Contrasted breeding strategies in four sympatric sibling insect species: when a proovigenic and capital breeder copes with a stochastic environment

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Summary

1. The evolution of strategies of resource acquisition and allocation is often considered to be closely dependent on the degree of environmental variability. Within this framework, female insects that experience stochastic fluctuations in the availability of their egg-laying sites in time or space can be expected to be fully synovigenic (i.e. they start maturing eggs after a delay once reaching adulthood), which allows them to tailor their reproductive investment to variations in the resource. Proovigenic females (that have most of their eggs already mature at the onset of their adult life, which corresponds to a capital breeding strategy), on the contrary, should have an advantage when the availability of the egg-laying sites is predictable. There is, however, a dearth of empirical studies testing these predictions.

2. Here, we tested the hypothesis that four phytophagous insect species of the genus *Curculio*, which coexist on a strongly fluctuating resource that they exploit for egg-laying purposes, would all be synovigenic as strict proovigeny should be counterselected. The resource consisted of the acorns of oak trees *Quercus* spp. We conducted field surveys to determine the date of adult emergence in each weevil species and the ability of newly emerged females to produce eggs. We also analysed the stable isotope profile of wild-caught females as a proxy for their feeding activity. Finally, we tested females under laboratory conditions for their ability to produce mature eggs when not fed and investigated whether dietary intake influenced their longevity.

3. Taken together, our results show that, contrary to the usual predictions, the four weevil species that were all exposed to a markedly fluctuating environment exhibited sharply contrasting strategies of resource acquisition and allocation: three species were synovigenic, while the fourth was proovigenic. Unexpectedly, therefore, our findings show that a strict capital breeding species might not always be counterselected in a temporally stochastic environment. They further suggest that fluctuations in the environment should not promote a sole, optimal strategy of energy acquisition and allocation to reproduction but instead should favour their diversification.

Key-words: *Curculio* spp., Curculionidae, energy allocation, fecundity, income breeding, stable isotopes, synovigeny

Introduction

Current theories in evolutionary ecology predict that a single optimal pattern of resource acquisition and allocation to reproduction should be favoured in a given environment, as an evolutionary response to environmental productivity, seasonality or unpredictability (Perrin & Sibly 1993; Ellers, Sevenster & Driessen 2000; Ellers & Jervis 2004; Houston *et al.* 2006; Fischer, Taborsky & Dieckmann 2009; Stephens *et al.* 2009; Fischer, Dieckmann & Taborsky 2010). These strategies have been finely investigated among insects from

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physiological processes to population dynamics through field surveys, laboratory experiments and theoretical modelling (Boggs 1997; Ellers & van Alphen 1997; Rivero-Lynch & Godfray 1997; Casas *et al.* 2000, 2009; Papaj 2000; Rosenheim, Heimpel & Mangel 2000; Jervis, Ellers & Harvey 2008; Richard & Casas 2009; Rosenheim 2011).

In insect populations exposed to stochastic fluctuations of their egg-laying sites (which constitute a limiting and fluctuating resource for population growth), proovigeny should be precluded (i.e. the females have all of their eggs mature at the onset of their adult life; Jervis *et al.* 2001; Jervis, Ellers & Harvey 2008; Jervis & Ferns 2004). Instead, the strategy of more synovigenic females, starting adult life with some immature eggs, can be expected to be optimal owing to the flexibility of the laying behaviour induced: such a strategy enables females to adjust the amount of energy allocated to reproduction to the perceived availability of the oviposition sites encountered (Heimpel 1998; Jervis & Ferns 2004; Richard & Casas 2009). There is evidence of a high egg maturation rate induced by such encounters (Casas *et al.* 2009), which might lower the cost for these females of being transiently egg-limited (Rosenheim, Heimpel & Mangel 2000). Such flexibility can be even increased by egg resorption that confers to synovigenic females the capacity of reallocating the energy from reproduction towards maintenance whenever current conditions are unfavourable because of either poor egg-laying sites or lack of food availability (Rivero-Lynch & Godfray 1997; Rosenheim, Heimpel & Mangel 2000; Jervis *et al.* 2001; Jervis, Boggs & Ferns 2005; Richard & Casas 2009). The flexible allocation of nutrients towards lifetime reproduction might be linked to the capacity for synovigenic females to fuel their gametes with energy gained concurrently through adult feeding, which corresponds to an income breeding strategy. Adult feeding has been reported in many synovigenic species among Hymenoptera and Lepidoptera (Jervis *et al.* 2001), and there is evidence of a negative relationship between the dependency on nutrients acquired at the adult stage and the ovigeny index that is the proportion of eggs produced throughout life that are mature at adult emergence (Jervis, Boggs & Ferns 2005; Jervis, Ellers & Harvey 2008). Yet the link between synovigeny and income breeding is complex because females synovigenic insect species have been shown to incorporate nutrients of both larval and adult origin into their eggs, thereby possibly using a mixed strategy, depending on the nutrient category, the feeding history or even ageing (Rivero & Casas 1999; Rivero, Giron & Casas 2001; O'Brien, Fogel & Boggs 2002; Casas *et al.* 2005; Jervis, Ellers & Harvey 2008).

In contrast, strict proovigeny corresponds to a capital breeding strategy as all nutrients incorporated into the eggs have been acquired at the larval stage. Females emerging with significant egg loads have a limited ability to disperse before ovipositing owing to their weak thoracic musculature and to the limited amount of energy available for flight (Dixon, Horth & Kindlmann 1993; Tammaru & Haukioja 1996; Jervis, Boggs & Ferns 2005). Proovigeny and capital breeding strategies are thus expected to be optimal when the local

availability of laying sites is both stable and predictable (Ellers & Jervis 2004).

While the proportion of eggs mature at emergence is well documented in holometabolous insects and shows empirical evidence of a continuum between strict proovigeny and synovigeny (Jervis, Ellers & Harvey 2008), the key role of the environmental fluctuations or of their steadiness in the evolution towards either extremes remains largely unexplored. This study aims to start filling this gap by investigating natural communities composed of four insect sibling species, all of which belong to the same genus and are exposed to the same, natural environment characterized by strong between-year fluctuations in the number of available egg-laying sites. Under this temporally stochastic environment, these species should all exhibit a synovigenic and income-like strategy, while none is expected to be strictly proovigenic and capital breeder.

We focused on insect communities composed of four weevil species – *Curculio glandium* (Marsham), *Curculio elephas* (Gyllenhal), *Curculio pellitus* (Boheman) and *Curculio venosus* (Gravenhorst) (Coleoptera, Curculionidae) – that are specialized on oak trees (*Quercus* spp.) in southern Europe, coexist on the same individual host plants (Hoffmann 1954; Coutin 1992; Hughes & Vogler 2004) and use oak acorns as their egg-laying sites (Venner *et al.* 2011). Females deposit eggs into oak acorns from early June to the end of the summer following their emergence as adults. Each larva fully develops into a unique, mature acorn before self-extracting from that fruit and burrowing into the soil in the fall (Venner *et al.* 2011). The fully mature larvae then enter diapause for variable periods of time (from 1 to 4 years), depending on the species and even on individuals within the same species (Coutin 1992; Menu & Debouzie 1993; Venner *et al.* 2011). Following that period, larvae experience full metamorphosis before emerging above-ground and breeding the same year. Because oaks are known to be 'masting' species, i.e. ones that produce seeds massively but intermittently, the availability of the resource suitable for larval development (i.e. mature oak acorns) varies considerably and partly randomly from 1 year to the next (Kelly & Sork 2002; See Appendix S1, Fig. S1, in Supporting Information). The spatial distribution of acorn availability is also heterogeneous, because trees located near each other are only partly synchronized (Liebhold *et al.* 2004). Each year, the population growth of the four weevil species might be severely limited whenever mature acorns are lacking locally, that is, in the spatial range that can be explored during the breeding season by adult weevils from the same population (Venner *et al.* 2011). The four weevil sibling species that exploit the same egg-laying sites and experience the same, unpredictable environment are thus expected to be synovigenic, which might allow them to cope optimally with the marked spatio-temporal fluctuations of the acorns.

Adults of the four weevil species under study do exclusively breed the year they emerge, even if the resource is locally depleted (which can occur in years when there are almost no oak acorns, neither on the tree on which an individual has matured nor on neighbouring trees). They must therefore be

able to escape from poor environmental conditions by moving to trees producing more acorns. Accordingly, adults need to emerge well before the laying period, so that they have enough time to prospect for suitable laying sites. As the energy required for this early dispersal is likely to be unavailable for oogenesis (according to the oogenesis-flight syndrome theory), very limited amounts of teneral reserves might be allocated towards reproduction (Jervis, Boggs & Ferns 2005; Zera 2005; Lorenz 2007). Accordingly, adult females should be synovigenous and could require additional energy to mature their eggs that they would obtain through feeding.

We conducted both field surveys and several experiments under controlled laboratory conditions for each species to examine these predictions following four steps: (i) we checked whether adults do emerge much before ovipositing by determining the emergence date of adults in the field and comparing it with the laying period, (ii) we determined whether the four species are synovigenic by estimating the ability of newly emerged females to produce eggs, (iii) to test whether they do feed as adults, first we analysed the stable isotope profile of wild-caught females and second we conducted a laboratory experiment to compare the longevity of adult females that were either unfed or fed a standard diet and finally, (iv) we checked under laboratory conditions the inability of unfed females to allocate energy towards reproduction, i.e. their inability to mature eggs.

Materials and methods

DETERMINATION OF THE EMERGENCE DATE OF ADULTS FROM THE FOUR WEEVIL SPECIES, SURVEYED YEARLY BETWEEN 2008 AND 2010

Following larval diapause and metamorphosis, adults emerge from the soil to take part in a single breeding season. In these species, females lay eggs in acorns from June to September. Each year between 2008 and 2010, we consistently surveyed the emergence date of adults of the four species in two natural communities located 30 km apart near Lyon in France, each community being found on isolated oak trees [site A (*Quercus robur*): N45°35′; E5°01′; site B (*Quercus petraea*): N45°45′; E5°16′]. Dormancy strategies vary greatly from one species to another (ranging in duration from 1 to 3 years): while *C. elephas* adults mostly emerge over the first 2 years following larval development, the number of years spent diapausing underground is fixed for the three other species (i.e. almost 2 years for *C. glandium* and almost 3 years for *C. pellitus* and *C. venosus*), irrespective of annual field conditions (Venner *et al.* 2011).

Here, we were surveying the emergence of adults from five consecutive larval cohorts (2005–2009). To do this, during each of these 5 years, we harvested all mature acorns that had dropped off the two oak trees studied onto a net laid on the soil over half the surface covered by the tree. These acorns were placed in wire-netting boxes in an outdoor arena that allowed us to collect and count daily all mature weevil larvae that emerged spontaneously from the acorns during the emergence season, i.e. from mid-August to the end of December. The larvae were then randomly assigned to several covered, water-permeable plastic receptacles that had previously been filled with sifted soil and buried under each host tree. The five cohorts were assigned to separate devices, which allowed larvae from a known cohort and tree

to develop in confined, semi-natural conditions until the adults emerge. Yearly from 2008 to 2010, the devices were surveyed on a weekly basis from early March. As soon as an adult was detected in one of the receptacles, we started a daily survey of all of them that continued until no further adults had emerged in either device during at least eight consecutive days. After identifying the species and the sex, each newly emerging adult weevil was weighed (to the nearest 0·1 mg, balance: Scaltec SBA 32): no significant difference was detected for any species in the weight of adults belonging to the different annual larval cohorts (see Appendix S2; Fig. S2).

CHARACTERIZATION OF FEEDING ACTIVITY IN THE FIELD

Isotope analysis of biological samples is an appropriate method for obtaining information about the diet of animals (Hood-Nowotny & Knols 2007), notably in natural populations (Markow, Anwar & Pfeiler 2000). The stable isotope ratios of nitrogen and of carbon, in particular, are influenced by the diet of animals to a large extent (DeNiro & Epstein 1978, 1981; Inger & Bearhop 2008). Capital and income resources can be identified with the use of naturally stable isotope profiles (Fischer, O'Brien & Boggs 2004; Warner *et al.* 2008; Wessels, Jordan & Hahn 2010). In this study, we used this property to explore the feeding activity of adults of the four weevil species in the field. In holometabolous insects, the larval and adult stages present very contrasting morphological and physiological traits and are mostly exposed to distinct environments. Throughout their underground life, none of the four weevil species does feed, which means that the tissues of a newly emerging adult are entirely derived from nutrients acquired from the oak acorn at the larval stage. The isotope signature of an emerging adult therefore only reflects the isotope composition of the oak acorn in which it developed. Moreover, because after becoming adult, no further moulting occurs, metabolically inactive tissues, such as the keratinized exoskeleton (integument), retain this larval isotope signature throughout the insect's life. Field and laboratory observations justified our assumption that adults do not feed on acorns. Thus, any change detected in the isotope profile of metabolically active tissues such as muscles, genital organs or fat body is likely to be due to adult feeding. By analysing the isotope profile of both the exoskeleton and internal tissues of wild-caught females, we attributed any absolute difference between these pairs of measures to adult feeding. This allowed us to use each adult female as her own control and thus to circumvent any possible variability in the isotope profiles of individual oak acorns, i.e. the larval food resource. We focused on the weevil community at the site A, where we sampled oak acorns as well as adults of the four weevil species both at emergence and later in the breeding season.

Sampling

For each insect species, we compared the isotope signature of newly emerged individuals captured before they had displayed any feeding activity with that of adults trapped on the oak tree between June and September 2009. Newly emerged individuals were obtained using the same experimental design as for determining the emergence date and were collected individually and freeze-dried at −20 °C. The other adult weevils were captured on the oak trees by beating branches with a wooden stick within the first hour after sunrise (Schauff 1986). Insects were collected on a white sheet laid under the

tree and were individually frozen at −20 °C after their sex and species were identified on the basis of morphological criteria (Hoffmann 1954). We examined the isotope profile of individual oak acorns and its variability across trees and years: eight acorns per tree were harvested from three different trees at site A, which was repeated in 2007 and 2009. Acorns were sampled at the time of the larval development of the insects, and they too were stored at −20 °C prior to analysis.

Sample preparation

Adult females were dissected under a binocular microscope (Zeiss STEMI-C, Illkirch, France) to collect separately the keratinized tissues (elytra) and the soft tissues (ovaries and the fat body, avoiding the gut). The acorns were dissected to retrieve the kernel, which constitutes the sole food source of weevil larvae. The insect and plant samples were then dried in the oven (Memmert Celsius 2005) at 60 °C for 48 h, after which the acorns were crushed using Stainless Steel Beads and shaken for 30 s at 25 Hz (Tissue Lyser; Qiagen, Courtaboeuf, France). We took four 2 mg ($\pm$0·2 mg) samples from each acorn and placed each of them in a tin capsule. The elytra and internal tissues of each adult insect were sampled separately the same way (four repeats per tissue per insect, each weighing 250 $\pm$ 50 μg). Overall, 44 newly emerged females (11 *C. elephas*, 15 *C. glandium*, 10 *C. pellitus* and eight *C. venosus*) and 49 live-trapped females (nine *C. elephas*, 10 *C. glandium*, 15 *C. pellitus* and 15 *C. venosus*) were analysed. As a larva depends on a single acorn for its entire development, and because the isotope signature of oak acorns was found to be very variable (see Appendix S3; Fig. S3), we computed for each insect the difference between its keratinized tissue – resulting from its larval diet – and its internal tissue that was assumed to reflect its adult diet.

Stable isotope analysis

Stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were measured by continuous flow stable isotope ratio mass spectrometry using an Isoprime mass spectrometer (Isoprime Ltd, Cheadle, UK) interfaced with a Eurovector EuroEA3028-HT elemental analyser (EuroVector, Milan, Italy). Carbon and nitrogen isotope ratios were expressed as δ in parts per thousand (‰) and referenced to Pee Dee Belemnite and atmospheric air, respectively. The analytical precision achieved for tyrosine, triphenylamine and ascorbic acid standards analysed along with the samples was better than 0·1‰ for $\delta^{13}C$ values and better than 0·3‰ for $\delta^{15}N$ values.

INFLUENCE OF FOOD INTAKE ON THE LONGEVITY OF THE FEMALES

We tested in the four weevil species the extent to which dietary intake by adult females influences their survival. Females were collected the day they emerged and were placed individually in Plexiglas rearing boxes (5·8 × 4·4 × 5 cm). These boxes were kept in a room at constant temperature (22 $\pm$ 1 °C), hygrometry (60 $\pm$ 5% relative humidity)

and under artificial light simulating the natural photoperiod ($\pm$ 15 min). Adults were randomly assigned to one of three dietary groups as follows: distilled water alone (diet 1: D1); sugar solution (sucrose 10%, diet 2: D2); and sugar (10%) plus fresh chestnut pollen (ca. 5 mg, 'Percie du sert' Penne d'Agenais, France; diet 3: D3). The number of individuals provided with the various diets was as follows: *C. elephas* (D1: *n* = 31, D2: *n* = 34 and D3: *n* = 26), *C. glandium* (D1: *n* = 11, D2: *n* = 10 and D3: *n* = 10), *C. venosus* (D1: *n* = 7, D2: *n* = 8 and D3: *n* = 8) and *C. pellitus* (D1: *n* = 5, D2: *n* = 8 and D3: *n* = 6). Adults were supplied *ad libitum* with their respective diet: water and the sugar solution were both provided using a piece of cotton wool soaked in 2 mL of the dietary solution that was renewed every 3 days. Pollen was compressed in a fine gauze bag hung at the top of the rearing box and was renewed weekly. We checked daily whether adult females were still alive until all of them naturally died.

OOGENESIS IN FOOD-DEPRIVED FEMALES

To test whether females required feeding before maturing eggs, we first dissected newly emerged females of the four species (*C. elephas*: *n* = 17, *C. glandium*: *n* = 20, *C. pellitus*: *n* = 13 and *C. venosus*: *n* = 12) under a binocular microscope to count the number of chorioned eggs in their ovaries. Second, to test the ability of unfed females to produce mature eggs, 10 *C. glandium* and 11 *C. elephas* females were provided with distilled water (D1) *ad libitum* for 8 days and were then sacrificed and dissected. *C. venosus* and *C. pellitus* were not included in this experiment, because of the too small sample size available.

STATISTICAL ANALYSIS

To find out whether adults from the four species differed with regard to the time of year they emerged, we used a generalized linear mixed model with a Poisson distribution of error (lme4 package). We tested the effect of the interaction between the species and the week in the year when an adult emerged. In this analysis, we considered the site (i.e. the oak trees A and B) and the year of adult emergence as random effects.

To assess the effects of the tree or of the year on the relationship between $\delta^{15}N$ and $\delta^{13}C$, we used the multivariate analysis of variance (MANOVA), which allows the response variable to be multidimensional (Tomassone *et al.* 1988). We used MANOVA to test whether the isotope enrichment differed between the four insect species. To do this, we computed for each individual the absolute difference between the keratinized tissue and the internal tissue in the $\delta^{15}N$ and in the $\delta^{13}C$ isotope profiles ($|\Delta\delta^{15}N|$ and $|\Delta\delta^{13}C|$).

We analysed the effect of the diet on the longevity of adult females with a generalized linear model assuming a Gamma distribution of the residuals (inverse link). All analyses were performed with the R free software environment (Ihaka & Gentleman 1996; http://cran.at.r-project.org).

Results

EMERGENCE DATE OF ADULTS WITHIN THE YEAR

As shown in Fig. 1, the comparative kinetics of the emergence dates clearly reveals two distinct patterns (interaction between the species and the week in the year when an adult emerged $\chi^2_{d.f.=66} = 2725$; $P < 0.0001$) that showed consistency across years and for the two communities: three
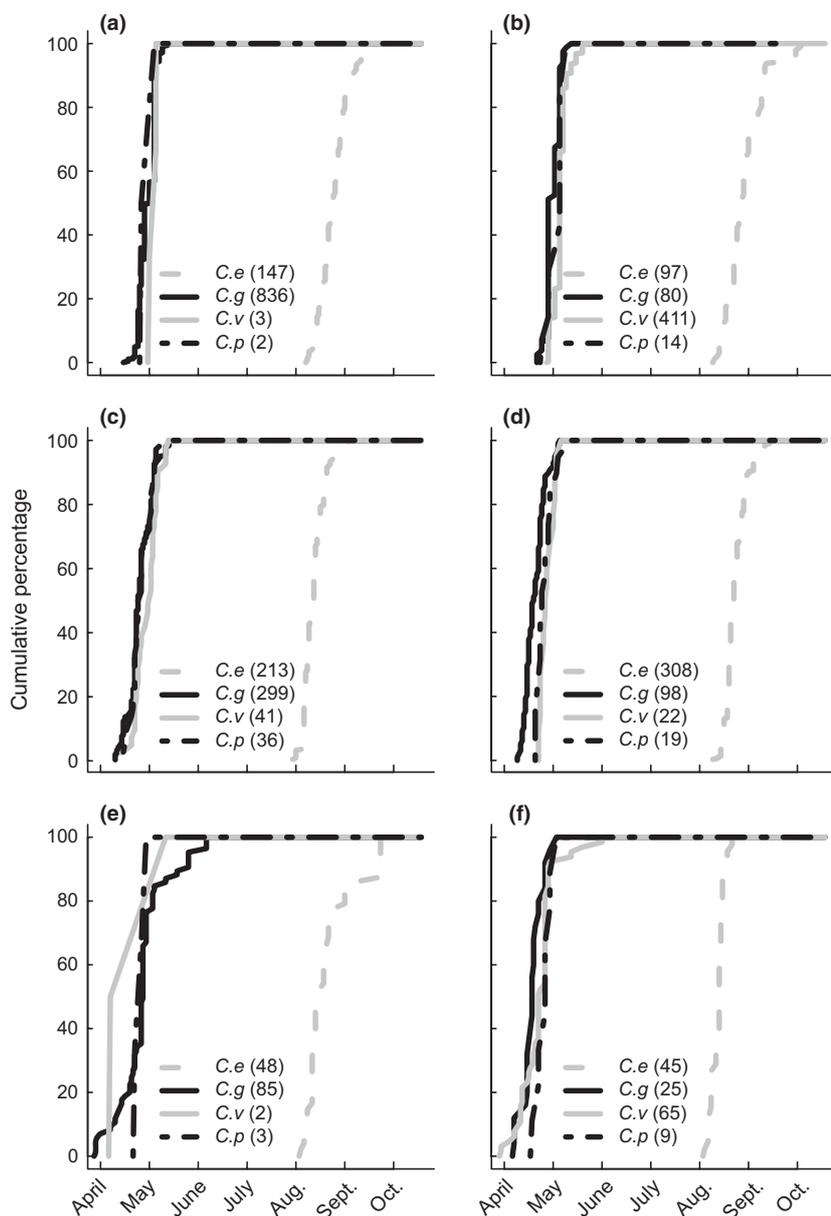
Fig. 1. Timing of the adult emergence in the four weevil species investigated. The cumulative abundance of emerging adults of each species is shown at the site A in 2008 (a), 2009 (c) and 2010 (e) and at the site B in 2008 (b), 2009 (d) and 2010 (f). Three of these species, *Curculio venosus, Curculio pellitus* and *Curculio glandium,* emerged synchronously and early in the season, while the emergence date of the fourth (*Curculio elephas*) differed markedly.

species (*C. glandium, C. pellitus* and *C. venosus*) emerged simultaneously in April, at a time when oak pollen was being produced (Bonnet Masimbert 1984), that is 2 months before egg laying, whereas the *C. elephas* females emerged much later in the year, in August during their laying period.

FEEDING ACTIVITY IN THE FIELD

The isotope signature of oak acorns varies considerably from year to year and between oak trees, as is shown by the $\delta^{15}N$ and $\delta^{13}C$ isotope ratios (MANOVA, years: $F_{2,\,41} = 19\cdot0$; $P < 0\cdot001$; trees: $F_{6,\,84} = 20\cdot6$; $P < 0\cdot001$; interaction: $F_{2,\,41} = 3\cdot82$; $P = 0\cdot03$; see Appendix S3; Fig. S3). The $|\Delta\delta^{13}C|$ and $|\Delta\delta^{15}N|$ values differed significantly between the newly emerged adults (emerging) and adults captured later in the field (trapped) in three of the four weevil species (*C. venosus, C. pellitus* and

C. glandium), indicating that individuals from these three species had fed at the adult stage. In contrast, no such difference was found in *C. elephas*, suggesting that adult females belonging to this species had not fed in the field until the time of egg laying (Fig. 2; Table 1; see Appendix S4; Fig. S4).

INFLUENCE OF FOOD INTAKE ON THE LONGEVITY OF THE FEMALES

We found that the longevity of adult weevil females varies from one species to another depending on diet (diet: $\chi^2_{d.f.=2} = 26$; $P < 0\cdot0001$; species: $\chi^2_{d.f.=3} = 40$; $P < 0\cdot0001$; diet × species interaction: $\chi^2_{d.f.=6} = 23$; $P < 0\cdot0001$; Fig. 3). These results were consistently observed at the two sites studied ($\chi^2_{d.f.=1} = 0.016$; $P = 0\cdot842$). Under controlled laboratory conditions, the longevity of *C. elephas* females was not
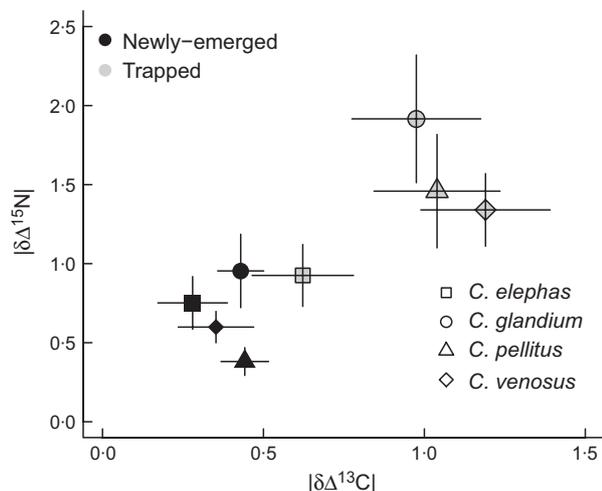
Fig. 2. Isotope signature displayed by females of the four weevil species when they emerge and at the time of breeding. $|\delta\Delta^{15}N|$ and $|\Delta\delta^{13}C|$ are the absolute differences, computed for each individual, between the isotope ratio of its keratinized tissue – of larval origin – and that of its internal, soft tissues (mean ± SE; newly emerged adults: *Curculio elephas* $n = 11$; *Curculio glandium* $n = 15$; *Curculio pellitus* $n = 10$; *Curculio venosus* $n = 8$; adults caught later in the breeding season: *C. elephas* $n = 9$; *C. glandium* $n = 10$; *C. pellitus* $n = 15$; *C. venosus* $n = 15$).

influenced by their diet ($\chi^2_{d.f.=2} = 1.182$; $P = 0.264$) and averaged 20.46 ± 13.27 days (mean ± SD; $n = 91$). *C. glandium* females lived the longest when fed a mixed diet: 123.40 ± 57.53 days (mean ± SD; $n = 10$) vs. only 12.45 ± 4.8 days (mean ± SD; $n = 11$) when unfed. Feeding also improved the longevity of *C. venosus* and *C. pellitus* females, similar for the two species (diet: $\chi^2_{d.f.=2} = 17$; $P < 0.0001$; species: $\chi^2_{d.f.=1} = 0.0001$; $P = 0.98$; diet × species interaction: $\chi^2_{d.f.=2} = 0.13$; $P = 0.83$) and lived 43.3 ± 33.74 (mean ± SD; $n = 30$), vs. only 10.75 ± 3.4 days when unfed.

OOGENESIS IN FOOD-DEPRIVED FEMALES

Dissection of ovaries of newly emerged females showed that except *C. elephas*, none of them had mature eggs at the day of their emergence (*C. glandium*: $n = 20$, *C. pellitus*: $n = 13$ and *C. venosus*: $n = 12$). *Curculio elephas* females had 3.82 mature eggs at emergence, on average (± 0.3 SD; $n = 17$ females). In the same way, only *C. elephas* females were able

to produce mature eggs after 8 days without any nutrient intake (6.3 ± 3.9 mature eggs, mean ± SD; $n = 10$ females). By contrast, none of the 11 *C. glandium* 8-day-old adult females started maturing eggs when unfed.

Discussion

Theoretical evolutionary ecology studies usually predict that when the reproductive success of insects is limited by unpredictable variations in their laying sites, synovigenic ovarian development can be expected, because this should give them considerable flexibility in the way they acquire energy and allocate it to survival or reproduction (Ellers & Jervis 2003; Jervis, Ellers & Harvey 2008). Despite this prediction, we show that four weevil species that occur together in the same communities and exploit the same egg-laying sites (oak acorns) – the availability of which fluctuates markedly over time – display radically different strategies of resource acquisition and allocation. Three of these species (*C. glandium*, *C. venosus* and *C. pellitus*) are strictly synovigenic and possess characteristics consistent with an income breeding strategy, while the fourth (*C. elephas*) is proovigenic and a capital breeder.

No significant between-year variation was detected over the 3-year survey and at the two sites, neither in the timing of adult emergence of each species across the breeding season nor in the fresh weight of adults at emergence (Fig. 1; see Appendix S2; Fig. S2). These findings suggest that the strategies followed by the four weevil species are robust to variations in the environmental conditions met each year by the different larval cohorts, and corroborate the consistency found previously in the patterns of dormancy and in the period of egg laying during the year found in these species (Venner *et al.* 2011). Three of the four species studied (*C. glandium*, *C. venosus* and *C. pellitus*) emerge in April, which is well before the breeding period begins in June (Venner *et al.* 2011): this 2-month interval may be long enough for them to escape from the original tree and to find a more suitable breeding site. Suitable trees can probably be identified from early April, when oak trees flower (Bonnet Masimbert 1984). Our results show that these three early-emerging species all are synovigenic (i.e. their ovigeny index equals 0), and they further suggest that these ones might be income breeders. Indeed, females of these three species all have empty ovaries at emergence, and in the field they feed as adults, as demonstrated by the isotopic signature of wild-caught females (more specifically, by the difference between isotope ratios in the

Table 1. Variability in the relationship between $|\Delta\delta^{15}N|$ and $|\Delta\delta^{13}C|$ across the four weevil species

| Species | Mean$|\Delta\delta^{13}C|$ ± (SE) | | Mean$|\Delta\delta^{15}N|$ ± (SE) | | $F_{(d.f.,d.f.)}$ | P |
	Newly emerged	Trapped	Newly emerged	Trapped		
Curculio venosus	$0.352 \pm (0.11)$	$1.190 \pm (0.20)$	$0.598 \pm (0.09)$	$1.340 \pm (0.22)$	$F_{(2,20)}5.1972$	$0.015*$
Curculio pellitus	$0.442 \pm (0.07)$	$1.039 \pm (0.19)$	$0.381 \pm (0.08)$	$1.458 \pm (0.35)$	$F_{(2,22)}4.2018$	$0.028*$
Curculio glandium	$0.429 \pm (0.07)$	$0.975 \pm (0.20)$	$0.954 \pm (0.23)$	$1.916 \pm (0.40)$	$F_{(2,22)}7.3868$	$0.003**$
Curculio elephas	$0.280 \pm (0.10)$	$0.622 \pm (0.15)$	$0.751 \pm (0.16)$	$0.925 \pm (0.19)$	$F_{(2,17)}1.7565$	0.202^{NS}

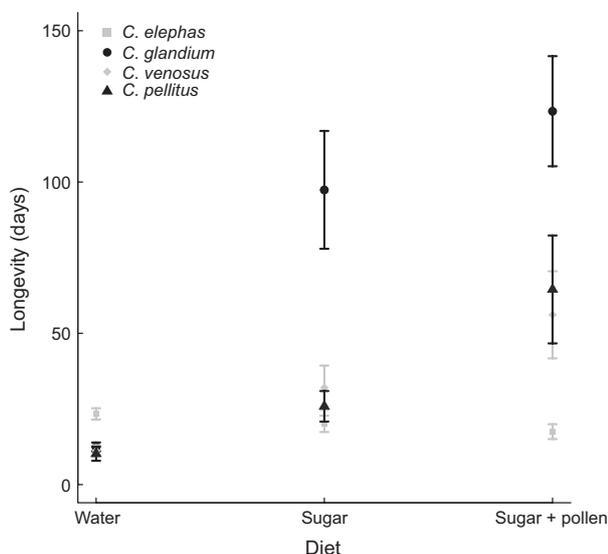NS, not significant, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Fig. 3. Influence of diet on the longevity of females (mean ± SE). While the longevity of *Curculio elephas* females (water: *n* = 31, sugar: *n* = 34 and sugar + pollen: *n* = 26) seems to be unaffected by their diet, the life span of *Curculio glandium* females markedly increases with an enriched diet (water: *n* = 11, sugar: *n* = 10 and sugar + pollen: *n* = 10). Feeding also improves, albeit to a lesser extent than *C. glandium*, the survival of *Curculio venosus* (water: *n* = 7, sugar: *n* = 8 and sugar + pollen: *n* = 8) and *Curculio pellitus* (water: *n* = 5, sugar: *n* = 8 and sugar + pollen: *n* = 6), with no difference being detected between these two species.

keratinized and soft tissues). Moreover, when females belonging to each of these three species are deprived of food in the laboratory, they have a very short life span (around 10 days), which is incompatible with the 2-month interval they have to survive in the field before egg laying starts (Venner *et al.* 2011). Furthermore, we also show that unfed *C. glandium* females approaching death were unable to produce mature eggs in the laboratory. Taken together, these observations suggest that when the females of these three species emerge, they do not have enough energy stored to enable them even to survive until the breeding season, let alone have energy available for allocation to ovarian development. Yet some specific nutrients stored from larval reserves could be incorporated into eggs, and the three synovigenic weevil species could act as both capital and income breeders, as it has been shown experimentally in other insects (Rivero, Giron & Casas 2001; Casas *et al.* 2005). This question deserves further investigations into the isotope signature of eggs or into the fecundity of females reared with various diets.

In contrast, if females of these three species are given a rich diet based on sugar plus pollen, their life span is comparable to that observed in the field (Venner *et al.* 2011). The life span of adults of two species (*C. venosus* and *C. pellitus)* fed a rich diet was extended by about 2 months. This would give them the advantage of prior access to the egg-laying sites and allow them to breed within a short time interval early in the season (Venner *et al.* 2011). *Curculio glandium* females fed on a rich diet increased their adult life span by up to 4 months, which allowed them to lay eggs over a longer interval, in accordance

with field observations [from June to September; (Venner *et al.* 2011)]. The major differences observed in the way energy is allocated to adult survival obviously explain the disparate longevities among oak weevil species and largely contribute to the strong temporal partitioning of their use of the communal resource. Such partitioning seems to constitute one of the major mechanisms underlying the coexistence of species that compete with each other in a fluctuating environment. Hence, under the general niche theory, stable coexistence is predicted among competing species whenever their ecological traits are sufficiently distinct from each other to lower the competition between them relative to that occurring within species, which enables any species within a community to grow whenever it becomes rare (Chesson 2000; Chesson *et al.* 2004). The intra-annual partitioning mechanism actually seems to be involved in the coexistence of these four weevil species (Venner *et al.* 2011).

Although the fourth species, *C. elephas*, exploits the same unpredictable environment as the other three species, it is clearly a capital breeder. The between-year fluctuations in the availability of oviposition sites could even be increased for this species: while years of high resource abundance could provide all weevil species with unlimited egg-laying sites, *C. elephas* would suffer more than the other species on years of low acorn production as the resource is likely to be already used by early species at the time of their emergence. In spite of this environment highly variable between years, we found that *C. elephas* adults emerge in August when females readily start laying eggs (Venner *et al.* 2011): females have mature oocytes as soon as they emerge, when oak acorns are already mature and ready for exploitation by weevil larvae. *Curculio elephas* adult females do not seem to need to feed, because the stable isotope profile of wild-caught females remained unchanged from their larval stage. Moreover, when these females were given a rich diet, their life span was not longer than that of unfed individuals. Nor did they require feeding during adulthood to produce mature oocytes. To summarize, these proovigenic females can breed successfully drawing solely on energy of larval origin, which corresponds to a capital breeding strategy (Jervis, Ellers & Harvey 2008).

Curculio elephas mature larvae are much heavier than those of the three other species when they leave the acorn (Pélisson *et al.* 2011), and similarly, *C. elephas* newly emerging adults are heavier than the other species (see Appendix S2, Fig. S2): besides their greater body size, they may therefore have greater energy reserves acquired during the larval stage than the other three species. This interspecific difference between adults composing the community a given year is unlikely to be due to heterogeneous conditions experienced by the distinct larval cohorts, which might occur owing to their distinct life cycles (Venner *et al.* 2011). Indeed, we did not detect any variation in the weight of emerging adults across years, for any species (see Appendix S2). This suggests that the quality of acorns, possibly varying between years, does not impact the energetic status of adults at emergence. Thus, ecophysiological characteristics of the breeding strategy should also be consistent across years for a given species. This energy seems

to be rapidly and entirely allocated to reproduction as soon as the adult emerges, which should make this species very efficient in exploiting acorns when local resources are abundant. However, when the local resource is scarce, the adults that emerge might fail to breed owing to their poor ability to move towards more favourable, but distant sites (because they are carrying excess body reserves destined for ovarian development that prevent them from dispersing spatially (Jervis, Boggs & Ferns 2005) and/or because they do not have enough time to prospect). We suggest that such a capital breeder strategy can only be efficient in a fluctuating environment if it is combined with other life-history traits that buffer unpredictable temporal environmental fluctuations. Thus, *C. elephas* is the only weevil species in this system that spreads significantly the emergence of adults belonging to the same larval cohort over several years, which has been interpreted as a form of bet-hedging (Menu, Roebuck & Viala 2000; Menu & Desouhant 2002; Gourbière & Menu 2009; Venner *et al.* 2011). This strategy ensures that at least some of the individuals that share the same genotype will encounter favourable local conditions and go on to breed successfully. Adults emerging when the resource is locally abundant should be able to maximize their lifetime reproductive success, while individuals emerging in a year when the resource is depleted would be sacrificed. In *C. elephas*, capital breeding might have evolved alongside with this bet-hedging strategy. In contrast, the other three species do not spread significantly their adult emergence over several years (Venner *et al.* 2011), and they can be expected to exploit the oviposition sites less efficiently than *C. elephas* when these are locally abundant: hence, adults from these early-emerging species face a significant risk of mortality during the prolonged interval between emergence and breeding, and they have to find food before they can breed. In contrast, these three species may be more efficient than *C. elephas* in taking advantage of the spatial variability of resource availability when resources are locally absent. The co-occurrence of both pro- and synovigenic species sharing the same fluctuating environment provides evidence that the different species cope in various ways with the spatial and temporal heterogeneity in the egg-laying site availability. This may be a key aspect of niche partitioning and of the coexistence of species that compete for the same fluctuating resource.

Insects display multiple strategies of acquiring energy and allocating it to different activities, and these have been studied in great detail (see Strand & Casas 2008 for a review among parasitoids). These strategies show great plasticity in response to numerous factors, including environmental stress (Wessels, Jordan & Hahn 2010), the quality of energy resources (Fischer, O'Brien & Boggs 2004) and diet or metabolism (O'Brien, Boggs & Fogel 2004; Min *et al.* 2006). At the individual level, there may be several strategies ranging from income to capital breeding, depending on the nutrient considered (Casas *et al.* 2005). Our work was not designed to reveal differences at such a fine scale, but it still provides the first report of the simultaneous occurrence of synovigeny and proovigeny breeding strategies in a community of sibling species that are competing with each other for the same fluctuating resource. Unlike previously proposed hypotheses, our result show an exception to the conventional wisdom that environmental stochasticity necessarily selects for synovigenic and income breeding insects and selects against proovigeny and capital breeding. Our findings suggest that environmental stochasticity does not favour the evolution of a single optimal breeding strategy, but may instead favour the diversification of these strategies. Our work should encourage future studies designed at testing this hypothesis.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Figure S1. Between-year fluctuations of the acorn crop at the two sites.

Figure S2. Between-year comparison of fresh adult weight.

Figure S3. Representation of isotope signature of oak acorns.

Figure S4. Representation of relative differences between keratinized tissues and internal tissues of females.

Appendix S1. Between-year fluctuations of the acorn crop.

Appendix S2. Between-year comparison of the fresh weight of adult weevils.

Appendix S3. Isotope signature of oak acorns.

Appendix S4. Relative differences between keratinized tissues and internal tissues of females.

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