Oecologia (2002) 132:167–174
DOI 10.1007/s00442-002-0969-6

ECOPHYSIOLOGY

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Bet-hedging for variability in life cycle duration: bigger and later-emerging chestnut weevils have increased probability of a prolonged diapause

Received: 10 October 2001 / Accepted: 29 April 2002 / Published online: 15 June 2002

Abstract Diversified bet-hedging for life cycle duration is defined as within-generation variability in cycle length expressed by a single genotype maximising mean geometric fitness. Such plasticity is not predictive, i.e. it is not a response to cues from the environment that has a predictive value for the decision at hand. In evolutionary terms, diversified bet-hedging is perceived as an adaptation to environmental stochasticity. However, clear evidence of bet-hedging is scarce and exists only for a few desert plant species and one desert bee. In temperate insects, diversified bet-hedging for life cycle duration has been suspected in the chestnut weevil, but proximate factors responsible for individual variation are still unknown. From field experiments, we show that the frequency of the long cycle depends on larval weight and on the date when a larva abandons the fruit, but not on larval burying depth in the soil. Since the two first factors are known to depend on food and temperature and cannot lead to predictive plasticity, we give evidence of bet hedging in this temperate species. Indeed, despite a cost associated with prolonged diapause (extra mortality and loss of reproductive opportunity), a previous study showed that plasticity for life cycle duration, such as discussed in this paper, maximises mean geometric fitness and persistence probability in the chestnut weevil. We propose the hypothesis that the variation in life cycle duration depends on individual variability of metabolic resources such as lipids.

Keywords Coin-flipping plasticity · Risk-spreading · *Curculio elephas* · Dimorphic variation · Dormancy evolution

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Introduction

In many insect species (Waldbauer 1978; Ushatinskaya 1984; Tauber et al. 1986; Danks 1987, 1992; Hanski 1988; Menu 1993b; Menu and Debouzie 1993; Roux et al. 1997; Danforth 1999; Menu et al. 2000) but also in other organisms such as plants (e.g. Venable 1989; Philippi 1993a, 1993b; Clauss and Venable 2000), crustaceans (Ellner and Hairston 1994; Hairston et al. 1995, 1996) and tropical fishes (Wourms 1972), life cycle duration varies within the population. Certain individuals of the same generation reproduce after 1 year and others after 2 or more years because of prolonged dormancy.

Theoretical studies on variability in life cycle duration have been conducted both in plants (e.g. Cohen 1966; Venable and Lawlor 1980; Bulmer 1984; Ellner 1985a, 1985b; Philippi and Seger 1989; Philippi 1993a, 1993b; Clauss and Venable 2000) and in insects (Walker 1986; Wiener and Tuljapurkar 1994; Menu et al. 2000). Some studies (e.g. Philippi 1993a, 1993b; Danforth 1999; Menu et al. 2000; Clauss and Venable 2000) proposed bet hedging as an explanation of such variation. Bet hedging is a strategy maximising mean geometric fitness by minimising fitness variance at the cost of lower arithmetic mean fitness (Seger and Brockmann 1987; Philippi and Seger 1989; Roff 1992; Hopper 1999). Such risk-spreading can be achieved by a single phenotype that avoids risks (conservative bet-hedging) or by phenotypic variation expressed by a single genotype (diversified bet-hedging).

As noted by Hutchinson (1996), "Biologists who are used to thinking in terms of maximisation of individual fitness are often perturbed that a seed (or an insect) should agree not to germinate (emerge as adult) immediately when its own chances of reproducing are lower if it spends a year in dormancy. In fact, there is no parent-offspring conflict because what counts is how many copies of a gene survive, not numbers of direct descendants." From such considerations, adaptive coin-flipping strategies and diversified bet-hedging can be considered as a kind of kin selection (Yoshimura and Jansen 1996) or

more generally as an example of Dawkins' (1976) selfish gene.

Diversified bet hedging can be generated either by coin-flipping plasticity (Cooper and Kaplan 1982; Kaplan and Cooper 1984; Menu and Debouzie 1993; Menu 1993a; Menu et al. 2000) or by maternal effects which need not be a metaphorical "coin-flip", such as effects of seed position and weight in mother plants (Philippi 1993b). But whatever mechanism is supposed, if bet hedging is viewed as an adaptation to environmental unpredictability (Hopper 1999), a short cycle (SC) or long cycle (LC) must be expressed as a responsiveness to unpredictive proximate environmental factors (i.e. factors without predictive value for the decision at hand) (Menu and Debouzie 1993; Menu 1993a; Menu et al. 2000).

According to the review by Hopper (1999), work on insect life history traits does not produce clear evidence of bet-hedging. However, his review did not consider species with prolonged diapause (Menu and Debouzie 1993; Menu 1993a) which, however, are good models with which to test bet-hedging theory. Indeed, most of these species live in environments with a high level of stochasticity (Menu and Debouzie 1993; Menu 1993a; Danforth 1999; Menu et al. 2000), a condition in which bet-hedging is expected to evolve (Hopper 1999).

However, clear field demonstrations of diversified bet-hedging for life cycle duration are scarce and limited to a few desert plants (Venable 1989; Philippi 1993a, 1993b; Clauss and Venable 2000) and one desert bee (Danforth 1999). In desert plants, seed weight and position of seed on the mother plant influence dormancy duration (Philippi 1993a, 1993b), and in the bee *Perdita portalis* (Danforth 1999), year of emergence depends on larval weight.

Evidence of bet hedging is still scarcer in temperate organisms and it is not clear whether conclusions drawn from desert species can be applied to these organisms. In the chestnut weevil, *Curculio elephas*, a European species, diversified bet-hedging has been suggested and ultimate factors for this have been identified (Menu and Debouzie 1993; Menu 1993a, 1993b; Menu and Debouzie 1995; Menu et al. 2000). However, the proximate factors responsible for unpredictive plasticity leading to bet-hedging are still unknown. Since it is known that environmental conditions during prediapause development control induction and/or duration of winter diapause (for reviews, see Tauber et al. 1986; Danks 1987), such prediapause conditions should influence also the duration of the life cycle (either short with only winter diapause or long including prolonged diapause).

Therefore, the aim of this study was to test, in the field, the assumption that life cycle duration in the chestnut weevil is correlated to larval weight and exit date from chestnut, two factors related to food availability (Desouhant 1997; Desouhant et al. 2000) and temperature (Manel and Debouzie 1997) during prediapause development in the fruit. As suggested by Menu and Debouzie (1993), we tested also the effect of larval burying depth on prolonged diapause frequency (PDF). Our results and previous studies (Menu and Debouzie 1993; Menu 1993a; Menu et al. 2000) provide evidence of diversified bet-hedging. Some ultimate factors for life cycle duration have been previously studied (Menu and Debouzie 1993; Menu 1993a; Menu et al. 2000) but the present paper also discusses other specific selective factors.

Materials and methods

Study site and chestnut weevil biology

All the experiments described in this paper were conducted in a chestnut weevil population infesting an isolated chestnut tree in the region of Lyon [Saint-Just Chaleyssin, 30 km south-east of Lyon at 300 m altitude; see Debouzie et al. (1993) for site description]. The soil in this site is clayey.

The chestnut weevil, *Curculio elephas*, attacks both chestnuts and acorns, and adults emerge from mid August to early October (Menu 1993a). Eggs are laid inside the chestnuts and larvae feed in fruits until the end of prediapause development. Mature larvae leave the fruit in October and burrow into the ground where they overwinter in diapause (Menu 1993b; Menu and Debouzie 1995). Just after burying, larvae build a cell in the soil in which they stay without moving until adult emergence (Colizza 1929). Adult emergence is spread over 3 or 4 years due to prolonged diapause in certain larvae; however, most adults emerge after one (SC individuals) and 2 years (LC individuals) (Coutin 1961; Menu and Debouzie 1993).

Contrary to a previous hypothesis (Menu 1993b), the postdormancy development (i.e. normal development) occurs in the field from March of the first year both in SC and LC individuals, and the switch between pupation or prolonged diapause occurs only after this phase of normal development, i.e. from June to July of the first year (Soula and F. Menu, in review). Before pupation, we cannot identify the larvae with respect to their life cycle phenotype (SC or LC), because of an absence of morphological criteria distinguishing them. Furthermore, because of high individual variability in postdormancy development rate (Menu 1993b), larvae in postdormancy development, pupae and adults, but also larvae in prolonged diapause, are in the soil from July to September.

Larvae collection

Each year, from 1993 to 1995, 10,000–12,000 chestnuts were collected under the chestnut tree and kept in the field in a cage to protect against predators until the end of larval emergence from the chestnuts. The chestnut weevil larvae were collected daily and uniformly deposited on the ground in metallic wire-netting boxes with covers (10×10×60 cm; net mesh 1 mm). Every day, the larvae not buried after 1 day were recorded and eliminated (Table 1). The boxes were filled with soil from around the chestnut tree and were buried under the chestnut tree (experiment 1) or 2–4 m from the chestnut tree canopy (experiments 2 and 3, in order to decrease infection by entomopathogenic fungi which occurred in the experiment 1). The boxes were buried 2 months before larval burying in order to decrease the effect of soil perturbation. The boxes protected larvae from predation, mainly by moles, wood mice and invertebrates >1 mm. In order to carry out the experiment in standard conditions, the ground inside the boxes was previously screened and stones and wood scraps removed.

Larval vertical position in the soil

Our first aim was to test whether plasticity for life cycle duration results from the effect of vertical variation in soil micro-environmental factors. Therefore, as a first step, we studied field variabili-

Table 1 No. of insects available for each experiment and their survival rates. *NL* Total no. of larvae collected after exit from chestnuts (these larvae were deposited on the soil of boxes buried in the soil near to the chestnut tree; see text), *NNB* no. of larvae not buried in the soil, *M* mortality rate before burying (=NNB/NL), *NB* no. of larvae buried in the soil (NB+NNB=NL), *NS* no. of larvae (experiment 1) or cumulative no. of larvae and pupae (experiment 2 with six boxes) found in the soil when boxes were removed {for experiment 2 with ten boxes and experiment 3, *NS* cumulative no. of adults [short cycle (SC) individuals] and the no. of larvae in prolonged diapause [long cycle (LC) individuals]}, *NSC* no. (among NS) of SC individuals (either larvae for experiment 2 with six boxes or adults for experiment 2 with ten boxes and experiment 3), *NLC* no. of LC larvae with prolonged diapause (NLC+NSC=NS); *SS* survival rate in the soil (=NS/NB), *NM* no. of months larvae spent in the soil before removal, *MS* monthly survival rate (=geometric mean estimated over the no. of months NM spent in the soil)

Experiment (years)	NL	NNB	M	NB	NS	NSC	NLC	SS	NM	MS
Experiment 1 (1993–1994)	1,319 (5 October–19 December 1993)[a]	45	0.034	1,274 (30 boxes)[b]	555 (13–14 June 1994)[c]	–	–	0.436	8 (5 October 1993–14 June 1994)[g]	0.901
Experiment 2 (1994–1995)	1,258 (29 September–15 November 1994)[a]	63	0.05	449 (6 boxes)[b]	326 (3–6 July 1995)[c]	280[d]	46[d]	0.726[e]	9 (29 September 1994–6 July 1995)[g]	0.965
				746 (10 boxes)[b]	329 (30 September 1995)[c]	253	76	0.441[f]	12 (29 September 1995–30 September 1996)[g]	0.934
Experiment 3 (1995–1996)	1,468 (10 October–27 November 1995)[a]	36	0.024	1,432 (21 boxes)[b]	519 (7–11 October 1996)[c]	275	244	0.362[f]	12 (10 October 1995–11 October 1996)[g]	0.918

[a] Date of emergence
[b] No. of boxes used
[c] Date of removal
[d] Estimated nos. (see Materials and methods)
[e] Survival before pupation (see text)
[f] Total survival (see text)
[g] Period larvae spent in soil before removal

ty in larval burying depth. Thus, the aim of the experiment 1 (Table 1) was to study vertical distribution of larvae in the soil before the switch to pupation or prolonged diapause.

On 13–14 June 1994, all boxes were removed and we noted the vertical position of each larva and pupa in the soil. Since no larvae were found deeper than 30 cm in the 60-cm-deep boxes, only six burying-depth classes were defined: 0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20–25 cm and 25–30 cm.

Influence of larval vertical position on life cycle duration

The first experiment revealed variability in larval burying depth in the soil (see Results). Therefore, the aim of the experiment 2 was to determine whether variability in larval vertical position leads to variation in life cycle duration, as was predicted by Menu and Debouzie (1993). In experiment 2, we estimated also the importance of larval mortality after exit from the chestnut. Indeed, a high mortality which occurred unpredictability in some years could select for bet-hedging in life cycle duration (see Selective factors for bet-hedging in the Discussion).

In his review on prolonged insect diapause, Ushatinskaya (1984) stated that the LC with prolonged diapause occurs deeper in the soil than the SC. However, such a statement must be considered carefully, because of the rarity of studies and bias in experimental designs. For example, according to Boyce (1936), his own data on *R. completa* do not allow such a conclusion to be drawn because of confounding factors. To avoid bias, one must avoid experimental designs that remove larvae from soil and group them in boxes containing soil by class of burying depth before pupation, and estimating PDF after adult emergence at the laboratory. Indeed, a preliminary study showed that such an artificial design strongly increases larval mortality and modifies conditions for insect development and larval weight supposed to influence the probability of prolonged diapause. Another bias in design is burying boxes containing insects at different depths in the soil because: (1) larvae are constrained to stay at a fixed burying depth independent of their natural "choice", and (2) it is impossible to collect adults emerging from boxes kept far from the soil surface without perturbing environmental factors such as the O_2/CO_2 ratio (suspected to influence diapause development; Ushatinskaya 1984). A possibility is to remove the boxes after adult emergence, but the PDF estimate could be biased since, in the soil, dead adults can be destroyed by fungi and/or small ants (personal observation). The design described below avoids all the above problems.

We observed in experiment 1, that larval burying depth varied from 2 to 30 cm and that 98% of the larvae were found between 2 and 20 cm deep in the soil. Therefore, to facilitate placing the boxes in the soil and their removal from the soil, we limited their depth to only 20 cm (boxes: 10×10×20 cm).

First, six boxes randomly chosen over the 16 initially used were removed on 3–6 July 1995 (i.e. just before the switch between pupation or entry into prolonged diapause, Soula and F. Menu, in review) to record the vertical distribution (Di) of the whole immature population (larvae plus pupae). At this point, we could not distinguish between the larvae with a SC or LC because of an absence of morphological criteria for distinguishing them. The other ten boxes were kept in the soil until the end of adult emergence (Table 1 and see below).

Second, on 30 September 1995 (i.e. after adult emergence had occurred from 11 August to 23 September 1995), we removed the ten boxes left in the soil (Table 1) and estimated the distribution of vertical position of the LC larvae (DLC) in prolonged diapause (at this date all the larvae in the soil were in prolonged diapause). For Di and DLC, ten classes of larval burying depth were defined: from 0–2 cm to 18–20 cm.

Mortality of larvae in prolonged diapause between the two removals (on July and September) was negligible (see Results). Therefore, to obtain the distribution of immatures with a SC (DSC), we subtracted the larvae in prolonged diapause of DLC from Di to give the proportion of the total number of larvae that were buried in the six and ten boxes (449 vs. 746, respectively). We found 76 larvae in prolonged diapause in the ten boxes removed on 30 September 1995; however, as only 449 larvae were initially buried in the six boxes removed on 3–6 July 1995 (distribution Di) vs. 746 in the ten boxes removed on 30 September 1995 (distribution DLC), we subtracted only 46 (=76/746×449) larvae in prolonged diapause from Di to estimate DSC.

Table 2 Nos. for estimation of prolonged diapause frequency and larval survival in the soil in experiment 3 according to the cohort (larval exit period from fruits) and larval weight. No adult was found dead in the soil. *W1* ≤80 mg, 80<*W2*≤100 mg, *W3* >100 mg; for other abbreviations, see Table 1

		W1≤80 mg	80<W2≤100 mg	W3>100 mg
Cohort 1 (10–21 October)	No. of larvae buried in the soil	166	256	240
	No. of larvae in prolonged diapause[a]	15	24	33
	No. of adults produced after 1 year[b]	24	49	65
Cohort 2 (21–31 October)	No. of larvae buried in the soil	161	210	246
	No. of larvae in prolonged diapause[a]	10	37	47
	No. of adults produced after 1 year[b]	22	51	32
Cohort 3 (1–27 November)	No. of larvae buried in the soil	72	40	41
	No. of larvae in prolonged diapause[a]	32	28	18
	No. of adults produced after 1 year[b]	22	7	3

[a] LC individuals; [b] SC individuals

Influence of larval weight and exit date from fruits on PDF

The purpose of this third experiment was to test, in the field, if larval weight and date when the larva abandons the chestnut influences the probability of prolonged diapause (experiment 3, Tables 1, 2).

Each morning the larvae that had emerged from chestnuts were weighed in the field (Mettler ME30 balance; precision: ±0.1 mg) and classified into three weight classes: (1) larvae weighing <80 mg (W1), larvae between 80 and 100 mg (W2), and larvae weighing >100 mg (W3). We defined also three cohorts (exit date period, Table 2). The three boxes used per weight class and per cohort (1 and 2), contained similar numbers of larvae. All the boxes contained a maximum of 90 larvae. Preliminary experiments showed that no interaction between larvae occurred in soil with the design used. According to previous data on the pattern of larval exit date (Menu and Debouzie 1995), we predicted a low number of larvae in the third cohort; consequently, we used only one box per weight class for this cohort.

The next year (1996), emergence from each box was recorded. After the emergence period (early October), all the boxes were removed to note the number of larvae in prolonged diapause and dead adults in the soil (Table 2). PDF was defined as the ratio of the number of larvae in prolonged diapause to the total number of insects (emerged and dead adults in the soil+larvae in prolonged diapause). Such a PDF is calculated after the switch to pupation or prolonged diapause (see Chestnut weevil biology).

Results

Influence of larval vertical position in the soil on life cycle duration (experiments 1 and 2)

The experiments 1 and 2 showed variability in larval burying depth in the soil. In 1994 (experiment 1), the larvae burrowed 2–30 cm deep in the soil, with 98% (*n*=555 alive larvae, Table 1) of them found between 2 and 20 cm (Fig. 1). Only one larva (0.2%) was buried deeper than 25 cm. The larvae buried on average at a depth of 8.4±0.2 (±SE) cm in the soil and 50% of larvae buried at a depth lower than 7 cm.

In 1995 (experiment 2), 333 immature individuals (of which 326 were alive and seven dead; Table 1) including 267 larvae (260 alive and seven dead) and 66 pupae (all alive) were found at a depth of 2–20 cm and 92% of immature individuals buried from 2 to 18 cm in the 20-cm-deep boxes. Experiment 2 showed that, in contrast to the suggestion of Menu and Debouzie (1993), variability in larval vertical position did not generate life cycle duration variation. Indeed, the cumulative distribution (DLC) of larvae with prolonged diapause (LC individuals, *n*=76) did not differ significantly from the distribution of larvae with a SC (i.e. DSC) (SC individuals, *n*=287, i.e. 333 minus 46 larvae in prolonged diapause, see Materials and methods and Table 1) (Fig. 2, Smirnov test: *D*=0.2, *P*=0.99). The mean DSC of immature larvae was 13.3±0.2 cm (*n*=287) (Fig. 2) and was not significantly higher than the mean of DLC (i.e. 12.9±0.4 cm, *n*=76; one-tailed *t*-test: *t*=–0.73, *P*=0.23, power >0.999 with δ=5 cm).

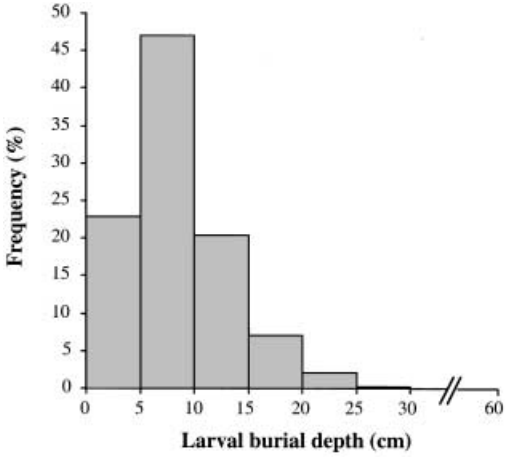

Fig. 1 Distribution of larval burial depth in a clayey soil (%) in the chestnut weevil (1994 data, experiment 1); *n*=555

A total of 243 adults (SC individuals) emerged from the ten boxes in the soil and after they emerged, we found 76 larvae in prolonged diapause (as indicated above) and ten dead adults in the soil (243+10=253; Table 1). The 4% (=10/253) of dead adults (i.e. SC individuals) were found, as were the pupae, at a depth of between 2 and 20 cm. PDF, estimated after 1 year in the soil, was 0.23 (=76/329).

Since, in insects, survival during diapause is higher than during development (Tauber et al. 1986; Danks

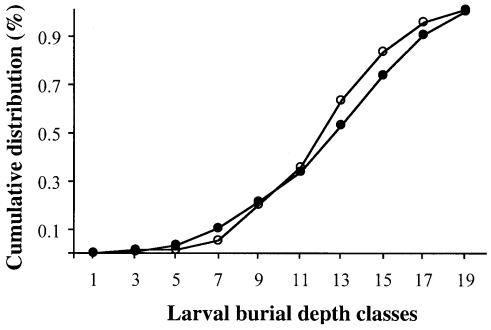

Fig. 2 Cumulative distributions (%) of larval burial depth in a clayey soil in the chestnut weevil (1995 data, experiment 2): Comparison between larvae with prolonged diapause [○ long cycle (LC) individuals, *n*=76] and larvae without prolonged diapause [● short cycle (SC) individuals, *n*=287]

1987), the monthly survival of larvae in prolonged diapause equalled at least the monthly survival before pupation, i.e. 0.965 (see Pupation and larval survival). Therefore, in experiment 2, the survival of larvae in prolonged diapause between 3 July (the date of removing the first boxes) and 30 September (the second removal) equalled at least 0.90 [=$(0.965)^3$]. Consequently, the number of larvae in prolonged diapause that died during this period was only nine at most {=[76/(1–0.10)]–76}. This low number of dead larvae gives us confidence in our estimate of DSC (see Materials and methods).

Influence of larval weight and exit date from fruits on PDF

Experiment 3 showed that globally, the PDF averaged 47.0% and ranged from 31.2 (W1≤80 mg, cohort 2) to 85.7% (W3>100 mg, cohort 3) (Fig. 3A, Table 2). A logistic regression (binomial error, logit link) showed that the PDF significantly increases with the larval weight (χ^2=7.50, 2 *df*, *P*=0.02; Fig. 3A, Table 2) and with the date of burrowing (χ^2=39.69, 2 *df*, *P*<0.0001; Fig. 3A, Table 2). Interaction between the two explanatory variables is nearly significant (χ^2=9.39, 4 *df*, *P*=0.052) and it seems that contrary to cohort 2 and 3, no weight effect exists for the first cohort.

Pupation and larval survival

Above we discussed the proximate factors of diversified bet-hedging. Now we discuss information on mortality factors selecting for bet hedging in life cycle duration (see Selective factors for bet-hedging).

Mortality before burying (measured by percentage of the larvae deposited on the soil and not buried after 1 day) ranged from 2.4% to 5% (Table 1). Experiment 3 showed that there was no effect of cohort (χ^2=5.64, 2 *df*, *P*=0.06) on this mortality but a significant influence of weight (χ^2=7.5, 2 *df*, *P*=0.02; about 4%, 3% and 1%, respectively, in W1, W2 and W3 classes).

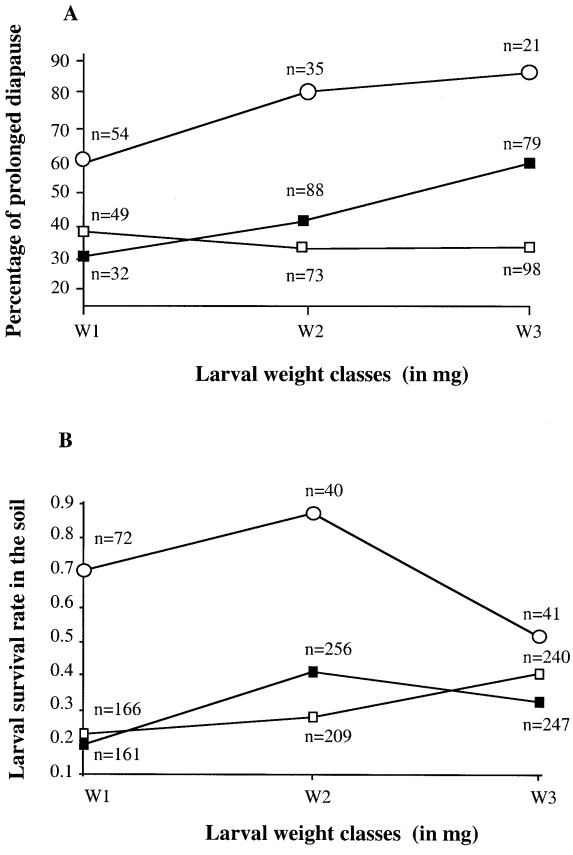

Fig. 3 A Percentage of prolonged diapause according to larval weight and date of exit from fruits. Dates are represented by three cohorts: □ cohort 1 (10–21 October 1995), ■ cohort 2 (22–31 October 1995), ○ cohort 3 (1–27 November 1995). Three weight classes are considered: ≤80 mg (*W1*), >80≤100 mg (*W2*), and >100 mg (*W3*). *n* represents the total number of surviving individuals after 1 year i.e. larvae in prolonged diapause (LC individuals)+adults (SC individuals). **B** Larval survival rate in the soil according to larval weight and date of exit from fruits. *n* No. buried larvae; for other abbreviations, see Fig. 3A

On the 15 June 1994 (experiment 1), no pupae were found in the soil but on the 3–6 July 1995 (experiment 2), the proportion of pupae was 20.2% (=66/326) of the total number of live immature individuals (pupae and larvae). Therefore, pupation occurs in the field between mid June and early July.

The total survival rates of immature individuals in the soil were low (Table 1). However, in experiment 2, the survival rate (S1) estimated from the six boxes removed on 3–6 July (i.e. during about 9 months) was higher, at on average 0.726 (*n*=449, SD=0.03 with six replicates), with a mean monthly survival rate equal to 0.965 [=$(0.726)^{1/9}$]. Since the frequency of pupae was small (20.2%, SD=0.13 with six replicates) on 3–6 July, S1 includes mainly survival before pupation. To simplify, it will be considered as an estimate of survival rate before pupation. The total survival rate (S2) in the soil (including pupation) estimated from the boxes removed on the 30 September 1995 (after adult emergence) equalled on average 0.441 (*n*=746, SD=0.10 with ten replicates)

(Table 1). In assuming constant monthly survival during pupation, the survival rate of immature individuals in the soil during pupation (3–6 July to 30 September 1995, i.e. about 3 months) should be 0.607 (=0.441/0.726). Therefore, with a total mortality estimated to be about 0.56 (=1–0.441), on average, at least 51% {=[(0.726×(1–0.607)/0.56]×100} of larvae died during the 3 months including pupation (3–6 July to 30 September) vs. at most 49% during the 9 months before pupation. We can provide confidence limits for this mortality during pupation since several replicates (boxes) are available (Table 1): at least 15–61% (with respect to the replicates used) died during pupation. These values are high since mortality during pupation occurs over only 3 months (vs. 9 months for mortality before pupation).

In experiment 3, we found an interaction between cohort and weight (χ^2=33.54; 4 *df*; P<0.0001) on total survival (including both adults, i.e. SC individuals and larvae in prolonged diapause, i.e. LC individuals) after burrowing in the soil (Fig. 3B, Table 2). On average, survival equalled 0.36 (=519/1432) and ranged from 0.31 (W1) to 0.38–0.39 (W2 and W3) and from 0.32 (cohorts 1 and 2) to 0.72 (cohort 3). The high survival rate observed in cohort 3 probably depends on the high frequency of larvae with prolonged diapause (Fig. 3A, B; Table 2). Indeed, in such LC larvae, pupation (critical period for survival, see above) did not occur during the first year but only from the second year.

Discussion

Being fat and late increases the probability of prolonged diapause

We have shown that the probability of a larva prolonging diapause increases with larval weight (the latter depending on metabolic resources consumed in prediapause development) and the date when the fruit is abandoned by the larva (the latter being related to time passed in the ground and then to energetic consumption).

Larval weight and emergence date from fruit depend on prediapause environmental conditions such as temperature (Manel and Debouzie 1997) and food (Desouhant 1997; Desouhant et al. 2000) in the chestnuts during summer and autumn. Such factors cannot reasonably "predict" quality (good or bad) of the next year, as this quality depends on soil drought in summer, chestnut and/or larval consumption (by human and animals) and larval attack by fungi during the next autumn (see the next section). Therefore, plasticity of life cycle duration dependent on larval weight (i.e. energetic resources) and exit date from fruits cannot evolve in response to predictive cues. Menu et al. (2000) showed, from a mathematical model, that such unpredictive plasticity maximises mean geometric fitness and probability of persistence in the chestnut weevil. Therefore, from these lines of evidence we conclude that adaptive plasticity in this species is diversified bet hedging.

Previous results (Desouhant et al. 2000) showed that a female spreads its offspring over several chestnuts at different times. Such an egg-laying strategy leads to individual variation in larval weight and emergence date from chestnuts in the offspring of a given female (Desouhant 1997) and consequently leads to variability in life cycle duration in a bet-hedging genotype.

A hypothesis for the mechanism underlying plasticity

Previous study showed that the adult performance of the chestnut weevil as measured by fecundity and longevity (Menu and Debouzie 1993; Soula and F. Menu, in review) is similar between adults which emerged after 1 (SC) and 2 years (LC). Furthermore, it is known that adult insect performance as indicated by egg production depends on metabolic resources such as lipids (Danks 1987). Therefore, these results and those presented in this paper are in accord with the hypothesis (hereafter called the "resource hypothesis") that larval phenotype (SC or LC) is influenced by individual metabolic resources (such as lipids) at the time of switching to pupation or prolonged diapause. Therefore, we assume that the LC phenotype compensates for an extra consumption of metabolic resources during prolonged diapause by a higher initial resource level. This hypothesis is supported by the fact that, in several species without prolonged diapause, such as the carabid *Nebria brevicollis*, it was shown that individuals that enter diapause exhibit a higher lipid level than those that develop directly (e.g. Penney 1969).

The metabolic resources available for a chestnut weevil larva depend on: (1) food availability during larval prediapause development in the chestnuts, and (2) consumption of metabolic resources during diapause and postdiapause in the soil. Since the time passed in the soil is shorter in larvae buried late, the cumulative consumption of these resources may be lower. Consequently, the larvae abandoning the chestnuts at a later date have a higher probability of a prolonged diapause as shown in this paper.

The bet-hedging concept based on the resource hypothesis produces, for the first time, a theoretical framework that is useful in clarifying the apparent diversity of factors influencing insect PDF (e.g. Ushatinskaya 1984; Tauber et al. 1986; Danks 1987; Hanski 1988). For instance, all the examples reviewed by Danks (1987, p 185) showed that different environmental factors may modify the expression of prolonged diapause during induction, diapause development, or even postdiapause development. Therefore, a challenge for the future may to investigate if such factors influence initial lipid levels during prediapause development and/or fat consumption during diapause and postdiapause development.

Even in assuming that metabolic resources control the switch to pupation or prolonged diapause, such plasticity is not predictive in the chestnut weevil since it maximises the potential fecundity of the LC phenotype but not its realised fecundity. For instance, during a bad year with soil drought during adult emergence (see below),

realised fecundity of the LC phenotype is low even if potential fecundity is equal to that of the SC phenotype (Menu and Debouzie 1993; Menu 1993a).

Menu and Debouzie (1993) suggested that vertical spatial variation in soil micro-environmental factors (acting as proximate factors) may also generate diversified bet hedging in the chestnut weevil. However, our results do not support such a hypothesis since the distribution of vertical position of SC and LC larvae are similar (Fig. 2). Our results also do not agree with Ushatinskaya's (1984) general statement that prolonged diapause in insects occurs when they are deeper in the soil. Therefore, in contrast to prediapause developmental variation, variability in vertical larval position cannot generate diversified bet-hedging in the chestnut weevil population that we studied. However, since life cycle duration probably depends on individual metabolic resources at the time of switching, it is not clear whether our conclusion can be generalised to other populations or species. Indeed, we can suppose that in the chestnut weevil population that we studied, variability in the O_2/CO_2 ratio in the soil was too low to lead to differential consumption of such resources as lipids and, consequently to variation in life cycle duration. In other populations or insect species overwintering in other kinds of soil and/or with a higher range of burying depth, conditions for life cycle duration variability could be met. Therefore, experiments avoiding bias (see Materials and methods) are needed for other populations and species.

We have shown correlations between different aspects of the biology of the chestnut weevil and the probability of prolonged diapause. Our correlative study suggests an influence of metabolic resources, but the exact underlying ecophysiological mechanisms should be studied experimentally (work now in progress).

Selective factors for bet hedging

Diversified bet hedging is viewed as a response to unpredictable variability in fecundity and survival. Previous data showed that variability in life cycle duration in the chestnut weevil is an adaptation to yearly and unpredictable variability in soil moisture during adult emergence (Menu 1993a; Menu et al. 2000), chestnut collecting by humans, and larval consumption by animals (Menu and Debouzie 1993; Menu et al. 2000). For example, in Lyon, in dry summers, the success rate of adult emergence from the soil is low. Furthermore, a high rate of chestnut collection by humans and/or larval consumption (before they have burrowed in the soil) by animals such as birds, wood mice or pigs decreases greatly the survival rate during prediapause development.

Data presented here permit discussion of other mortality factors selecting for variability in life cycle duration. Even in our design which protected against larval predation, at least 56% of the buried larvae died during the first year in the soil (experiment 2 with ten boxes and experiment 3). In our experiments, mortality during pupation (over 3 months) explains on average at least 51% of the annual mortality (over 12 months). Thus, the pupation phase is a critical period. High mortality in the soil in our protected design may be mostly due to infection by an entomopathogenic fungus that we identified as *Metarhizium anisopliae.* A greater rate of fungal infection in larvae in experiment 1 (1993–1994) explains the highest mortality in this experiment. The larvae of *Curculionidae* are infected by fungal conidia on the soil surface when they emerge from chestnuts (Gottwald and Tedders 1982, 1983). The fungi develop during larval postdiapause development and during pupation (personal observation). Therefore, stochastic annual variation in infection by fungi (due to annual variability in conidia production on the soil) may be another efficient selective factor for bet-hedging in life cycle duration. Conversely, despite its impact on overall larval survival, predation in the soil by wood mice, moles and ants (Menu and Debouzie 1993) is probably not a key factor for selection of bet-hedging for life cycle duration in the population that we studied. Indeed, since the distribution of vertical position of SC larvae is similar to that of larvae with a prolonged diapause, predation in the soil may be the same in the two kinds of larvae.

To conclude, evidence of diversified bet-hedging for life cycle duration both in desert organisms (Philippi 1993a, 1993b; Danforth 1999; Clauss and Venable 2000) and in a temperate species, the chestnut weevil, *Curculio elephas,* does not support the alternative hypothesis of a genetic polymorphism of pure diapause strategies. According to this latter hypothesis, no phenotypic plasticity in life cycle duration exists: a population may be composed of genotypes for either pure short life cycle (emergence after 1 year) or pure fixed long life cycle (for instance, emergence after 2 years). However, existence of diversified bet-hedging does not mean that genetic variability is absent in such a system. Indeed, plasticity (for example, a lipid level threshold indicating a switch to a prolonged diapause for a bet-hedging genotype) may be genetically variable, as seen for diapause induction in the cricket, *Allonemobius socius* (Roff and Bradford 2000). Results from genetic experiments conducted in the chestnut weevil are in accord with such a hypothesis (F. Menu, et al., in preparation).

Acknowledgements We are grateful to C. Bernstein, D. Debouzie, G. Driessen, S. Gourbiere and the two referees (D. Roff and one anonymous) for their helpful comments and J. Berthier and G. Valla for the identification of *Metarhizium anisopliae.* We thank also R. Grantham for help with the English and constructive remarks, J. M. Legay for allowing us to experiment on his property and A. Heizmann for technical help. This study was supported by the Centre National de la Recherche Scientifique (CNRS, UMR 5558, France) and by the French Environment Ministry (Comité Écologie et Gestion du patrimoine Naturel, grant 95082).

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