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Ecophysiological attributes of adult overwintering in insects: insights from a field study of the nut weevil, *Curculio nucum*

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Abstract. Diapausing insect species have evolved a great diversity of life cycles, although overwintering occurs at a single development stage within most species. Understanding why diapause has evolved towards a given life stage requires investigation of both the ecological and physiological attributes. Notably, it is suggested that adult overwintering is more energy-demanding than larval overwintering but it brings fitness gains by allowing adults to be synchronized with their seasonal requisites through an early spring emergence. This hypothesis is tested in field conditions in the nut weevil *Curculio nucum*, whose life cycle comprises an obligate 2-year, nonfeeding underground phase, including a larval, followed by an adult, overwintering. In this species, adult wintering leads to an early spring emergence; at first glance, however, this does not enhance synchronization between weevils and their host because adults emerge more than 1 month before starting to breed. It is suggested that adult overwintering ultimately evolved in response to the phenology of the host, by allowing females to oviposit in nuts before their full sclerotinization. Adult overwintering appears to be costly because adults postpone reproduction for 1 year, incur a significant weight loss and require feeding before egg laying. Surprisingly, lipids are unaffected during diapause, lipogenesis even being likely in the summer metamorphosis. These results suggest that the lipids involved in egg production may entirely come from the larval stages, whereas the other nutrients are acquired through adult feeding but this remains to be tested.

Key words. Complex life cycle, *Curculio nucum*, host phenology, larval or adult diapause, lipids, seasonal adaptation.

Introduction

Life cycles of many insect species comprise phases of active life alternating with forms of dormancy. Diapause is considered as one major trait of insect lifetime and is a major concern in numerous evolutionary studies devoted to understanding life history and strategies of resource acquisition and allocation (Denlinger, 1986; Mazaki & Wipking, 1994; Menu & Desouhant, 2002; Soula & Menu, 2005; Danks, 2006). Diapause is defined as a genetically-based developmental strategy that enables many temperate insect species, by lowering their metabolic needs, to survive adverse conditions and to be synchronized with their seasonal requisites (Tauber *et al.*, 1986; Danks, 1987; Hahn & Denlinger,

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2007). Diapause is considered to be a dynamic process comprising phases of lowered metabolic rate and development resumption, as well as initiation of post-diapause active life (Kostal, 2006).

Diapausing insect species have evolved a great diversity of life cycles in temperate regions, with considerable interspecific variations in the pattern of overwintering (Tauber *et al.*, 1986; Danks, 1987). Across species, dormancy is described at all life stages from eggs to adults but, at the species level, a single dormant period mostly occurs at a given development stage (Danks, 1987). In a large proportion of holometabolous insect species, overwintering diapause occurs in fully grown larvae, at the pre-pupal stage or pupal stage, and is generally terminated by a post-diapause metamorphosis. Overwintering at a pharate or fully developed adult stage appears to be less frequent and mainly occurs among Coleoptera and Hymenoptera species (Danks, 1987).

Understanding the diversity of life cycles among diapausing insects and the evolution of dormancy at a particular life stage requires the simultaneous exploration of its ecological and physiological consequences with respect to the lifetime development of organisms. Because food intake is often reduced or absent during diapause, organisms can only rely on stored nutrients to sustain this extended, often energy-demanding period and to resume development afterwards (Danks, 1987; Hahn & Denlinger, 2007). Both the amount and quality of nutrient reserves available at the onset of diapause are critical for sustaining diapause and post-diapause metabolism (Hahn & Denlinger, 2007). Lipids, directly acquired through diet or synthesized from proteins or carbohydrates, are among the most efficient forms of energy storing and are widely used as one major metabolic substrate of physiological processes underlying dormancy and development resumption (Downer & Matthews, 1976; Hahn & Denlinger, 2007). The course and the issue of diapause are known to be linked to the amount and quality of stored nutrients that are likely to differ among distinct life stages owing to their differing diets. Insects diapausing at distinct life stages are not only likely to assimilate and store different nutrients, but also they may have different metabolic needs to achieve post-diapause development; therefore, they may be constrained by depletion of distinct nutrient supplies. From an ecological perspective, diapausing at distinct life stages may also provide organisms with unequal ability to overcome environmental stress throughout diapause (i.e. cold, heat or drought) and diapausing at a specific stage may ultimately be governed by the most limiting environmental resource required for development resumption (Tauber *et al.*, 1986). Moreover, diapause is considered to be one major mechanism allowing synchronization of the life cycle with environmental seasonal variations, particularly when post-diapause active life requires a resource available only during a short time interval (Tauber *et al.*, 1986; Danks, 2006; van Asch & Visser, 2007). The evolution of diapause toward one particular life stage should therefore result from the best balance between costs and benefits at both ecological and physiological levels.

Studies comparing ecological and physiological attributes of diapause at distinct life stages are scarce and are limited to interspecific comparisons of adult versus larval overwintering (Eijs, 1999; Bosch *et al.*, 2001; Ellers & Van Alphen, 2002; Kemp *et al.*, 2004). These laboratory studies suggest that overwintering as an adult is energetically costlier than as a larva but provides adults with higher fitness returns through their earlier emergence in spring. Emerging early in the season should be adaptive because adults benefit from breeding early to find suitable conditions for egg laying and larval provisioning (Bosch *et al.*, 2001; Fordyce *et al.*, 2006), maximize time for offspring development when the favourable season is short (Danks, 2004) or increase their fitness through an extended breeding period (Eijs, 1999; Ellers & Van Alphen, 2002; Kemp *et al.*, 2004). This hypothesis remains untested in the field, however, probably due to the paucity of life cycles including overwintering at various life stages under the same environmental conditions.

Some weevil species of the genus *Curculio* sp. (Coleoptera: Curculionidae) have evolved a complex life cycle involving wintering diapauses at larval and adult stages, successively (Coutin, 1992). These phytophagous specialist insects infest perennial plants for larval development. Fully mature larvae burrow into the ground where they spend a wintering diapause. Metamorphosis occurs either during the next summer or after a prolonged larval diapause that may last up to 3 years. Then, depending on the species, adults either readily emerge from the ground at mid-summer and readily reproduce (e.g. the chestnut weevil *Curculio elephas*; Coutin, 1992; Menu, 1993a, b; Menu & Desouhant, 2002; Soula & Menu, 2005) or appear to extend their underground life by overwintering before emerging the next year (e.g. the acorn weevil *Curculio glandium*, the hazelnut weevil *Curculio nucum*; Martin, 1949; Coutin, 1992). Because *Curculio* species do not feed at all throughout their underground life, which is from mature larvae to emerged adults, the depletion of larval resources can be investigated through field studies during this extended nonfeeding period.

The nut weevil *C. nucum* (L.) (Coleoptera: Curculionidae), a specialist of hazelnut trees (*Corylus avellana* L.), appears to be able to display an extended 2-year life cycle, including one adult wintering in addition to larval wintering, with both phases being probably diapause-mediated (Martin, 1949; Coutin, 1992; AliNiazee, 1998). Such a complex, obligate life cycle possibly involves additional costs compared with a single-year life cycle, such as postponing breeding to the next year and thus reducing breeding opportunities, increasing energy depletion that impedes females' fecundity and increasing exposure to predators and thus, pre-reproductive mortality, throughout the extended underground phase. Moreover, adult wintering diapause could be energetically costlier than larval diapause but could in turn allow adults to be better synchronized with their host, as shown in a few Hymenopteran species (Tauber *et al.*, 1986; Eijs, 1999; Bosch *et al.*, 2001; Ellers & Van Alphen, 2002; Kemp *et al.*, 2004).

To test this hypothesis in *C. nucum*, a field study is used to obtain a quantitative description of the life cycle; the energy expenditure of larval overwintering is then compared with adult overwintering among individuals that are held under the same natural conditions. Field experiments are designed with four objectives: (i) confirming the obligate 2-year complex life cycle through field investigations of the timing of metamorphosis and of adult emergence; (ii) assessing the timing of adult emergence after adult overwintering and relating it to the oviposition period; (iii) testing whether adult overwintering is more a energy-demanding phase than larval overwintering by comparing dry weight variations of larvae and adults within the same period and under the same natural conditions; and (iv) testing whether lipids equally fuel adult and larval wintering phases because these nutrients are mostly used to sustain diapause and post-diapause development in insects (Downer & Matthews, 1976; Hahn & Denlinger, 2007) and should be a nonlimiting resource because hazelnuts provide larvae with a great amount of free fatty acids (Koksal *et al.*, 2006). In addition, weight and lipid variations are estimated during summer metamorphosis.

Materials and methods

Biology of the nut weevil

The nut weevil is a holometabolous phytophagous insect widely distributed throughout Europe and Asia; it is one major pest of hazelnut crops and the only weevil infesting this host plant (Martin, 1949; AliNiazee, 1998). Development appears to occur in a 2-year life cycle (Fig. 1a). In early summer (June), females oviposit within maturing hazelnuts (*Corylus avellana* L.), laying a single rich-yolk egg per nut, and larvae feed on the nut kernel to achieve their development. By the end of summer, mature larvae bury into the ground where they build an individual cell. After an overwintering larval diapause, most larvae undergo full metamorphosis during the next summer. Newly-formed adults then mainly overwinter before emerging on spring of the next year. Anecdotal observations report that a few adults emerge in the autumn after metamorphosis (Martin, 1949) but no reliable quantitative description is available. Adult females are reproductively immature at emergence and ovarian development is only attained from 1–2 months later, after a feeding period (Martin, 1949; Coutin, 1992; AliNiazee, 1998). *Curculio nucum* also shows some variability in its life cycle, due to a small, yet not quantified, proportion of individuals per cohort delaying metamorphosis from 1–2 years (Martin, 1949; Coutin, 1992; AliNiazee, 1998).

Field experiments: general design

Field experiments were conducted yearly from 2004 to 2006 on nut weevils from the same hedge of trees in a hazelnut plantation (Pollionnay, France, 420 m a.s.l., 45°46′N, 4°39′ E). Mature hazelnuts were harvested on trees each year between 26 July and 20 August and placed in a wire-netting box in a sheltered location nearby the trees. For the purpose of Experiments 1–4 (see below), naturally-emerging mature larvae were collected daily and were randomly assigned to, and allowed to freely burrow in, several covered, water-permeable plastic receptacles that had previously been filled with sifted soil and burrowed under the host tree.

Seasonal pattern of adult emergence and presence on hazelnut trees

Experiment 1. Eight hundred and seventeen mature hazelnuts were harvested in summer 2004 from which 162 larvae were collected. These larvae were randomly assigned to one of two receptacles (26 L, height 40 cm, diameter 44 cm). Natural emergences were monitored weekly daily between early April and end of June 2006.

Experiment 2. The presence of adult males and females on hazelnut trees was weekly sampled between 12 April and 15 July 2005 by means of a systematic striking of branches of the tree hedge with a wooden stick; insects were collected on white sheets laid out under the trees.

Assessing energetic demand and fat metabolism of adult and larval overwintering

Experiment 3. In summer 2005, 2073 hazelnuts were harvested and 517 larvae were collected. Of these, 210 and 231 larvae were randomly assigned to one of two receptacles that were monitored from fall 2006 until spring 2007 (Fig. 1b).

Experiment 4. In summer 2006, 3359 hazelnuts were harvested and 621 larvae were collected. Out of these, 147 larvae were randomly assigned to, and successfully buried into, one of ten water-permeable plastic tubes (3.14 L); of these tubes, seven had 15 larvae and three only had 14 larvae each. These tubes were located according to five neighbouring sites (two tubes per site) located nearby receptacles of Experiment 3.

Insect excavation (Fig. 1b). On 26 September 2006, pre-wintering adults and pre-wintering short-cycle larvae were systematically excavated from one randomly selected pot (Expiriment 3) and from five randomly selected tubes, one per site (Experiment 4), respectively. Excavated weevils were systematically weighed (to the nearest 0.1 mg), adults were measured using a binocular microscope (maximum body length without rostrum to the nearest 0.05 mm) and all individuals were immediately stored at −20 °C. The same operation was carried out on 19 April 2007 on the remaining receptacles (one pot from Experiment 3 and five tubes from Experiment 4).

Short-cycle larvae were the main concern of this study, and long-cycle larvae were excluded from the analysis due to low numbers: among 158 and 65 larvae that survived to their first winter in 2006 and 2007, 5.1% and 6.2% engaged in a prolonged larval diapause, respectively. Even if both sexes

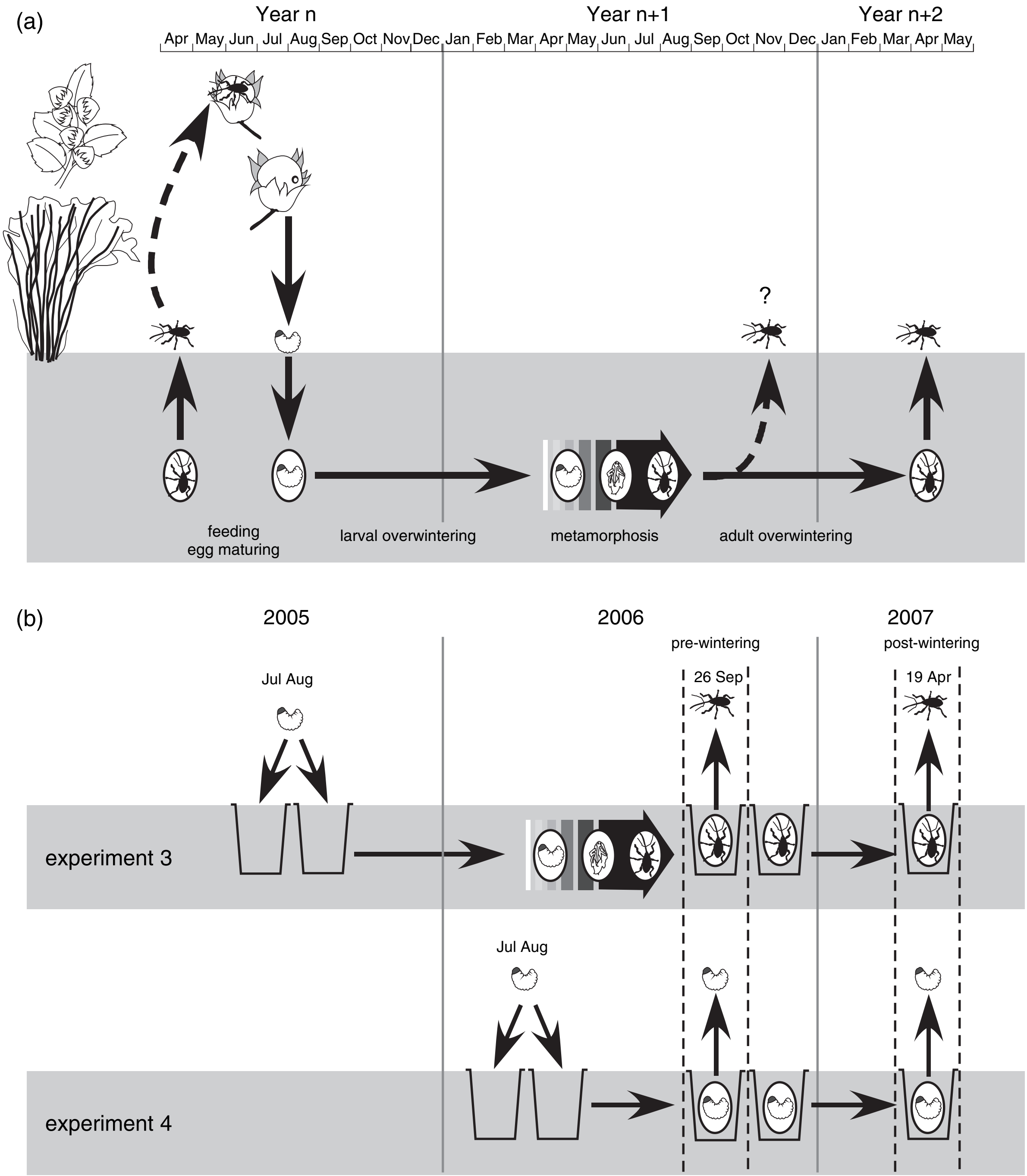

Fig. 1. (a) Life cycle of the nut weevil, *Curculio nucum*. In early spring of year *n*, adults emerge from ground and spend some time feeding and maturing eggs. In June, they mate and oviposit within fertilized, maturing nuts; larvae develops within the nut and mature larvae leave the fruit, drop on the ground and readily burrow into the ground where they build a single cell. They enter a larval, diapause-mediated, overwintering and most individuals undergo metamorphosis on the next summer (year $n + 1$). Once metamorphosis is completed, adults remain in their cell and overwinter until next spring (year $n + 2$) when they emerge from ground and start their active adult life. A few adults have also been reported to emerge in late summer after metamorphosis (year $n + 1$; Martin, 1949). This life cycle occurs yearly and may be extended over 4 years due to prolonged larval diapause of a few individuals per cohort (not shown here). (b) Design of field experiments 3 and 4, respectively. Both experiments were used to explore, under the same field conditions, energy depletion and lipid use of pre- and post-wintering larvae and adults (for details, see text). Larvae from cohorts 2005 and 2006 were allowed to burrow into receptacles previously filled with soil and placed nearby the host trees. Pre- and post-wintering adults from cohort 2005 and larvae from cohort 2006 were simultaneously excavated on 26 September 2006 and on 19 April 2007.

overwintered as adults (see below), only adult females were studied due to possible sex-related differences in nutrient storing and allocation toward reproduction (Karlsson & Wickman, 1990; Sibly *et al.*, 1997).

Analysis of dry weight variations. Dry weight variations provide a good estimate of the total nutrient depletion and hence, the energetic cost of overwintering. First, dry weight of an insect had to be inferred from wet weight according to the life stage (adult female or larva) and time in wintering phase (pre- or post-overwintering insect). For that purpose, 16 pre- and ten post-wintering females, and 14 pre- and 11 post-wintering randomly selected larvae, respectively, were weighed before and after they were maintained at 60 °C over 48 h. Influence of life stage, time in wintering phase and their interaction was tested on the dry weight-wet weight relationship using an analysis of covariance (ANCOVA). Second, variations of estimated dry weight during overwintering were tested on a separate sample that was also analysed for lipid content (15 pre- and 13 post-wintering females and 15 pre- and 15 post-wintering larvae; see below).

Analysis of total lipid content. To assess implication of lipids as source of energy for larval and adult wintering phases, total lipid content was measured on whole organisms, once the adult rostrum, legs, elytra and wings were removed, using the vanillin-phosphoric acid method of van Handel & Day (1988).

Assessing energetic demand and fat metabolism of summer metamorphosis

A first estimate of energy depletion associated with summer metamorphosis is given by comparing both dry weight and total lipid content of post-wintering spring larvae and pre-wintering adult females (15 larvae and 15 females excavated on 19 April 2007 and 26 September 2006, respectively).

Results

Curculio nucum: life cycle and seasonal distribution of adults

Life cycle. The time elapsed between larval burying and adult emergence was measured. No emergence occurred the year after larval burying, although pupation and metamorphosis were achieved by most individuals in summer of that year. Hence, of the 162 larvae buried in summer 2004, 41 adults freely emerged and none emerged before 18 April 2006 (Experiment 1; Fig. 2a). The pattern of emergence was similar for cohort 2005 that produced 155 adults alive in spring 2007 that started to emerge only from 14 April 2007 (Experiment 3). No larvae from cohort 2006 pupated before 19 April 2007 (Experiment 4; Fig. 1b), whereas most larvae from cohort 2005 had already completed their metamorphosis on 26 September 2006 (Experiment 3; Fig. 1b), with the remaining larvae (5.1%, $n = 158$) undergoing a prolonged larval diapause. In this studied population, therefore, the nut weevil has an obligate 2-year life cycle involving one larval wintering diapause followed by one summer metamorphosis and one adult wintering dormancy.

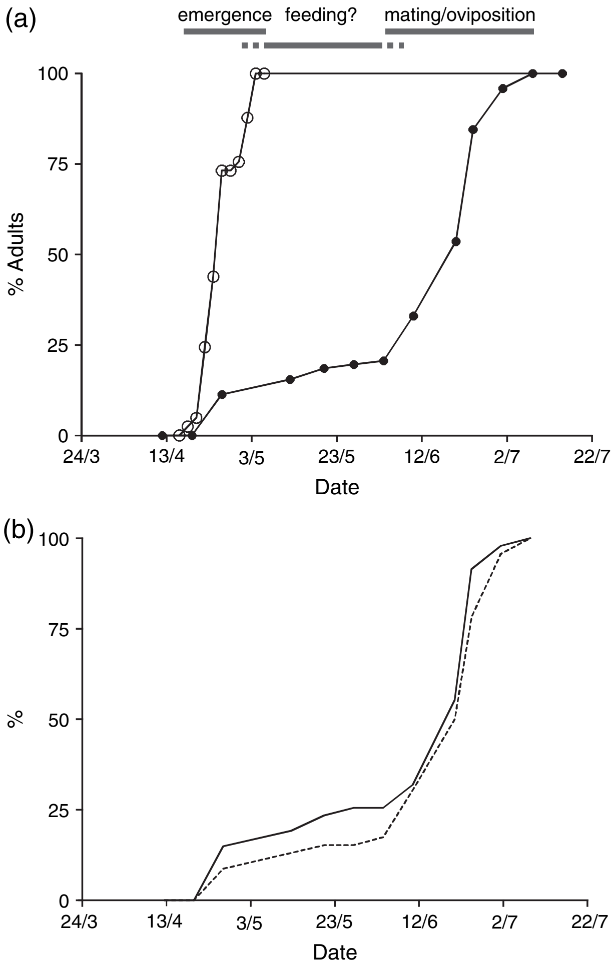

Fig. 2. (a) Seasonal distribution of adult nut weevil emergence from ground (open circles, 41 adults) and adult presence on hazelnut trees (full circles, 94 adults). After emergence, adults appear to leave the tree hedge before massively coming back more than 1 month later (54 days elapsed between the median values of adult emergence and presence on hazelnut trees). (b) Seasonal distribution of males (full line, $n = 47$) and females (dotted line, $n = 47$) on hazelnut trees. Males and females experienced a similar feeding period, strengthening the hypothesis that mating occurs afterwards.

Seasonal pattern of emergence and presence on host trees. Of the 65 larvae from cohort 2004, 41 adults were alive on spring 2006 and emerged naturally between 18 April and 3 May 2006, with 24 April as the median value (Fig. 2a). In 2005, we found 97 adult weevils on the tree hedge between 26 April and 8 July (median date 17 June, Experiment 2). Thus, assuming that emergence and presence patterns are stable over years on the studied tree hedge, 54 days elapsed between adult emergence and presence on the host tree.

Moreover, the adult sex ratio was balanced among weevils present on the host tree, and the two sexes had a similar distribution across the season (generalized linear model fitted on a binomial error: effect of sampling date: $F_{1,8} = 1.72$, $P = 0.23$; mean estimated proportion of males among adults over the season = 0.506; Fig. 2b).

Energetic demand and lipid metabolism

Wet weight–dry weight relationship. Water content significantly differed according to both life stage and time in the wintering phase (ANCOVA, life stage: $F_{1,47} = 6.96$, $P = 0.011$; time: $F_{1,47} = 10.75$, $P = 0.002$). Overall, larvae had greater water content than adult females, and pre-wintering insects of both life stages were less hydrated than post-wintering ones (linear regression model: pre-wintering larvae: dry weight = –2.75 + 0.49 × wet weight; post-wintering larvae: dry weight = –3.92 + 0.49 × wet weight; pre-wintering adult females: dry weight = –1.61 + 0.49 × wet weight; post-wintering adult females: dry weight = –2.78 + 0.49 × wet weight; $R^2 = 0.96$, $F_{1,47} = 938.53$, $P < 0.001$). Due to the strong relationship between dry and wet weight, dry weight of individuals was estimated in all subsequent analyses.

Overwintering phases. Dry weight variance as well as variance of total lipid content were homogeneous among pre- and post-wintering insects (dry weight: Levene test, larvae: $F_{1,28} = 0.97$, $P = 0.33$; adult females: $F_{1,26} = 0.18$, $P = 0.68$; lipid content: larvae: $F_{1,28} = 0.32$, $P = 0.58$; adult females: $F_{1,28} = 0.05$, $P = 0.83$). Controlling for their body size, females lost approximately 8.5% of their initial weight [pre-wintering females: dry weight = –25.50 (± 6.08 SE) + 7.86 (± 1.11 SE) × body length; post-wintering females: dry weight = –27.66 (± 6.17 SE) + 7.86 (± 1.11 SE) × body length; dry weight of pre- versus post-wintering females, Student‘s *t*-test: $t_{25} = 4.26$, $P < 0.001$; body length effect on dry weight, $t_{25} = 7.11$, $P < 0.001$; Fig. 3]. By contrast, no significant weight variation was found in larvae [estimated dry weight of pre-wintering larvae = 23.51 (± 1.20 SE) mg; post-wintering larvae = 22.86 (± 1.20 SE) mg; Student's *t*-test: $t_{28} = 0.38$; $P = 0.71$]. Total fat content corrected by dry weight did not significantly vary during larval overwintering [pre-wintering larvae = 124.79 (± 11.01 SE) μg mg^{-1}: post-wintering larvae = 125.61 (± 11.01 SE) μg mg^{-1}; $t_{28} = 0.053$, $P = 0.96$; Fig. 4a]. Adult females also showed no significant variation of lipids during overwintering [pre-wintering females = 208.70 (± 13.80 SE) μg mg^{-1}: post-wintering females = 224.97 (± 14.83 SE) μg mg^{-1}; $t_{28} = 0.053$, $P = 0.96$; Fig. 4b] and their lipid content neither varied according to their body length ($F_{1,25} = 0.546$, $P = 0.47$), nor interaction between body length and wintering time ($F_{1,24} = 0.442$, $P = 0.51$).

Summer metamorphosis. Dry weight was more variable in post-wintering larvae than in pre-wintering adult females (Levene test: $F_{1,28} = 6.29$, $P = 0.018$); adult females were 25% lighter than larvae [Mann–Whitney *U*-test: $U = 36$, $z = -3.173$, $P = 0.0015$; median dry weight (lower-upper quartiles): females = 17.61 mg (16.17–19.47); larvae = 23.50 mg (19.39–26.02)]. Variance of lipids (μg mg^{-1} dry weight) was homogeneous between post-wintering larvae and pre-wintering adult females (Levene test: $F_{1,28} = 0.07$, $P = 0.79$). Adult females had a greater content of lipids (larvae = 124.80 ± 11.38 μg mg^{-1} dry weight; adult females: 208.70 ± 11.38 μg mg^{-1} dry weight; $t_{28} = 5.214$, $P < 0.001$) and had even 31.7% more absolute fat content than larvae (larvae = 2799.3 ± 223.8 μg; adult females: 3685.4 ± 223.8 μg mg^{-1} dry weight; $t_{28} = 2.80$, $P = 0.009$).

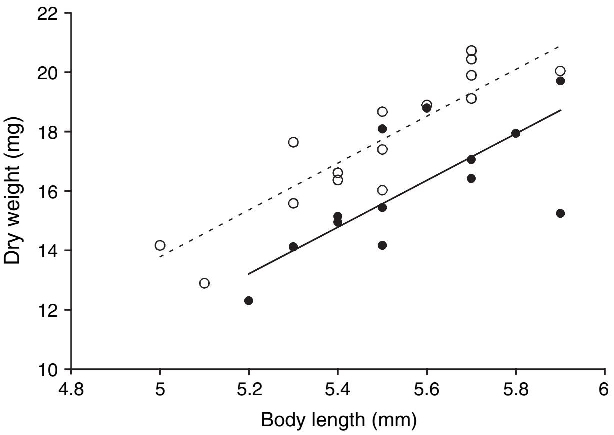

Fig. 3. Variations of dry weight between pre-wintering (open circles, $n = 15$) and post-wintering adult females (full circles, $n = 13$) according to their body length. Estimating dry weight variations is best achieved through a linear additive model including wintering time and body length ($F_{2,25} = 29.84$, $R^2 = 0.68$, $P < 0.001$) Controlling for their body length, females lost approximately 8.5% of their dry weight during the wintering period considered (Pre-wintering dry weight = –25.5 + 7.86 × body length (dotted line); Post-wintering dry weight = –27.66 + 7.86 × body length (full line); Student's *t*-test, $t_{25} = 4.26$, $P < 0.001$).

Discussion

In this field study, *C. nucum* shows an obligate 2-year life cycle because no adults emerge just after the summer metamorphosis; instead, most larvae emerge as adults 2 years after burying, with the remainder undergoing a prolonged larval diapause. This 2-year life cycle appears costly for two major reasons: first, despite their full metamorphosis during the first year, newly-formed adults postpone their breeding activity until the second year instead of breeding yearly. Everything else being equal, genotypes setting up one generation every 2 years should be readily counter-selected compared with genotypes breeding at a faster rate. Second, extending the pre-reproductive underground phase over several months could increase pre-reproductive mortality because of a greater risk of disease (due to entomopathogenic fungi *Beauveria bassiana*; Paparatti & Speranza, 2005) or predation by small mammals (Menu & Debouzie, 1993). Moreover, there are no obvious benefits from breeding every

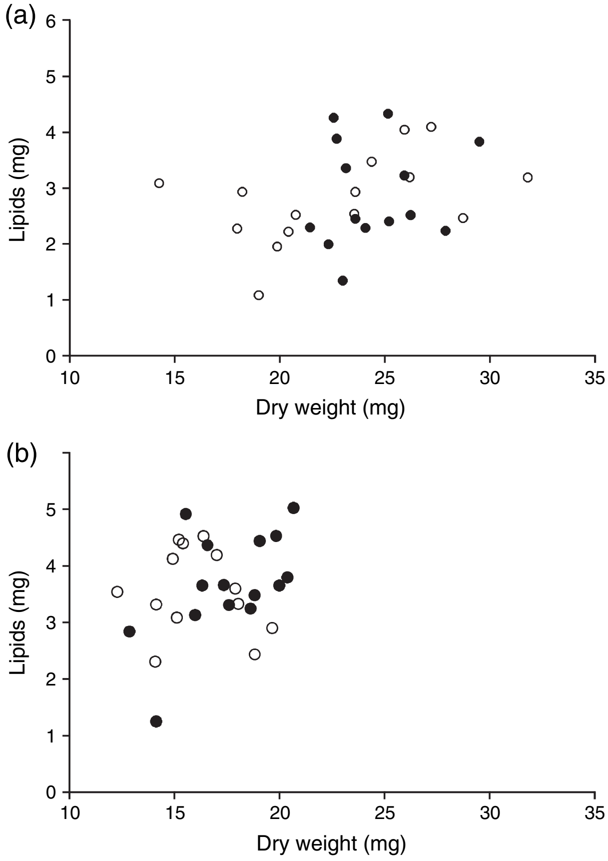

Fig. 4. Variations of absolute amount of lipids (mg per individual) between pre-wintering (full circles) and post-wintering (open circles) (a) larvae and (b) adult females with dry weight as a covariate. Lipids did not significantly vary during wintering, neither for larvae, nor for adult females despite their significant weight loss (for details, see text). By contrast, organisms significantly lost weight and gained lipids at metamorphosis.

2 years because hazelnut trees show no strong interannual variation of fruit production and thus provide *C. nucum* with a homogeneous resource over years (Germain, 1994).

What evolutionary forces triggered the evolution of such a complex life cycle? The obligate 2-year life cycle of *C. nucum* involves two successive overwintering, the first one at the larval stage and the second at the adult stage. In agreement with predictions, adult overwintering allows an early spring emergence of adults, from mid-April until early May. It is suggested that adult wintering diapause is the only way for weevil adults to emerge and breed early in the season but that it requires postponing emergence for 1 year after metamorphosis. Several observations corroborate this hypothesis: *C. nucum* are unable to perforate a mature nut to lay an egg inside and must therefore oviposit before July to avoid fully hardened nut shells (Martin, 1949; Germain, 1994). Such an early egg-laying could be impossible after a larval wintering diapause due to developmental constraints. The chestnut weevil *C. elephas* requires 7 months from larval post-diapause quiescence (late January) to metamorphosis (Menu, 1993b). Assuming that this development time is a physiological constraint common to *Curculio* spp. populations under the same latitudes and weather conditions, diapausing at the larval life stage only would have been strongly counter-selected in *C. nucum*. Moreover, early spring emergence associated with adult overwintering is also reported in a few hymenopteran and lepidopteran species (Eijs, 1999; Bosch *et al.*, 2001; Ellers & Van Alphen, 2002; Kemp *et al.*, 2004).

It is predicted that adult wintering, because it allows adult males and females to emerge in early spring, might also allow them to breed early in the season because of a tight synchronization with their vegetal host. This appears not be the case in the present study because a large delay occurs between adult emergence and female oviposition, as is suggested by adults' early departure from hazelnut trees (mid-end April), followed by their mass return more than 1 month later (June); the similar pattern of male and female presence on trees suggests that mating does not occur before this come-back. These observations confirm the quantitative study of Martin (1949) who shows that, in this species, ovaries are not fully mature before early June. Therefore, the present observations match neither of the classical evolutionary hypotheses that emerging early should be adaptive because adults benefit from breeding early and synchronously to find suitable conditions for egg laying and larval provisioning (Bosch *et al.*, 2001; Fordyce *et al.*, 2006), adults maximize time for offspring development when the favourable season is short (Danks, 2004) or they increase their fitness through an extended breeding period (Eijs, 1999; Ellers & Van Alphen, 2002; Kemp *et al.*, 2004).

Consequently, why do nut weevil adults spend so much time after emergence before mating and egg-laying? Upon weevil emergence, pollinated hazelnuts are already apparent, yet unfertilized, and thus they could allow an early oviposition (Germain, 1994). Nut fertilization only occurs from early June, depending on warm conditions, and nut kernel starts developing at that time. It is suggested first that egg-laying early within unfertilized oocytes may be risky because a large proportion of pollinated oocytes drops before fertilization (Germain, 1994), precluding eggs from developing successfully. Second, *C. nucum* would need to feed at adulthood before reproductive development takes place, as shown in a number of long-lived insect species where females mature rich-yolk eggs (Toepfer *et al.*, 2002, Wainhouse *et al.*, 2004). Third, such energy requirements would be increased due to the significant energy depletion occurring throughout adult overwintering. Several data are in agreement with this hypothesis: first adult overwintering is costly in *C. nucum* as shown by dry weight depletion, contrasting with the lack of significant dry weight variations among larvae under similar conditions. Second, several species overwintering as adult also need to replenish their reserves during a feeding period before females can mature eggs and oviposit (e.g. anautogenous mosquitoes *Culex pipiens*: Spielman, 1957; *Eustenopus villosus*: Connett *et al.*, 2001). Third, species diapausing at the larval stage only seem to save more larval-derived resources and to allocate them to a greater extent into reproduction. In *C. elephas*, female fecundity is not increased when adults are fed a sucrose diet, and egg maturation arises shortly after emergence (fecundity peaking approximately 8 days after emergence, Menu, 1992). Therefore, *C. elephas* females appear to depend entirely on larval-derived resources for reproduction, which coincides with the capital breeder strategy (Jervis *et al.*, 2008).

By contrast to *C. elephas*, the results suggest that *C. nucum* requires adult feeding for reproduction, thus being an income breeder at least for some nutrients. Emerging early in the spring should be adaptive either because it increases the feeding period, or because it allows adults to feed on a specific resource only available at the time of insect emergence. Further work is needed to disentangle these alternative hypotheses. Acquisition of some nutrients required for egg completion is shown to be restricted to a specific stage (Rivero *et al.*, 2001) and, more generally, recent studies highlight the need to explore nutrient acquisition and resource allocation strategies considering each nutrient class separately (Casas *et al.*, 2005). Paradoxically, in this preliminary physiological study, the nut weevil appears to be capital breeder for lipids. Lipids classically provide the most efficient means of storing energy (Downer & Matthews, 1976) and, in most diapausing species, they are involved as one major energy source for diapause and post-diapause phases irrespective of the life stage involved (Danks, 1987; Wipking *et al.*, 1995; Saunders, 2000; Ellers & Van Alphen, 2002; Fordyce *et al.*, 2006; Hahn & Denlinger, 2007). Whereas fat is the predominant fuel of diapausing insects and nuts are one lipid-rich substrate for larval development, lipids are surprisingly not the energy source of overwintering weevils: fat content remains steady among pre- and post-wintering larvae and adult females. In the present study, oxidization of lipids cannot be masked by any lipid uptake from an external source because the underground life is a strictly nonfeeding stage in this species. Alternatively, lipids can be consumed then restored through intermediary metabolism (Giron & Casas, 2003).

Broad information about physiological changes occurring during summer metamorphosis is provided by comparing post-wintering larvae and pre-wintering females. Larvae and adults obviously come from two distinct cohorts because their energy depletion needs to be compared under the same environment (i.e. during the same period in field conditions); nevertheless, variations are so large that they must be representative of physiological changes associated to summer metamorphosis. The loss of dry weight found between spring larvae and adult females entering diapause is consistent with a high level of metabolism and with costly physiological changes linked to metamorphosis of holometabolous insects (Nestel *et al.*, 2003; Wang *et al.*, 2006; Dutra *et al.*, 2007). Surprisingly, however, lipids drastically increase from larvae to adult females, by more than 31% of their initial absolute amount (65% if corrected by mg dry weight).

In this species and from the current field study, lipid saving and even lipid synthesis are observed during the underground phase. This suggests that teneral lipids may have a predominant role for adult active life in *C. nucum*. Lipids of larval origin stored in emerging adults are positively related to fitness components (Ellers and Van Alphen, 2002; Dutra *et al.*, 2007; Hahn & Denlinger, 2007). Hence, lipid concentration could be fixed upon adult emergence. In the nut weevil, teneral lipids could sustain the long pre-oviposition feeding period involving dispersal and flight and/or be further allocated to egg maturation. Adult males and females could be capital breeders for this nutrient class, similar to some hymenopteran species that cannot synthesize lipids as adults (Casas *et al.*, 2003, 2005; Giron & Casas, 2003). Adult spring diet in *C. nucum* appears to be mainly composed of carbohydrates (Martin, 1949; Coutin, 1992; AliNiazee, 1998); dietary proteins are also likely through pollen ingestion (M.-C. Bel-Venner, personal observation), a common feature among Coleoptera that triggers egg maturing in some species (Rana & Charlet, 1997; Wäckers *et al.*, 2007).

Studies combining field work and ecophysiology remain scarce among insects (Casas *et al.*, 2003, Wäckers *et al.*, 2007). The present study aims to fill this gap, based on a species whose life cycle extends over 2 years and includes one larval wintering diapause followed by one adult overwintering. It is shown that adult overwintering is more energy-demanding than larval wintering phase. Such a combined approach brings useful information to assist in our understanding of the evolution and diversity of life cycles among insects. Moreover, this field study is the first to quantify energy use of overwintering at distinct life stages; the lack of lipid use by any of the wintering life stages is an unexpected result. This must trigger the development of such field studies to rigorously reconsider energetic aspects of wintering dormancies.

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