Extended life cycle in the chestnut weevil: prolonged or repeated diapause?

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Abstract

Many insect species extend the life cycle of a part of their population over several years. The adaptive value of these long cycles is now well documented, but the physiological processes underlying them have been little studied. Long life cycles are usually viewed as resulting from prolonged diapause proceeding from a simple extension of the usual winter diapause. However, this hypothesis has not been greatly tested, and information is lacking for species with a larval diapause. The energetic cost of a prolonged larval diapause also needs to be measured, because the few published estimates of lipid consumption concern an imaginal diapause. It is not therefore clear whether the negligible lipid consumption observed during adult prolonged diapause can be extrapolated to larval diapause. From microrespirometry and lipid measures in the chestnut weevil, *Curculio elephas* Gyllenhal (Coleoptera: Curculionidae), we show that: (1) in contrast to the usual hypothesis, the long cycle does not result from an extension of larval winter diapause, but is due to a prolonged diapause occurring secondarily to a developmental phase, and (2) energy consumption during the prolonged diapause is not negligible, but is provided for by a higher initial lipid content in the long cycle individuals. The adaptive value of the observed cycle is discussed.

Introduction

A within-generation variation in life cycle duration (the mixing of short and long cycles) occurs in many insects, crustaceans, and plants (e.g., Cohen, 1966; Ushatinskaya, 1984; Powell, 1986; Tauber et al., 1986; Danks, 1987; Hanski, 1988; Menu & Debouzie, 1993; Hairston et al., 1995; Clauss & Venable, 2000). Several hypotheses for the adaptive value of the long cycle have been proposed (Tauber et al., 1986; Danks, 1987; Hanski, 1988), and recent work suggests that variability in life cycle duration represents bet-hedging, at least in some species such as the chestnut weevil, *Curculio elephas* Gyllenhal (Coleoptera: Curculionidae) (Menu & Debouzie, 1993; Menu, 1993a; Menu et al., 2000; Soula & Menu, 2003), the bee *Perdita portalis* Timberlake (Danforth, 1999), and the plant *Plantago insularis* Eastwood (Clauss & Venable, 2000). In the chestnut weevil, variation in life cycle duration decreases the risk of extinction due to unpredictable catastrophic events (Menu et al., 2000) such as soil drought (Menu, 1993a), high chestnut consumption (Menu & Debouzie, 1993), or larval attack by entomopathogenic fungi (Menu & Desouhant, 2002).

In contrast, the physiological processes underlying long cycles have been neglected, and eco-physiological studies are needed for many species. Indeed, the eco-physiology of prolonged diapause has only been studied in a few species such as the Colorado potato beetle, *Leptinotarsa decemlineata* Say, in which diapause occurs in the adult stage (Ushatinskaya, 1966, 1978, 1984; Tauber & Tauber, 2002). Ushatinskaya's statement (1984) that 'It is evident therefore that their [the Colorado potato beetles] . . . summer rest transforms into winter diapause without interruption; and in part of the population, into many-year superdiapause' probably explains why the long life cycle is usually viewed as resulting from a simple extension of the normal winter and/or summer diapause (Tauber et al., 1986;

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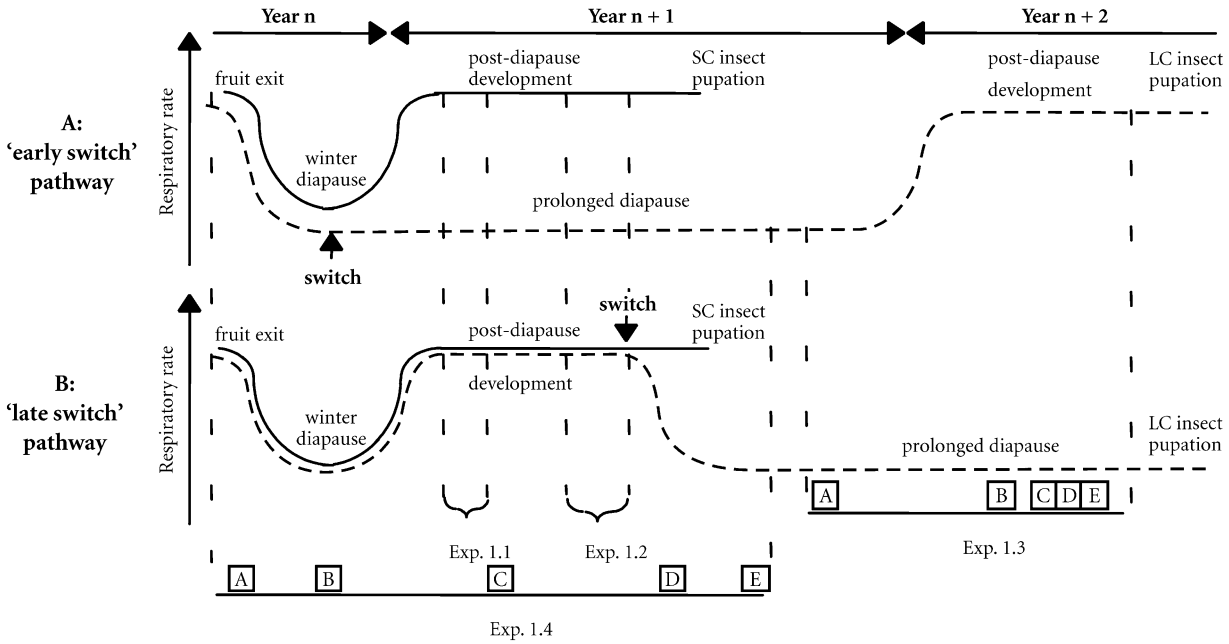

Figure 1 Schematic diagram of the expected mean respiratory rate variation for *Curculio elephas* larvae according to the two different life cycle hypotheses: (A) 'early switch' pathway vs. (B) 'late switch' pathway, and the relative position of respirometry experiments (indicated by abbreviations Exp. 1.1 to Exp. 1.4). Solid lines: short cycle (SC) insects; dotted lines: long cycle (LC) insects. When the solid and dotted lines are congruent, they are not superimposed, to facilitate reading; this does not mean that the respiratory rates of LC larvae are lower. The relative positions of the experiments are linked to the cycle phase, but not to calendar dates because cycle duration depended on insect storage temperature for each experiment (see Table 1). We show the respiratory rates expected in the laboratory at temperatures allowing a direct resumption of development after winter diapause [in the field, a post-diapause quiescence occurs up to March due to low temperatures (Menu, 1993b)].

Danks, 1987; Hanski, 1988; Menu, 1993a; Danforth, 1999). Such an extension of winter diapause to prolonged diapause is illustrated by the 'early switch' pathway in Figure 1A. However, Ushatinskaya (1984) presented no real evidence for her statement, and this hypothesis needs to be tested in more species, particularly those diapausing during the larval stage. Indeed, in such species, it is unclear whether a prolonged diapause involves similar physiological processes to the winter one (Roux et al., 1997). An alternative to the 'early switch' pathway is that all individuals (i.e., short and long-cycle insects) resume their development after the winter diapause, and that long-cycle individuals re-enter a second diapause afterwards (the 'late switch' pathway in Figure 1B). Although a secondary short diapause is known to occur in some species (Torchio & Tepedino, 1982), 'late switch' pathways, including a secondary prolonged diapause in the same (larval) stage that undergoes winter diapause, have never been proposed as an explanation for long cycles, probably since it is viewed as not parsimonious. Indeed, the cost of re-entering a second diapause following a development phase is probably high, and the selective advantage is not clear.

Another open question is: what energy cost is associated with prolonged larval diapause? Lipids are known to greatly influence both survival and reproduction in insects (Danks, 1987). According to Ushatinskaya (1966, 1978, 1984), Colorado potato beetle fat reserves are conserved very economically during prolonged diapause at the imaginal stage, resulting in amounts that allow female fertility after a long cycle to be comparable to fertility following a single overwintering. However, estimates of lipid consumption during a prolonged diapause are scarce, and it is unclear if results concerning imaginal diapause can be extrapolated to larval diapause. We have previously shown that chestnut weevils have a similar adult performance after 1 or 2 years of larval life in the soil (Soula & Menu, 2003), and that there is a positive relationship between the probability of prolonging the larval life cycle and larval fresh weight (Menu & Desouhant, 2002; see also Danforth, 1999 for the desert bee *Perdita portalis*). From these results, we propose that, in contrast to the Colorado potato beetle, cumulative consumption of the chestnut beetle's fat reserves after 1 year of prolonged diapause is not negligible, but that long-cycle individuals compensate for this supposed extra

energy consumption by accumulating additional initial lipid reserves. However, it is still not clear if individual fresh weight, as used in Menu & Desouhant (2002), is a good indicator of lipid reserves, because of possible individual variations in water content, independent of lipid content.

In the present paper we examine whether in the chestnut weevil: (1) larval prolonged diapause is a simple extension of winter diapause, and (2) long-cycle individuals compensate for an extra energy consumption by accumulating additional initial lipid reserves.

Materials and methods

Biological model: the chestnut weevil, *Curculio elephas*

Larvae develop in chestnuts from October to November, and then burrow into the soil to overwinter (Colizza, 1929; Coutin, 1960; Menu, 1993b). Some of the larvae pupate from July onwards the following year and emerge as adults from August to early October (short-cycle insects, henceforth termed 'SC'). Other larvae remain in the soil for 2–4 years before emergence (long-cycle insects, henceforth termed 'LC'). However, most weevils (about 95%) emerge after 1 or 2 years (Coutin, 1961; Menu & Debouzie, 1993). Winter diapause for SC larvae ends in the second fortnight of December, and its completion does not require exposure to low temperatures during winter (Menu, 1993b). In the field, from January onwards, SC larvae undergo a post-diapause quiescence due to low temperatures (below 6 °C), and morphogenesis only proceeds after March, without further interruption until adult emergence (Menu, 1993b). The developmental pathway followed by LC larvae is unknown.

The experiments described in this paper were conducted on the same isolated population as in Menu & Debouzie (1993), Menu (1993a), and Menu & Desouhant (2002). It is a well-defined weevil population (both spatially and genetically) situated near Lyon (France), and representative of other weevil populations in chestnut tree plantations in the Cévennes [similar juvenile distribution per fruit, adult emergence distribution, and variability in life cycle duration (see Debouzie & Menu, 1992; Oberli, 2001; Soula & Menu, 2003, and references therein for further details)].

Experimental design

Early or late switch pathway?

Determination of diapause and development phases. No known external morphological or behavioural criteria allow a discrimination between chestnut weevil larvae in diapause and those undergoing development. A preliminary study conducted in collaboration with L. Lavenseau and L. Peypelut examined imaginal wing disk morphogenesis as a possible discriminator between the two kinds of larvae, as described in Gardenne et al. (1989). Unfortunately, this study demonstrated this criterion to be impractical, because the wing disks only become easily distinguishable just before the pupal stage. Furthermore, studies of wing disks or other physiological or molecular markers are destructive, and cannot indicate the kind of cycle (short or long) that is being followed by living larvae. We therefore used individual respiratory rate measurements to specify the physiological status of the larvae, because it is known that oxygen consumption is lower during diapause than during development (e.g., for review see: Danks, 1987). Oxygen consumption was expressed as the volume of oxygen consumed per hour and per gram of fresh weight ($\mu l\ O_2\ h^{-1}\ gfw^{-1}$) (Scaltec SBA 30, Heiligenstadt, Germany, precision balance, ±0.1 mg). The design of our respirometer was similar to that used by Slama (1984).

Figure 1 shows the values from the respirometry measurement experiments, compared with the expected larval respiratory activities for the two hypothesized cycles. We compared physiological status (diapause vs. development) at 25 °C between SC and LC larvae just after winter diapause (Experiment 1.1, from February to March 1999) and immediately prior to SC pupation at 10, 15, and 20 °C (Experiment 1.2, from June to July 1999). Experiment 1.3 (from October 1999 to June 2000) investigated LC larvae from Experiment 1.2 at 20 °C, with five measurements (A, B, C, D, and E) after the pupation of SC individuals. Experiment 1.4 (from October 1999 to June 2000) comprised five series of measurements at 20 °C: (A) just after exit from fruits, (B) during winter diapause, (C) just after winter diapause (repetition of Experiment 1.1), (D) on LC larvae during SC pupation, and (E) on LC larvae about 1 month after SC pupation.

In all experiments, the larvae exited from chestnuts in the field. In Experiments 1.1 and 1.2, the larvae used for respirometry measurements were removed from custom-designed enclosures that had previously been installed in the soil of the study site, as described in Soula & Menu (2003). Once removed from their enclosure, the larvae were put into plastic boxes (10 × 10 × 10 cm) containing soil from the study site and brought to the laboratory for a storage period of 48 h (a duration sufficient to stabilize insect metabolism at the measurement temperature). In Experiment 1.4, after their exit from chestnuts, the larvae were directly stored in the laboratory in plastic boxes (10 × 10 × 10 cm) containing soil from the study site at 19.5 ± 0.5 °C, in order to avoid the usual quiescence following winter diapause.

Individual life cycle determination (short or long). The kind of individual investigated (SC or LC larva) was known only

a posteriori, that is, after the pupation of SC weevils (LC larvae failed to pupate in the first year). Therefore, in all the above experiments, after measurement of its respiratory rate, each larva was put into a specially constructed wire mesh 'pocket' (5 cm length, 1 cm diameter, mesh 1 mm^2) filled with soil, as in Desouhant et al. (2000). The larvae in their pockets were stored in the laboratory at 19.5 ± 0.5 °C, in plastic boxes (23.5 × 17.5 × 9 cm) filled with soil from the study site. After the emergence period of SC adults, the soil in the pockets was removed in order to identify: (1) live LC larvae, and (2) dead SC pupae, or SC adults that had failed to emerge or were dead.

Energy cost associated with a long cycle

Types of measurements and collection of larvae. Two types of measurements, oxygen and lipid consumption, were used to estimate the energy costs of the long cycle. We estimated cumulative oxygen consumption from the average respiratory rate values measured, to define the nature of the long life cycle (see above). Lipid consumption was evaluated in a collection of 2400 larvae collected daily as they emerged from chestnuts in the field in autumn of 2000, placed in 48 plastic boxes (23.5 × 17.5 × 9 cm) filled with soil (50 larvae/box), and stored in the laboratory at 19.5 ± 0.5 °C. For each of the following experiments, larvae were randomly sampled from each box.

Since we cannot discriminate morphologically between SC and LC larvae before the switch, and because lipid assessment is a destructive procedure, we could not investigate SC larvae alone, as distinct from LC. However, because the 'late switch' pathway has been validated (see respirometry results), we could estimate lipid consumption during development following winter diapause, using two measurements (Experiments 2.1 and 2.2 in Table 1), on a mixture of SC and LC larvae before pupation of the SC individuals.

After SC adult emergence, LC larvae were in prolonged diapause (see respirometry results). Therefore, a third series of measurements was made on the LC larvae (Experiment 2.3 in Table 1) to compare lipid levels between LC larvae in prolonged diapause and the mixture of SC and LC larvae before the pupation of SC individuals.

Finally, a fourth series of measurements on the LC larvae remaining in boxes (Experiment 2.4 in Table 1) allowed an estimate of the lipid consumption during prolonged diapause (performed at 2 months after Experiment 2.3 in order to avoid excessive mortality due to successive removal of larvae from the soil).

Procedure for assessment of lipid reserves. The larvae studied were cleaned with a brush. Their fresh weight was measured (Scaltec SBA 30, Heiligenstadt, Germany, precision balance ±0.1 mg), they were then dried at 60 °C for 48 h and their dry weight was recorded. Fat reserves were assessed by ether extraction (David et al., 1975). Each dried larva was placed in a sealed vial with 5 ml diethyl ether. After 5 days, the larva was washed with fresh ether, dried for 12 h, then weighed, and the amount of fat reserves was calculated by

Table 1 Larval fresh weight and water and lipid content before (Experiments 2.1 and 2.2) and after (Experiments 2.3 and 2.4) the switch to pupation or prolonged diapause. Means and proportions were compared between measurement dates (specified between parentheses) and tested for significance with a two-sided t-test

Measurement date	1 February 2001	4 April 2001	20 June 2001	27 August 2001
Experiment	2.1	2.2	2.3	2.4
Sample size (number of larvae)	20	61	60	64
Fresh weight	108.6 ± 4.6 mg	94.2 ± 2.6 mg	77.6 ± 2.5 mg	87.7 ± 3.0 mg[a]
	(2.1 vs. 2.2: P = 0.008)	(2.2 vs. 2.3: P<0.0001)	(2.3 vs. 2.4: P = 0.01)	
Water percentage	57.1 ± 0.5%	55.9 ± 0.7%	45.9 ± 0.9%	52.5 ± 1.3%[a]
	(2.1 vs. 2.2: P = 0.034)	(2.2 vs. 2.3: P<0.0001)	(2.3 vs. 2.4: P<0.0001)	
Dry weight before lipid extraction	46.9 ± 3.3 mg	41.5 ± 1.2 mg	41.3 ± 1.1 mg	38.6 ± 0.9 mg
	(2.1 vs. 2.2: P = 0.03)	(2.2 vs. 2.3: P = 0.90)	(2.3 vs. 2.4: P = 0.06)	
Lipid percentage on dry weight	50.0 ± 0.7%	35.1 ± 0.7%	44.4 ± 0.6%	42.6 ± 0.4%
	(2.1 vs. 2.2: P<0.0001)	(2.2 vs. 2.3: P<0.0001)	(2.3 vs. 2.4: P = 0.01)	
Lipid weight	23.4 ± 1.2 mg	14.5 ± 0.4 mg	18.2 ± 0.5 mg	16.4 ± 0.4 mg
	(2.1 vs. 2.2: P<0.0001)	(2.2 vs. 2.3: P<0.0001)	(2.3 vs. 2.4: P = 0.006)	

The two first samples correspond to a mixture of SC and LC larvae, measured before the switch to pupation or prolonged diapause (Experiments 2.1 and 2.2). The two last samples (Experiments 2.3 and 2.4) correspond to LC larvae only, measured after the switch. For each variable, the P-values correspond to comparative t-tests between different experiments.

[a]Soil in boxes was watered between Experiments 2.3 and 2.4 in order to avoid larval mortality due to dehydration. This explains the increase in fresh weight and water level between Experiments 2.3 and 2.4.

subtracting the dry weight after ether extraction from the dry weight before extraction.

Statistical analysis

T-tests were used to compare between measurement dates: larval mean oxygen consumption, mean larval weight (fresh weight, dry weight, lipid weight, see Table 1), and larval water proportions (see Table 1). The corresponding P-values apply to two-sided tests. For one-sided t-tests, we use P/2 instead of P. A one-way ANOVA was used (indicated by F in the text) to test the influence of period of measurement (five modalities) on mean oxygen consumption in LC larvae.

Results

Early or late switch pathway?

Measurements from Experiment 1.1 at 25 °C showed similar average respiratory rates for both kinds of larvae ($t = 0.20$, 81 d.f. = 81, $P = 0.84$). Mean oxygen consumption was 994 ± 14 (mean ± SE) µl O_2 h^{-1} gfw^{-1} for SC larvae ($n = 14$) and 1009 ± 29 µl O_2 h^{-1} gfw^{-1} for LC ones ($n = 67$). The 131 other larvae investigated all died before their diapause status could be determined and had a mean respiratory rate of 957 ± 24 µl O_2 h^{-1} gfw^{-1}. From a previous study (Menu, 1993b) we know that SC individuals had resumed development because of storage at 24.5 °C in the laboratory for the 2 days before measurements. So, since the SC and LC larvae had similar high mean values of oxygen consumption, we conclude that the LC larvae were also in development following winter diapause. This result supports the 'late switch' pathway (Figure 1B).

The results of Experiment 1.2 matched those of Experiment 1.1 because, whatever the temperature, SC and LC larvae had similar values of mean oxygen consumption (Figure 2: 10 °C: $t = 0.89$, d.f. = 73, $P = 0.38$; 15 °C: $t = 0.63$, d.f. = 88, $P = 0.53$; 20 °C: $t = 0.05$, d.f. = 84, $P = 0.96$), showing that both kinds of larvae are still in the development phase after winter diapause. Logically, oxygen consumption increased with temperature.

After SC pupation, the mean respiratory rate of LC larvae (Experiment 1.3) was stable over the five periods of measurement ($F_{4,135} = 1.52$, $P = 0.20$), and was much lower [345 ± 20 µl O_2 h^{-1} gfw^{-1} ($n = 140$) for all data pooled] than during the first year at the same temperature, just before the pupation of SC individuals [718 ± 27 µl O_2 h^{-1} gfw^{-1} ($n = 55$) at 20 °C, see Figure 2: $t = 33.8$, $P<0.001$]. The low respiratory activity in LC larvae indicates that they had entered into a second diapause at this time, as expected under the 'late switch' pathway hypothesis. Since this diapause was observed over at least 12 months, it must be viewed as a prolonged diapause.

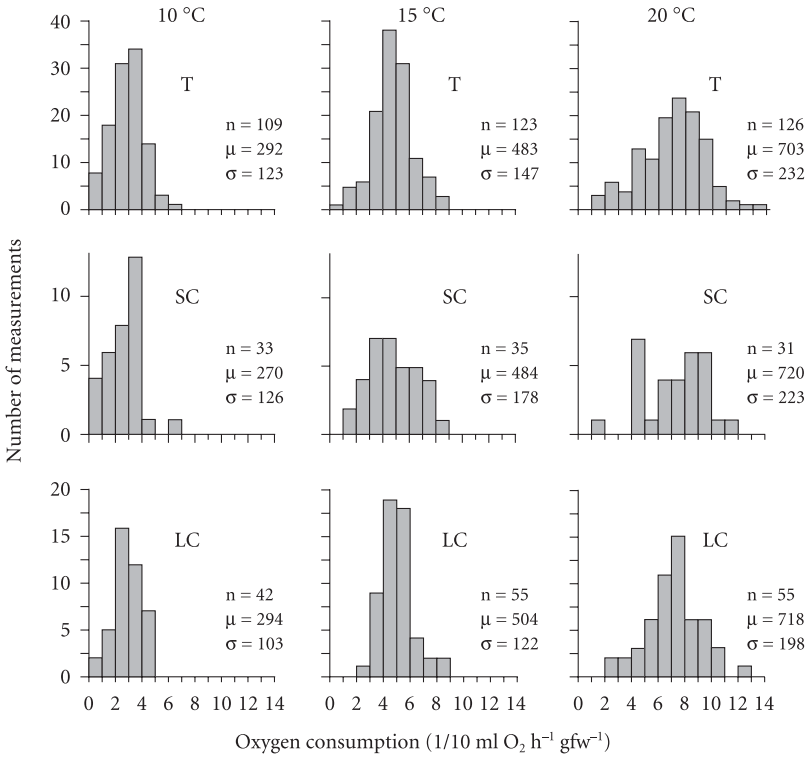

Figure 2 Distribution of larval respiratory rates in *Curculio elephas* measured from 4 June to 18 July 1999 at 10 °C, 15 °C, and 20 °C (Experiment 1.2). 'T' shows the respiratory activity distributions of all larvae measured. Among these insects, SC individuals pupated from 2 to 27 August 1999 (short cycle larvae) and LC individuals did not pupate in 1999 (long cycle larvae). Other larvae died before their physiological status was known. Sample size (n), average oxygen consumption (µ), and standard deviation (σ) are indicated for each histogram.

In Experiment 1.4 (at 20 °C), the data were pooled, as SC and LC larvae showed similar patterns of oxygen consumption. At 48 h after emergence from the fruit, mean larval oxygen uptakes (A in Figure 1) were high (1047 ± 49 µl O_2 h^{-1} gfw^{-1}, n = 50) but they decreased considerably after 38 days (B in Figure 1: 508 ± 25 µl O_2, n = 60; Student's t-test on logarithm-transformed data in order to equilibrate variances: t = 9.4, P<0.001), indicating that the larvae had entered winter diapause. Measurements conducted in March 2000 (C in Figure 1) confirmed the resumption of development observed for the same period in Experiment 1.1, since mean oxygen uptake (714 ± 37 µl O_2) was significantly higher than during the period B in Figure 1 (t = 4.63, P<0.001). Note that the average oxygen consumption during development following winter diapause in Experiment 1.4 (C in Figure 1) was lower than in Experiment 1.1 because the temperature of measurement was lower (20 °C in Experiment 1.4 vs. 25 °C in Experiment 1.1). The oxygen consumption of LC larvae during SC pupation (D in Figure 1: 537 ± 37 µl O_2, n = 21) was significantly lower than during period C (t = 4.45, P<0.001). Finally, the oxygen consumption of LC larvae about 1 month after SC pupation in June (E in Figure 1: 354 ± 24 µl O_2, n = 17), reached a low metabolism, similar to that seen in Experiment 1.3.

All our results are in accord with the 'late switch' pathway (Figure 1B). Therefore, prolonged diapause does not proceed from an extension of the winter diapause but occurs as a secondary event.

Energy costs associated with a long cycle

Cumulative oxygen consumption. In order to compare cumulative oxygen consumption between the first and second year in LC larvae, the cumulative oxygen volume consumed during the first year (until SC insect pupation) and second year (prolonged diapause) were calculated from laboratory observations at 20 °C. The cumulative oxygen consumption during the second year is an indication of the energy costs of a long cycle. We used the average values obtained in Experiment 1.4 as respiratory rates, i.e., 508 µl O_2 h^{-1} gfw^{-1} during the 3 months of diapause (October–December) and 714 µl O_2 h^{-1} gfw^{-1} during 4 months of development (January–April). Using these data, and neglecting the high metabolism just after exit from the fruits, the volume of oxygen consumed during the first year would be 3.2 l O_2 g^{-1}. If we suppose that the high oxygen consumption immediately after exit from the fruit (1047 µl O_2 h^{-1} gfw^{-1}; Experiment 1.4A) lasted for 15 days before the larvae entered diapause, the volume of oxygen consumed only increased to 3.4 l O_2 g^{-1}. During the second year, the mean respiratory rate approximates 354 µl O_2 h^{-1} gfw^{-1} (Experiment 1.4E) over 12 months (May of year n to April of year n + 1), and so the volume of oxygen consumed was 3.1 l O_2 g^{-1}, similar to that estimated for the 7 months of the first year.

Lipid consumption. Table 1 compares mean fresh weights, total dry weights, and lipid weights between four samplings of larvae measured at different periods. From this table, we can estimate lipid consumption.

Assuming a constant lipid consumption rate per month during development following the winter diapause, the 67.5% decrease in lipid during this period, from 15 December 2000 [date of winter diapause completion (Menu, 1993b)] to 4 April 2001 (before pupation) translates to a consumption of 18.4% per month. Similarly, under the assumption that the rate of lipid consumption is constant during prolonged diapause (strongly suggested by the constant respiratory rates over this period, see Experiment 1.3), the decrease in lipid percentage over 1 year in prolonged diapause is estimated at 53.1% (4.4% per month). By using: (1) the observed lipid consumption rate during prolonged diapause, (2) the lipid weights before (on a mixture of SC and LC larvae) and after (on LC larvae only) switch, and (3) a 49% proportion of LC larvae (number of identified LC larvae divided by the number of identified SC and LC larvae × 100), we calculated that larvae delaying development (LC larvae) have greater initial lipid reserves than individuals pupating during the first year (SC larvae). Indeed, just before the switch (on 4 April 2001), lipid weight was assessed at 20.5 mg and 8.7 mg, respectively, for LC and SC larvae. One year later, LC larvae just before pupation were found to contain 9.6 mg of lipid, very similar to the value (8.7 mg) for SC larvae. Non-lipid dry weight also decreases from 27 to 22 mg from 4 April to 27 August (Table 1). This suggests that other reserves are being utilized as well as lipids, as reported in other studies (e.g., Adenokun & Denlinger, 1985).

Discussion

The usual hypothesis to explain the extension of life cycle over several years is a prolonged diapause consisting simply of an extension of the winter diapause (see Introduction and Figure 1A). However, our data are not compatible with this hypothesis, but support a 'late switch' pathway (Figure 1B), in which a second diapause occurs after a development phase. This second diapause lasts longer (at least 1 year) than a simple winter diapause (3 months; Menu, 1993b) and so must be viewed as a prolonged diapause. This result suggests that the larval prolonged diapause corresponds to physiological processes that are independent of those underlying winter diapause, and

which could be governed by different environmental factors. This hypothesis is supported by the differential influence of temperature and/or moisture on winter diapause, and prolonged diapause frequency in the chestnut weevil (Menu, 1993b).

Our results suggest that the usual definition of prolonged diapause should be changed from diapause lasting more than 1 year (e.g., Ushatinskaya, 1984; Tauber et al., 1986; Danks, 1987) to diapause lasting for 1 or more years, resulting in the spreading of adult emergence over at least 2 years. Therefore, the maximum duration for a prolonged diapause given in the literature (e.g., reviews of Ushatinskaya, 1984, and Danks, 1987) must be revisited, at least for certain species. For instance, the maximum duration in *C. elephas* is not 4 years as indicated in Ushatinskaya (1984) but 3, because the minimum duration of a prolonged diapause is 1 (this paper) and not 2 years. However, prolonged diapause, occurring secondarily during the first year (this paper), results in an adult emergence spreading over 4 years (Coutin, 1961; Menu & Debouzie, 1993).

In the chestnut weevil with larval diapause, as in the Colorado potato beetle with imaginal diapause (Ushatinskaya, 1978), oxygen consumption is lower during a prolonged diapause than during the winter diapause. However, in contrast to Ushatinskaya's statement, we show that the cumulative energy consumption during prolonged diapause is high, but is compensated-for by extra lipid reserves in the LC larvae. Therefore, the relationship between prolonged diapause frequency and larval fresh weight previously shown in the field by Menu & Desouhant (2002) does not result from differences in water content, but from variations in lipid resources. The lipid compensation in LC larvae (this paper) is probably the physiological explanation for the observed similar adult performance (e.g., fecundity and longevity) of short and long cycle individuals previously shown in Soula & Menu (2003). The selective value of this energy compensation is clear because: (1) the chestnut weevil is a pro-ovogenic species (Menu, 1992) in which egg production in an LC individual depends on the food consumed 2–4 years before reproduction, and (2) long-term selection for bet-hedging (against short cycle strategies) does not affect the outcome if LC individuals have a lower adult performance than SC ones, except if the bad years are very stressful (F. Menu, unpubl. modelling data). In contrast, when the adult performances are similar, bet-hedging is selected over a large range of environmental stochasticity (Menu et al., 2000). Therefore, energy compensation allows bet-hedging genotypes to decrease the risk that the long cycle morph might emerge as an adult without enough lipid resources for reproduction.

Two alternative hypotheses can be proposed to explain the observation that LC larvae are fatter than SC ones. The first is that some larvae are 'LC-programmed' in the chestnuts by environmental factors other than food, and accumulate more energy reserves in the fruit in preparation for a prolonged diapause. In this case, the higher lipid level in LC larvae may be a consequence of the switch, but not a cause. However, with such a mechanism, an 'early switch' pathway is expected, because a 'late switch' pathway is probably associated with the cost of re-entering a second diapause, and we found no selective advantage associated with a 'late switch' under the 'LC programmed' hypothesis. Furthermore, complementary data do not support the strict 'LC programmed' hypothesis, since soil moisture and/or temperature subsequent to larval development in the fruits influences long life cycle frequency (Menu & Debouzie, 1993; Soula, 2002).

The second hypothesis is that the switch occurs as late as possible, directly in response to the lipid reserves available at the end of larval development following winter diapause. This supposes that in some years, an exceptionally high lipid consumption occurs randomly [with respect to moisture and/or temperature in the soil, see e.g., Thompson & Davis (1981)], and that the lipid consumption rate during a prolonged diapause is less sensitive to between-year environmental variability than that during development (as there is a lower metabolic activity during diapause). Under such conditions, a 'late switch' depending on energy reserves may decrease the risk that long cycle individuals might not have enough lipids to reproduce as adults. If this hypothesis is correct, moisture and/or temperature in the soil may influence prolonged diapause frequency (Menu & Debouzie, 1993), by modifying lipid consumption (an experiment is in progress to test this hypothesis).

To conclude, evidence that prolonged diapause occurs secondarily in the chestnut weevil cycle changes the usual conception of prolonged diapause and opens the field for new evolutionary hypotheses. Furthermore, whatever the actual underlying mechanism, evidence of lipid compensation in long cycle larvae definitively demonstrates the adaptive value of variability in life cycle duration for the chestnut weevil.

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