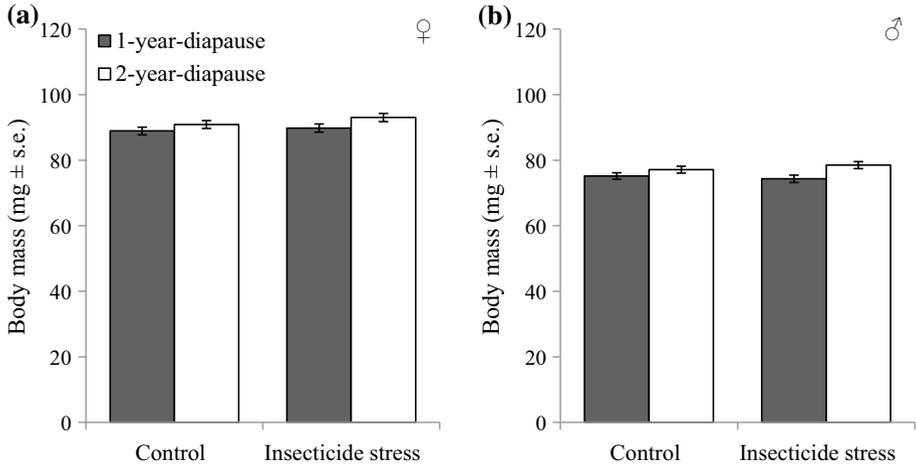


Fig. 6 Paternal diapause duration (2-year-diapause/1-year-diapause) and treatment (insecticide stress/control) effects on emergence body mass (mg ± s.e.) on **a** female and **b** male offspring. White bars represent the emerged adult body mass of progeny descending from 2-year-diapause fathers and dark bars from 1-year-diapause fathers

$p=0.282$) or males ($F_{2,564}=0.07$, $p=0.798$). On average, 2-year-diapause fathers produce 3% (2.6 mg) bigger female offspring and 4% (3.1 mg) bigger male offspring when compared to 1-year-diapause fathers (Fig. 6a, b).

Discussion

Prolonged diapause has been proposed to be a risk-spreading strategy which allows individuals to skip unfavourable conditions (Menu and Debouzie 1993; Menu et al. 2000). This risk-spreading strategy, however, might not be equally beneficial to both sexes as we show that prolonged diapause had sex-specific effects on fertility, offspring quality and offspring life-history traits in the Colorado potato beetles. Prolonged diapause lowered female fertility while no effects were found for the males (Figs. 1, 2). We also found that 2-year-diapause females produced offspring with low survival (Tables 1, 2), underlying that for females this strategy can be harmful if we also account for the high mortality risk often associated with the prolonged diapause (Sims 1983). We also show that prolonged diapause had transgenerational effects as parental diapause duration affected offspring' life-history traits. These effects were mostly positive as two-year-diapause parents produced or sired offspring with higher emergence body mass than 1-year-diapause parents (Figs. 4, 6). Higher adult body mass and size are often associated with higher overwintering survival and reproduction (Matsuo 2006; Hahn and Denlinger 2007; Piironen et al. 2011). In addition, 2-year-diapause mothers produced offspring with shorter development times (Fig. 3), opposite to 2-year-diapause fathers that sired offspring with longer development times when compared to 1-year-diapause fathers (Fig. 5). Although there were changes in the offspring life-history traits, the 2-year-diapause parents did not prime their offspring to have higher stress tolerance and increase their chemical insecticide stress tolerance.

Prolonged diapause had negative effects on female fertility. 2-year-diapause mothers produced fewer offspring with lower larvae-to-adult survival, but they produced offspring with shorter development time and higher body mass when compared to 1-year diapause females. Trade-off between prolonged dormancy and fecundity has been demonstrated also in European cherry fruit fly (*Rhagoletis cerasi*) (Moraiti et al. 2012) and many studies have reported that older mothers produce offspring with lower larvae-to-adult survival or shorter lifespan (the Lansing effect) (Hercus and Hoffmann 2000; Marshall et al. 2010; Lind et al. 2015). The negative effects of prolonged diapause could be due to a decline in general physiological condition (Hercus and Hoffmann 2000) such as lower lipid reserves (Danks 1987; Košťál 2006) or oxidative stress (Ushatinskaya 1966, 1978). Our physiological data suggest that the 2-year-diapause mothers had lower reduced to oxidized glutathione (i.e. GSH/GSSG) ratio which could mean decreased defence against free radicals, and thus increased cellular oxidative stress and/or DNA damage (Asensi et al. 1999). Increased or decreased activities of oxidative stress biomarkers could also lead to trade-offs and have negative effects on fertility. Alternatively, the negative fertility effects could stem from age effects as the prolonged diapause means increased chronological age and increased life span. It is well known that increasing age in females of *Callusobruchus maculatus*, *Drosophila serrata* and *Callosobruchus chinensis* decreases fertility, egg size and egg hatching (Fox 1993; Fox and Dingle 1994; Hercus and Hoffmann 2000; Yanagi and Miyatake 2002; Fox et al. 2003). Hercus and Hoffmann (2000) have also shown that age effects can be present and transferred through multiple generations, as grandmothers' age has been

associated with the decreased egg hatching rate of their grand-offspring. Insects also build up fat body reserves to survive the diapause, sometimes also at the expense of other body tissues such as reproductive tissues. While prolonging diapause does allow females to skip unfavourable seasons it carries costs by means of lower fertility and decreased offspring survival.

Opposite to females, 2-year-diapause males sired an equal amount of offspring as 1-year-diapause males. In addition, paternal diapause duration had no effect on the larvae-to-adult survival of their offspring. A few previous studies have tried to investigate both maternal and paternal age effects and have found that ageing in males has no or small effects on fertility compared to females (Butz and Hayden 1962; Fox et al. 2003). Paternal effects have been suggested to be smaller than maternal effects because the former are mediated via maternal responses and thus females can mitigate negative paternal effects (Crean and Bonduriansky 2014). Ageing does not have such a strong effect on male fertility as on females due to the low costs of producing sperm compared to eggs (Royer and McNeil 1993). Therefore, the negative paternal effects can be mitigated by the 1-year-diapause females in our experiment. We are also aware that we might overestimate positive paternal effects since we did not include the combination of 2-year-diapause mothers and fathers. We also did not let females to choose between the males of different diapause duration. However, it has been shown in many species that females tend to prefer chronologically older males (Beck and Powell 2000; Beck et al. 2002).

The prolonged diapause carried also positive life-history effects and we found that both 2-year-diapause mothers and fathers produced or sired offspring with higher emergence body mass (Figs. 4, 6). This suggests that parents who have prolonged their diapause contribute positively to their offspring fitness and thus the low fertility in females might result from the progeny size-number trade-off (Clark et al. 2001). Alternatively, large offspring body mass could result from a differential survival during the larval period. This is unlikely as this effect is present in both parents. We also found that 2-year-diapause mothers produced offspring with shorter development times, opposite to 2-year-diapause fathers that sired offspring with longer development times when compared to 1-year-diapause parents. The differences in the development times are small. The larvae were grown in the 23 °C at the laboratory conditions without natural enemies. In nature, these differences are likely much bigger. Mixed evidence of parental age effects on the development time of the offspring have been reported before ((Halle et al. 2015) and references therein). We show that the maternal and paternal diapause duration can affect development time differently. Paternal prolonged diapause effects can be mitigated via maternal effects, which could partially explain the difference between the parental age effects on the development time of the offspring. Together these life-history results suggest that, in general, parental prolonged diapause can be advantageous for the offspring, as higher body mass has been associated with higher overall fitness, fertility, overwintering survival and mating success (Stearns 1992; Kingsolver and Huey 2008; Lehmann et al. 2012). In addition, 2-year-diapause mothers might be able to compensate for lower fertility by producing bigger offspring.

Prolonged parental diapause was not associated with increased chemical stress tolerance of offspring as suggested by the literature (Kirkwood and Austad 2000). This could be due to the fact that the Colorado potato beetle is infamous for developing resistance against different insecticides (Alyokhin et al. 2008; Mota-Sanchez and Wise 2019). It is possible that the lack of differences may be due to the population we used, which may be exceptionally resistant to organophosphate insecticides (Margus 2018). Beetle populations from the USA have been reported to have a high frequency of the organophosphate insecticide resistance-associated (S291G) mutation (Piiroinen et al. 2013). Thus, our results may underestimate

the effects of prolonged diapause on offspring stress-tolerance in more susceptible populations. Alternatively, stress tolerance (incl. chemical stress) that has been associated with longevity (Kirkwood and Austad 2000) might not decouple with prolonged diapause. Insecticides usually target very specific sites and it is possible that prolonged diapause does not induce changes in these sites. However, 2-year-diapause parents did also not produce offspring with decreased stress tolerance, suggesting that other prolonged diapause effects did not explain insecticide exposure results. Although, we assessed that 2-year-diapause mothers produced offspring with lower larvae-to-adult survival, this did not decrease the survival after the insecticide exposure.

Conclusions

Skipping one breeding season by prolonging diapause can carry a cost in females but not in males, which may have population-level consequences. As a result, females with prolonged diapause, and hence lower fertility and lower offspring survival, are less likely to contribute as much to population growth and dynamics as younger females with higher fertility. In turn, prolonged diapause in males carried no costs and it could be still a strategy for them to skip unfavourable environmental conditions and thus decrease the risk of the reproductive failure. Although the ability to spread the risks over multiple generations carried sex-specific fertility and fitness costs, it can be still important strategy for pest species which often live under detrimental environmental conditions, and contribute to the invasion rate (Mahdjoub and Menu 2008) and thus invasion success.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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