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MATE CHOICE, PREDATION AND CHEMICAL DEFENSE IN TWO SPECIES OF ALPINE LEAF-BEETLES

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Thèse présentée à la faculté des sciences de l'Université de Neuchâtel pour l'obtention du grade de docteur ès sciences

2000

IMPRIMATUR POUR LA THESE

Mate choice, predation and chemical defense in two species of alpine leaf beetles

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UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel sur le rapport des membres du jury,

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Neuchâtel, le 3 août 2001

Le doyen:

J.-P. Derendinger

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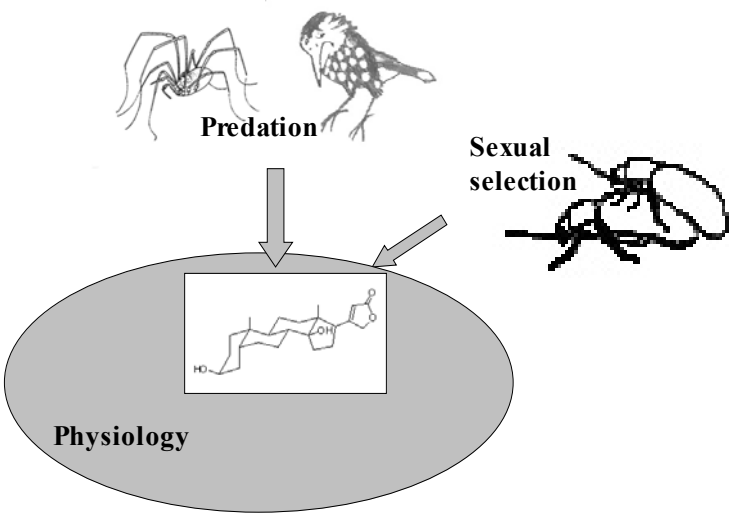
MATE CHOICE, PREDATION AND CHEMICAL DEFENSE IN TWO SPECIES OF ALPINE LEAF BEETLES: SYNTHESIS OF THE THESIS

Introduction

Defensive traits such as toxic compounds most likely appear in response to pressures applied by natural enemies. These pressures are supposed to drive the evolution of these traits, together with various physiological constraints which may act antagonistically (Fig. 1). A secondary mechanism that acts on the evolution of toxicity may arise from sexual selection if individuals benefit from mating with a well-defended partner. Such a benefit could be represented by the transfer of valuable compounds (nutrients, toxins) from the male to the female during copulation, the increased protection from predators while *in copula* with a well-defended partner, or the "good genes" that the progeny would inherit from the chosen partner. In all cases, there is the need for signals that would honestly display the quality of each individual. These signals would be used by individuals to assess the benefit of mating with a specific partner. Hence, mechanisms of sexual selection may influence the evolution of defensive traits. Mate choice for partners that are resistant to parasites has been widely demonstrated (Hamilton and

Zuk 1982, Møller 1990). However, little is known about sexual selection for traits related to resistance to other natural enemies. As initially described by Darwin (1871), sexual selection concerns "the advantage which certain individuals have over others of the same sex and species solely in respect of reproduction". Among the various mechanisms of sexual selection described (Andersson 1994, Andersson and Iwasa 1996, Birkhead and Biggins 1997), mate choice is probably most important. Hence it is of particular interest to study the relationship between defense-related traits and sexual selection mechanisms.

Fig. 1: The different selective forces that can influence the evolution of chemical defense



The biological system

The genus *Oreina* includes 14 species (Lohse & Luche 1994) living in European mountainous areas (Alps, Pyrénées, Jura, Vosges). The active period of these beetles is no longer than four months per year and can be as short as two months, because snow is covering their habitat for the rest of the year. As soon as they emerge at spring, the beetles start to feed and mate. Approximately three weeks later, the females start to larviposit or oviposit (only two species). The larvae develop to the fourth instar during summer. Before the long

overwintering period, post-larvae and adults bury into the ground close to their host-plants (Eggenberger pers. com., Kalberer 2000). The larvae will emerge as adults in mid-summer of the second year, while second-year overwintering adults emerge in the spring when the snow melts. The newly emerged adults can be distinguished from those that overwintered as adults by their elytrae that remain soft for three days after emergence. In *O. gloriosa* the sex ratio at emergence in the laboratory was balanced (Eggenberger pers. com.). However in both *O. gloriosa* and *O. cacaliae*, the sex ratio of beetles sampled in the field was male biased, with up to 84 % of males in one sampling of *O. cacaliae* (Kalberer, Knoll, Nessi, pers. com.). This unbalanced pattern might be due to differential survival or activity of males vs. females. These data originate from field samples of beetles that were active (feeding and mating) on the leaves of their food plants, and should thus represent the individuals that contribute to reproduction. These beetles constitute the main part of the population, and inspection on the ground under the food plants revealed the presence of few beetles, but no mating.

In the two species we studied (*O. gloriosa* and *O. cacaliae*), it is unlikely that a substantial nutrient and toxin-rich spermatophore would be transferred from male to female during copulation. Indeed, males do not seem to loose much weight during mating, whereas in moth species were nuptial gifts have been observed, males can transfer up to 25% of their body weight as a spermatophore to the female (Wiklund, pers. com.). In one experiment we found that males had the same average weight at the beginning of the season

and three weeks later, the standard deviation being the same for the two measures. During these three weeks, 80% of the individuals mated and most of the males had experienced several matings (average number of matings per individual is 1.7 in *O. cacaliae* and 5.9 in *O. gloriosa*). Additionally, the body weight of males measured at the end of the season did not correlate with their number of matings.

Oreina adults are aposematic with blue and green metallic coloration of their elytrae and bright red wings. They live in high numbers and densities in host-plant patches. As is indicated by their bright coloration, these beetles use chemical defense against their predators. When disturbed, they secrete a defensive liquid that covers the pronotum and the sides of the elytrae. This liquid is bitter tasting, and when a bird takes a beetle into its mouth, it immediately rejects the beetle, which remains unharmed and survive this experience. The secreted liquid is not resorbed after emission, and three weeks are needed for the defensive gland to regenerate 80% its content (Eggenberger & Rowell-Rahier 1993).

The different species of *Oreina* are either monophagous or oligophagous on two main plant families: Apiaceae or Asteraceae. Host-plants are used for larval and adult food, mating, oviposition and shelter. In some species the host-plant is also responsible for the chemical protection of the beetles against their predators. These species acquire defensive compounds (pyrrolizidine alkaloids (PAs)) from their food-plant, and store them in their defensive glands (Rowell-Rahier *et al.* 1991, Pasteels *et al.* 1992). This strategy is

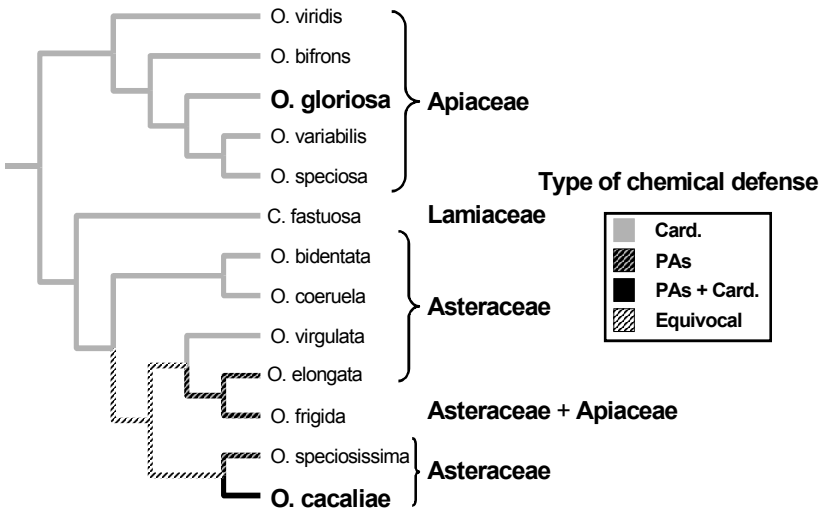
called “sequestration”, and it most likely derived from the plesiomorphic strategy of toxin neosynthesis (also called *de novo* synthesis) (Fig. 2, Hsiao and Pasteels 1999). Neosynthesis of cardenolides occurs in most of the *Oreina* species (Van Oycke *et al.* 1987), and some species benefit from both strategies (Dobler and Rowell-Rahier 1994, Pasteels et al. 1995). *O. cacaliae* is the only species of this genus that is only defended by sequestered-PAs and has lost the capacity to synthesise cardenolides. Cardenolides and PAs are thought to be the deterrent factors of *Oreina*’s secretions.

A previous study showed that there is no distance attraction of males by females *O. cacaliae* (Kalberer 2000), which makes it unlikely that pheromones are used in this species. As these beetles live in very high densities on their food-plant, it may be confusing for each individual to perceive pheromonal messages from all other individuals around; this way of communication is described mostly in species that have dispersed distributions, and that need long distance attraction to find their mates. Hence in *Oreina*, contact chemicals or visual cues could be involved in mate assessment.

The reproductive strategies in the genus *Oreina* range from oviparity to viviparity, including all intermediates (Dobler and Rowell-Rahier 1996). Larvae of viviparous species such as *O. gloriosa* and *O. cacaliae* are not chemically-protected at birth, but they soon acquire the same type of defensive compounds as their parents. However, the larvae do not emit defensive secretion, and PAs and/or cardenolides are spread within their body. Hence, contrarily to adults, a larva attacked by a predator would not survive

the attack. Predators of adults could be birds or small mammals. Predators of larvae are mostly arthropods (Carabidae, Staphylinidae, arachnids). The harvestman *Mitopus morio* is very abundant on the food plants of *Oreina*, and seems to be a major predator of larvae (Jeanbourquin 1999). It is possible that *Oreina* larvae could be the main food of individual harvestmen that specialise on hunting on the plants where only *Oreina* live.

Fig. 2. Phylogeny and chemical defense strategies of the genus Oreina obtained from sequencing data (redrawn from Hsiao and Pasteels 1999)



The questions

O. gloriosa and *O. cacaliae* have been extensively studied, thus their life history, chemical defense, population structure and dispersion are well understood. Also, they are easy to collect in very high numbers at known field sites. Furthermore, these two species can be found

either in monospecific or in mixed populations. The aim of my study was to investigate several potential selective pressures that may influence the evolution of chemical defense in these beetles; in this context we investigated three main questions:

- Is mate choice a potential selective pressure for defense-related traits in the laboratory? (Chapter 1),
- Is there indeed a relation between mating success and toxicity in *Oreinas* in nature? (Chapter 2),
- Which of the two types of defences is more deterrent to predators, and are generalist predators locally adapted to each of these defences? (Chapter 3).

Additionally, the Appendix presents results documenting the cost of the two types of defense, the intra-individual variation in toxicity, and the pattern of regeneration of defensive secretions. It contains data that will not be published separately, but are relevant to interpret the results described in Chapters 1 to 3.

The three chapters of this thesis correspond to manuscripts that will be submitted to international journals for publication. Hence I ask the reader to apologise for the repetitions that occur in the text, especially in the introduction and material and methods sections.

Summary of the results

Evidence for the use of defensive compounds for sexual purposes is scarce. However sexual selection might have some importance in the evolution of defense-related traits. The present study reports a parallel analysis of defense-related traits and mate choice in two sister species of leafbeetles differing in their type of chemical defense. *Oreina gloriosa* produces autogenous cardenolides, whereas *O. cacaliae* sequesters pyrrolizidine alkaloids (PAs) from its food plant. We analysed order of mating and number of matings as measures of individual sexual success. The relationship between these measures and body weight and toxicity (i.e. concentration of defensive secretions) was investigated. Only in *O. gloriosa* the mating success of individuals correlated with the concentration of defensive compounds. This pattern implies that sexual selection intervenes in the evolution of defensive traits in this species whereas this would not be the case in *O. cacaliae*. Although the process responsible for this pattern remains hypothetical, we propose that reciprocal male and female choice of sexual partners might be implicated.

We also analyzed the mating pattern for several traits, including defense-related traits in the field. This study reveals that mating is not random in these species. In both species, body weight and volume of the defensive secretion produced were important factors in mating pattern, and in *O. gloriosa* age also played a role.

The concentration of defensive secretion did not influence the mating pattern in the field. We discuss the ability and the need of these beetles to evaluate the defensive capacity of their mates. In the field, male-male competition for the access to females might favor heavy males, but may render mate choice for toxicity secondary.

The harvestman *Mitopus morio* is a major predator of the leafbeetles *Oreina gloriosa* and *O. cacaliae* at the larval stage. We investigated both learning and local adaptation of *M. morio* towards these two preys, by conducting choice experiments. We found that to some extent *O. cacaliae* was better defended than *O. gloriosa*. We propose that the pyrrolizidine alkaloids (PAs) contained in *O. cacaliae* are more deterrent to this predator than *O. gloriosa*'s cardenolides. Deterrence by PAs did not occur at first, but after prior experience, which we interpret as a dose-dependent response to avoid intoxication. Thus, there would be some post-ingestion deterrence, caused by the toxic effect of PAs. Moreover, *O. gloriosa* did not gain protection from toxic plant material in its gut content.

Discussion and perspectives

The abundance of insect chemical defences sequestered from the host plant or synthesised *de novo* by the insect (Bowers 1990, Whitman *et al.* 1990) suggests that natural enemies influence the evolution of phytophagous insects. The deterrent effect of defensive compounds for predators and parasitic insects is well documented (Euw *et al.* 1967, Fink and Brower 1981, Rowell-Rahier *et al.* 1995), whereas little is known about the efficiency of these compounds for resistance to other classes of parasites such as protozoa, or viruses. However the main kind of enemy that applies its selective pressure on a species will strongly influence the type of defense that is developed. It is commonly assumed that exposure to parasites could be quasi constant and very diverse. In this regard, resistance to parasitism was proposed to shape the evolution of sexual display and mating preference in various species (Hamilton and Zuk 1982, Møller 1990, Ridley 1996). Predation is commonly not considered, as attacks by predators are supposed to be rare and unpredictable. However, in aposematic herbivorous insects such as *Oreina* leafbeetles which live in high density, predation is most likely an important pressure. Aposematic signal has probably little effect on parasites, but is very efficient in the learning of aversion by predators (Guilford 1990). Hence in such a system, chemical defense could have evolved in response to predators, more than to parasites. Another particularity of the *Oreina* genus is that in most of the

species (i. e. the cardenolides-producing species), the defensive compounds are produced and stored very locally in the defensive glands, and are not spread within the body. Furthermore, the defensive liquid is emitted only when the insect is touched or disturbed. This supports the notion that this type of defense is mainly directed at predators or parasitoids, but not at other types of enemies (internal parasites for instance). In laboratory rearing of field-collected *Oreina*, survival was very high, which might indicate that parasitism is low. On the other hand, potential predators may be birds and mammals (shrews, foxes, Mustelidae) for adult beetles, and arthropods (carabids, staphylinids, spiders and myriapods) for larvae (Jeanbourquin 1999). Densities of larval predators on the host-plants were as high as 60 individuals per plant per week of capture. As a larva would not survive the attack by a predator, one may wonder why they also produce or sequester defensive compounds. Larvae are often found in large numbers on a same leaf, and have been shown to be attracted to plants that already host other larvae (Conconi and Nessi, unpublished data). As the females lay their larvae within the same plant patches, it is not excluded that sib-selection might be the advantage for larvae to be chemically defended. Also field data of capture-recapture suggest that the survival is high in *Oreina* adults (Knoll and Rahier 1997, Conconi and Nessi unpublished data). In these beetles that have a two years life-cycle it is important to keep predation low until males mate and females larviposit. This starts nine months after the emergence of the beetle (after overwintering), at the earliest. It is possible that

some females may live a third year and continue to reproduce. These might be the females we observe (about 20 %) laying only a few larvae at spring and then dying a few weeks later. The genus *Oreina* offers great potential for understanding how various selective pressures may influence the evolution of chemical defense, thanks to the different kinds of chemical defense that occur in species of *Oreina*. We found differences concerning mate choice and larval predation between two of these species. In order to be able to draw stronger evolutive conclusions concerning the evolution of chemical defense in this genus, there would be the need to extend similar experiments to other *Oreina* species, that use either PAs or cardenolides for their defense. It would be especially interesting to analyse also these patterns in species (*O. elongata* and/or *O. speciosissima*) that have the capacity to both synthesise cardenolides and sequester PAs, depending on which host-plant they feed.

Several other questions arose from this study, which would be most valuable to develop in further research. For instance, it would be most interesting to investigate in detail the actual process of mate choice. Indeed we found indications for both male and female choice of sexual partners; female choice is commonly analysed in the literature, whereas male choice and reciprocal male and female choice seems poorly documented.

As multiple mating was found to correlate with concentration of cardenolides in the secretions of *O. gloriosa* only, there is the need to know the advantage of polyandry in both species. Especially an exploration of sperm competition mechanisms would enable to

reveal the advantage of mating first for males, the mate guarding process, and the reason why the mating frequency and the different phases of mating were so different between *O. gloriosa* and *O. cacaliae*.

Another interesting point to investigate is the effective relationship between concentration of defensive substances in the secretions and its effectiveness (i.e. deterrent efficiency towards predators). In the same way, the dose-dependant deterrent effect of PAs proposed in Chapter 3 could be tested by using purified substances that can be purchased. Although these patterns would probably vary according to the predator species, such a study would provide good information on the significance for an *Oreina* beetle to be well-defended.

Also a behavioural experiment could be designed to test our hypothesis that recently disturbed individuals remain quiet during the time that their defensive secretions regenerate, and that individuals in poor physical condition (old, parasitized, etc..) secrete less volume of defensive secretion with low concentration of defensive compounds.

Finally, courtship or sexual display by means of pheromones or, more likely, cuticular tastes, is still hypothetical and further observation of this process would allow to better link defense and sexual display. It remains unclear whether the defensive secretions of *Oreina* beetles can directly act as sexual signals for mate quality, or if associated traits are used for this purpose.

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MATE CHOICE AND TOXICITY IN TWO SPECIES OF LEAF BEETLES WITH DIFFERENT TYPES OF CHEMICAL DEFENSE

by

E. Labeyrie[1] and M. Rahier[1]

Running head: Labeyrie and Rahier – Mating pattern and toxicity in two leaf-beetles

ABSTRACT

Evidence for the use of defensive compounds for sexual purpose is scarce. However sexual selection might have some importance in the evolution of defense-related traits. The present study reports a parallel analysis of defense-related traits and mate choice in two sister species of leafbeetles differing in their type of

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chemical defense. *Oreina gloriosa* produces autogenous cardenolides, whereas *O. cacaliae* sequesters pyrrolizidine alkaloids (PAs) from its food plant. We analysed order of mating and number of matings as measures of individual sexual success. The relationship between these measures and body weight and toxicity (i.e. concentration of defensive secretions) was investigated. In *O. gloriosa* the mating success of individuals correlated with the concentration of defensive compounds. This pattern implies that sexual selection intervenes in the evolution of defensive traits in this species whereas this would not be the case in *O. cacaliae*. Although the process responsible for this pattern remains hypothetical, we propose that reciprocal male and female choice of sexual partners might be implicated.

Keywords: sexual selection, mating pattern, chemical defense, sequestration vs. *de novo* synthesis, mate choice, assortative mating, cardenolide, pyrrolizidine alkaloid, *Oreina*, Chrysomelidae.

INTRODUCTION

Although the relationship between resistance to parasites and sexual selection has been widely demonstrated in several organisms (Hamilton and Zuk 1982, Møller 1990), very little is known about the role of other natural enemies on sexual selection. Sexual selection was initially described by Darwin (1871) as "the advantage which certain individuals have over others of the same sex and species solely in respect of reproduction". Many authors have since proposed and described various mechanisms of sexual selection (Andersson 1994, Andersson and Iwasa 1996, Birkhead and Biggins 1997), among which mate choice is important and widespread. Defensive traits such as toxic compounds most likely evolve in response to predation pressure, and may be constrained by various physiological parameters. However, an additional mechanism of sexual selection for toxicity may arise if individuals benefit from mating with a well-defended partner. An alternative possibility would be that toxicity is a reliable trait for assessing overall mate quality because it is costly (Andersson and Iwasa 1996, Zahavi 1975). However, the assessment of the cost of chemical defense is difficult, which makes this hypothesis very difficult to test. Evidence for the use of defensive compounds for sexual purposes is scarce, and in most of the cases it concerns plant-derived compounds (Nishida and Fukami 1990, Trigo and Brown 1990, Dussourd *et al.* 1991, Amano *et al.* 1999). Sexual selection for defensive compounds may occur in cases were the individuals of the choosing

sex benefit from this choice; this is the case for example in the moth *Utetheisa ornatrix* (Dussourd *et al.* 1991) where females choose males advertising for a toxin rich spermatophore, a nuptial gift that females use for defending their offspring. In turnip sawflies, the more distasteful females have a higher mating success (Amano *et al.*1999), which may indicate male choice for toxic females. Although poorly documented, male choice for females may occur when males are in some ways limited in their number of matings and if the quality of their mates strongly influences their fitness.

Two means of chemical defense occur within the alpine genus *Oreina* Chevrolat (Coleoptera, Chrysomelidae). Some species, like *O. gloriosa* defend themselves by synthesising autogenous cardenolides (Van Oycke *et al.* 1988, Eggenberger and Rowell-Rahier 1993). Other species like *O. cacaliae*, are defended by pyrrolizidine alkaloids (PAs) acquired from their food-plant, and which they store in defensive glands (toxin sequestration, Rowell-Rahier *et al.* 1991, Pasteels *et al.* 1992). In none of these species defensive compounds are directly provided to offspring by their mother (Eggenberger and Rowell-Rahier 1992, Dobler and Rowell-Rahier 1994). This study reports a parallel analysis of defense-related traits and mate choice. The underlying assumption is that sexual success should reflect mate quality, i.e. the capacity of mates to produce a large number of offspring with high genetic fitness. Good quality might include having a high concentration of defensive compounds, as this trait determines the resistance to predators. Body weight is another factor of potential importance, as it may determine

the physical condition of the beetles (i.e. fat reserves, aptitude to feed, possible increased fecundity of females). We suppose that high quality individuals would be pursued for mating more often and/or earlier. For both *O. gloriosa* and *O. cacaliae*, we report laboratory data on:

- behavioural observations of mating events
- the order of mating and the number of matings as measures of sexual success of individuals, in relation to body weight and concentration of the defensive secretions.
- the degree of assortative mating in relation to body weight and chemical defense.

This study provides evidences that mating success correlates with concentration of defensive compounds in *O. gloriosa*. This pattern implies that sexual selection intervenes in the evolution of defensive traits in this species whereas this would not be the case in *O. cacaliae*. Although the process responsible for this pattern remains hypothetical, mate choice is proposed to operate.

MATERIAL AND METHODS

Collection of beetles. For both species, we collected 60 adults of each sex. *Oreina cacaliae* was sampled near La Fouly in the Val Ferret (Valais, Swiss Alps, 45.56 N, 7.05 E, alt.1500m), in early May, when individuals emerge from the snow and start feeding on *Petasites paradoxus* (Asteraceae). Individuals of this species were sexed using sexual dimorphism of the tarsi (Lohse& Luche 1994). *Oreina gloriosa* were sampled in Saas Grund (Valais, Swiss Alps, 46.08 N, 7.57 E, alt. 1800m) at the beginning of June, when they start feeding on their food plant *Peucedanum ostruthium* (Apiaceae). As sexual dimorphism of tarsi does not exist in this species, beetles were sexed using weight polymorphism: previous studies have shown that females are heavier than males, and weight distribution of both sexes has been documented (Eggenberger, pers. com.). We thus had the possibility to assess the sex of individuals with a 95% interval of confidence. A confirmation of sex has been done during the experiment by mating observation; only in two cases we discovered a wrong assessment of sex. For minimum disturbance during transportation to the laboratory, beetles were placed in plaster-bottomed boxes (insuring high humidity level) with plenty of leaves of their food-plant. All the beetles still alive after the end of the experiment were released at their respective field site.

Experimental procedure. The experiment was conducted from June 1st to 30, 2000 for *O. gloriosa*, and from May 3 to 30, 2000 for *O. cacaliae*. These dates corresponded to the mating period in the field, as reported by previous field observations in the last four years (Steffi Knoll, Nicole Kalberer and Luca Nessi pers. com.).

For each species, we placed 30 males and 30 females in each of two 30x50 cm trays. This approximately reflected the natural density on plant patches in the field. The beetles were individually marked with correction fluid (Tipp-Ex) and labels for bee queens on the elytrae. The marking code could easily be read without disturbing the insects. The collection of defensive secretions and weighting were done for all insects within two days after the end of the experiment. The trays had wet filter paper at the bottom to keep humidity, and were placed in the laboratory at room temperature (temperature ranged from 19 to 25°C) away from direct sunlight. Fresh food-plants were provided every day for food and shelter. We recorded every mating by careful observation every four hours for all the period of the experiment. Because preliminary observations indicated that matings are on average six hours long in both species, we assumed that nearly all copulations had been noted. During six nights of nocturnal observation, we confirmed that copulations are very rare at night (one mating observed at 4 AM); hence no survey was done between 11 PM to 6 AM. Each mating pair was carefully removed from the tray and kept in a vial until the partners separated. Afterwards, the beetles were released back in their tray in order to

keep a constant insect density over the experiment. At the end of the experiment, each beetle was weighed and milked for its defensive secretions as described below. As every copulation was recorded, chronological order of mating (i.e. first mating observed is number one, etc…), number of copulations and assortative mating could be determined. This allowed us to correlate these mating patterns to measured traits of each individual in order to assess whether sexual selection occurs. To estimate the duration of the different phases of mating, 12 mating pairs of *O. gloriosa* and 13 of *O. cacaliae* were observed every hour for the whole duration of the mating.

Collection of defensive secretions. Sampling of defensive secretions was done by holding the insect under the microscope and gently hitting its pronotum and elytrae with fine forceps until secretions cover the cuticle. The drops were collected with a calibrated capillary glass tube, and the volume was measured using a graduated lens. Each secretion was stored individually in 150 µl of methanol in a –80°C freezer, until chromatographic analysis for concentration was performed. All beetles were weighed to the nearest 10^{-4}g.

Sample preparation and chromatographic analysis of cardenolides. Samples were prepared and the concentration of total cardenolides in the secretions of *O. gloriosa* was determined by reverse-phase HPLC as described by Eggenberger and Rowell-Rahier (1993). We only diverged from their method by using a Varian, Star chromatography

workstation system with automated injection. We used ouabain as internal standard, thus the concentration values obtained are expressed as µg equivalent ouabain/µl. The minimum detected value was 0.95 µg/µl, and standard deviation equalled 0.7% of the mean. We also dispose of the UV spectra for each peak.

Sample preparation and chromatographic analysis of PAs. The samples were prepared and the concentration of total PAs in the secretions of *O. cacaliae* were determined by capillary GC as described by Rowell-Rahier *et al.* (1991). We only diverged from their method by using a HP1-MS 30m x 0.025mm x 0.25µm column, and senecionin as external standard. The concentration values obtained are expressed as µg equivalent senecionin /µl**.** The minimum detected value was 0.01 µg/µl, and standard deviation equalled 6.4% of the mean. GC/MS was used to confirm that the peaks corresponded to PAs.

Statistical analysis. Relationships between traits, order of mating and assortative mating for each trait were investigated using linear models using SPSS 10.0 software. Comparisons of means were performed by 2-sample t-tests. When necessary, the data were log-transformed to fit model assumptions (amount of cardenolides in *O. gloriosa*, mating number and concentration in *O. cacaliae*).

Fidelity and random mating simulation. From our results we suspected that matings are not random in *O. gloriosa*, because certain

combinations of individual partners were observed several times. In order to see if this pattern could happen under random mating assumptions, we performed a simulation using the S-PLUS 2000 software. As the experiment was divided in two trays in which the beetles were isolated from the ones of the other tray, the simulation was performed to represent the situation in one single tray. The program displayed two lists of 182 numbers (there was approximately 182 = 384/2 matings in each tray) randomly chosen between 1 and 30 (since each tray contained 30 individuals of each sex). Each list represented beetles of one sex, and mating events were represented by the two numbers on a same line. The program then displayed the number of "matings" occurring once, twice, or more. We repeated this simulation 300 times, and recorded the mean numbers of expected matings in each category, and their standard error (Fig.1). A goodness-of-fit Chi-square test was performed to see if the differences between observed and calculated values were significant; for this test we used only two categories (once the same partner and more than once) because the expected values were too small in the last two categories.

RESULTS

Sample size. In 2000, the respective numbers were 384 and 77 mating events. Because of technical problems we were able to obtain the concentration of defensive secretions only for 31 males and 28 females of *O. gloriosa* and 18 males and 7 females of *O. cacaliae*. Thus we know the concentration of both partners for only 155 mating events in *O. gloriosa* and 12 mating events in *O. cacaliae*. In *O. gloriosa* all males mated and only 3 % of the females did not mate, whereas in *O. cacaliae* 28 % of males and 59% of females remained unmated.

Mating observations. These observations required approximately 12 hours of survey. Mean length of mating was similar in both species (6.1± 2.8 hours and 6.7 ± 2.4 hours in *O. gloriosa* and *O. cacaliae* respectively (Fig. 2)). We distinguished two phases: one is true copulation (i.e. male copulatory organ fully inserted into female reproductive tract), the other is referred as "guarding" (i.e. male being installed on the female's back as during true copulation, but without penetration of copulatory organ). Although variable, the duration of the phases of mating were different in the two species: in *O. gloriosa* true copulation lasted usually less than one hour; on the contrary, in *O. cacaliae* all mating time consisted of true copulation. Neither of the two species showed

a courtship phase. In both species, males often tried to mate with females that were running away. Most of the time these unwilling females were successful in escaping. Direct competition for access to mates was not evident in males nor females. Although in some cases potential male competitors were approaching a mating pair, and even climbing on the back of the male already in place, this never caused the current copulation to end.

Order of mating. In neither of the sexes of *O. gloriosa* there was a relationship between the order of first mating and body weight (linear regression $P = 0.402$ for males and $P = 0.132$ for females), nor was there a correlation between the order of first mating and secreted volume (regression $P = 0.187$ for males and $P = 0.561$ for females). That is, beetles that mated first did not have a higher weight, nor volume of defensive secretions. There was a negative relationship between order of the first mating of the individuals and concentration of defensive secretions (Fig. 3, in males $F = 6.394$, $R^2 = 0.186$, $P = 0.017$; in females males $F = 5.308$, $R^2 = 0.194$, $P = 0.031$). In *O. cacaliae* there was no relationship between the order of mating and either secreted volume ($P = 0.167$ for males and $P = 0.363$ for females) or concentration ($P = 0.876$ for males and $P = 0.172$ for females), nor between order of mating and body weight of females ($P = 0.676$). However there was a positive relationship between order of mating and body weight of males (Fig. 4, $F = 4.37$, $R^2 = 0.11$, $P<0.05$). Note that in both species there was no relationship between weight, secreted volume and concentration of

defensive secretions. *O. gloriosa* females had more concentrated defensive secretions than males (means = 22.93 ± 14.64 and 16.62 ± 12.74 µg/µl respectively, $p<0.001$, $t = -6.14$), while no such difference was found in *O. cacaliae.*

Assortative mating. In *O. gloriosa* there was no significant relationship between the weight of males and the average weight of their female partners ($P = 0.98$) and *vice versa* ($P = 0.82$), nor between concentration of males' and average concentration of the secretions of their female partners ($P = 0.25$) and *vice versa* ($P = 0.34$). In *O. cacaliae* there was no significant relationship between any traits of males and traits of their female partner ($P = 0.521$ for weight, $P = 0.164$ for volume, $P = 0.151$ for concentration).

Number of copulations and measured traits. Multiple mating of males and females was frequent in both species. The mean number of mating per individual was 5.91 for males and 5.98 for females of *O. gloriosa*, and 1.71 for males and 1.88 for females of *O. cacaliae* (Fig. 5). The maximum number of matings for a single individual was 17 for a male and 16 for a female of *O. gloriosa*, and 5 for a male and a female of *O. cacaliae.* In *O. gloriosa*, the number of copulations was not linked to weight ($P = 0.082$ for females, $P = 0.661$ for males), secreted volume ($P = 0.920$ for females, $P = 0.411$ for males). In females only there was a positive relationship between number of copulations and concentration of defensive secretions (Fig. 6, $F=5.02$, $R^2=0.16$, $P<0.05$). This was not the case in males

of this species (P = 0.481). In *O. cacaliae* the number of copulations was not linked to weight (P = 0.403 for females, P = 0.660 for males), secreted volume (P = 0.818 for females, P = 0.206 for males), nor concentration of defensive secretions (P = 0.475 for females, P = 0.570 for males).

Fidelity and random mating simulation. Remating with the same partner was never observed in *O. cacaliae*. In *O. gloriosa* 21% of the mating events observed occurred between mates that had already mated with each other or that would eventually remate together later on (82 couples out of 384, Fig. 1). Moreover, there were 12 matings between mates that met three times, and 4 matings between mates that met four times together. Note that in the figure these numbers were divided by two to represent the situation in only one of the two trays. The simulation shows that if the beetles choose their mates randomly, there would be significantly more matings occurring between partners that would encounter only once, and less matings repeated between same partners ($X^2 = 32.01$, one degree of freedom, $P < 0.001$).

DISCUSSION

Fidelity as represented by "remating", i.e. mating that occurred between individual mates that have already mated together or that will remate together later, was observed in 21% of the *O. gloriosa* couples but never in *O. cacaliae.* This pattern indicates that mating is not random in this species, and certain combinations are favoured. Such a phenomenon is poorly documented in invertebrates. In the experiment, the beetles were very active, and it is unlikely that they remated with the same partner because of proximity within a tray. Also the remating of individuals did not occur soon after the first encounter, and other mating might intervene in between. The mechanism for these individual choice remains to be established. One adaptive explanation of this behaviour is that good combination of the genes of two individuals could enhance the fitness of their offspring. However there is the need to further investigate the advantage of mating several times with the same partner.

In *O. gloriosa* heavier individuals did not mate before the lighter ones, whereas in *O. cacaliae* the first males that mated where heavier than males that mated later. In *O. cacaliae* this means that the small males mate earlier and heavy males later. This unexpected pattern might be due to the fact that some beetles first fed and then mated later in the period of the experiment. In neither of the species

there was significant assortative mating for weight, nor did the number of matings correlated to weight. Thus, contrarily to our expectations, body weight did not seem to be an important factor in mate quality.

In another experiment we found that males had the same average weight at the beginning of the season and three weeks later, the standard deviation being the same for the two measures. During these three weeks, 80% of the individuals mated and most of the males had experienced several matings (average number of matings per individual is 1.7 in O. cacaliae and 5.9 in O. gloriosa). Also since the body weight of males measured at the end of the experiment was not correlated with the number of matings, it is unlikely that males loose much weight at each mating.

In *O. gloriosa* there was a negative relationship between order of first mating and concentration of defensive secretions, which means that the first individuals to mate were better defended than later mating individuals. This could indicate females' choice for toxic males and males' choice for toxic females. Hence by their choice, individuals would increase the survival of their offspring and thus their own fitness. This earlier mating of well-defended individuals did not occur in *O. cacaliae* of either sex.

Multiple mating is frequent in insects (Walker 1980, Hosken and Blankenhorn 1999). In *Oreina*, the reason for polyandry is probably not sperm replenishment, since females *O. cacaliae* can have their all set of ova fertilised by a single mating (Dobler and Rowell-Rahier 1996). However the literature proposes many other

potential advantages that may explain superfluous mating activity; these include transfer of nutrients, increased protection of females from predators, reduction of energy loss from male harassment and genetic benefits increasing the fitness of offspring (Walker 1978, Tregenza and Wedell 1998, Hosken and Blanckenhorn 1999, Bernasconi and Keller 2001). In our experiment, as a given effect of balanced sex-ratio, mean number of mating per individual was not different between sexes. This might not be true in nature where sex ratio was biased towards males. On average, *O. gloriosa* individuals mated nearly 3.5 times more than *O. cacaliae*. If we relate this to the difference in length of true copulation, which was dramatically higher in *O. cacaliae* than in *O. gloriosa*, we may hypothesise that *O. gloriosa* males transfer less sperm or other material (accessory gland secretion) to the female (Micholitsch *et al.* 2000, He and Tsubaki 1992). This would enable males of this species to mate more often than males of *O. cacaliae*. Other phenomena such as the need to "guard" females for limiting access to competing males could also be responsible for such observations (Dickinson 1995, Facundo 1999), although we have no evidence for it. The advantage of multiple mating in these species has to be investigated further.

In *O. gloriosa,* the more concentrated were the defensive secretions of the females, the higher was their number of copulations and thus their sexual success. Here again this might be due to male choice for female defensive potential, this choice being made possible by the artificially balanced sex-ratio in the experiment. Though poorly documented, male choice for females has been shown

to operate in several taxa, and not only in species in which sex ratio is biased towards males (Amano *et al.* 1999, Waring-Wilde 1996, Cunningham and Birkhead 1998, Gwynne and Bailey 1999). For instance if males are limited in the number of mating they can afford, then it can be worth for them to choose the best female to mate with. In *O. gloriosa* where each mating lasts on average six hours, we cannot exclude that this situation might occur; by choosing the best-defended females, males increase their fitness by means of an enhanced survival of their progeny.

A smaller variation in levels of chemical defense could reflect a higher selection, driven by sexual selection and/or predation (Labeyrie and Rahier Chapter 3, Rowell-Rahier *et al.* 1995). From our data we calculated that standard deviation/mean = 71% in *O. gloriosa* and 159% in *O. cacaliae*. In *O. gloriosa* there is a significant heritability of toxicity (i.e. concentration of defensive secretions) in cardenolides from mother to offspring (average heritability = 0.5, Eggenberger and Rowell-Rahier 1992), indicating that toxicity is genetically determined in this species. Thus in this species, there is the potential for the "good-gene" theory of mate choice to operate. On the other hand in *O. cacaliae*, toxicity is probably highly related to host plant content in PAs, as it is the case in other sequestering species (Isman 1977, Isman *et al.* 1977, Brower *et al.* 1984, Bowers 1992). In this species, there is also certainly some part of genetic variation in the physiological sequestering efficiency or in the ability to choose the best food-plant, but this might be minor compared to the high variation in the host-plant

content in PAs. The individual rank of toxicity is not conserved throughout seven samplings of their secretions during a six-week experiment (see Appendix). This indicates that individual level of concentration of PAs in the secretion is not mainly due to intrinsic genetic background. If this is true, their would not be the possibility of the good-gene mate choice process to occur in *O. cacaliae*. One might notice that all the other examples of sexual selection in relation to chemical defense concern sequestering species. However in these studies this could be linked to the presence of nuptial gift given by males to females, either toxin-rich spermatophore or significant "good-defensive genes". In some other species, females use their defensive compounds to protect their eggs once laid, whereas the larvae of the viviparous species *O. gloriosa* and *O. cacaliae* are not defended at birth (Eggenberger 1993, Dobler and Rowell-Rahier 1994). These direct benefits of mating with a well-defended partner do not seem to occur in *O. cacaliae*.

It is a possibility that toxicity would be a sexually-selected trait because it is costly and thus reliable for assessing male overall quality (Zahavi 1975, Andersson and Iwasa 1996). Since toxin sequestration is often thought to be less costly than *de novo* synthesis (Pasteels *et al.* 1990, Bowers 1992), this could explain why mate choice for toxicity does not exist in *O. cacaliae*, whereas it could evolve in *O. gloriosa*.

The mean length of mating was approximately the same in both species. However in *O. gloriosa* true copulation was very short (less than one hour) whereas in *O. cacaliae* it lasted the all mating

time. The current knowledge of the reproductive biology of these species do not allow to interpret this difference. Some studies report cases in which duration of copulation is associated with insemination and fertilisation rates (Micholitsch *et al.* 2000). Spermatophore size has also been shown to increase with time between matings (He and Tsubaki 1992). In our study it is unlikely that a large toxin-rich spermatophore would be transferred from male to female during copulation, because males do not loose weight dramatically during copulation (Labeyrie and Rahier, Chapter 2 and unpublished data). Although there was no conspicuous courtship behaviour, a use of pheromones or cuticular tastes for sexual display is possible (Edwards and Seabrook 1997, Shu *et al.* 1999, Ruther *et al.* 2000). Indeed, for toxicity and mate choice to be associated, there is the need of a cue that beetles may use for assessing toxicity of potential mates. Such signals need to honestly correlate to the quality of the individual (concentration of defensive compounds in our case). As far as we can visually observe, the beetles only emit their defensive liquid when disturbed by a predator, but not when they mate. This makes unlikely that concentration could directly be assessed by mates before or during mating. However, as defense glands are under neural regulation (Schooneveld *et al.* 1992), it is a possibility that very small amounts of secretions are released from the glands for sexual purpose. Other authors have proposed that defensive secretion may directly have a pheromonal effect, providing that glands are not sealed and volatile compounds might diffuse continuously (Attygalle *et al.* 1991, Eggenberger and Rowell-Rahier

1993). Also in some species the defensive compounds were shown to be precursors of sexual pheromones (Trigo and Brown 1990, Dussourd *et al.* 1991, Amano *et al.* 1999); in these cases it is unsure whether predation or sexual selection or both were responsible for the acquisition of these molecules. We do not have any evidence for this process in *Oreina* leafbeetles.

Even though we are unsure on the process and signals involved in the mating pattern for toxicity found in *O. gloriosa*, this pattern reflects a phenomenon that may contribute to the evolution of defensive traits. Reciprocal male and female choice for well-defended partner is a possible process that could lead to this pattern.

ACKNOWLEDGEMENTS

We are grateful to Pr. Bruno Betschart, Thomas Degen, Laure-Emmanuelle Perret and Ted Turlings for their support in chemical analysis, Yves Borcard, Erick Campan, Loïc Didillon, Håkan Häggström and Christina Tamò for helping in lab work, Betty Benrey, Wolf Blankenhorn, Pierre-Yves Claudin and Karl Gotthard for useful comments and Jacqueline Moret for advice in statistical analysis.

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FIGURES

Fig. 1. Observed and calculated numbers of mating events occurring with a partner met one to four times. Calculated numbers were obtained by a simulation assuming random mating. Note that standard errors of theoretical means are to small to be visualized on the figure.

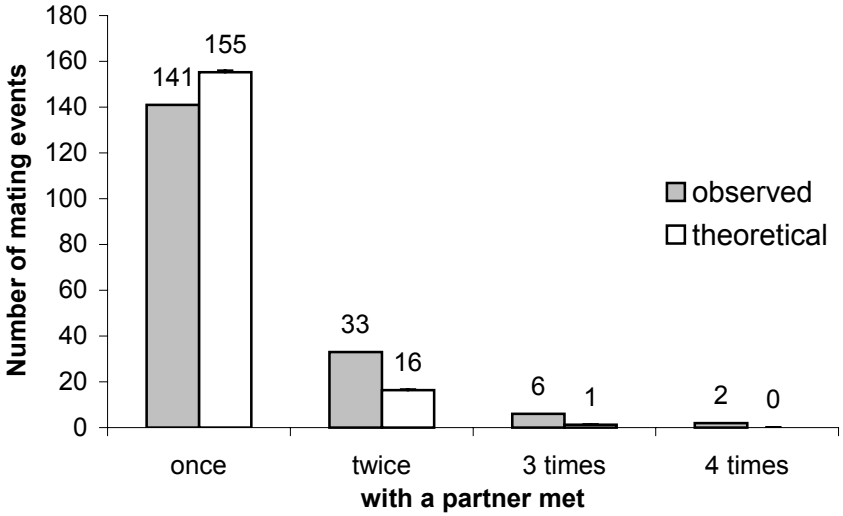


Fig. 2. Mean mating duration in *O. gloriosa* and *O. cacaliae*

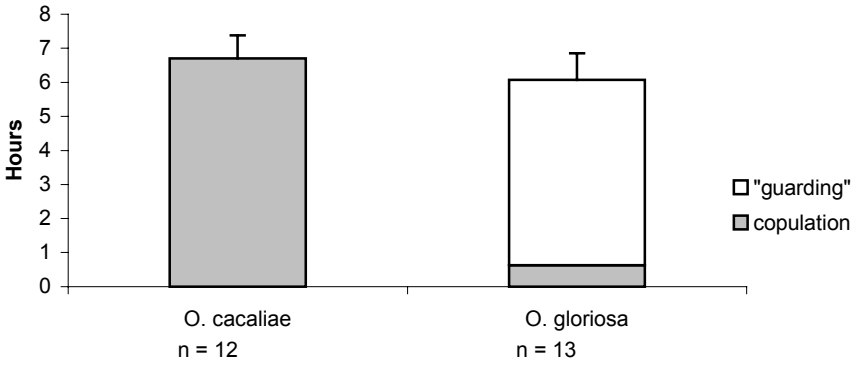


Fig. 3. Linear regression between order of mating and concentration of defensive secretions in *O. gloriosa* males and females

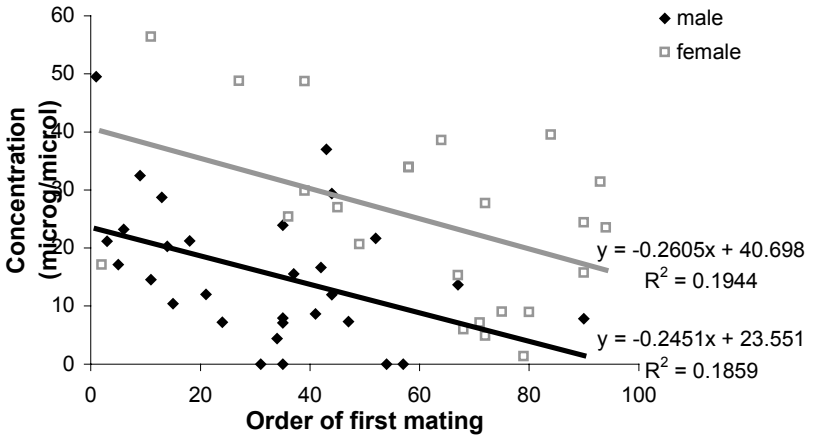


Fig. 4. Relationship between the order of mating and body weight in *O. cacaliae*

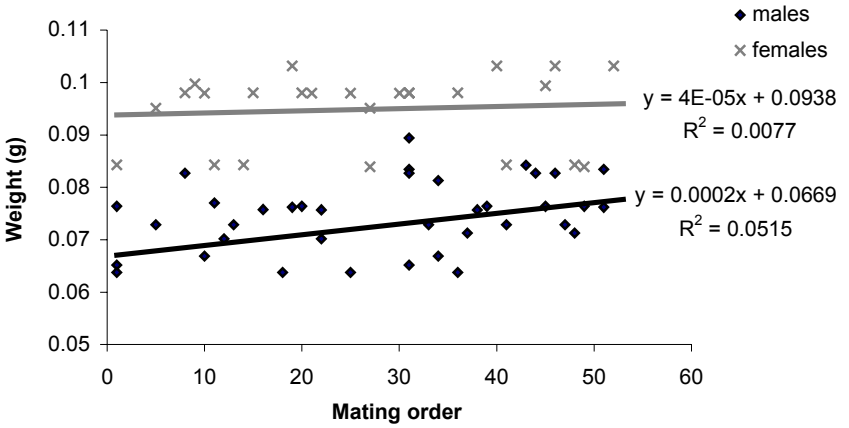


Fig. 5. Mean number of matings in males and females of *O. gloriosa* and *O. cacaliae*

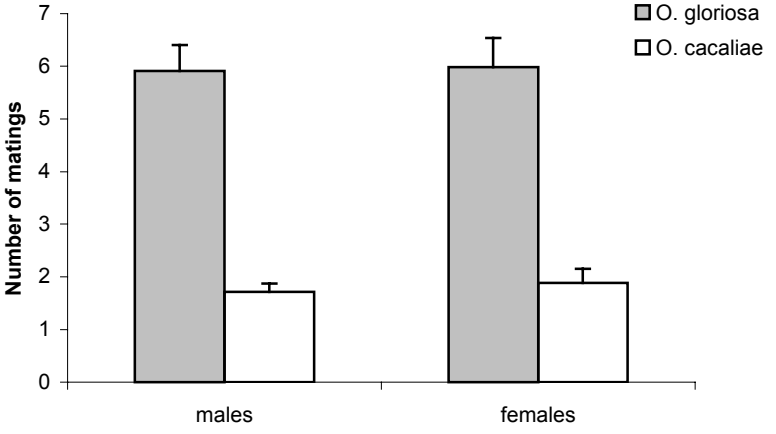
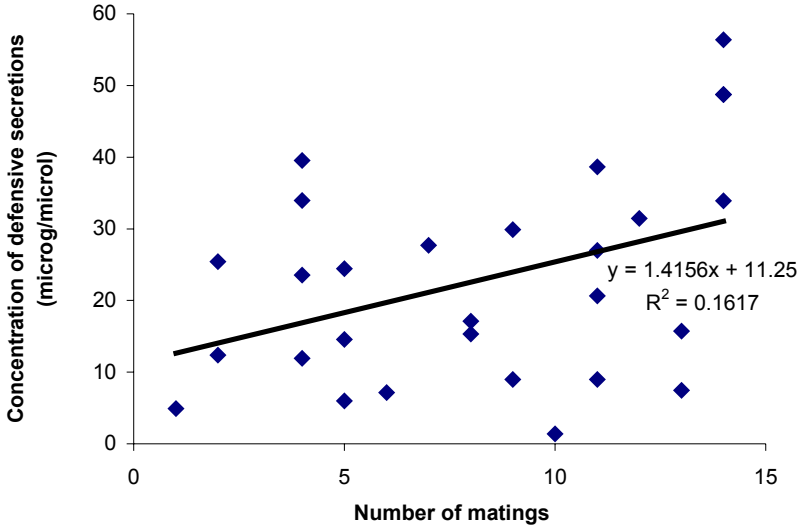


Fig. 6. Relationship between number of matings and concentration of defensive secretions in *O. gloriosa* females



FIELD MATING PATTERN IN RELATION TO DEFENSE-RELATED TRAITS IN ALPINE LEAF-BEETLES

by

E. Labeyrie[2] and M. Rahier[1]

Running head: Labeyrie and Rahier – Mating pattern, competition and toxicity in two leaf-beetles

ABSTRACT

Very few studies have focused on the importance of sexual selection in the evolution of chemical defense. In the alpine leafbeetles genus *Oreina*, two types of chemical defense are used for deterring predators. Some species like *O. gloriosa* produce autogenous cardenolides, whereas other species like *O. cacaliae* sequester pyrrolizidine alkaloids (PAs) from their food plant. We analyzed the mating pattern for several traits, including defense-

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related traits. This study reveals that mating is not random in these species. In both species, body weight and volume of the defensive secretion produced were important factors in mating pattern, and in *O. gloriosa* age also played a role. The concentration of defensive secretion did not influence the mating pattern in the field. We discuss the ability and the need of these beetles to evaluate the defensive capacity of their mates. In the field, male-male competition for the access to females might favor heavy males, but may render mate choice for toxicity secondary.

Keywords: sexual selection, male-male competition, chemical defense, sequestration *vs. de novo* synthesis, mate choice, assortative mating, cardenolide, pyrrolizidine alkaloid, *Oreina*, Chrysomelidae.

INTRODUCTION

Very few studies have focused on the importance of sexual selection in the evolution of chemical defense (Trigo and Brown 1990, Nishida and Fukami 1990, Dussourd *et al.* 1991, Amano *et al.* 1999). The predation pressure is commonly thought to be entirely driving the evolution of defensive traits. Although predation initiated the apparition of the defenses, sexual selection may play an important role in the evolution of defensive traits once established. A wide variety of insect species exploit chemical defenses to protect themselves against potential natural enemies (Euw *et al.* 1967, Eisner and Eisner 1991, Bowers 1992). The defensive compounds may either be autogenously synthesized (*de novo* synthesis, Van Oyke *et al.* 1987), or acquired by feeding (sequestration) (Euw *et al.* 1967, Brower *et al.* 1984, Nishida and Fukami 1990, Rowell-Rahier *et al.* 1991, Pasteels *et al.* 1992). The evolutionary relationship of sequestration and *de novo* synthesis has been extensively explored in Chrysomelid beetles (Pasteels *et al.*, 1984, 1990, Dobler *et al.* 1996, Hsiao and Pasteels 1999); in this group, sequestration has evolved from the plesiomorphic strategy of *de novo* synthesis. These two means of chemical defense occur within the alpine genus *Oreina* Chevrolat (Coleoptera, Chrysomelidae). Some species, like *O. gloriosa*, synthesize autogenous cardenolides whereas other species, like *O. cacaliae*, sequester the pyrrolizidine alkaloids (PAs) from

their food-plant in their glands and use these substances for their own defense. The sequestering species thus highly depends on its food-plant for its defense against predators.

We run an analysis of the mating pattern for age, body weight and defense related traits (volume and concentration of defensive secretion), which are likely to be important factors in fitness and thus in mate choice. We thus investigated in field-sampled individuals of both species:

- the relationship between traits,
- mating pattern for volume and concentration of defensive secretions,
- mating pattern for body weight,

In an additional laboratory experiment, we tested the effect of age in the mating pattern of *O. gloriosa*.

MATERIAL AND METHODS

Collection of beetles. Two categories of beetles were collected in each species: mating couples (n=31 in *O. gloriosa*, n=25 in *O. cacaliae*), and beetles that were not mating at the sampling time (31 beetles of each sex in *O. gloriosa*, 25 in *O. cacaliae*). This procedure allows for an instantaneous representation of the mating population in the field. Thanks to the large sample-size, we assumed that individuals of the mating group mate on average more often or longer than the average of single individuals. Adults *O. cacaliae* were collected near La Fouly in the Val Ferret (Valais, Swiss Alps, 45.56 N, 7.05 E, 1500m above see level), in early May on *Petasites paradoxus* (Asteraceae). Individuals of this species were sexed using sexual dimorphism of the tarsi (Lohse& Luche 1994). *O. gloriosa* were sampled in Saas Grund (Valais, Swiss Alps, 46.08 N, 7.57 E, 1800m above see level) and were collected on their single food plant *Peucedanum ostruthium* (Apiaceae). As sexual dimorphism of tarsi does not exist in this species, beetles were sexed using weight polymorphism: females are heavier than males, and weight distribution of both sexes was known from previous studies (Eggenberger pers. com.). We thus had the possibility to assess individuals sexes with a 95% confidence interval; in another study this method gave 2% of error. The sampling date corresponded to the peak time of mating in the field (June 16, 1999 for *O. gloriosa*,

May 3, 2000 for *O. cacaliae*). At that time, the sex ratio was biased towards males. For transportation to the laboratory, each mating pair was placed in a separate vial, and each single beetle in an individual vial in order to minimize disturbance. All the vials were plaster-bottomed to insure high humidity, and provided with a piece of fresh leaf of the food-plant. All the beetles were released at their respective field site after the end of the experiment.

Measures. Each beetle was milked for its defensive secretion within 24 hours after arrival in the laboratory; this was done by holding the insect under the microscope and gently hitting its pronotum and elytrae with fine forceps until drops of secretion appeared from the gland openings. The drops were collected with a calibrated glass capillary, and the volume was measured using a graduated lens. Each secretion was stored individually in 150 µl of methanol in the freezer. Then the beetles were weighed to the nearest 10^{-4}g.

Sample preparation and chromatographic analysis of cardenolides. A preliminary trial revealed that the spectrophotometric method described by Dobler and Rowell-Rahier (1994) is not sensitive enough for analyzing individual secretions. Thus the samples were prepared and the concentration of total cardenolides in the secretions of *O. gloriosa* was determined by reverse-phase HPLC as described by Eggenberger and Rowell-Rahier (1993). We only diverged from their method by using a Varian, Star chromatography workstation system with automated

injection. We used ouabain as internal standard, thus the concentration values obtained are expressed as µg equivalent ouabain/µl. The minimum detected value was 0.95 µg/µl, and standard deviation equalled 0.7% of the mean. We also dispose of the UV spectra for each peak.

Sample preparation and chromatographic analysis of PAs. The samples were prepared and the concentration of total PAs in the secretions of *O. cacaliae* were determined by capillary GC as described by Rowell-Rahier *et al.* (1991). We only diverged from their method by using a HP1-MS 30m x 0.025mm x 0.25µm column, and senecionin as external standard. The concentration values obtained are expressed as µg equivalent senecionin /µl**.** The minimum detected value was 0.01 µg/µl, and standard deviation equalled 6.4% of the mean. GC/MS was used to confirm that the peaks corresponded to PAs.

Effect of age on mating pattern in O. gloriosa. Beetles of this species mate several times, and mating with a virgin partner may influence the probability of fecundation by this partner. A choice experiment was performed in order to determine whether the newly-emerged beetles are more prone to mate than beetles that emerged the previous year (Fig. 1). The young individuals came from laboratory rearing (larvae that over-wintered and pupated in an incubator with temperature and photoperiod matching the natural conditions). They emerged as adults at the end of July. Twenty-three young males and 20 young females were placed in a tray with same

numbers of males and females collected in the field in early June and kept in semi-natural conditions in the laboratory; these beetles were thus at least one-year old; they emerged at the end of the previous summer, over-wintered as adults, and got out again in June. Individuals of the two age-groups were color-labeled on their ventral side. All the matings were observed and then removed from the tray. As this experiment required constant observation, it was stopped when 50% of the individuals had mated.

Statistical analysis. Multiple discriminant analysis (MDA) were performed on standardized weight, secreted volume and concentration data using S-PLUS 2000 software. This analysis enables to visually separate groups of individuals from their traits, but do not constitute a statistical test. Statistical comparison of means were carried out using 2-samples t-tests, and linear regression was used to determine relationships between traits and assortative mating for each trait (SPSS 10.0 software). When necessary, data were log-transformed to fit model assumptions (secreted volume in *O. gloriosa*, secreted volume and concentration in *O. cacaliae*). In the choice experiment, the number of old and young mating insects was compared with a test of goodness-of-fit chi2.

RESULTS

Sample size. *W*e collected 31 and 25 mating couples of *O. gloriosa* and *O. cacaliae* respectively, and 31 and 25 single beetles of each sex in *O. gloriosa* and *O. cacaliae* respectively. However, because of technical problems, we were only able to measure concentration of 14 mating and 12 non-mating females, 12 mating and 14 non-mating males of *O. gloriosa*, and 23 mating and 8 non-mating females and 19 mating and 17 non-mating females of *O. cacaliae*. Among these, we have concentration data for 11 mating couples of *O. gloriosa* and 19 mating couples of *O. cacaliae*.

Multiple discriminant analysis. For *O. gloriosa* the MDA allowed the separation of sexes, of mating and non-mating males, and to a lesser extent of mating and non-mating females (Fig. 2a). The first discriminant variable (2.87*Weight + 0.23*Volume + 0.09*Concentration, discriminant correlation = 0.94) separated groups mostly based on weight. Thus mating males look heavier than non-mating males, but mating females were not different from non-mating females. The second discriminant variable separates groups mostly based on volume of secretions data (-0.08*Weight + 1.20*Volume + 0.34*Concentration); this axis indicates that mating males secrete more volume of defensive secretions than non-mating males, and that in females this pattern is reversed. The second and

third discriminant variable did not separate the data as well as the first axis (discriminant correlation = 0.50 and 0.22 respectively), and the third discriminant variable did not allow any interesting separation of the groups. For *O. cacaliae*, the MDA also separates males and females, mating and non-mating males, as well as mating and non-mating females (Fig. 2b). Again the first discriminant variable (2.06*Weight - 0.26*Volume + 0.03*Concentration, discriminant correlation = 0.88) allows to separate sexes mostly based on weight. Hence mating males tend to be heavier than non-mating males, while mating and non-mating females were not separated by their weight. The second discriminant variable separates groups mostly from volume data (-0.13*Weight – 1.31*Volume + 0.49*Concentration), but neither this variable nor the third discriminant variable allowed any interesting separation of the groups, and their correlation with the data was low (discriminant correlation = 0.43 and 0.31 respectively).

Relationship between traits. In *O. gloriosa*, there was no significant relationship between body weight and secreted volume ($P = 0.096$, $F = 2.89$) nor between weight and concentration of defensive secretions ($P = 0.541$, $F = 0.378$). However a negative relationship was found between secreted volume and concentration ($F = 4.85$, $P < 0.05$, $R^2 = 0.09$). In *O. cacaliae*, there was a significant positive relationship between weight and concentration ($F = 7.84$, $P < 0.01$, $R^2 = 0.11$), a negative relationship between secreted

volume and concentration ($F = 33.9$, $P < 0.001$, $R^2 = 0.34$), but no relationship between weight and secreted volume.

Mating pattern in relation to volume of defensive secretions. In *O. gloriosa* mating males secreted significantly larger volumes of defensive secretions than non-mating individuals ($t = 3.08$, 2-tailed $P<0.01$), whereas mating and non-mating females did not differ from each other (Fig. 3a). In *O. cacaliae*, mating and non-mating males did not differ significantly from each other, but mating females secreted larger volumes than non-mating females ($t= -2.15$, $P<0.05$) (Fig. 3b). In neither of the species, the secreted volume differed between sexes. Overall, *O. gloriosa* produced a larger volume of defensive secretions than *O. cacaliae* ($t =-7.00$, $P<0.001$).

Mating pattern in relation to concentration of defensive secretions. In *O. cacaliae* we found no effect of concentration on mating pattern. In contrast, in mating pairs of *O. gloriosa*, we found a trend in the relationship between female and male concentration ($r^2 = 0.39$, $P = 0.04$). However this trend to homogamy is only supported by a single extreme couple out of 11.

Mating pattern in relation to body weight. For *O. gloriosa*, as indicated by the MDA (Fig. 2a), mating males were heavier than non-mating males (Fig. 4, $t= 4.29$, $P<0.001$), whereas mating and non-mating females did not significantly differ in their body weight. Only in this species we found a positive relationship (homogamy) between the weight of males and the weight of their female partner

(Fig. 5, R^2=0.34, P<0.001). In *O. cacaliae*, mating males were also heavier than non mating males (Fig. 4b) (t= 2.25, P<0.05), but there was no homogamy.

Effect of age on mating pattern in O. gloriosa. A total of 22 mating pairs was observed in this experiment. One year-old males mated more than newly-emerged males (18 *vs.* 4 respectively, goodness-of-fit chi2 P=0.003) (Fig. 6), and the opposite trend occurred in the females (6 *vs.* 16 respectively, goodness-of-fit chi2 P=0.03).

DISCUSSION

Our results indicate that very few males *O. gloriosa* mate soon after emerging at the end of the summer (only 4 out of 22). The cuticle of the young individuals hardens within a few days, and the genitalia of the males may not be sclerified enough for copulation (personal observation). Hence, in nature, most of the newly emerged males would not be able to mate before the following spring. On the contrary, the young females appeared to mate more than old ones. Several processes may be proposed for explaining the advantage for males to mate with virgin females (Simmons *et al.* 1994), and for virgin females to mate more than old females; however nothing is known about these processes in *Oreina*.

In both *O. gloriosa* and *O. cacaliae*, the sex ratio of beetles sampled in the field was male biased, with up to 84 % of males in one sampling of *O. cacaliae* (Kalberer, Knoll, Nessi, pers. comm.). These data originate from sampling of beetles that were active (feeding and mating) on the leaves of their food plants, and should thus represent the individuals that participate to reproduction. These beetles constitute the main part of the population, and inspection on the ground under the food plants revealed the presence of few beetles but no mating.

The assortative mating for body weight in *O. gloriosa* might be due to males competing for the largest receptive females, that may have a higher fecundity as it is the case in other Coleoptera. The heaviest males might be stronger and win the opportunity to mate with the largest females, or females may choose heaviest males, which might explain the homogamy for weight. This is not a surprising result since male choice has been reported in a wide variety of taxa (see Gwynne and Bailey 1999, Ihara and Aoki 1999), and similar assortative mating for weight and size has been found in other Coleoptera (Berstein and Berstein 1998, Harari *et al.* 1999). It is not unlikely that strong males both compete with rivals and choose the best females, although the conventional expectation would be that females are the exclusive choosing sex because the sex ratio was male-biased. Both male-male competition and female choice could result in the observed pattern that mating males are heavier than non-mating males.

Field and laboratory observations revealed that females can avoid mating by running away. Hence for males it might be easier to mate with the less active females. From our present sampling, we observed that mating females of *O. cacaliae* secrete less volume of defensive secretions than non-mating females. The volume of defensive secretion that the insect produces when disturbed may be associated with the activity of individuals. Indeed in *O. cacaliae* we have some preliminary evidence that the inactive individuals might produce a smaller volume of secretion. We hypothesize that beetles that have been disturbed not long ago hide and stay less active for a

certain time in order to regenerate their gland content faster. Thus females during this regenerating phase would be more easy to mate with because of their reduced activity. This hypothesis could explain the mating pattern for secreted volume observed in *O. cacaliae*. In *O gloriosa* we do not have any evidence for this, but it could also be the case. The higher secreted volume of mating males may reflect also in this species that these males are in better condition; thus these males would be more likely to win the competition for access to females, and/or females might choose these males for their good condition.

Concentration of toxin is commonly considered as the deterrent factor for predators, more than the secreted volume or total amount of toxin in the secretion. Eggenberger and Rowell-Rahier (1993) reported that the defensive secretions of *O. gloriosa* differ quantitatively and qualitatively between males and females, and that mated females had more concentrated secretions than virgin females. They further hypothesise on a pheromonal action of defensive secretions, as suggested by Attygalle *et al.* (1991). This could be adaptive because no additional metabolic pathway would be required for pheromone biosynthesis and receptor proteins could be already present in the form of enzymes of the biosynthetic pathway. Since beetles do not emit their defensive secretions for courting nor mating, pheromonal action of secretions has been suggested to be based on the slow leakage of gland contents, assuming defensive glands are not hermetically sealed (Attygalle *et al.* 1991). There is the possibility that the defensive secretions could be used as contact

signal for assessing mates quality. We could also attribute the difference of secretions of virgin *vs.* mated females to metabolic changes in the resource allocation (from defense to production of larvae), related to mating status.

In a laboratory experiment designed to investigate mate choice, we observed a mating pattern in relation to concentration of defensive secretions in *O. gloriosa* but not in *O. cacaliae*, which was most likely due to both male and female choice. No such pattern could be observed from our field sampling of mating *vs.* non-mating beetles. The main difference between these two experiments was the sex ratio; indeed field samplings showed that the sex ratio of beetles that were active on the surface of the leaves is highly biased towards males (Knoll and Kalberer pers. com), whereas in the laboratory experiment we imposed a balanced sex ratio. This was done in order to observe mate preference without being confounded by competition. Indeed if males have to compete with other males for access to females, they may have to be less selective and mate with any available female. This may be the reason why in this study there was no mating pattern for toxicity in the field sample of *O. gloriosa*. Hence in nature, mate choice may not favour well defended individuals. However it remains possible that other processes of sexual selection (sperm competition, etc…) would play an important role in the evolution of defensive traits.

ACKNOWLEDGEMENTS

We are grateful to Pr. Bruno Betschart, Thomas Degen, Laure-Emmanuelle Perret and Ted Turlings for their support in chemical analysis, Yves Borcard and Loïc Didillon for helping in fieldwork and Jacqueline Moret for advice in statistical analysis. We also thank Betty Benrey and Karl Gotthard for their useful comments on the manuscript.

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FIGURES

Fig. 1. Hypothetical life cycle of Oreina beetles in Saas Grund and Val Ferret. At spring, the beetles that overwintered as adults emerge from the ground and mate. The females start to lay larvae three weeks later. On July, young beetles that owerwintered as post-larvae emerge from the ground, and the virgin females mate with old males. On mid-August, adults and larvae bury into the ground for overwintering.

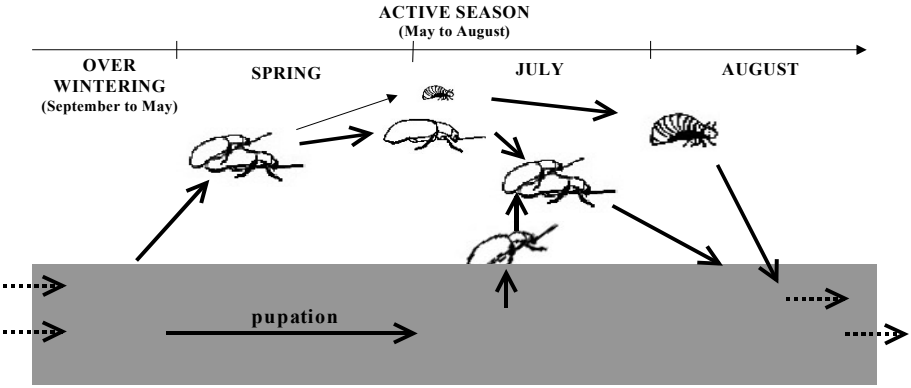
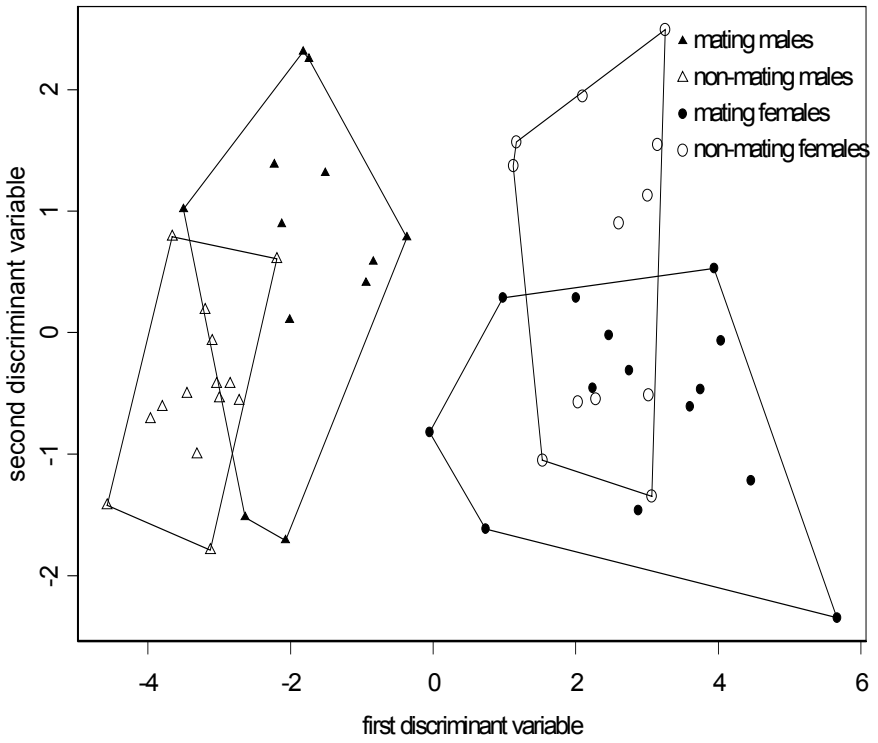


Fig. 2. Multiple discriminant analysis performed on body weight, and volume and concentration of defensive secretions of *O. gloriosa* (2 a), and *O. cacaliae* (2 b)

2 a: Multiple discriminant analysis in *O. gloriosa*



2 b: Multiple discriminant analysis in *O. cacaliae*

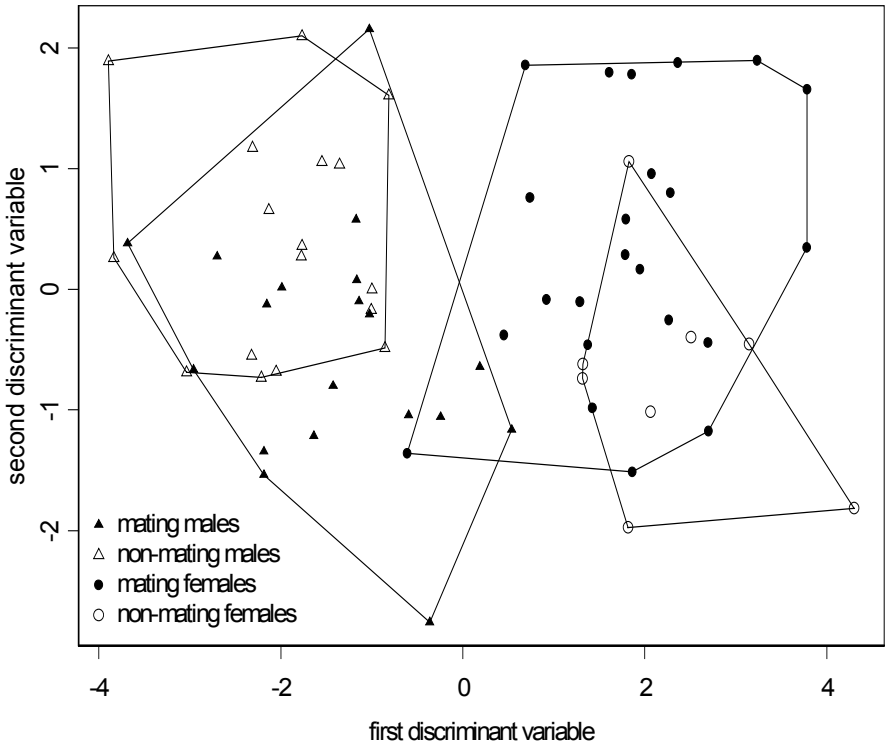
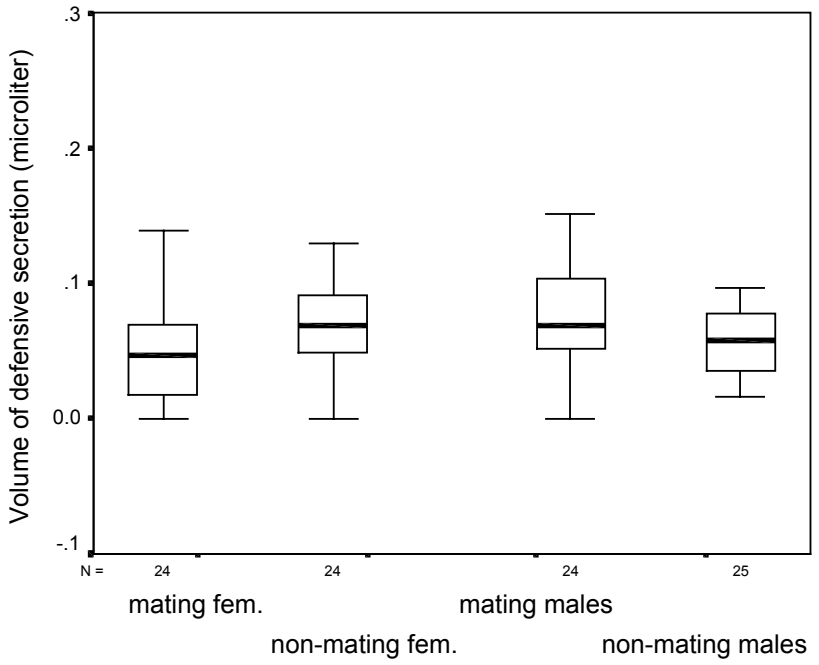


Fig. 3. Volume of defensive secretions produced by mating and non-mating males and females. Boxplots represent the median (line across the box), quartiles, and extreme values. The box represents the interquartile range which contains 50% of the values.

3a. *O. gloriosa*



3b. *O. cacaliae*

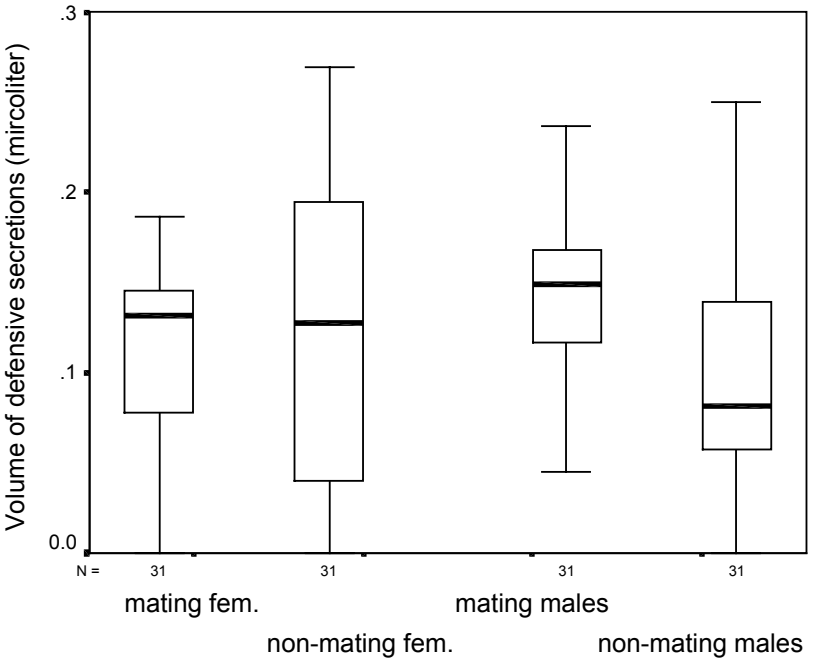
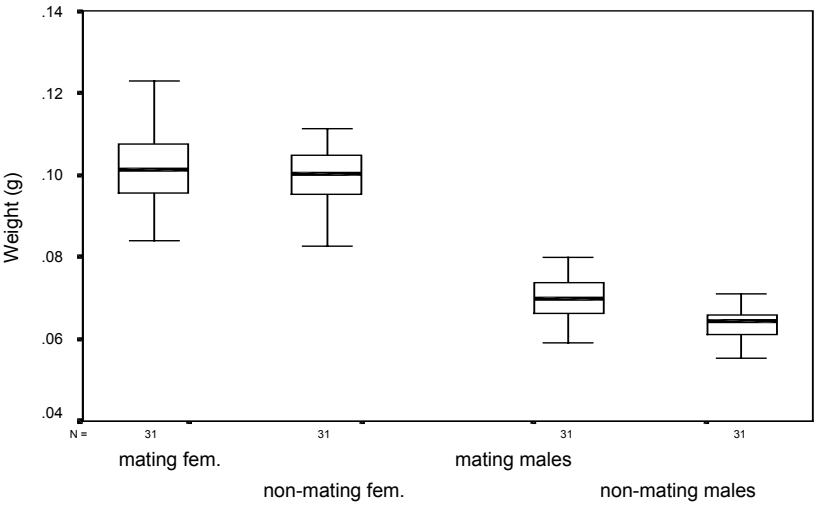


Fig. 4. Body weight of mating and non-mating males and females

4a. *O. gloriosa*



4b. *O. cacaliae*

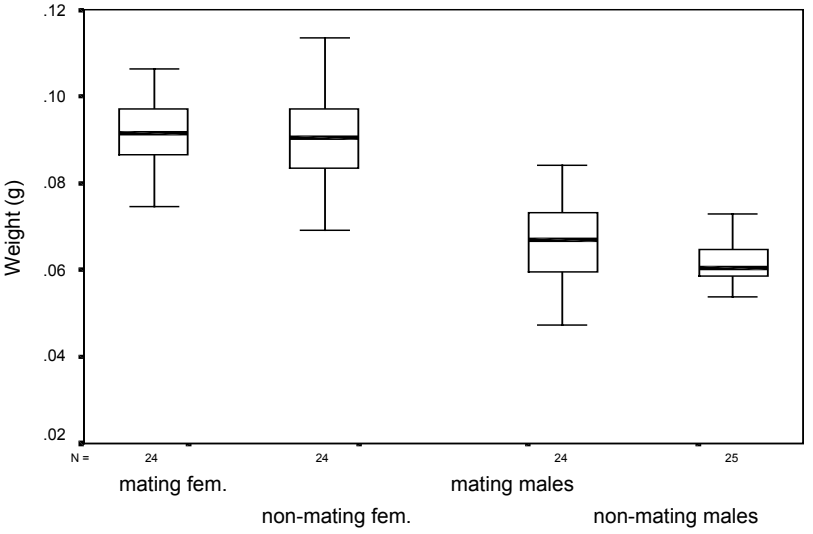


Fig. 5. Relationship between body weight of males and females in *O. gloriosa* mating couples

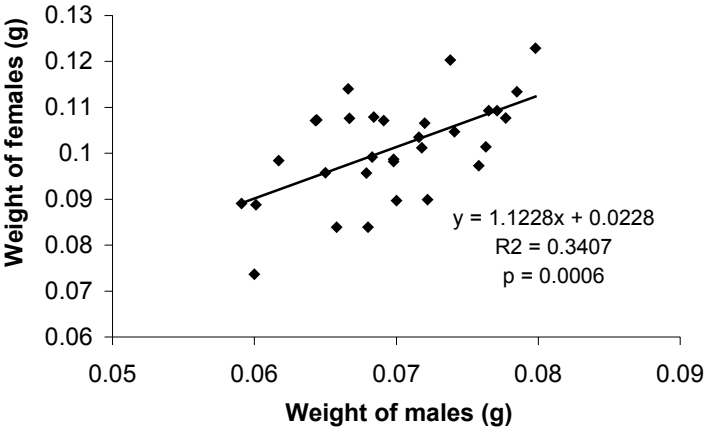
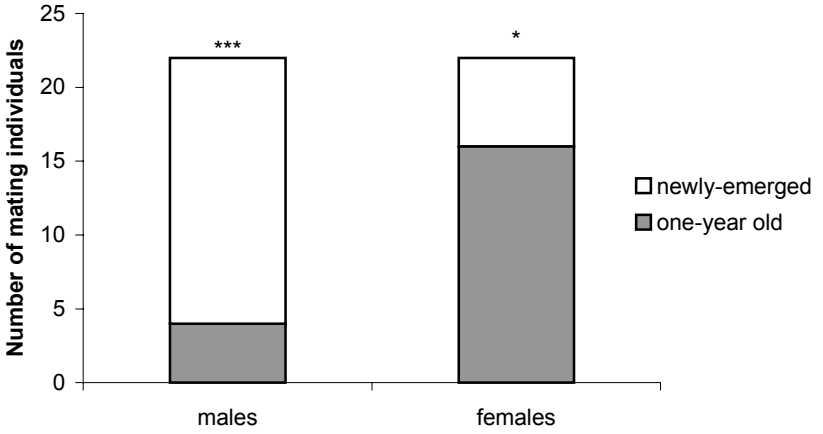


Fig. 6. Number of mating observed for newly-emerged and one-year old males and females *O. gloriosa*



AN INVESTIGATION OF LEARNING AND LOCAL ADAPTATION OF A GENERALIST PREDATOR TOWARDS TWO CHEMICALLY DEFENDED PREYS

by

E. Labeyrie[3] and M. Rahier[1]

Running head: Labeyrie and Rahier –Feeding preferences of predators of *Oreina* larvae

ABSTRACT

The harvestman *Mitopus morio* is a major predator of the leafbeetles *Oreina gloriosa* and *O. cacaliae* at the larval stage. We investigated both learning and local adaptation of *M. morio* towards these two preys, by performing choice experiments. We found that

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to some extent *O. cacaliae* was better defended than *O. gloriosa*. We propose that the pyrrolizidine alkaloids (PAs) contained in *O. cacaliae* are more deterrent to this predator than *O. gloriosa*'s cardenolides. Deterrence by PAs did not occur at first, but after prior experience which we interpret as a dose-dependent response to avoid intoxication. Thus there would be some post-ingestion deterrence, caused by the toxic effect of PAs. Moreover, *O. gloriosa* did not gain protection from its gut content with toxic plant material.

Keywords: predation, generalist predator, *Mitopus morio*, Opiliones, *Phalangidae*, *Oreina*, Chrysomelidae, Coleoptera, local adaptation, chemical defense

INTRODUCTION

Predators that feed on chemically-defended prey require behavioural and/or physiological adaptations that are typically associated with specialised predator species. However generalist predators are also known to discriminate between prey (Roger *et al.* 2000, Theodoratus 1999). If one kind of toxic prey is frequent in some habitat, generalist predators could evolve detoxification pathways and become locally adapted to this prey. Predators of the leaf-beetle genus *Oreina* Chevrolat (Coleoptera, Chrysomelidae) offer a good potential to study local adaptation of generalist predators, since different *Oreina* species have different defense strategies, although the appearance and life history of the species are otherwise similar. The different *Oreina* species can either occur in sympatry or in monospecific groups, thus the predators sampled in *Oreina* sites may either encounter the two species together, only one of them, or none. *Oreina* adults and larvae live in very high density on the host-plants where they feed, rest and reproduce, whereas other herbivorous insects are very rare on these plants (Knoll, Nessi pers. com.). This makes them a valuable resource to predators. The predators of the adult stage are birds and small mammals (Conconi pers. com.), whereas the harvestman *Mitopus morio* (Arachnida, Opiliones, Phalangiidae) is a major predator of larvae (Jeanbourquin 1999). *M. morio* is distributed across the northern hemisphere

(Martens 1978, Hillyard and Sankey 1989). Whereas other predators (i.e. carabids, staphylinids, spiders and myriapods) have been recorded only on the ground, *M. morio* is the only one found on the vegetation, and often observed while waiting for prey on the food-plants. Adult *Oreina* beetles are highly aposematic, with metallic blue and green coloration of their elytrae, with bright red wings. Larvae of some species, like *O. cacaliae*, are also warningly coloured with bright yellow pronotum and shiny black body, whereas larvae of other species, like *O. gloriosa* are not that conspicuous (brownish). Both adults and larvae of these species are chemically defended against their predators (Rowell-Rahier *et al.* 1995), using two different kinds of substances. Most of the species, like *O. gloriosa*, produce a mixture of cardenolides that are biosynthesised de novo (Pasteels *et al.* 1992), whereas others, like *O. cacaliae,* use plant-sequestered pyrrolizidine alkaloid N-oxides (PAs) (Pasteels *et al.* 1988, Rowell-Rahier *et al.* 1991, Ehmke *et al.* 1999). Various studies describe toxic effect of both cardenolides and PAS, and their deterrent properties (Euw *et al.* 1967, Fink and Brower 1981, Nelson *et al.* 1981). Several authors also proposed and noticed that the toxic effect was very variable from one predator species to another (Fink and Brower 1981). In the genus *Oreina*, PAs sequestration is thought to be a derived strategy evolved from *de novo* production of cardenolides (Dobler *et al.* 1996, Hsiao and Pasteels 1999). We ask whether this evolutionary change is linked to increased protection. For this we addressed the following questions:

1. how do generalist predators react when given the choice between known and unknown prey species?
2. could they evolve local adaptation to certain abundant prey?
3. do harvestmen show a learning capacity after experiencing a bad prey?
4. which of two types of toxins (cardenolides vs. PAs) is most efficient against larval predators? A previous study has shown that PAs are more efficient than cardenolides for deterring bird predators (Rowell-Rahier *et al.* 1995). Would cardenolides be most efficient against arthropod predators?
5. *O. gloriosa* feeds on *Peucedanum ostruthium* (Apiaceae), which contains toxic coumarins (Hadacek 1994, Luca Nessi unpublished data). Does this species gain some protection from the presence of toxic plant material in its gut, as proposed by Brower (1984).

MATERIAL AND METHODS

Animals and collection sites. The prey used for predation trials were third and fourth instar larvae of *O. gloriosa* and *O. cacaliae*. These two species are sympatric at the Val Ferret site (Wallis, Swiss Alps, 45.56 N, 7.05 E, 1500m above see level). *O. gloriosa* occurs alone in Saas Grund (Wallis, Swiss Alps, 46.08 N, 7.57 E, 1800m above see level) and *O. cacaliae* alone in Guebwiller (Vosges, France, 47.55 N, 7.13 E, 700m above see level). *O. gloriosa* and *O. cacaliae* were collected on their food-plants, *Peucedanum ostruthium* (Apiaceae) and *Adenostyles alliariae* (Asteraceae) respectively. Harvestmen were chosen as predators for these experiments because field observations and previous bioassays show that they are important predators of *Oreina* larvae in the field (Häggström *unpublished data*, Jeanbourquin 1999). They were sampled at four field sites: the three *Oreina* sites listed above, and also near Neuchâtel (Switzerland, 46.59 N, 6.55 E, 800m above see level) in a site were there are no *Oreina* beetles (table1). They were kept in individually-labelled plastic boxes with plaster bottom to insure high humidity level. *A posteriori* determination showed that all but two specimens were *Mitopus morio* (Fabricius).

Choice experiment between O. gloriosa and O. cacaliae. Thirty harvestmen were sampled at each site and brought to the

laboratory immediately. They were fed ground meat for three days in order to standardise their "appetite status". After these three days, each individual was given the choice between one *O. cacaliae* larva and one *O. gloriosa* larva of approximate same size. These larvae originated from laboratory rearing. We recorded which larva was eaten first; we also noted if the second larva, which was left with the harvestman during 24 hours, was eaten within this time. To investigate the learning potential of *M. morio*, each individual harvestman was tested three consecutive times ("consecutive experiment", test 1, 2 and 3). For this experiment we used *M. morio* from Saas Grund and Neuchâtel. We recorded the choice in each test and if the choice changed between tests for individual harvestmen. For tests 1 and 3, but not test 2, we recorded if the second larva was eaten too within 24 hours.

Experiment of predator deterrence by larval food plant P. ostruthium. Forty-five harvestmen collected in Saas Grund on *P. ostruthium* were offered the choice between one fed and one starved *O. gloriosa* larva. *O. gloriosa* larvae were collected at the same site. One group had *ad libitum* access to the food-plant and an other group was starved 24 hours before the experiment. We recorded which larva was eaten first, and if the second larva was eaten within 24 hours.

Statistical analysis. Numbers of harvestmen making one choice vs. the other were compared by goodness-of-fit chi-square

test. The Bonferoni correction was used for the analysis of the consecutive experiment. Fishers exact test were performed for comparing the number of harvestmen that changed their choice between tests, and the number that died, providing that they have previously eaten *O. gloriosa* vs. *O. cacaliae*. The S-PLUS 2000 software was used for all the analyses.

RESULTS

Choice experiment between O. gloriosa and O. cacaliae.

First choice. Independently of their origin, as many harvestmen chose *O. gloriosa* as *O. cacaliae* at their first choice (Fig. 1, goodness-of-fit chi^2 P = 0.83 for Guebwiller, 0.24 for Val Ferret, 0.32 for Saas Grund, and 0.85 for Neuchâtel; one degree of freedom). In three cases the two larvae were eaten very fast and we were not able to tell which larva was consumed first. Some individuals did not eat any of the two larvae.

Total number of larvae eaten after 24 hours. Twenty four hours later, most of the harvestmen had eaten the second larva, except the harvestmen from Guebwiller (Fig. 2). The total number of *O. gloriosa* and *O. cacaliae* larvae eaten were respectively 13 and 17 by harvestmen from Guebwiller, 27 and 26 by harvestmen from

Val Ferret, 26 and 23 by harvestmen from Neuchâtel and 16 and 15 by harvestmen from Saas Grund.

Consecutive experiment for harvestmen from Val Ferret. The harvestmen from this site significantly preferred *O. gloriosa* at the third test (21 vs. 5, $X^2 = 9.85$, one degree of freedom, P = 0.006 including Bonferoni correction), but not at the first and second test (Fig. 3b, P = 0.68). Further analysis showed that individuals that had eaten *O. cacaliae* in one test change their choice in the next test more often than the ones that have eaten *O. gloriosa* (Fig. 4, Fisher's exact test two-sided between tests 1 and 3 P=0.03; between tests 2 and 3 P= 0.024; between tests 1 and 2 not significant).

Consecutive experiment for harvestmen from Guebwiller. The *M. morio* from this site had no significant preference for *O. gloriosa* or *O. cacaliae* at test 2 (Fig. 3a, 9 vs. 3 respectively, goodness-of-fit chi^2 P = 0.08), nor at test 3 (5 vs. 4, P = 0.74). Previous feeding on *O. gloriosa* or *O. cacaliae* did not influence consecutive choice (Fisher exact tests not significant between tests 1 and 2, 2 and 3 nor 1 and 3). The number of harvestmen from Guebwiller that made a choice decreased from test 1 to 3 (n= 21 at test 1, n= 13 at test 2, n= 8 at test 3). Indeed several opiliones (n=12) stopped feeding or died. The proportions of *O. cacaliae* vs. *O. gloriosa* larvae eaten by these harvestmen and by individuals that remained alive did not differ (Fisher exact test, P = 0.16).

Experiment of predator deterrence by food plant P. ostruthium. Gut content seemed to have no effect on *M. morio* feeding choice. There was no difference in the feeding preferences between fed larva or starved larva of *O. gloriosa* (respectively 23 vs 16: chi^2 P = 0.26, one degree of freedom); 6 harvestmen did not eat at all.

DISCUSSION

Fed and starved *O. gloriosa* larvae were equally chosen by *M. morio,* and there was no effect of gut content in harvestmen deterrence. The fact that *O. gloriosa* larvae feed on a toxic plant does not seem to be useful for deterring its main predator. Thus it is unlikely that this species has specialised on its only food plant *P. ostruthium* for gaining better protection against predators. More likely the specialisation occurred for enabling the use of an abundant resource with little interspecific competition. *P. ostruthium* grows in large patches in mountain areas, and are mainly attacked by *O. gloriosa* adults and larvae. The only other herbivorous insects to have been observed on *P. ostruthium* are homopterans (Nessi pers. com.). Thus *O. gloriosa* does not seem to face inter-specific competition for food, and they can live in very high density.

This suggests that *O. gloriosa* has specialised on its only food plant *P. ostruthium* for other reasons than gaining better protection against predators.

Against our expectations on the potential adaptation of *M. morio* to the chemical defense of local prey, these harvestmen never showed any preference in their first choice. Moreover after 24 hours, most of the harvestmen have eaten the two larvae, except the ones from Guebwiller (a difference which remains unclear). For

harvestmen from Val Ferret, Neuchâtel and Saas Grund, this observation shows that there was no strong preference, and individual harvestmen did not consistently prefer one species over the other. Hence *Oreina* larvae are not well defended against harvestmen, despite the defensive substances contained in their body. However, there is the possibility that PAs and /or cardenolides are most efficient for deterring other predators of the larvae, such as carabids, staphylinids, or spiders, or even parasitoid flies (tachinids), which may participate to the predation pressure under which defensive compounds are maintained.

Thus if any difference in effectiveness between toxin exists in this system, deterrence would rely on a dose-dependence process. Such a phenomenon would lead to a random feeding pattern until a certain toxicity threshold is reached, and then careful discrimination of prey while running detoxification process. *M. morio* from *O. cacaliae* sites has efficient PAs-detoxification pathway, contrarily to the ones from sites without PAs-defended prey (Häggström *et al.*, in prep.). The harvestmen from Guebwiller were used to feed on *O. cacaliae* in the field, but not on *O. gloriosa*. They did not show a preference in any of the three tests, which indicates that the cardenolides of *O. gloriosa* are not efficient for deterring even non-adapted predators. Harvestmen from Val Ferret that are used to feed on both species avoided *O. cacaliae* at the last test only. This might reflect that they have to wait a certain time before ingesting PAs again. Thus harvestmen might become choosy only after ingestion of a threshold amount of toxins that make them sick. These

individuals may have to eat *O. gloriosa* before eating *O. cacaliae* again, because eating *O. cacaliae* would make them reach this toxicity threshold of PAs. However it is unclear why there is this difference of feeding pattern between harvestmen from Guebwiller and Val Ferret.

Naive harvestmen (from Neuchâtel) were not *a priori* deterred by any of the two species of prey. The reason why they did not discriminate in their first choice can either be that both species are suitable prey, or because they needed to learn which was the best prey. Indeed the toxic compounds are spread within the body of the *Oreina* larvae, and thus may not be perceived from the outside. Only *M. morio* sampled in Val ferret supposedly had prior encounter with both kinds of larvae, but even these harvestmen did not choose at first. It is uncertain whether they could forget previous experience with distasteful prey, and some invertebrate predators are known to have a very good long-term memory (Hare and Eisner 1993).

In general *M. morio* that have eaten *O. cacaliae* in one test are more likely to change their choice (i.e. switch to *O. gloriosa*) in the next test than the ones that had eaten *O. gloriosa*. Again this indicates a post-ingestion deterrence, caused by the toxic effect of PAs. Both cardenolides and PAS were shown to have deterrent properties against various predators (Euw *et al.* 1967, Fink and Brower 1981, Nelson *et al.* 1981). For instance cardenolide-defended *O. speciosissima* larvae have been shown to be more deterrent to ants than grasshopper legs (Rowell-Rahier and Pasteels 1990). Our results indicate that the cardenolides of *O. gloriosa* have

little effect for deterring the generalist *M. morio*, whereas the PAs of *O. cacaliae* do, to some extent. This result is similar to the pattern for deterrence reported by other studies using ants as predators of larvae and naive birds as predators of adults *Oreina* (Rowell-Rahier and Pasteels 1990, Rowell-Rahier *et al.* 1995). Hence PAs seem more efficient than cardenolides to deter generalist predators of the different life stages of *Oreina*. In field sites where both *Oreina* species occur, like in Val Ferret, *O. cacaliae* could thus be advantaged over *O. gloriosa*. Also *O. cacaliae* might need better chemical protection than *O. gloriosa* because their activity rhythm and the anatomy of their host-plant renders them more vulnerable to generalist predators. Indeed they spend days and nights on the leaves of their food plant whereas *O. gloriosa* only move and feed on the leaves during a few hours at night, staying most of their time sheltered in the leaf sheath of *P. ostruthium* (Luca Nessi unpublished data).

ACKNOWLEDGEMENTS

We thank Yves Borcard, Pierre-Yves Claudin, Julien Delnatte, Jérôme Jourde, Franck Laruelle for helping in the experiment, Jacqueline Moret for advice in statistical analysis and Karl Gotthard and Håkan Häggström for useful comments on the manuscript.

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TABLE AND FIGURES

Table 1. The four collection sites for *M. morio*: *Oreina* species and toxins potentially experienced by harvestmen from these sites

	Val Ferret	**Saas Grund**	**Guebwiller**	**Neuchâtel**
Oreina species	*O. gloriosa*	*O. gloriosa*		none
	O. cacaliae		*O. cacaliae*	
Toxins potentially experienced by predator at this site	cardenolide and PAS	cardenolide	PAs	none

Fig. 1. First choice of harvestmen from each site for *O. gloriosa* vs. *O. cacaliae*

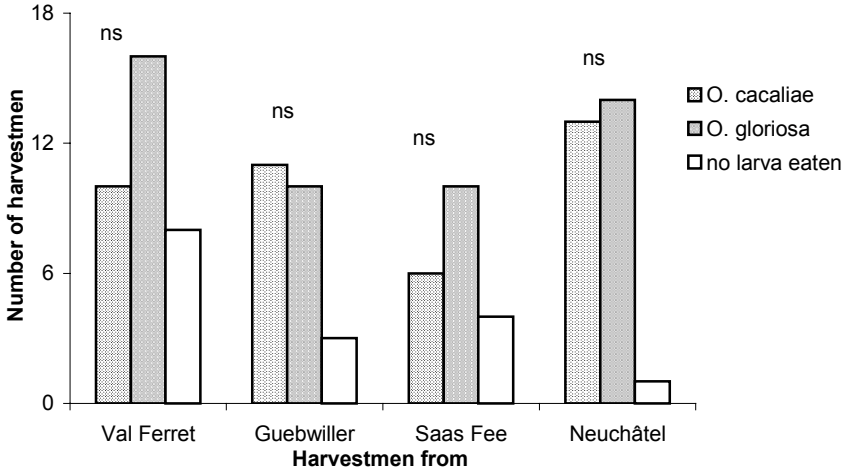


Fig. 2. Number of harvestmen from each site that have eaten the second larva after 24hours

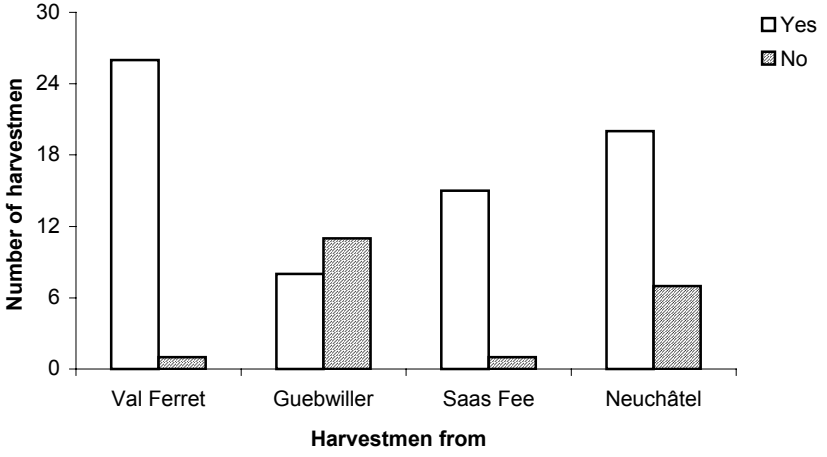
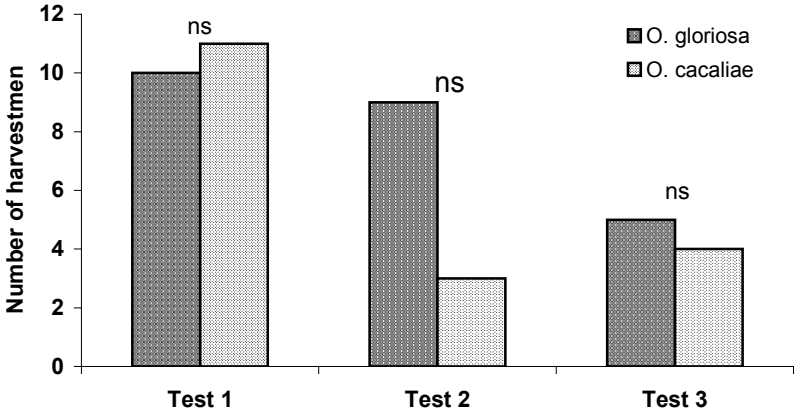


Fig. 3. Number of harvestmen that chose *O. gloriosa* vs. *O. cacaliae* as first choice at tests 1, 2 and 3.

a. Harvestmen from Guebwiller



b. Harvestmen from Val Ferret

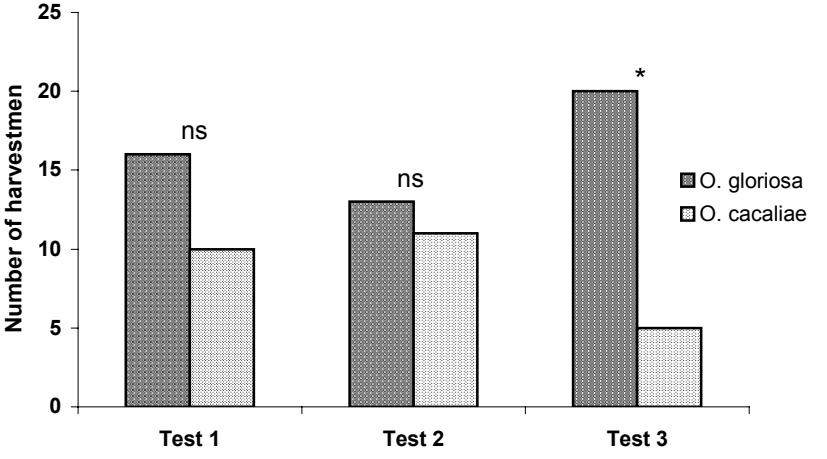


Fig. 4. Number of harvestmen from Val Ferret that changed their choice between tests

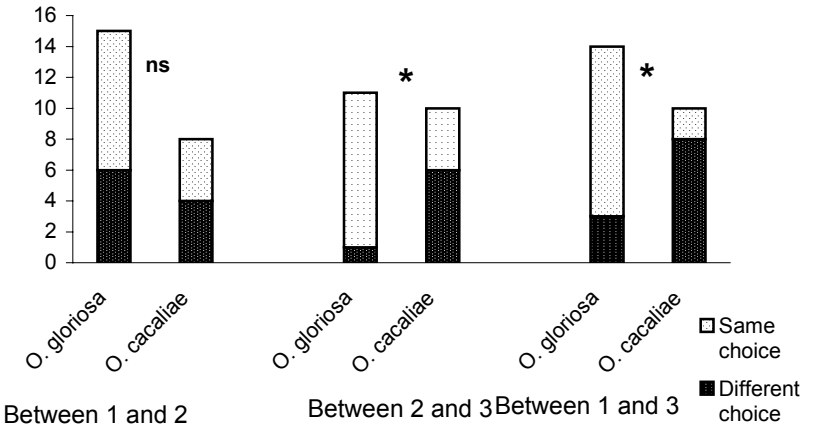
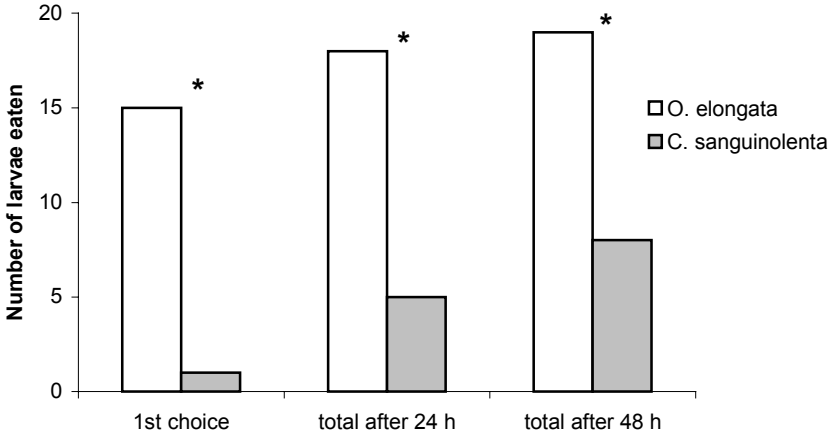


Fig. 5. Total number of *O. elongata* and *C. sanguinolenta* eaten by the harvestmen



APPENDIX: REGENERATION OF DEFENSIVE SECRETIONS AND COST OF TWO TYPES OF CHEMICAL DEFENSE

This section describes some experiments designed for investigating the cost of PA-sequestration *vs.* cardenolide synthesis and the intra-individual variation in toxicity.
However most of the results obtained fail to demonstrate any cost of chemical defense, and not many conclusions could be drawn from them. Only two parts (regeneration of secretions and relationship between survival and secreted volume) will later be integrated in the previous chapters for publication.

INTRODUCTION

Chemical defenses have been extensively studied in plants and animals during the last decades (Mc Key 1974, 1979, Berenbaum and Feeny 1981, Pasteels *et al.* 1984, Eisner and Eisner 1991, Bowers 1992, Sime *et al.* 2000), and much progress in this area was made possible by the development of highly sensitive quantitative techniques such as chromatography. Although seasonal, sexual and inter-individual variation in toxicity are well documented

(Eggenberger and Rowell-Rahier 1992, 1993, Pasteels *et al.* 1992, Dobler and Rowell-Rahier 1994), very few studies follow the toxicity level of the same individual at different times. A better knowledge of this intra-individual variation could however be very important. For instance it would allow to attribute to each individual a value of defensive capacity that might be more representative than that of a single measure. Although the cost of chemical defense in insects is a central question in evolutionary research, it has rarely been studied properly.

Alpine leaf-beetles in the genus *Oreina* Chevrolat (Coleoptera, Chrysomelidae) fit particularly to this kind of study because the adults secrete a defensive liquid that covers the pronotum and the sides of the elytrae when scared. Hence toxicity can easily be determined several times for a same individual without arming the beetle. Chemical defense differs between species in this genus; some species like *O. gloriosa* produce autogenous cardenolides (*de novo* synthesis, Van Oycke *et al.* 1987), whereas other species like *O. cacaliae* acquire pyrrolizidine alkaloids (PAs) from feeding on their main food-plant *Adenostyles alliariae* (sequestration, Euw *et al.* 1967, Brower *et al.* 1984, Nishida and Fukami 1990, Rowell-Rahier *et al.* 1991). In the phylogeny of the genus *Oreina* (Hsiao and Pasteels 1999), *O. gloriosa* is among the group of species which synthesize cardenolides and feed on Apiaceae. One other group of species have switched to Asteraceae but still synthesize cardenolides on their own. Three other species have the apomorphic capacity to sequester host-plant PAs, but have

conserved the ability to produce cardenolides. Finally *O. cacaliae* is the only species of this genus that have lost this ability and is only defended by host-plant PAs. This last event might indicate that:

1. cardenolides synthesis is costly
2. PAs are more efficient or at least as efficient as cardenolides for deterring predators.

Previous studies reported that in the autogenous species (*O. gloriosa*) toxicity is heritable and at least partially genetically determined (Eggenberger and Rowell-Rahier 1992), whereas in the sequestering species (*O. cacaliae*) toxicity is thought to be mostly determined by environmental factors linked to the host-plant content in PAs, as it is the case in other sequestering species (Isman 1977, Isman *et al.* 1977, Brower *et al.* 1984, Bowers 1992). However in this case there may also exist some genetic variation in the sequestering efficiency, or in the ability to choose the food-plant the more favorable to sequestration. In this context we aimed to answer the questions:

- 1. in both species, can we say that one beetle is more toxic than one other, or is toxicity highly variable in time for each given individual?
- 2. in the sequestering species, what is the part of individual *vs.* plant-derived variation?
- 3. is gland content regenerated faster by sequestration *vs. de novo* synthesis ? That is could toxin sequestration enable to discard more frequent disturbances from predators than *de novo* synthesis?

- 4. can we find any cost of defensive secretions? Two other methods were used to further compare the cost of chemical defense in these two species: one estimation of the trade-off between growth and chemical defense as proposed by Bowers (1992), and one investigation of increased food-intake in response to disturbance. The hypothesis is that sequestration is cheaper than *de novo* synthesis, as proposed by several authors (Pasteels *et al.* 1990, Bowers 1992), but to date there is no evidence for it.

MATERIAL AND METHODS

Collection of beetles. *O. cacaliae* were collected near La Fouly in the Val Ferret (Valais, Swiss Alps, 45.56 N, 7.05 E, alt. 1500m), on May 21, 2000 on *Adenostyles alliariae* (Asteraceae). Individuals of this species were sexed using sexual dimorphism of the tarsi (Lohse& Luche 1994). *O. gloriosa* were sampled in Saas Grund (Valais, Swiss Alps, 46.08 N, 7.57 E, alt. 1800m), on June 3rd, 1999 on their single food plant *Peucedanum ostruthium* (Apiaceae). As sexual dimorphism of tarsi does not exist in this species, beetles have been sexed using weight polymorphism: females are heavier than males, and weight distribution of both sexes was known from previous studies (Eggenberger, unpublished data). We thus had the

possibility to assess individuals sexes with a 95% interval of confidence. A *posteriori* checking was possible when females started to larviposite; only two females were initially sexed as males. The beetles have been carefully transported to the laboratory in cooled vials, and provided with fresh leaves of food-plant. The beetles that were still alive after the end of the experiment were released at their respective field site.

Sampling of defensive secretions. Secretion were artificially induced by hitting the pronotum with fine forceps. Pronotal and elytral secretions were taken up individually in calibrated capillary glass tubes (0.25 mm external diameter) and quantified using a graduated lens. Each secretion was stored individually in 150 µl of 100% methanol in the freezer for further analysis of concentration.

Sample preparation and chromatographic analysis of cardenolides. Samples were prepared and the concentration of total cardenolides in the secretions of *O. gloriosa* was determined by reverse-phase HPLC (Fig. 1a) as described by Eggenberger and Rowell-Rahier (1993). We only diverged from their method by using a Varian, Star chromatography workstation system with automated injection. The concentration values obtained are expressed as µg equivalent ouabain/µl.

Sample preparation and chromatographic analysis of PAs. The samples were prepared and the concentration of total PAs in the

secretions of *O. cacaliae* were determined by capillary GC (Fig. 1b) as described by Rowell-Rahier *et al.* (1991). We only diverged from their method by using a HP1-MS 30m x 0.025mm x 0.25µm column, and senecionin as external standard. The concentration values obtained are expressed as µg equivalent senecionin /µl**.**

1. Experiment to compare regeneration speed and intra-individual variation in toxicity. For this experiment, we collected 30 males and 29 females *O. cacaliae* and 31 males and 35 females *O. gloriosa*. Back to the lab, each beetle was weighted to the nearest 10^{-4}g, "milked" for its defensive secretions (T0), and finally installed in individually-labeled plastic box with a plaster bottom to insure high humidity level. Fresh food-plant was provided in excess at least once a week. The beetles were kept in cooled incubators with regular photoperiod (15:9) and temperature (18 :15) following natural conditions; boxes position inside the incubators were randomized at least once a week. The defensive secretions of each beetle were again sampled repeatedly every week for 6 weeks (T1 to T6), and for *O. cacaliae* only, beetles were weighted at this occasion and the number of larvae laid by each female was recorded. In *O. cacaliae*, three groups of 10 beetles were defined in each sex. The individuals within a group were provided with pieces of the same leaf of *A. alliariae*. This was done for investigating the importance of plant variation in PAs content.

2. Calculation of comparative cost of chemical defense. The data used for this calculation originate from 25 females and 27 males *O. gloriosa* and 27 females and 36 males *O. cacaliae* field-sampled as described in Labeyrie and Rahier (Chapter 2). The cost of chemical defense has been investigated by plotting classes of body weight against both total amount of toxins in the secretions and concentration per body weight (C_W = amount/body weight), as proposed by Bowers (1992). According to this author, if chemical defense is costly, there should be a trade-off between levels of chemical defense and growth. Hence a cost of sequestration or *de novo* synthesis of defensive compounds would be revealed by a negative correlation of chemical concentration (C_W) with body weight.

3. Experiment for assessing food intake compensation for disturbance cost in O. gloriosa. We designed this experiment for assessing the cost of producing defensive secretion, in terms of increased food intake and decreased larval production. Thirty females *O. gloriosa* have been field-collected, then installed in individual boxes. Every three days, they were all given pieces of leaves of 13 cm2 for food, and half of them were milked for their defensive secretions (disturbed lot). When giving fresh plant, the old piece of leaf was carefully removed, labeled and dried. The surface eaten by each beetle was calculated after image analysis (NIH image 1.62b7 software).

Statistical analysis. Relationships between traits and regeneration speed were analyzed by linear models (SPSS 10.0 software). In the investigation of the individual part of toxicity, ranks have been attributed to each sampling of each individual, for secreted volume and concentration. This was done in order to see if one given individual remains better defended than another, and this all across the season. Each individual thus has 7 values of ranks for volumes, but less than 7 ranks for concentration because we have not obtained measures of concentration for each sampling time of each individual. In *O. cacaliae*, the effect of plant variation on volume and concentration of PAs in defensive secretions was not statistically tested because graph representation showed that there was no difference between groups. The relationships between body weight and amount of toxins in the secretions, and between body weight and C_W were tested by t-test and Pearson correlation coefficient for *O. gloriosa*, and Mann-Whitney test and Spearman correlation coefficient for *O. cacaliae* because in this species the variables were not normally distributed. Comparisons of food-intake and larvae production of disturbed *vs.* undisturbed *O. gloriosa* were done by two sample t-test. Some of the data were log-transformed to fit model assumptions (secreted volume in *O. gloriosa*, secreted volume and concentration in *O. cacaliae*).

RESULTS

Relationship between traits of lab-followed beetles. In both sexes of *O. cacaliae*, no relationship was found between weight at T0, mean rank of concentration and survival. Also there was no relationship between mean rank of concentration and mean rank of secreted volume. In females, mean rank of secreted volume was positively related to weight at T0 (Fig. 1, $p = 0.001$), and to survival (Fig.2, $p<0.001$). In males, no such trend was found. In *O. gloriosa*, there was no relationship between weight at T0 and survival, nor between survival and mean rank of volume, nor between weight at T0 and mean rank of volume in females. In males mean rank of volume increased with weight at T0 (Fig. 3, $P<0.05$). Because of technical problems, we have not been able to obtain the concentrations of the defensive secretions of *O. gloriosa* for T0, T1, T2 and T3. We replaced mean T0 values by mean concentrations obtained from beetles collected in the same condition for another experiment (Chapter 2).

Regeneration speed of defensive secretions. In both species, volume (Fig. 4 a and b) and concentrations (Fig. 5 a and b) of defensive secretions decreased in time. Both species were able to produce 67-68% of the volume produced previous week, and in *O. cacaliae* 64% of the concentration produced previous week . The regression slopes for secreted volumes did not differ significantly between the two species, nor between sexes of a same species.

Concentrations of cardenolides in *O. gloriosa*'s secretions decreased faster than concentrations of PAs in *O. cacaliae's* secretions (Fig. 6). Concentrations of cardenolides dropped from 22 µg/µl at T0 to less than 0.95 µg/µl (detection threshold) at T4, whereas PAs concentration first increased from 0.49 µg/µl at T0 to 1.82 at T1, and then decreased to 0.19 µg/µl at T4. The regression slopes for concentration did not differ significantly between sexes in *O. cacaliae* nor *O. gloriosa*.

Intra-individual variation in toxicity. The individual ranks of secreted volume were not conserved throughout the six weeks of the experiment, in none of the two species. The individual rank of concentration in *O. cacaliae* was not conserved in time neither. This analysis was not possible in *O. gloriosa* because of lack of concentration data from T0 to T3.

Effect of food-plant vs. individual variation in O. cacaliae. No effect of diet group could be found concerning the volumes (Fig. 7 a and b) nor concentrations of defensive secretions of males nor females within each week (Fig. 8 a and b).

Comparative cost of chemical defense. In both species, there were no significant relationships between weight and total amount of defensive compounds in the secretions, but C_W decreased when body weight increases (Fig. 9a and b, Pearson correlation coefficient = -0.31, P = 0.03 in *O. gloriosa*, Spearman correlation coefficient = -

0.33, P = 0.04). A slight trends to decreasing amounts for increasing weight was observed in *O. cacaliae* (Spearman correlation coefficient = -0.32, P = 0.054). The C_W was a factor 100 larger in *O. gloriosa* than in *O. cacaliae* (means respectively 3.10^{-3} and 10^{-5}); at equal body weight, *O. gloriosa* beetles produce 100 times more weight of defensive compounds than *O. cacaliae*. Mean amounts of defensive compounds in individual secretion were 2.559 µg in *O. gloriosa* and 0.009 µg *O. cacaliae*. Mean concentrations of defensive compounds in individual secretion were 22.94 µg/µl in *O. gloriosa* and 0.11 µg/µl in *O. cacaliae*.

Assessment of food intake compensation for disturbance cost in O. gloriosa. Disturbed individuals ate significantly less surface of leaf than undisturbed individuals (p=0.009). There was no difference in the number of larvae laid by disturbed *vs.* undisturbed females.

DISCUSSION

Relationship between traits of lab-followed beetles. In *O. cacaliae*, the body weight of the beetles did not condition their defensive capacity (PAs concentration in the secretions) nor survival. As PAs are acquired by feeding, it is surprising that beetles that eat a lot (and though should be heavy) do not have more PAs in their secretions. There might be some physiological threshold particular to each individual and not related to its weight and food-intake, which prevents direct relationship between weight and concentration. Females that had a high body weight at T0 produced on average more volume of defensive secretion and survived longer. It seems that secreted volume is linked to the physical condition of the beetles, though the exact process for it remains unsure. It is unclear why no such trend was found in males.

Regeneration speed of defensive secretions. In both species, the volumes of defensive secretions decreased in time, at the approximate same speed. Only one week was sufficient for regenerating about 67-68% of secreted volume. Similar regeneration speed was reported in the *de novo*-alkaloid-producing 7-spot ladybird (Holloway *et al.* 1991). Regeneration of concentrations was faster in *O. cacaliae* than in *O. gloriosa*. It is not surprising since *O. cacaliae* are known to store PAs with a two-compartment system (Rowell-

Rahier *et al.* 1991): PAs ingested are first diffused in the whole body of the insect, and then transferred and stored into the glands until use. Hence after a disturbance, glands of *O. cacaliae* are fast replenished by the PAs of the body compartment, whereas *O. gloriosa* needs to re-synthesize its defensive cardenolides. This process might explain why concentrations of PAs are the highest at T1 (Fig. 4); after a first disturbance, the glands of *O. cacaliae* refill with PAs contained in large quantity in the body of the beetle. After the second disturbance, the gland content is also regenerated from body content in PAs, but since the beetle had only a week of feeding, not as much PAs could be obtained. Rowell-Rahier *et al.* (1991) proposed that PAs-sequestration enables to discard more frequent disturbances, and our data may confirm this hypothesis.

Intra-individual variation in toxicity. The individual ranks of secreted volume were not conserved through the six weeks of the experiment, in none of the two species. The individual ranks of concentration in *O. cacaliae* were not conserved neither. This result means that there is no definitely "good " or "bad" individuals concerning defensive capacity. In *O. gloriosa*, toxicity (cardenolides concentration) has been shown to be heritable (Eggenberger and Rowell-Rahier, 1992) and genetically determined. By lack of concentration data we have not been able to see if this could be verified in our experiment.

Effect of food-plant vs. individual variation in O. cacaliae. No effect of diet group could be found concerning the volumes nor concentrations of defensive secretions of males nor females. This indicates that the quality of the food-plant ingested does not strongly influence the defensive capacity of *O. cacaliae* beetles, contrarily to what occurs in other insects (Isman 1977, Brower *et al.* 1984). Also the leaves we used might not differ very much in their PA content. Although we used leaves from different individual plants, we did not determine the concentration of PA in these leaves. The inter-individual variation (error bars on Fig. 7 and 8) in secreted volumes and PAs concentration is larger than the inter-plant effect (means on Fig. 7 and 8); both secreted volume and concentration may be determined by the current physiological condition of the individual, rather than the host plant quality.

Comparative cost of chemical defense. The production of total amount of defensive compounds for the secretions is the factor which is supposed to be potentially costly in the secreting process. In *O. gloriosa*, this factor was not affected by body weight. Thus the individuals that invest in growth (size, fat reserve or other fitness-related trait) are not obliged to reduce their defensive expenses. In *O. cacaliae* there was a light relationship between these traits (though only significant at the 5.4 level of confidence). This may indicate some trade-off between growth and defense, imposed by a cost of PAs-sequestration. However further interpretation is not possible because of the very light significance of this trend. We

conclude that either kinds of chemical defense do not seem very costly by this measure. From the predator point of view, C_W is the factor that is likely to play a role in deterrence. As C_W decreased when body weight increased, it is possible that the largest individuals would be less efficiently defended. However for further hypothesis in this direction, we need data on the relationship between C_W and activity (i.e. deterrent effect for predators). At equal body weight, *O. gloriosa* beetles produce 100 times more weight of defensive compounds than *O. cacaliae*. This might mean that higher quantities of cardenolides *vs.* PAs are needed for deterring predators. Mean amounts and mean concentrations of defensive compounds in individual secretion were respectively 284 times and 208 times larger in *O. gloriosa* than in *O. cacaliae*. A higher concentration of cardenolides *vs.* PAs may be needed for deterrence. Indeed, comparative studies on predator repellence by *O. cacaliae vs. O. gloriosa* indicated that the first are better defended than the second (Rowell-Rahier and Pasteels 1990, Rowell-Rahier *et al.* 1995, Labeyrie and Rahier Chapter 3). It is likely that, at equal concentration, PAs are more repellent than cardenolides. Several authors suggested that sequestration is less costly than *de novo* synthesis for two principal reasons:

1. In sequestration, toxic compounds are directly acquired from food-plant; this saves precursor molecules for other potential needs (Pasteels *et al.* 1990, Bowers 1992)
2. In this group where sequestration evolved from de novo synthesis, only slight modification in the specificity of

two pre-existing enzymes would be needed for acquiring PAs from food (Pasteels *et al.* 1990). Thus there would be no additional cost of enzyme production.

In this system, in addition to these arguments, defense based on PAs could be less costly than cardenolides-based defense because a smaller amount of compound is efficient for repelling predators. However we have no evidence for this as we failed in finding any costs in any of the two strategies.

Assessment of food intake compensation for disturbance cost in O. gloriosa. We expected that disturbed individuals would eat more than undisturbed beetles because of their increased energetic expenses. The opposite pattern was observed in our experiment. This suggests that disturbed insects stop feeding for a while because of disturbance stress. It also means that these insects feed basically all the time and disturbance also induces a cost by reducing the feeding time. However we have not been able to demonstrate any cost of secretion. Further investigation should be done on survival and larvae production at the following breeding season.

ACKNOWLEDGEMENTS

We are grateful to Pr. Bruno Betschart, Thomas Degen, Laure-Emmanuelle Perret and Ted Turlings for their support in chemical analysis, Alex Aebi, Yves Borcard and Loïc Didillon for

helping in laboratory and field work and Jacqueline Moret for advice in statistical analysis.

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FIGURES

Fig. 1. Linear regression between mean rank of secreted volume and weight at T0 in females of *O. cacaliae*

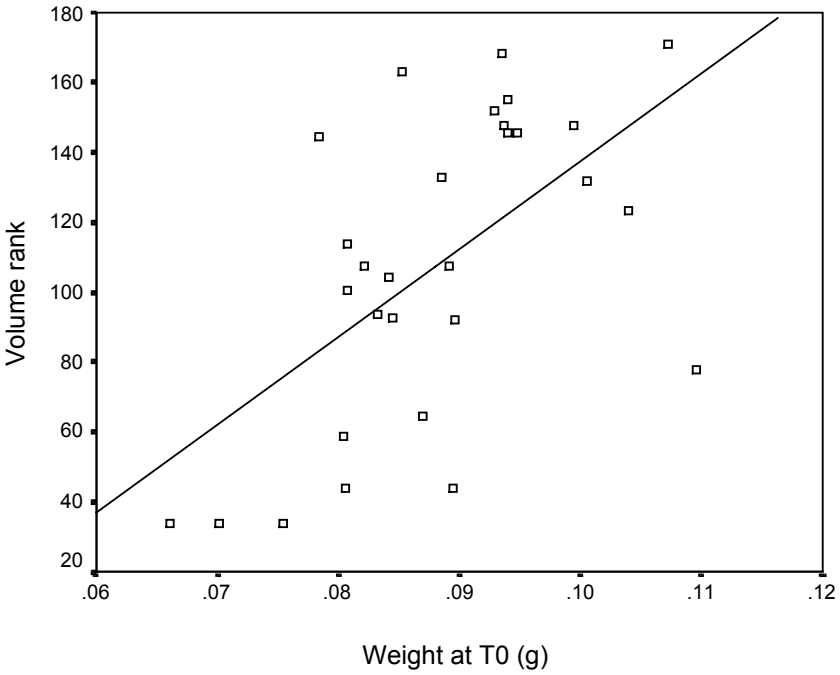


Fig. 2. Linear regression between mean rank of secreted volume and survival in females of *O. cacaliae*

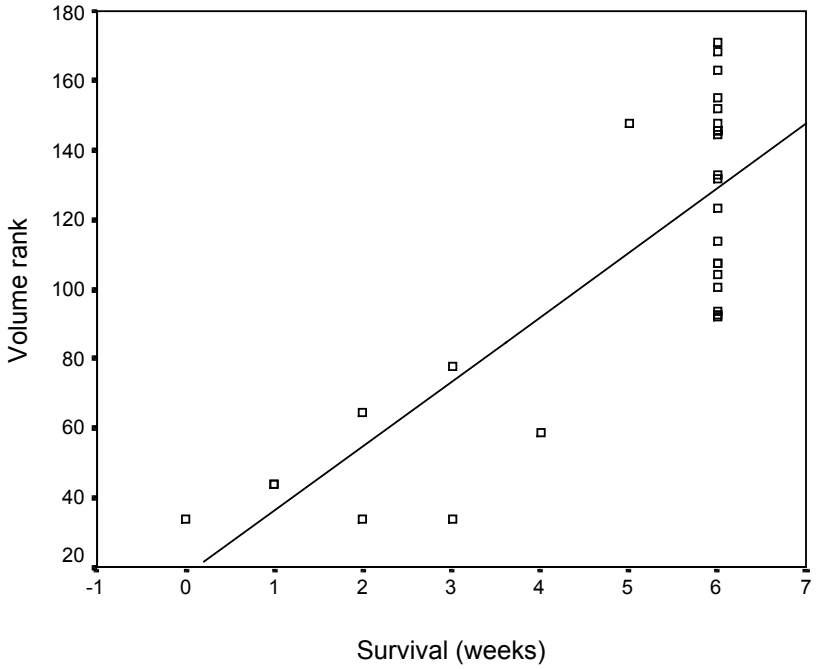


Fig. 3. Linear regression between mean rank of volume and weight at T0 in males of *O. gloriosa*

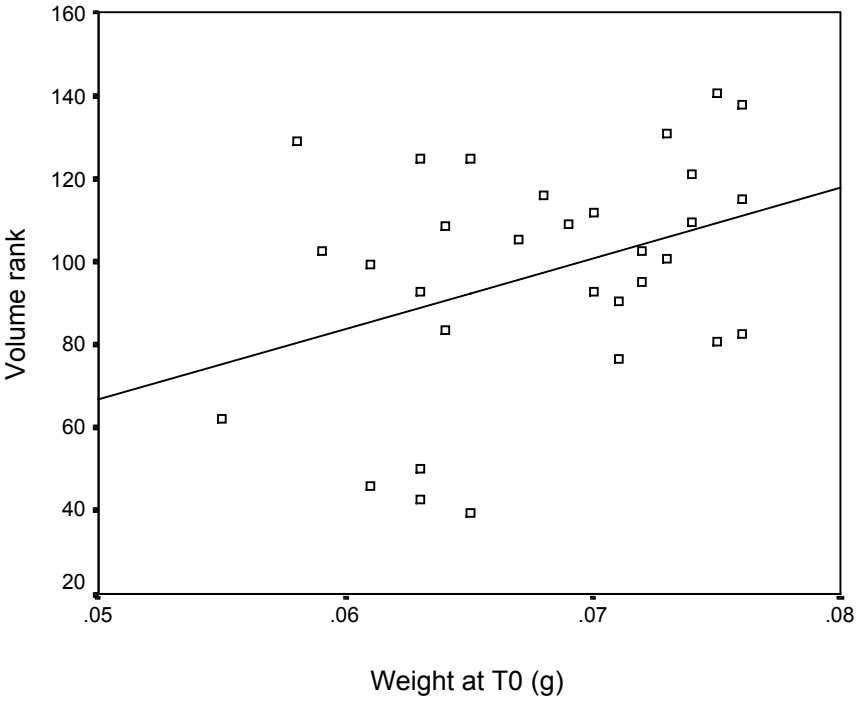
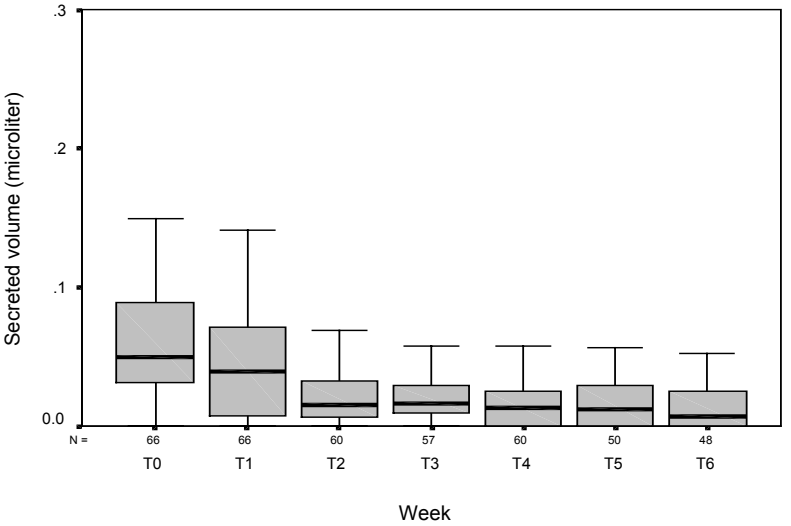


Fig. 4. Volumes of defensive secretions every week

4a. *O. gloriosa*



4b. *O. cacaliae*

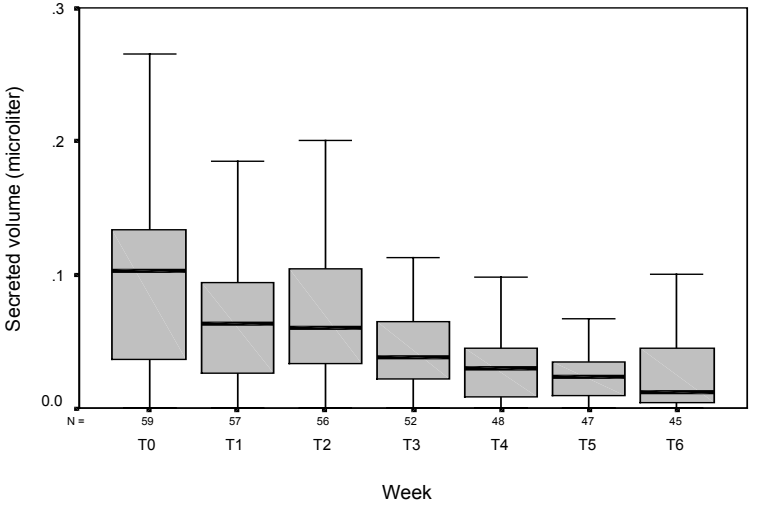
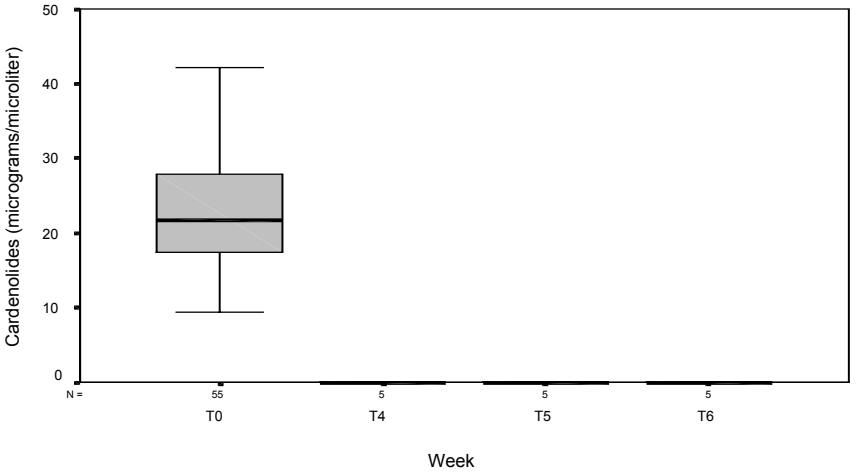


Fig. 5. Concentrations of cardenolides and PAs in defensive secretions every week

5a. *O. gloriosa*



5b. O. cacaliae

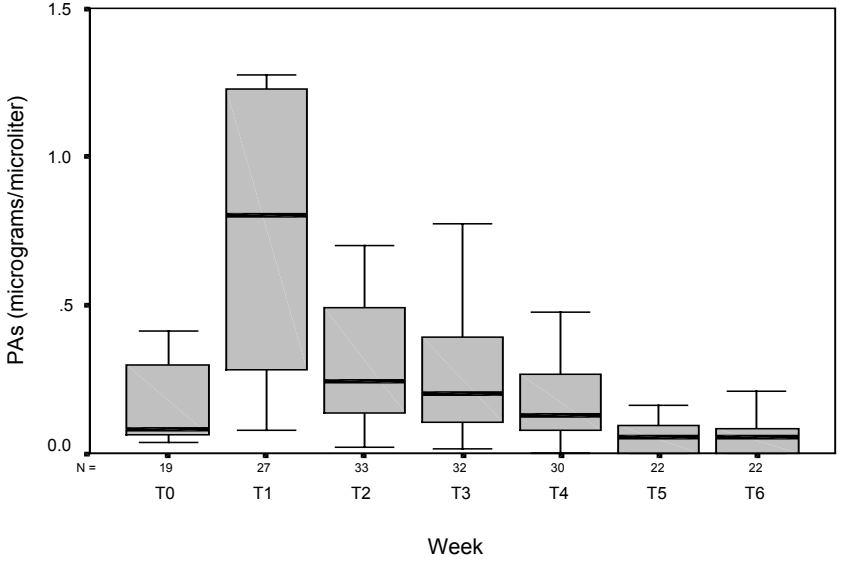


Fig. 6. Concentrations of cardenolides in *O. gloriosa*'s secretions and PAs in *O. cacaliae's* secretions at each sampling time

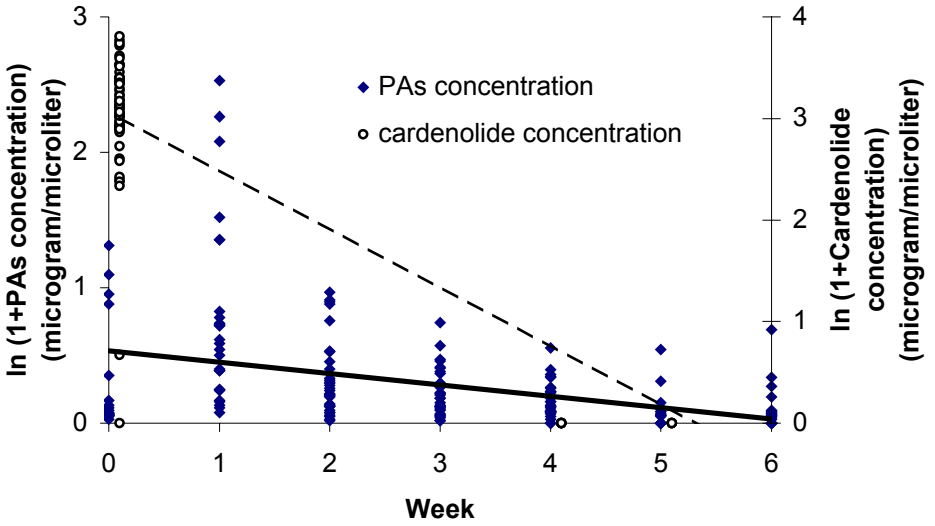
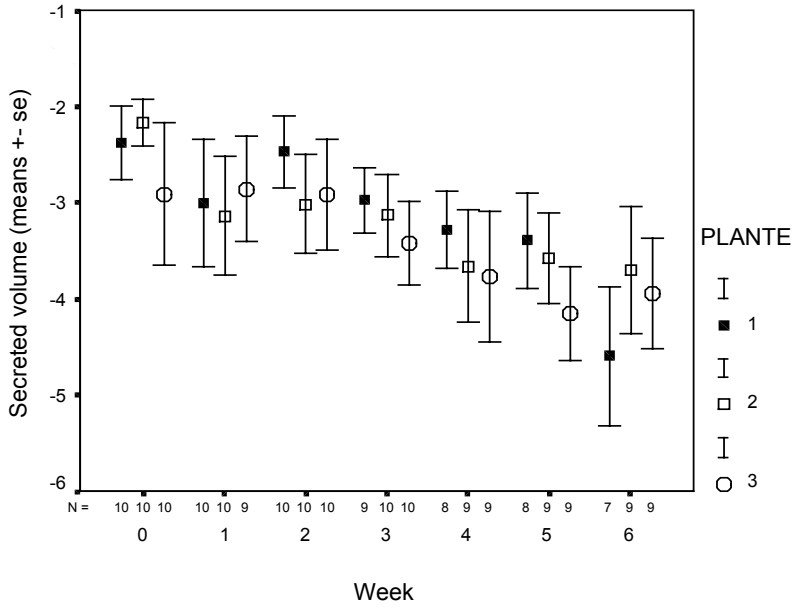


Fig. 7. Secreted volumes and diet groups

7 a. Females



7b. Males

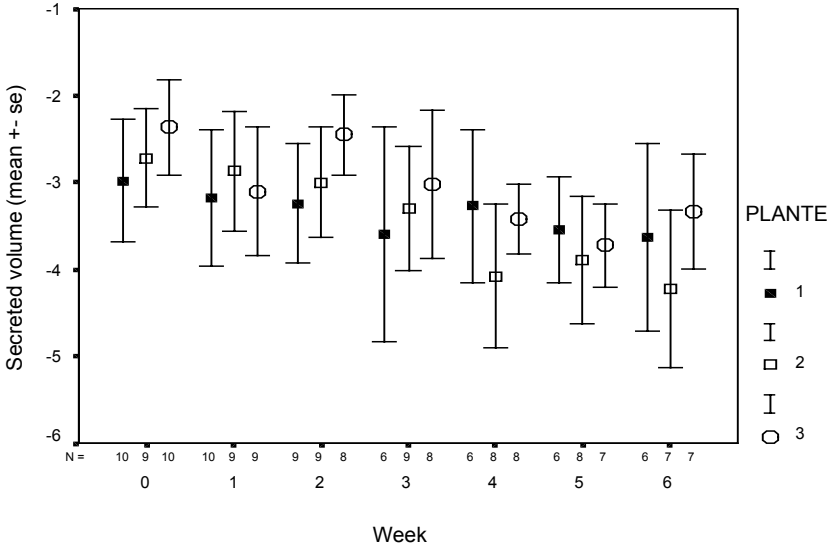
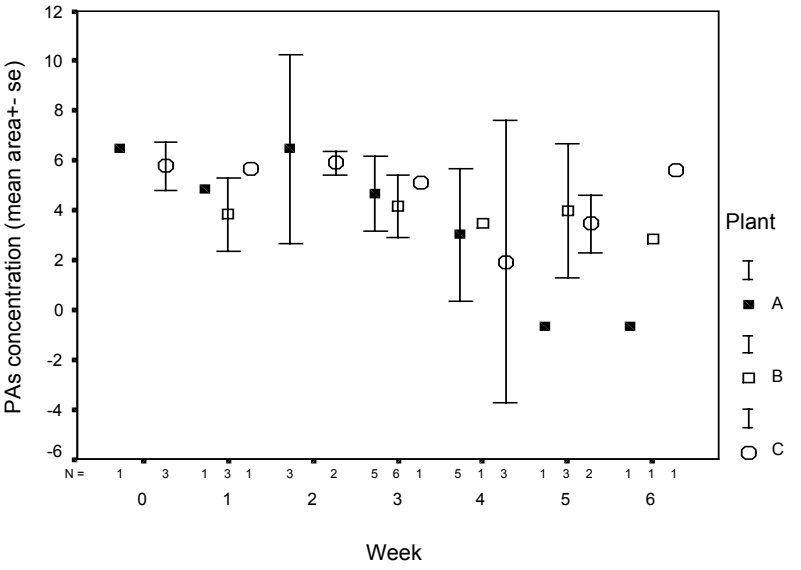


Fig. 8. PAs concentrations and diet groups

8 a. Females



8b. Males

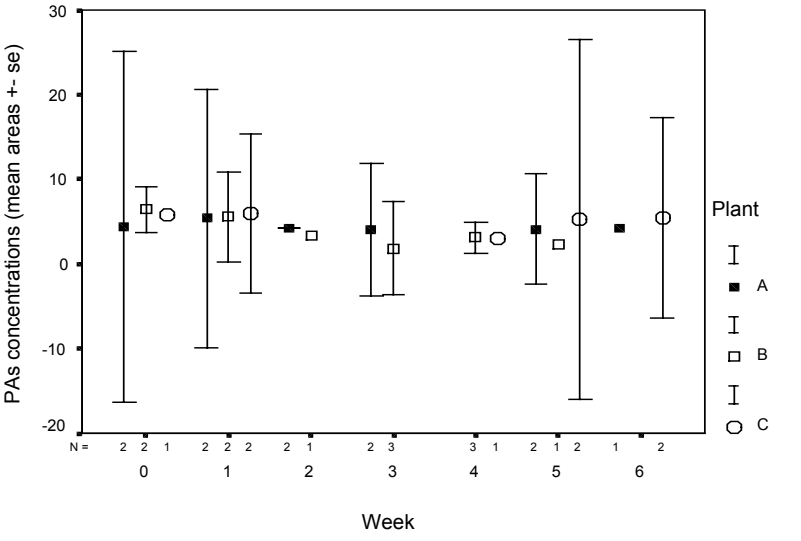
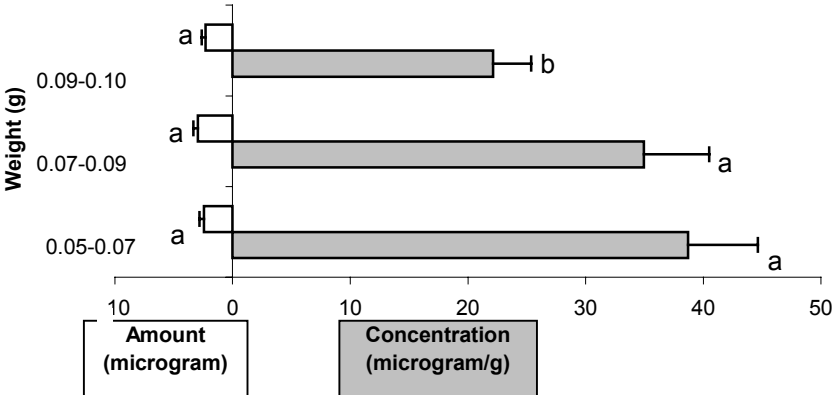
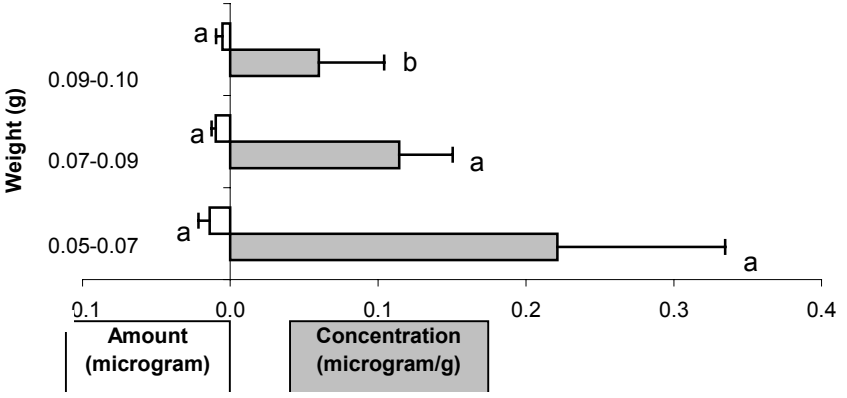


Fig. 9. Relationships between body weight and total amount of defensive compounds in the secretions, and body weight and C_W (body weight and C_W are represented as mean + standard error)

9a. *O. gloriosa*



9b. *O. cacaliae*



CURRICULUM VITAE

1) Etat civil

Estelle LABEYRIE

Née le 8 février 1973, à Paris
Nationalité: Française
Célibataire, un enfant né le 22.10.1999 à Boudevillier (Suisse)

2) Etudes supérieures (spécialisation en Ecologie et Evolution)

1990: **Baccalauréat C** Lycée de Grasse (06)

1990/91: **DEUG A 1ère année** Université Paris XI (Orsay)

Option: Mathématiques

1991/92: **DEUG B 1ère année** Université Paris XI (Orsay)

1992/93: **DEUG B 2nd année** Université de Brest

1993/94: **Licence BOP** Université de Brest

Option: Paléontologie

1994/96: **Maîtrise BOP** Université Montpellier II

Option: Génétique et Evolution
Principaux cours: génétique des populations, statistiques et analyse de données, écologie fonctionnelle et évolutive, paléontologie, anglais

1996/97: **D.E.A. Evolution et Ecologie** Université Montpellier II

Option: Génétique évolutive
Principaux cours: écologie fonctionnelle et évolutive, génétique évolutive, statistiques et

analyse des données, biogéographie, phylogénie moléculaire, écologie tropicale

1997/2001: **THESE** Université de Neuchâtel, Suisse

3) Expérience d'enseignement (4 années, 216 heures de cours, 4 étudiants stagiaires)

1. **Responsabilité des TP d'Ecologie Animale et d'Entomologie pour les étudiants de 3ème année:**
   Volume horaire: 38 h/an
   Contenu: - Indice Biologique Global Normalisé d'un cours d'eau, sorties Gastéropodes terrestres, faune cavernicole, successions des communautés d'insectes dans les bouses de vache et les arbres morts
   - 6 séances de projets (dispositif expérimental, expériences de terrain, analyse des données, rédaction d'un rapport et présentation orale) sur les chrysomèles (Coléoptère) du genre *Gastrophysa*

2. **Assistance aux TD de "Méthodes quantitatives en Ecologie" (bases statistiques pour étudiants de 3ème année):**
   Volume horaire: 16 h/an
   Contenu: - Initiation à l'utilisation des logiciels Excel, Statview et SPSS, notions de dispositif expérimental, graphiques, t-test, ANOVA, régression linéaire
   Responsable du cours: Dr B. Benrey, Maître assistante

3. **Encadrement du stage de terrain "Ecologie terrestre" à Banyuls/mer (juin 1998):**
   Volume horaire: 1 semaine
   Contenu: - Initiation à l'entomofaune méditerranéenne, mini-projet sur les recolonisations par l'entomofaune de zones incendiées
   Responsable du cours: D. Conconi, doctorant

4. **Responsabilité d'un mini-projet pour deux étudiants de 3ème année (mai 2000):**
   Volume horaire: 3 jours
   Contenu: Planification, expérience, analyse des données, et rédaction d'un rapport sur les performances d'*Oreina cacaliae* (Coléoptère) sur l'une ou l'autre de leurs plantes–hôtes.

5. **Responsabilité de 2 stagiaires d'été:**
   Juillet et août 1999: un stage de maîtrise (Loïc Didillon, Grenoble)
   Juillet 2000: un stage niveau maîtrise (Julien Delnatte, Montpellier)

4) Expérience de recherche avant la thèse

(9 stages dans 8 groupes de recherche, plus de 25 mois de recherche au total dont 17 mois outre-atlantique)

1. Biologie et comportement de la fourmi champignonniste *Acromyrmex octospinosus*

Date: 1990

Durée: 2 mois

Description: Initiation à la recherche. Comportement des fourmis face à un appât empoisonné.

Techniques utilisées: - Elevage et marquage de fourmis
- Observations de terrain

Responsable et structure d'accueil:
Luc GOMEL, I.N.R.A., Guadeloupe

2. Assistance pour l'étude comportementale d'un groupe de grands dauphins (*Tursiops truncatus*)

Date: 1994

Durée: 10 jours

Description: Suivi et observation d'un groupe de grands dauphins sédentaires de l'île de Sein (Bretagne)

Techniques utilisées: Collecte de données comportementales sur les mammifères marins

Responsable et structure d'accueil:
Céline LIRET, Océanopolis et Université de Brest

3. Analyse de la relation entre le nombre de sarcelles d'hiver (*Anas crecca)* en hibernation et la réduction des surfaces de gagnage en Camargue.

Date: 1994

Durée: 6 semaines

Description: Analyse de données: étude de la réduction de la biomasse de graines disponible dans différents biotopes de l'unité fonctionnelle du Saint Seren, liée à la diminution des surfaces de gagnage entre 1950 et 1990. Ceci a été mis en

relation avec les fluctuations des effectifs de sarcelles d'hiver observées dans cette zone.

Techniques utilisées: Logiciels: Statgraphics, Quattro pro, Microsoft Office, analyse de variance

Responsable et structure d'accueil:
Alain TAMISIER, C.E.F.E., CNRS, Montpellier

4. Effet des substances stimulantes de contact sur le comportement d'oviposition de *Papilio polyxenes* (Lepidoptera)

Date: 1995

Durée: 6 semaines

Description: Assistance aux manipulations de chimie, élevages et observations comportementales

Techniques utilisées: Observations comportementales et techniques d'élevage, initiation aux techniques d'HPLC, et logiciel associé, initiation aux techniques d'isolation des molécules actives

Responsable et structure d'accueil:
Paul FEENY, Department of Ecology and Systematics, Cornell University, Ithaca NY, USA

5. Etude des relations entre différentes espèces de mouches simulides (Diptera) et les champignons Trichomycetes associés.

Date: 1995/96

Durée: 10 mois

Description: Suivi des prévalences d'infection par sept espèces de champignons Trichomycetes dans les espèces de simulides hôtes locales. Culture et isolation de *Smittium simulii*, et démonstration de la possibilité d'induire expérimentalement l'infection de larves saines avec des trichospores issus de cultures. Détermination expérimentale de la durée nécessaire à la sporulation d'un jeune thalle. Ces données ont donné lieu à une publication.

Techniques utilisées: Techniques de culture axénique, microdissection, microscopie et microphotographie en contraste de phase, lames permanentes, systématique des Simuliides et des Trichomycetes

Responsable et structure d'accueil:
Daniel P. MOLLOY, Field laboratory of Cambridge, Biological Survey, New York State Museum, USA

6. Stage de maîtrise – Etude comparative du comportement alimentaire dans deux espèces de Curculionides (Coleoptera, genre *Eudiagogus*) sur leurs plantes hôtes spécifiques (genre *Sesbania*)

Date: 1996

Durée: 2 mois.

Description: Mise en relation des comportements alimentaires de deux espèces de charançons avec le type de défense chimique de leur plante.

Techniques utilisées: Observations comportementales d'insectes, utilisation de techniques vidéo pour observations comportementales, analyse de données: analyse de variance avec Statistix et SAS, quantification de carbone et azote dans les plantes

Responsable et structure d'accueil:
M. Hossaert-McKey et L. Ceballos, Laboratoire de coévolution, C.E.F.E., CNRS, Montpellier

7. Stage principal de DEA: L'acide formique dans la relation passiflore/fourmis/herbivores.

Date: 1997

Durée: 7 mois

Description: Les plantes de *Passiflora glandulosa* sont patrouillées par des fourmis opportunistes qui se nourrissent du nectar extrafloral, lequel contient de l'acide formique. Cette étude s'intéressait au rôle potentiel de ce composé dans les relations plante-fourmis-phytophage, en essayant de répondre aux questions suivantes:

1. Quels sont les rythmes de production de nectar et de l'acide formique qu'il contient pendant un cycle nycthéméral? Quels sont les rythmes d'activité des fourmis associées?
2. Quelles sont les différences dans les profils de sécrétion des nectaires floraux, extra floraux et sépalaires?
3. Y a-t-il modification de la teneur en acide formique induite par l'attaque des phytophages?
4. Quelles sont les réactions des fourmis vis à vis de l'odeur ou du goût de l'acide formique?
5. Quel est le degré de spécificité des relations fourmis-passiflore?
6. La défense biotique est-elle efficace dans les mutualismes de type opportunistes?

Techniques utilisées: observation d'insectes sur le terrain et en tests expérimentaux au laboratoire, travail de terrain en Guyane française: prélèvements de nectar et fourmis, dosage d'acide formique par spectrophotométrie.

Responsables et structure d'accueil:

M. Hossaert-McKey et L. Pascal, Laboratoire de coévolution, C.E.F.E., CNRS, Montpellier

8. Stage annexe 1 de DEA – Influence du rang dans la nichée chez la mouette tridactyle *Rissa tridactyla*.

Date: 1997

Durée: 2 semaines

Description: Analyse de données de capture-recapture. Le rang dans la nichée n'est pas retenu comme facteur explicatif des paramètres de survie et de recapture.

Techniques utilisées: bases de modélisation grâce au programme Surge

Responsables et structure d'accueil: Jean-Yves Monnat et Emmanuelle Cam, Université de Bretagne Occidentale (Brest)

9. Stage annexe 2 de DEA – Parasitisme intestinal et asymétrie fluctuante chez le crache-sang *Timarcha maritima* (Coleoptera).

Date: 1997

Durée: 2 semaines

Description: Démonstration d'une relation négative entre charge parasitaire et asymétrie fluctuante chez les mâles, qui pourrait être attribuée à la mise en place de couteux mécanismes de résistance aux parasites au détriment de la stabilité développementale.

Techniques utilisées: - mesure de l'asymétrie fluctuante grâce à un mesuroscope Nikon

Responsables et structure d'accueil: Frédéric Thomas et François Renaud, Université de Montpellier

5) Autres expériences, loisirs et intérêts

► LANGUES ETRANGERES ET LOGICIELS UTILISES

- Anglais *(lu, parlé et écrit couramment)*
- Russe *(notions de trois années scolaires)*
- Allemand *(initiation)*

• Word, Excel, Powerpoint pour Windows et Macintosh, Quattro Pro, Statgrafics, Statistix, Statview, SPSS et Adobe photoshop *(utilisation courante)*

• programmation en basique, modélisation (Surge), analyse d'image (NIH Image), SAS, S-PLUS *(notions)*

► AUTRES EXPERIENCES

• 1999 à 2001: Enseignement de la danse traditionnelle (40 heures par an pendant 3 ans)

• 1991: Marquage et protection des tortues luth à l'écloserie des Hattes (2 mois)
Yalimapo, Guyane française

• 1992: Guide nature dans une réserve naturelle (1 mois)
Société d'Etude et Protection de la Nature en Bretagne

• 1992: Animation d'un stand « Fête de la science » à Paris

• 1992 à 1994: Participation active au Cercle des Etudiants Naturalistes Brestois

Responsable de la projection hebdomadaire de films naturalistes, et participation à l'organisation et animation de sorties, weekends et exposition

► LOISIRS ET AUTRES INTERETS

• Observation de la nature (botanique, entomologie, invertébrés marins, mycologie,...)
• Danse, randonnée, plongée sous-marine, escalade
• Photographie (macro, micro et portraits)
• Ethnobotanique, ethnologie

6) Travaux scientifiques

Travaux publiés (7 dont 4 en préparation):

1. Labeyrie E., Molloy D. P. and Lichtwardt R. W. 1996. An Investigation of Harpellales (Trichomycetes) in New York State Blackflies (Diptera: Simuliidae). *Journal of Invertebrate Pathology* 68, 293-298

2. Labeyrie E., Pascal L., Delabie J., Orivel J., Dejean A., and Hossaert-McKey M. A protection of *Passiflora glandulosa* (Passifloraceae) against herbivory: impact of ants exploiting extrafloral nectaries - ***Accepté dans*** *Sociobiology* en février 2001

3. Hossaert-McKey M, Orivel J., Labeyrie E., Pascal L., Delabie J. and Dejean A. Differential associations with ants of three co-occurring extrafloral nectary-bearing plants - ***Accepté dans*** *Ecoscience* en mars 2001.

4. Labeyrie E., Blankenhorn W. and Rahier M. Mating pattern and chemical defense in two species of alpine leafbeetles (Coleoptera, genus *Oreina*). *In prep*.

5. Labeyrie E. and Rahier M. Mate choice of defense-related traits in aposematic leafbeetles (Coleoptera, genus *Oreina*). *In prep.*

6. Labeyrie E. and Rahier M. An energetical investigation of different ways of being bitter. *In prep.*

7. Labeyrie E. and Rahier M. How naive and experienced generalist predators respond to the choice between two chemically defended preys. *In prep*.

Colloques, séminaires et allocutions (5 présentations orales, 1 poster):

1. Labeyrie E. and Rahier M. Mating and toxicity in two species of alpine leafbeetles. Présentation orale au meeting de la Société Suisse de Zoologie, Neuchâtel, Février 2001

2. Labeyrie E. Mate choice in relation to toxicity in alpine leafbeetles. Séminaire donné comme "invited speaker" par Pr. Blanckenhorn, Zürich, Avril 2001

3. Labeyrie E. Selection sexuelle, prédation et toxicité chez les chrysomèles alpines. Séminaire donné comme "invited speaker" par Dr. Després, Grenoble, Juin 2001

4. Labeyrie E. Les relations plante-insectes. Allocution publique à l'occasion de l'inauguration des nouveaux bâtiments de l'université de Neuchâtel. Mai 2001

5. Labeyrie E. and Rahier M. Mating and toxicity in two species of alpine leafbeetles (genus *Oreina*). Poster présenté à International Society of Behavioural Ecology Congress, Zürich, Aout 2000

6. Labeyrie E. and Rahier M. Selection sexuelle et défense chimique chez 2 espèces de chrysomèles alpines. Présentation orale au Petit Pois Déridé, Orsay, Aout 2001