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Overwintering strategy of two weevils infesting three gorse species: When cold hardiness meets plant–insect interactions

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ABSTRACT

The cold hardiness of two closely related weevil species, *Exapion ulicis* and *E. lemovicinum* was studied in relation to their life cycles. These two seed-eating weevils reproduce on *Ulex* plant species with different fruiting phenologies. *E. ulicis* lays eggs in spring and overwinters as an adult while *E. lemovicinum* lays eggs in autumn and overwinters as a larva. Adult weevils were collected in natural populations of Brittany (Western France) and characterized with morphological and molecular tools before experiments. We showed that both weevil species exhibited low supercooling points (SCPs) with mean seasonal values below $-17\,^{\circ}\mathrm{C}$. Fresh mass, moisture content and sex were not correlated to supercooling ability. Weevils died upon freezing and the lower lethal temperatures (LLT) were within the range of SCP, indicating that both species are freezing intolerant. Comparison between species for SCP, LLT and survival to exposure at $-8\,^{\circ}\mathrm{C}$ in winter showed a higher cold resistance for *E. ulicis* than for *E. lemovicinum*. In addition, the seasonal evolution of cold hardiness differed depending on the species. These features suggest that response to cold of weevils is linked to their life cycles, and thus to the life history of their host plants.

1. Introduction

Insects are poikilothermous organisms that have to adapt, either behaviourally or physiologically, to survive cold winters. Among them, weevils (Coleoptera, Curculionoidea) represent one of the most diverse groups in terms of species richness (Kuschel, 1995) and their ability to adapt to variable environmental conditions probably contributed to their worldwide expansion. In weevils, as in many other insect species, three major strategies are employed to survive hard winter conditions (Leather et al., 1993). The first consists of migrating to habitats where the temperature will be more favourable (e.g. *Anthonomus pomorum*, Toepfer et al., 2000). The second is to locally avoid adverse conditions and to enter a quiescent state or diapause (Tauber et al., 1986; Denlinger, 2008), which is characterized by a decrease in or cessation of metabolism and an increased resistance to cold (e.g. *Curculio elephas*, Menu and Desouhant, 2002; *Exechesops leucopis*, Matsuo, 2006). In the third strategy, insects can remain active during thermally favourable periods, and employ complex mechanisms to overcome cold and freezing. Indeed, they can survive either by "freezing tolerance" (they withstand the formation of extracellular ice in their tissues) or by "freezing avoidance" (whereby they prevent freezing through depression of the supercooling point (SCP) of their body fluids) (Salt, 1961; Zachariassen, 1985; Lee, 1991; Danks, 1996). Freezing-avoidant species are also called "freezing-intolerant" since they always die upon freezing. Freezing-avoidant insects predominate in temperate climates; freezing-tolerant species are more abundant in continental and/or northern regions where winter is long and predictable, with probabilities of extreme frost events (Leather et al., 1993; Bale, 1996). Among weevils, both strategies have been reported (freezing avoidance: *Rhynchaenus fagi*, Coulson and Bale, 1996; *Palirhoeus eatoni*, van der Merwe et al., 1997; *Hypera punctata*, Watanabe and Tanaka, 1997; *Cylas formicarius*, Kandori et al., 2006; *Anthonomus grandis*, Suh et al., 2002; Suh and Spurgeon, 2007; freezing tolerance: *Bothrometopus elongatus*, *B. parvulus*, *B. randi*, *Ectemnorhinus marioni*, *E. similis*, van der Merwe et al., 1997). However, to our knowledge, no study has yet been performed to evaluate the relative role of phylogenetic constraints and ecological adaptation in their cold hardiness strategy. Comparisons of phylogenetically close insect species that live in sympatry but exhibit different overwintering strategies may help answer this question.

In Brittany (Western France), two weevil species that are morphologically and genetically very close exhibit different life cycles relative to their host plant phenology (Barat et al., 2007,

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2008). *Exapion ulicis* (Forster) and *E. lemovicinum* (Hoffmann) (Curculionoidea, Apionidae) are seed predators of three gorse species (Fabaceae, Genisteae) that differ in their fruiting period. The fruiting peak of *Ulex europaeus*, the host plant of *E. ulicis*, is in spring, whereas *U. gallii* and *U. minor*, the host plants of *E. lemovicinum*, fruit during autumn and winter. In Western Europe, the geographical distribution of *Exapion* species is thought to follow the native distribution of gorse species: the Iberian Peninsula and European Atlantic coast, i.e. places where winter frost is common, but where extreme temperatures (below −10 °C) are rare. In Western Europe, *U. europaeus* is more widespread than *U. gallii* or *U. minor*, but the three gorse species, and thus the two weevil species, can frequently be encountered in sympatry. In addition, *U. europaeus* has recently invaded numerous regions of Southern Hemisphere where it is considered as a weed (Lowe et al., 2000).

In some invaded countries, *E. ulicis* has been introduced as a biological control agent to reduce gorse seed production, but its efficiency was limited by the fact that *E. ulicis* only lay eggs in spring, while *U. europaeus* produces pods both in spring and in autumn (Hill et al., 1991). For this reason, it would have been useful to introduce an autumn egg-laying weevil such as *E. lemovicinum*, but our previous results have shown that this last species is restricted to the non-invasive gorse species *U. minor* and *U. gallii*, and does not infest autumnal pods of *U. europaeus* (Barat et al., 2007). However, this strict association between closely related seed-eating weevils and host plant with different fruiting periods emphasises the interest of a basic comparison of their life cycles.

The two weevil species exhibit numerous common behaviours. Both weevil species lay eggs within their host's young green pods; larvae feed on seeds and develop until the adult stage within the pods. Adults are released with pod dehiscence and feed on vegetative parts and flowers of *Ulex* species if the temperature is high enough. Below 9 °C, adults of both species are immobile. The main difference between the two weevil species lies in the timing of their life cycle: *E. ulicis* lay eggs in early spring, during the peak of pod production of *U. europaeus* (Barat et al., 2007, 2008). Adults are released at the beginning of summer, stay in reproductive diapause in autumn and winter (where they are present on gorse branches), and reproduce the following spring. This species is described as univoltine by Hoddle (1991). *E. lemovicinum* lay eggs in autumn within pods of *U. gallii* or *U. minor* (Barat et al., 2007, 2008). Adults are released next spring and reproduce next autumn. As a consequence, this species overwinters mainly in the larval stage within gorse pods. However, some adults can be encountered on gorse branches in winter, but those individuals from the previous season are thought to be at the end of their life.

Consequently, although adults of *E. ulicis* and *E. lemovicinum* may be observed on gorse branches all year round, *E. ulicis* overwinters as an adult, whereas *E. lemovicinum* overwinters as a larva. Besides, *E. ulicis* and *E. lemovicinum* have approximately the same small body size (about 3 mm) – an important feature to resist cold (Lee, 1991) – and often live in sympatry. The association between weevils and gorses in Brittany thus offers a rare opportunity to compare cold hardiness of two similar insect species living in the same climatic conditions but differing in the seasonality of their life cycle in relation to their host plant's fruiting period.

The main aim of this study is to make a comparison of cold hardiness of *E. ulicis* and *E. lemovicinum* in regard to their ecology and life cycles in Brittany. The questions were: (i) which strategy do *Exapion* weevils use to survive sub-zero temperatures? (ii) do *E. ulicis* and *E. lemovicinum* differ in their responses to cold? and (iii) can these differences be related to their life cycle and therefore to their host plant fruiting phenology? To answer these questions, individuals of the two species were collected at different seasons in natural populations, and several aspects of their cold hardiness were tested and compared. Only adults were studied because it is the only stage that was present in the same season for the two species.

As supercooling ability is one of the main features of the freezing-avoidant strategy, the measurement of the supercooling point (SCP) is a key parameter to know if a species is freezing-tolerant or freezing-avoidant. However, it is not sufficient to correctly describe cold hardiness strategies. For example, it has been known since a long time that freezing-avoidant species may endure a non-freezing cold death during long or short exposures to temperature above their SCP (e.g. Knight et al., 1986; Renault et al., 2002). Moreover, numerous factors, either physiological (body mass, moisture content, gut content) or environmental (seasonal variations) may also play an important role in resistance to freezing temperatures (e.g. Lee, 1991; Košťál and Šimek, 2000; Watanabe, 2000). In order to assess the freezing tolerance status of the two species, we thus compared their SCP, fresh masses and moisture contents in different seasons. We also measured traits more directly related to survival in winter, such as lower lethal temperature, survival to long exposure to −8 °C and resistance to starvation. This set of weevils' cold hardiness features is discussed in an ecological context, notably in relation to plant–insect interactions.

2. Materials and methods

2.1. The study locations

Sampling was done in six locations in Brittany. In four of them, two gorse species bearing different *Exapion* species were in sympatry (Fig. 1). The climate in Brittany is temperate: data collected by Meteo France from 1961 to 1990 show that mean temperature varies from 5.1 °C in January to 18.3 °C in July in Rennes (the closest weather station from our inland locations), and from 5.8 °C in January to 18.3 °C in August in Saint-Malo (the closest weather station from our coastal locations). The mean number of days below 0 °C varies from 38.8 in Rennes to 13.1 in Saint-Malo. The mean number of days below −5 °C varies from 4.3 in Rennes to 0.9 in Saint-Malo. The temperature of −8 °C chosen for the lethal time experiment corresponds to the lowest temperature weevils can experience more than 1 day in the study locations.

2.2. Sampling

Adults of *E. ulicis* were collected on *U. europaeus* and adults of *E. lemovicinum* were collected on *U. gallii* (no weevils were sampled on *U. minor*, due to the very low infestation rate of this species). For *E. ulicis*, adults are abundant all year long. For *E. lemovicinum*, however the abundance is low. It is at a maximum in summer, after pod dehiscence, and at a minimum in winter, after the end of the laying-eggs period. In 2005, the number of *E. lemovicinum* adults collected in winter only allowed the measure of the SCP. A new winter sampling of both species was done in winter 2009. We obtained enough *E. ulicis* to estimate lower lethal temperature (LLT) and survival time, and enough *E. lemovicinum* weevils to estimate LLT. The recollection of the weevils took several days, and during the sampling process, the collected weevils were kept in "field-like" condition, i.e. with water and food supply and at the mean temperature of the study month.

Due to the difficulty of species identification in apionid weevils, we identified the insects collected both with morphological and molecular criteria. Morphological identification was made with the keys provided by Alonso-Zarazaga (Alonso-Zarazaga and Lyal, 1999, and pers. com.). Molecular identification was made using the mitochondrial diagnostic sites described in Barat et al. (2008).