

ORIGINAL ARTICLE

Transgenerational phenotypic plasticity of diapause induction and related fitness cost in a commercial strain of the parasitoid *Aphidius ervi* Haliday

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Abstract Diapause is an adaptation that insects have evolved to synchronize their life cycle with that of seasonal climatic changes and resources availability. However, cues for its induction are not always clear and, in some cases, a maternal effect may be involved. At the population level, just a part of the individuals may exhibit diapause with important consequences in terms of winter survival. Moreover, clear indicators of diapause state are difficult to identify. Diapause induction was thus investigated in the aphid parasitoid species *Aphidius ervi* Haliday (Hymenoptera: Braconidae) developing in the aphid *Sitobion avenae* (Hemiptera: Aphididae) at four crossed photothermal regimes (16 °C and 8 °C, 16:8 h L:D and 8:16 h L:D), and during 2 successive generations. We analyzed the reliability of changes in mummy color to assess for the diapausing state compared to dissections, and we measured parasitoid morphological and physiological traits. We observed that the proportion of dark brown mummies increased after one generation under low photothermal regime compared to other regimes. No diapause was recorded at 16 °C, 16:8 h L:D, while we observed 16.2% and 67.5% diapause incidence at 8 °C, 8:16 h L:D, at 1st and 2nd generation, respectively. Diapause induction is thus increased by short day-length conditions and low temperatures as well as by maternal effects. All parasitoid life-history traits (weight, size, fat content, water content, egg-load, and longevity) were affected by the photothermal regime and/or the generation. These results raise new questions on the environmental thresholds needed to induce diapause and on survival and adaptation potential of commercially available parasitoid strains in different environments.

Key words dormancy; life-history traits; mummy color; photothermal regimes; transgenerational plasticity

Introduction

Adaptive phenotypic plasticity allows organisms to respond to environmental changes at different times of their life-cycle (Sinclair *et al.*, 2003; Pigliucci, 2006). As tem-

perature is one of the main factors affecting ectotherms development and activity (Hallman & Denlinger, 2019), facultative diapause has evolved as a particular type of developmental phenotypic plasticity. Winter diapause is usually induced by well-defined environmental cues, such as a photoperiod and temperature decrease, that precede the arrival of unfavorable conditions (Tauber *et al.*, 1986). It is distinguished from quiescence which is a direct and short-term response to stressful conditions (Tauber *et al.*, 1986).

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In temperate areas, the proportion of individuals that undergo diapause increases as the time left for growth and development before the arrival of harsh conditions becomes limited. However in a population, not all the individuals will respond to environmental cues inducing diapause with the same intensity (i.e., at the same threshold), resulting in reaction norm variability among genotypes (Bradford & Roff, 1993; Hopper, 1999). In temperate climates most aphid parasitoids arrest their growth or activity before winter time and lie dormant inside the dead host cuticle (Langer & Hance, 2000; Polgár & Hardie, 2000). However, among parasitoids associated with anholocyclic cereal aphids under mild winter only a proportion of the population undergoes true diapause, the other part can remain active during winter if climatic conditions allow these parasitoids to terminate their development (Polgár *et al.*, 1995; Langer & Hance, 2000; Tougeron *et al.*, 2017b).

The recent work of Andrade *et al.* (2016) showed that the increase in average winter temperatures could change the composition of parasitoid communities in cereal crops probably because some species undergo changes among the proportions of individuals which enter diapause. A rapid shift in aphid–parasitoid communities was observed in Western France over the last three decades due to modifications in overwintering strategies of insects (Tougeron *et al.*, 2018a) and probably due to top-down and bottom-up effects (Chidawanyika *et al.*, 2020). This may be reflecting the loss of diapausing stage by some species, or at least indicating that conditions for diapause induction are no longer encountered. Some parasitoid strains show low levels of diapause, either because they have been maintained in constant summer-like rearing conditions (e.g., commercially available strains), because they have evolved with very mild or no winter conditions in their area of origin, or because of adaptation to climate-warming (Garipey *et al.*, 2015; Tougeron *et al.*, 2019).

The aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) is a generalist parasitoid of aphids which can be found in numerous cultivated crops around the world and in which commercial strains are also available for biological control (Van Emden & Harrington, 2017). In *A. ervi*, diapause occurs at the prepupae instar inside the aphid host cuticle (called a “mummy”). In this species, continuous exposure to 15 °C at a photoperiod of 9:15 h L:D (light : dark) for three consecutive generations produced an increase in the number of diapausing individuals up to a maximum of 42.2% (Langer & Hance, 2000). In addition to direct perception of environmental cues by the diapausing generation, these results imply the existence of a maternal effect. Such transgenerational effects in which

conditions perceived by one generation induce diapause in the following generation have already been described in numerous other species such as in mosquitoes (Lacour *et al.*, 2014) or fly parasitoids (Saunders, 1965). In the case of *A. ervi*, a previous paper suggested that diapause levels and life-history traits can be modulated by conditions experienced by precedent generations, due to transgenerational plasticity (TGP) (Tougeron *et al.*, 2020). Among other mechanisms, TGP can involve epigenetics (Jablonka & Raz, 2009), which is known to act on phenotypic expression, including diapause (Reynolds, 2017). Acclimation within and across generations is generally thought to be adaptive for insects (Basson *et al.*, 2012). Adaptive acclimation effects through TGP on overwintering strategies have been shown in overwintering insects, such as in the grain aphid *Sitobion avenae* (Hemiptera: Aphididae) (Powell & Bale, 2008).

Life-history traits are also subject to phenotypic plasticity (Travis, 1994) and their expression can be influenced by diapausing states or by diapause-inducing cues. If diapause is adaptive to face adverse conditions, it also represents a fitness cost in term of survival of diapausing stages and for emerging adults (Hance *et al.*, 2007). For instance, the parasitoid *Asobara tabida* (Hymenoptera: Braconidae) showed a negative relationship between the time spent in diapause and egg load in adults (Ellers & Van Alphen, 2002). For the aphid parasitoid *Praon volucre* (Hymenoptera: Braconidae), diapause is also associated with a reduction of adult longevity (Colinet *et al.*, 2010). In that case, diapausing mummies present a higher lipid mass than non diapausing ones. This storage of lipids could be used both to ensure survival during a long period of diapause but also as energy resources for the production of eggs after diapause. We can therefore expect exacerbated trade-offs between survival and fertility as shown for *A. tabida* (Ellers & Van Alphen, 2002), or between lipid storage and diapause (Varpe & Ejsmond, 2018). Moreover, costs also arise from the need to resist cold exposure during overwintering (Langer & Hance, 2000; Hodkova & Hodek, 2004). Thus fitness related traits (e.g., female fecundity) may be affected either by cold storage or by diapause itself, even over several generations (Ellers & Van Alphen, 2002; Levie *et al.*, 2005). Life-history traits such as fecundity, survival, size, energetic reserves, and developmental time are therefore usually measured to evaluate parasitoid fitness (Godfray, 1994; Roitberg *et al.*, 2001) and consequence of cold exposure or diapause (Colinet *et al.*, 2010; Ismail *et al.*, 2013).

Within insect populations that need to overwinter, color polymorphism has been commonly reported (Leather *et al.*, 1995). In the case of *A. ervi*, mummies have been

described as light brown, intermediate and dark brown. Previous research stated that dark brown mummies usually correspond to a state of diapause (Brodeur & McNeil, 1989b; Krespi *et al.*, 1994; Polgár *et al.*, 1995; Langer & Hance, 2000). In another aphid parasitoid, *Praon volucre*, dark colored mummies are always correlated to a significant delay/arrest in development of the parasitoid with also significant reduced metabolic rates whose are characteristic of diapausing insects (Colinet *et al.*, 2010). However, in a related species *Aphidius rhopalosiphi* (Hymenoptera: Braconidae), mummy color characteristics was not found as a reliable marker for identifying diapause and is most probably a phenotypic variation only linked to cold resistance (Legrand *et al.*, 2004b,c). In consequence, plasticity in the color of the parasitized mummy seems to be very species-specific, must be taken with caution when considering diapause indicators and must be completed with other, more reliable methods such as mummy dissections.

In this study, we aimed to test whether diapause levels could vary depending on the photothermal regime and how this effect can be amplified during successive generations of a commercial strain of the parasitoid *A. ervi*. Entering diapause under spring-like or summer-like conditions should be avoided because of expected costs, unless competition is too high (Tougeron *et al.*, 2017a). Transgenerational plasticity (acclimation) in response to environmental cues might be expressed because it should be adaptive and have benefits regarding fitness. Plastic response allows rapid responses to changing conditions and it is this aspect we wanted to test over a very small number of generations. We explored mummy coloration patterns and emergence delays as indicators of the state of diapause. Moreover, we evaluated how photothermal regimes could influence the value of a set of fitness-related parasitoid life-history traits. We hypothesized that low temperatures and photoperiods would increase the number of diapausing parasitoids, and that diapause levels would be higher at the second generation experiencing such conditions. We also predicted that fitness would be reduced at low temperature and short photoperiod, due to physiological costs induced by these conditions.

Materials and methods

Biological material and insect rearing

Cereal aphids *S. avenae* were obtained from Koppert (The Netherlands), and they were maintained on wheat seedlings (*Triticum aestivum* L.) in 0.3 m³ cages under laboratory conditions (18 °C, 16:8 h L:D, 65% ± 5% rel-

ative humidity). Parasitoids *A. ervi* were purchased from Viridaxis S.A. (Belgium) and reared on *S. avenae* under the same laboratory conditions. The experiment was conducted in the laboratory of Earth and life Institute, Biodiversity Research Centre, Université catholique de Louvain, Belgium.

We checked for the presence of the facultative endosymbionts *Hamiltonella defensa*, *Serratia symbiotica* and *Regiella insecticola* in our aphid culture because their presence could affect parasitism rates and diapause (Douglas, 1998; Mushegian & Tougeron, 2019). The infection status was verified using diagnostic PCRs with primers *16SA1* (5'-AGAGTTTGATCMTGGCTCAG-3') and *PASS cmp* (5'-GCAATGTCTTATTAACACAT-3') (Attia *et al.*, 2016; Fukatsu *et al.*, 2000). None of the facultative symbionts we tested were found in our aphid clone.

Parasitism and photothermal regimes

Parasitoid females used in the experiment were less than 48 h old and naive. In order to ensure mating, each female was maintained for 24 h at 18 °C with 1 male in an Eppendorf tube (1.5 mL) and fed with 50% honey solution. After mating, each female was presented a batch of 10 aphids *S. avenae* (3–4 d old, L2 instar) in a glass petri dish (Ø 4.5 cm) and stings were observed directly. Once parasitized, batches of 10 aphids were removed from the petri dish and transferred to a 4–6 d old fresh wheat seedlings cultured in glass vials (centrifuge tube 50 mL). The top of glass vial was closed by mesh cloth for ventilation. Diapause induction, developmental delay and life-history traits of *A. ervi* were analyzed under four crossed photothermal regimes; 2 temperature regimes (8 °C and 16 °C) and 2 photoregimes (16:8 h L:D and 8:16 h L:D), over 2 consecutive generations. These conditions match the range of temperatures and photoperiod parasitoids encounter in the field in Belgium at the end of autumn and induced diapause in a related species *A. rhopalosiphi* (Legrand *et al.*, 2004a; Tougeron *et al.*, 2017b). Parasitized aphids were distributed in four different incubators and were kept at these conditions until mummy formation and adult emergence, following the protocol of Tougeron *et al.* (2019b). For each photothermal regime and generation, 200 aphids were parasitized (by 20 different females) and we managed to obtain between 80 and 191 mummies. After measuring the desired parameters on the 1st parasitoid generation, the 2nd generation was obtained from parents that experienced the same treatment in the same way as described above.



Fig. 1 Classes of mummies based on perceived color (cuticle of parasitized aphid containing a L3 larvae/prepupae of *A. ervi*), according to Colinet *et al.* (2010).

Diapause induction, development time, and mummy coloration

Mummies were kept upon formation separately in small plastic tubes under their respective photothermal regimes. Mummies were characterized as light brown, intermediate and dark brown following the categories described in previously published work (Colinet *et al.*, 2010) and presented in Figure 1.

Day-degrees necessary for an individual insect's development was calculated from developmental time of egg laying to mummy formation and after mummy formation to development of adult at different photothermal regimes for 2 generations. Developmental time on a day-degree (DD) time scale was calculated as: $DD = DT(T - T_0)$, where DT is the calculated developmental time in days, T is the temperature in $^{\circ}\text{C}$, and T_0 is the threshold temperature for development of *A. ervi*, which is 2.2°C (Sigsgaard, 2000). We also measured mummy formation date, time from egg-laying to emergence and time from mummy formation to parasitoid emergence.

Parasitoids which did not emerged 3 weeks after mummy formation were shifted to 16°C . If they did not emerged after 3 more weeks (total of 6 weeks), mummies were dissected to determine if they contained alive larvae still in the state of diapause or dead ones (Tougeron *et al.*, 2017b; Colinet *et al.*, 2010). After dissection, alive larvae were considered to be under diapause as the delay of emergence was over 3 weeks once placed at 16°C , for which quiescence should be naturally interrupted to continue development. This helped to test the reliability of the diapause identification method based on the coloration of the mummy.

Trait measurements

We measured success of emergence, adult longevity, fecundity, and sex ratio at each photothermal regime and for each generation. For survival, 20 male and 20 female parasitoid adults were individually placed in Eppendorf tube (1.5 mL) and fed with a 50% honey solution at 20°C ,

as done by Levie *et al.* (2005), and for each treatment. Survival of each adult parasitoid was monitored twice a day until the last parasitoid died. To measure potential fecundity of *A. ervi*, <24 h emerged females were freeze-killed at -20°C and dissected ($n = 20$ for each treatment) and the number of eggs at emergence was counted as described by Lins *et al.* (2013).

For morphological traits, 40 additional 1-d-old adult parasitoids (20 males and 20 females) were killed by cold exposure at -20°C for 10 min and we then measured size, fresh mass, dry mass, fat mass, and fat contents (Ismail *et al.*, 2010), for each treatment. Fresh mass was measured using a Mettler-electrobalance Me22 (sensitivity: $1\text{ }\mu\text{g}$). Size was measured using the length of the left hind tibia as a standard estimation for aphid parasitoids (Godfray, 1994; Ismail *et al.*, 2012). Digital pictures of hind tibia were captured with Nikon 4500 camera mounted on a stereomicroscope. The images captured were analyzed by *ImageJ* v. 1.48 software (Wayne Rasband, USA). The dry mass was measured after the samples/adults were oven dried for 3 d at 60°C . Each sample was left in Eppendorf tube containing 1 mL of extracting solution (chloroform: methanol solution 2:1) on agitator for 2 weeks (Terblanche *et al.*, 2004; Colinet *et al.*, 2006). Adults were then placed in an air oven for 12 h at 60°C to eliminate residues of the extracting solution and to obtain the lean dry mass. Fat mass and fat contents were calculated using the formulas:

$$\text{Fat mass} = \text{Dry mass} - \text{Lean dry mass},$$

$$\text{Fat contents} = \text{Fat mass}/\text{Lean dry mass},$$

following Terblanche *et al.* (2004), Colinet *et al.* (2006).

Water mass was determined as the difference between fresh and dry mass. The water content (WC) was calculated as a proportion of dry mass using the formula:

$$\text{Water Content} = \text{Water mass}/\text{Dry mass}$$

following Colinet *et al.* (2006)

Statistical analyses

Coloration patterns were compared among generations and photothermal regimes using a chi-square test of independence, by considering the numbers of dark vs. white mummies for each tested condition. Intermediate coloration forms were ignored in this analysis but were counted and displayed in the graphic representation. This was done to analyze the differences in proportions of the two most contrasted colorations (dark vs. white).

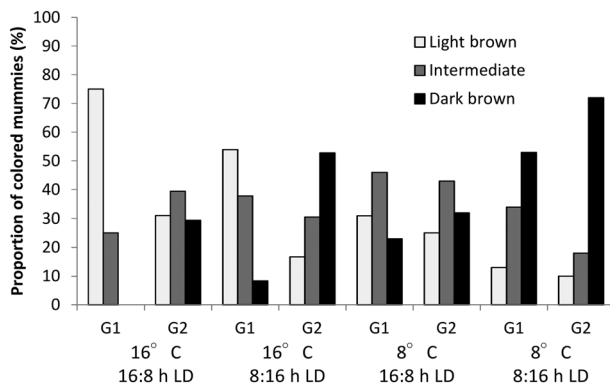


Fig. 2 Percentage of mummies emerged during 1st and 2nd generation at different photothermal regimes. $N = 1291$ mummies.

Emergence patterns were fitted to a survival logistic-rank model (using package *survival*) by adding a censoring factor for nonemerged living parasitoids inside mummies. The effects of the generation, of the photoperiod and the temperature regimes, as well as their three-way interaction were considered and models were tested using Wald chi-squares using the Anova function of the package *car* (Fox & Weisberg, 2011). Life-history trait data were compared among generations, photoperiod and the temperature regimes and their three-way interaction using Linear Models and tested using a likelihood-ratio chi-square method (Fox & Weisberg, 2011). However, biological interpretation could not be made on two-way interactions of photoperiod and temperature with generation, because parasitoids from G2 are necessarily coming from the same photoperiod $\times$ temperature regime as those of G1. Tukey's HSD *post hoc* tests were performed to differentiate means. Previous work with similar experimental design on the same species did not find any "brood" effect, so all female lines and families were treated equally (Tougeron *et al.*, 2020). Statistical analyses were performed using R (R Core Team, 2019).

Results

Mummies characteristics

The percentage of brown colored mummies (individuals usually considered under diapause or dormant state) increased from generation 1 to generation 2, all regimes put together (Fig. 2, $\chi^2 = 84.542$, $df = 1$, $P < 0.001$), and it also increased at photoregimes under low photoperiod (8 : 16 h L:D) (Fig. 2, $\chi^2 = 162.87$, $df = 3$, $P < 0.001$). At least 72% of the mummies were brown at the second

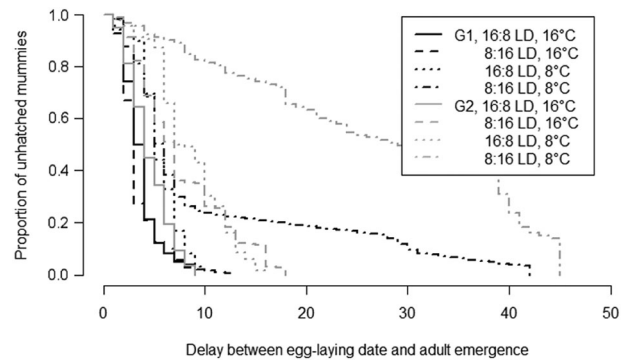


Fig. 3 Emerging pattern of 1st and 2nd generations *A. ervi* at different photothermal regimes. $N = 1309$ parasitoids.

generation of the most stringent regime of 8 °C 8:16 h L:D (Fig. 2).

Emergence delay

Emergence patterns were significantly affected by the photoperiod ($\chi^2 = 350.4$, $df = 1$, $P < 0.001$), the temperature regime ($\chi^2 = 773.9$, $df = 1$, $P < 0.001$), and were different between generations ($\chi^2 = 286.96$, $df = 1$, $P < 0.001$). There was an interaction effect between photoperiod and temperature ($\chi^2 = 157.2$, $df = 1$, $P < 0.001$) but also between photoperiod and generation ($\chi^2 = 55.6$, $df = 1$, $P < 0.001$), meaning that the photoregime did not affect development time in the same way depending on the considered generation (Fig. 3). Overall, parasitoids took longer to develop at the second generation than at the first one (10.05 ± 0.43 d vs. 5.98 ± 0.26 d, respectively). Parasitoids also took longer to develop at 8 °C, 8:16 h L:D (15.66 ± 0.84 d) than at all of the other regimes (4.12 ± 0.10 , 5.61 ± 0.22 and 6.82 ± 0.19 d for 16 °C, 16:8 h L:D, 16 °C, 8:16 h L:D and 8 °C, 16:8 h L:D, respectively).

At 16 °C, all parasitoids from the 180 mummies emerged within 3 weeks, while a proportion of the mummies did not emerge at 8 °C, especially for the second generation placed at 8 °C, 8:16 h L:D (up to 67.5%) (Table 1). Out of both photoregimes at 8 °C, 123 (38 during 1st generation and 85 during 2nd generation) parasitoids did not emerged from their mummies after 3 weeks. After being shifted to 16 °C, 52 parasitoids emerged rapidly, 39 parasitoids were found dead and 32 were still alive at the prepupae instar after three other weeks. Parasitoids that were found alive after mummy dissection were considered as diapausing individuals. Diapause levels reached 1.2% and 1.5% at 8 °C, 16:8 h L:D (for G1 and G2, respectively) and 4.7% and 23.8% at 8 °C, 8:16 h L:D (for G1 and G2, respectively) (Table 1). All the mummies that

Table 1 Emerging pattern of *A. ervi* adults during 1st and 2nd generation, at different photothermal regimes. Values in brackets are percentages. Total counts of mummies from different colors are displayed. The number of mummies from which nothing emerged after 3 weeks, the number of mummies from which parasitoids emerged when put back at 16 °C and the number of dead and alive parasitoids in the remaining mummies are also indicated.

Regime	Generation	Total no. mummies	Light Brown	Intermediate	Dark brown	Mummies emerged	Mummies not emerged	Emerged at 16 °C	Dead	Alive (diapause)	Sex ratio (♂:♀)
16 °C, 16:8 h L:D	1	180	135 (75.0)	45 (25.0)	0 (0)	180 (100.0)	0 (0)	0	0	0	1 : 0.84
	2	180	56 (31.1)	71 (39.4)	53 (29.5)	180 (100.0)	0 (0)	0	0	0	1 : 1.14
16 °C, 8 : 16 h L:D	1	180	97 (53.9)	68 (37.8)	15 (8.3)	180 (100.0)	0 (0)	0	0	0	1 : 0.68
	2	180	30 (16.7)	55 (30.6)	95 (52.7)	180 (100.0)	0 (0)	0	0	0	1 : 0.96
8 °C, 16:8 h L:D	1	168	52 (31.0)	75 (44.6)	41 (24.4)	161 (95.83)	7 (4.17)	0	5	2	1 : 1.24
	2	132	33 (25.0)	57 (43.2)	42 (31.8)	101 (76.2)	31 (23.8)	15	14	2	1 : 0.87
8 °C, 8 : 16 h L:D	1	191	24 (12.6)	65 (34.0)	102 (53.4)	160 (83.8)	31 (16.2)	15	7	9	1 : 0.81
	2	80	8 (10.0)	15 (18.8)	57 (71.2)	26 (32.5)	54 (67.5)	22	13	19	1 : 0.44

Table 2 Day-degrees necessary for egg-to-mummy and mummy-to-adult development at different photothermal regimes.

Photothermal regimes	Egg-mummy		Mummy-adult	
	G1	G2	G1	G2
16 °C, 16:8 h L:D	165.6	151.8	122.2	150.4
16 °C, 8 : 16 h L:D	179.4	193.2	141.0	197.4
8 °C, 16:8 h L:D	191.4	197.2	40.6	65.8
8 °C, 8 : 16 h L:D	232.0	243.6	92.4	121.8

were dissected and in which a diapausing parasitoid was found were brown in color.

At the first generation, emergence peaks occurred at day 3, 4, 5, and 5, with 72, 52, 41, and 33 parasitoids, respectively, at 16 °C, 16:8 h L:D, 16 °C, 8:16 h L:D, 8 °C, 16:8 h L:D, and 8 °C, 8:16 h L:D, respectively. At the second generation, emergence peaks occurred at day 4, day 7, day 6, and day 18, with 35, 28, 25, and 10 parasitoids, respectively, at 16 °C, 16:8 h L:D, 16 °C, 8:16 h L:D, 8 °C, 16:8 h L:D, and 8 °C, 8:16 h L:D, respectively (Fig. 3).

Results revealed that day-degrees for egg-to-mummy formation were the highest at 8 °C, 8:16 h L:D, whereas lowest day-degree requirements were recorded at 16 °C, 16:8 h L:D. For mummy-to-adult duration, highest day-degree requirement was observed at 16 °C, 8:16 h L:D, while lowest day-degrees requirement was observed at 8 °C, 16:8 h L:D, for both generations (Table 2).

Life-history traits

There were significant differences in mean value of most of the measured life-history traits among photoperiod, temperature regimes, generations, with interaction effects of these factors (LMs, Tables 3 and 4). For all traits but fat mass, there were significant interaction effects of photoperiod and temperature, meaning that the effect of photoperiod depended on the tested temperature, and reversely (Table 3). No generation effect was found on tibia size, dry weight, and water content. Overall and all photoregimes put together, mean trait value of all other traits and longevity of both males and females were higher for the first than for the second generation. Egg-load was the only trait that was observed higher at the second generation. The effect of generation on fresh weight and longevity of both males and females was different depending on the photothermal regime tested (three-way interaction effect). For both generations, mean trait values were overall different among regimes at 16 °C and

Table 3 Analysis of variance for life-history traits for the different treatments. Note that a photothermal regime, as described in the method section, corresponds to a photoperiod $\times$ temperature interaction.

Trait	Treatment	DF	LR χ^2	$P (> \chi^2)$
Fresh weight	Photoperiod	1	0.17	0.68
	Temperature	1	10.45	<0.001***
	Potoperiod $\times$ temperature	1	35.59	<0.001***
	Generation	1	8.87	<0.001***
	Generation $\times$ photoperiod	1	0.69	0.40
	Generation $\times$ temperature	1	0.08	0.78
	Generation $\times$ photoperiod $\times$ temperature	1	7.69	<0.01**
Dry weight	Photoperiod	1	15.05	<0.001***
	Temperature	1	53.57	<0.001***
	Potoperiod $\times$ temperature	1	37.47	<0.001***
	Generation	1	1.66	0.20
	Generation $\times$ photoperiod	1	2.74	0.10
	Generation $\times$ temperature	1	1.47	0.22
	Generation $\times$ photoperiod $\times$ temperature	1	0.14	0.71
Water contents	Photoperiod	1	24.21	<0.001***
	Temperature	1	39.46	<0.001***
	Potoperiod $\times$ temperature	1	8.40	<0.01**
	Generation	1	1.44	0.23
	Generation $\times$ photoperiod	1	0.46	0.50
	Generation $\times$ temperature	1	1.51	0.22
	Generation $\times$ photoperiod $\times$ temperature	1	5.76	<0.05*
Fat mass	Photoperiod	1	21.32	<0.001***
	Temperature	1	12.62	<0.001***
	Potoperiod $\times$ temperature	1	1.47	0.23
	Generation	1	5.03	<0.01**
	Generation $\times$ photoperiod	1	2.72	0.09
	Generation $\times$ temperature	1	1.39	0.24
	Generation $\times$ photoperiod $\times$ temperature	1	0.99	0.31
Fat contents	Photoperiod	1	0.003	0.9
	Temperature	1	103.50	<0.001***
	Potoperiod $\times$ temperature	1	14.80	<0.001***
	Generation	1	7.50	<0.001***
	Generation $\times$ photoperiod	1	12.34	<0.001***
	Generation $\times$ temperature	1	8.60	<0.001***
	Generation $\times$ photoperiod $\times$ temperature	1	3.90	0.05
Tibia size	Photoperiod	1	90.06	<0.001***
	Temperature	1	0.54	0.46
	Potoperiod $\times$ temperature	1	25.54	<0.001***
	Generation	1	1.71	0.19
	Generation $\times$ photoperiod	1	97.70	<0.001***
	Generation $\times$ temperature	1	51.40	<0.001***
	Generation $\times$ photoperiod $\times$ temperature	1	5.40	<0.05*
Longevity male	Photoperiod	1	4.96	<0.05*
	Temperature	1	437.80	<0.001***
	Potoperiod $\times$ temperature	1	5.63	<0.01**
	Generation	1	76.01	<0.001***
	Generation $\times$ photoperiod	1	0.90	0.34

(to be continued)

Table 3 Continue.

Trait	Treatment	DF	LR χ^2	$P (>\chi^2)$
Longevity female	Generation $\times$ temperature	1	6.81	<0.05*
	Generation $\times$ photoperiod $\times$ temperature	1	9.09	<0.05*
	Photoperiod	1	4.60	0.03
	Temperature	1	771.90	<0.001***
	Potoperiod $\times$ temperature	1	33.91	<0.001***
	Generation	1	50.21	<0.001***
	Generation $\times$ photoperiod	1	2.56	0.11
	Generation $\times$ temperature	1	33.91	<0.001***
Egg load	Generation $\times$ photoperiod $\times$ temperature	1	43.52	<0.001***
	Photoperiod	1	11.10	<0.001***
	Temperature	1	68.60	<0.001***
	Potoperiod $\times$ temperature	1	7.10	<0.001***
	Generation	1	3.10	<0.05*
	Generation $\times$ photoperiod	1	0.27	0.60
	Generation $\times$ temperature	1	30.96	<0.001***
	Generation $\times$ photoperiod $\times$ temperature	1	0.10	0.80

Note: Regarding two-way interactions including the generation factor: parasitoids from G2 are necessarily coming from the same photoperiod $\times$ temperature regime as those of G1.

* ($p < 0.05$) ** ($p < 0.01$) *** ($p < 0.001$)

regimes at 8 °C, although complex interactions were observed (Table 4).

Noticeably, during the 2nd generation at 8 °C, 8:16 h L:D, minimum fresh weight (0.15 mg) was recorded. This very low value of fresh weight also corresponds to very low values of dry weight, tibia size and fat-content. Sex-ratio was male-biased for most of the treatments, especially at 8 °C, 8:16 h L:D (Table 4).

Discussion

Surprisingly, we observed very low levels of diapause in *A. ervi* after mummy dissections, under all of the photothermal regimes tested. As expected, maximum diapause incidence was observed at 8 °C, 8:16 h L:D, which represents conditions typically inducing diapause in aphid parasitoids from temperate areas (Langer & Hance, 2000; Tougeron *et al.*, 2017b, 2020). However, it concerned only around 25% of the mummies exposed to these conditions while Christiansen-Weniger and Hardie (1997) observed 100% diapause induction in field-collected populations of *A. ervi* after one generation at 15 °C and 10:14 h L:D. The small proportion of diapausing parasitoids we found could be due to the commercial strain of *A. ervi* we used in this experiment and that has been maintained at constant temperatures and photoperiods for several years for mass release by the Viridaxis Company. Under continu-

ous controlled rearing conditions, it is possible that this population has lost a part of its ability to enter diapause (Tougeron *et al.*, 2020). Releasing parasitoids with poor genetic diversity into the fields could impair biological control strategies and long-term establishment of natural enemies, because of low diapause levels and/or low resistance to adverse environmental conditions. Such an observation was already made for *Binodoxys communis* (Hymenoptera: Braconidae) which showed poor capacity to enter diapause (less than 0.8%) and had probably lost that ability due to long period of quarantine rearing (Garipey *et al.*, 2015). Here we used 8 °C, but probably lower temperature can be more effective at inducing diapause, and differences among populations are to be expected due to local adaptations of overwintering strategies (Tougeron *et al.*, 2018b).

Mummy characteristics such as coloration or cuticle thickness have been used to discriminate between diapausing and nondiapausing mummies but have not always proved to be reliable (Brodeur & McNeil, 1989a; Krespi *et al.*, 1994; Polgár *et al.*, 1995; Legrand *et al.*, 2004c). Dark mummies usually show a significant reduction in metabolic rate, which is typical of diapausing insects (Tauber *et al.*, 1986; Hahn & Denlinger, 2007; Colinet *et al.*, 2010). However, usually few developmental data is available to provide support for this statement, and the reliability of such cue may vary across species. At low temperature and short-day length, we observed

Table 4 Mean $\pm$ SE for life-history traits parameters at different photothermal regimes.

Photothermal regimes	Generation	Fresh weight (mg)	Dry weight (mg)	Water contents	Fat mass (mg)	Fat contents	Tibia size (mm)	Longevity (days)		Egg load
								Males	Females	
16 °C, 16:8 h L:D	1	0.36 $\pm$ 0.11 ab	0.09 $\pm$ 0.004 a	4.00 $\pm$ 0.20 ab	0.030 $\pm$ 0.001 a	0.50 $\pm$ 0.03 b	0.46 $\pm$ 0.01 d	9.6 $\pm$ 0.65 cd	12.5 $\pm$ 0.48 cd	94.90 $\pm$ 8.11 b
16 °C, 8 : 16 h L:D	1	0.29 $\pm$ 0.01 c	0.06 $\pm$ 0.004 b	4.81 $\pm$ 0.26 a	0.027 $\pm$ 0.002 ab	0.71 $\pm$ 0.06 a	0.64 $\pm$ 0.01 a	11.3 $\pm$ 0.54 c	13.9 $\pm$ 0.43 c	83.65 $\pm$ 8.30 bc
8 °C, 16:8 h L:D	1	0.32 $\pm$ 0.01 bc	0.10 $\pm$ 0.003 a	3.07 $\pm$ 0.07 c	0.025 $\pm$ 0.001 abc	0.31 $\pm$ 0.02 cd	0.56 $\pm$ 0.01 bc	18.2 $\pm$ 0.29 a	25.5 $\pm$ 0.45 a	92.55 $\pm$ 11.21 b
8 °C, 8 : 16 h L:D	1	0.39 $\pm$ 0.01 a	0.11 $\pm$ 0.004 a	3.67 $\pm$ 0.12 bc	0.021 $\pm$ 0.002 bc	0.24 $\pm$ 0.02 de	0.64 $\pm$ 0.01 ab	17.9 $\pm$ 0.28 a	21.8 $\pm$ 0.59 b	43.70 $\pm$ 8.65 cd
16 °C, 16:8 h L:D	2	0.31 $\pm$ 0.08 bc	0.09 $\pm$ 0.003 a	3.49 $\pm$ 0.12 bc	0.029 $\pm$ 0.001 a	0.49 $\pm$ 0.02 b	0.58 $\pm$ 0.01 abc	7.6 $\pm$ 0.34 e	11.8 $\pm$ 0.33 d	145.9 $\pm$ 14.19 a
16 °C, 8 : 16 h L:D	2	0.29 $\pm$ 0.01 c	0.07 $\pm$ 0.003 b	4.69 $\pm$ 0.29 a	0.019 $\pm$ 0.001 c	0.43 $\pm$ 0.03 bc	0.58 $\pm$ 0.01 abc	8.5 $\pm$ 0.31 de	11.8 $\pm$ 0.36 d	146.6 $\pm$ 12.38 a
8 °C, 16:8 h L:D	2	0.31 $\pm$ 0.01 bc	0.09 $\pm$ 0.003 a	3.46 $\pm$ 0.13 bc	0.024 $\pm$ 0.002 abc	0.36 $\pm$ 0.03 bcd	0.54 $\pm$ 0.01 c	14.0 $\pm$ 0.39 b	20.7 $\pm$ 0.37 b	60.85 $\pm$ 12.88 bcd
8 °C, 8 : 16 h L:D	2	0.25 $\pm$ 0.01 d	0.10 $\pm$ 0.002 a	3.12 $\pm$ 0.10 c	0.018 $\pm$ 0.001 c	0.22 $\pm$ 0.02 e	0.49 $\pm$ 0.04 d	0.00 f	0.00 e	16.30 $\pm$ 8.6 d
HSD		0.059	0.021	0.838	0.007	0.142	0.074	1.74	1.81	47.125

Note: Means sharing similar letters are not statistically significant at $P = 0.05$ among and within generations.

higher percentages of dark brown mummies and a clear generational effect on mummy coloration and development timing of *A. ervi*. Our results revealed that at the most autumn-like regime (8 °C, 8:16 h L:D), during 1st and 2nd generation, percentage of dark brown mummies was higher as compared to other photothermal regimes. The mummies which were dissected and in which parasitoids were found in diapause were all brown in color. According to Legrand *et al.* (2004a), change in mummy color could be an adaptation to overwintering, but not necessarily a direct consequence of diapause. In a related aphid parasitoid species, *A. rhopalosiphi*, dark mummies showed significantly higher water content, survival at low temperature and lower supercooling point values than pale mummies, but the proportion of colored mummies was not linked to diapause induction (Legrand *et al.*, 2004b). Taken together, our results suggest that because the proportion of dark-brown colored mummies increased after one generation at 8 °C 8:16 h L:D, diapause levels could be high. However, as we only found a relatively small proportion of diapausing parasitoids using the dissection method, we cannot fully conclude on the reliability of the mummy coloration method.

The observed proportion of diapausing mummies, as well as development times, were higher for the 2nd generation at 8 °C, 8:16 h L:D. In the same way, a generation effect was also observed for the coloration of the mummies with brown mummies being more frequent in the second generation. Such a potential maternal effect was already reported for instance for *Cotesia vestalis* (Hymenoptera: Braconidae) (Hao *et al.*, 2013) but also for *Trichogramma* (Hymenoptera: Trichogrammatidae) parasitoids (Voinovich *et al.*, 2015) even on multiple generations (Reznik & Samartsev, 2015) and is usually observed for mosquitoes (Denlinger & Armbruster, 2014; Lacour *et al.*, 2014). Adaptive role of TGP and mechanisms facilitating TGP are increasingly becoming elucidated (Mousseau & Dingle, 1991; Sgrò *et al.*, 2016; Donelson *et al.*, 2018). Conditions perceived by females may influence contents of the egg which acts on the developmental plasticity of the embryo, or act on the next generation through changes in hormonal contents or epigenetic mechanisms (Denlinger, 2002; Uller, 2008; Reynolds, 2017). Such maternal effect would prepare offspring to better face incoming environment, which is known to happen in the natural environment (Mousseau & Dingle, 1991). Although unlikely to happen under the nonlethal conditions we tested—because selection on diapause can only occur if parasitoids that do not enter diapause die (before reproduction)—nonrandom mortality at G1 is always possible so phenotypic changes observed within just one generation could either be due to TGP or to selection.

We found a male-biased sex-ratio for all conditions but more pronounced for the most stringent regime. However, it is possible that more females underwent diapause under our experimental conditions and so were part of the individuals that did not emerge rapidly and were dissected at the larval stage. Male biased sex ratios have been reported in some insects emerging from diapause, such as *Lucilia caesar* (Diptera: Calliphoridae) (Ring, 1971), *A. tabida* (Kraaijeveld & van Alphen, 1995) and *Diprion pini* (Hymenoptera: Diprionidae) (Geri *et al.*, 1995). Low temperatures are also known to bias sex ratio in favor of males, which do not require as much resources for their development than females (Langer *et al.*, 2004; Zamani *et al.*, 2007). More recently in *A. ervi* it was found that females reared at 14 °C produced more males than females reared at 20 °C (Tougeron *et al.*, 2020). However, this does not explain why the progeny of *A. ervi* was male biased in our nondiapausing parasitoids. It has been suggested that high male proportion can be linked to laboratory conditions (Heimpel & Lundgren, 2000).

Time spent in diapause usually induces a higher mortality among diapausing pupae but also decrease egg load, fat reserves, and dry weight of the emerging adult females due to physiological trade-offs (Ellers & Van Alphen, 1997). Indeed, parasitoids have to deal with limited amount of resources provided by their host. Earlier researches showed such a trade-off between the resources allocation to reproduction and survival (Ellers & Van Alphen, 1997; Ellers *et al.*, 2000). In our case, there was a reduction in most of the fitness-related traits in parasitoids exposed to 8 °C, 8:16 h L:D regime, especially at the second generation. With the increase in development time, the emerging adults were stressed and tibia length was shorter. Although the link between diapause and fitness could only be indirectly assessed, because not all individuals underwent diapause at 8 °C, 8:16 h L:D, our results confirmed that exposure to diapause-inducing cues (photoperiod and temperature combination) has fitness consequences (Zhou *et al.*, 1995; Ismail *et al.*, 2014).

We showed a reduction in size and fresh weight of adults under the 8 °C, 8:16 h L:D regime for the second generation. Developmental temperature may modulate size of parasitic wasps (Nealis *et al.*, 1984; Bazzocchi *et al.*, 2003; Ichiki *et al.*, 2003) and fitness indicators, such as longevity and fecundity. In addition, fat reserves have been shown to be positively correlated with size and mass (Cloutier *et al.*, 1981; Visser, 1994; Ellers *et al.*, 2001; Frère *et al.*, 2011). We have also shown that the longevity of adults was negatively affected by low photoperiod–temperature conditions. These findings can be compared with similar results in the literature (Renault *et al.*, 2002; Colinet *et al.*, 2006; Amice *et al.*, 2008). It is possible that

females conserve most of their energy for their fecundity at low temperature, at the expense of their longevity, due to the trade-off longevity–fecundity (Ellers & Van Alphen, 2002; Michaud & Qureshi, 2006). We suggest that females invested a part of their fat reserves in fecundity that could have been invested in longevity to resist the effects of cold storage, although egg-load was also negatively affected by low photothermal regimes, at both generations.

It can be predicted that change in phenology through modifications in diapause induction threshold can have dramatic consequences because if the appropriate environmental conditions are not reached, the proportion of individuals that may enter in diapause will be greatly reduced, threatening parasitoid survival in the field. How parasitoids will adapt their seasonal ecology to climate warming and climate unpredictability, and what will be the consequences for aphid–parasitoid interactions and biological control still remain challenging questions (Tougeron *et al.*, 2019). Determining possible changes of threshold levels needed to induce diapause and their consequences on a set of life-history traits of emerging generation are of tremendous importance to better address these issues.

Acknowledgments

The authors are highly indebted to the Higher Education Commission of Pakistan for providing financial support to Muhammad Mubashir Saeed throughout the study period and the Biodiversity Research Center, Université catholique de Louvain, Belgium for providing the necessary facilities to carry out the research. KT was supported by the F.R.S.-FNRS. The authors are also thankful to the anonymous reviewers for their valuable comments that helped to improve the manuscript.

Disclosure

This paper is part of research publication from the PhD degree of Muhammad Mubashir Saeed. The author(s) declare that there is no conflict of interest.

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Manuscript received January 14, 2020

Final version received April 16, 2020

Accepted April 21, 2020