



"The butterfly and the wasp : host-parasitoid relationship between *Boloria eunomia* and *Cotesia eunomiae*"

Choutt, Julie

ABSTRACT

Global environmental change affects not only species, but also their interactions, e.g. host-parasitoid relationships, exceedingly common in nature. *Boloria eunomia* is a well-known vulnerable butterfly species, but its parasitoid had never been profoundly investigated. In this thesis, we focused on the relationship with one of its parasitoid, the *Cotesia eunomiae* wasp. Firstly, the relationship was investigated from the host side. The study of the impact of habitat quality on the parasitoid prevalence revealed that suboptimal habitats may be used by the butterfly to decrease the mortality due to parasitoids (enemy-free space). Then, based on a literature review, we studied how ecological and/or morphological factors of Lepidoptera caterpillars explain why some species are parasitized by a larger set of Braconidae parasitoids. Secondly, we undertook different studies to study the relationship from the parasitoid side. An olfactometric study did not succeed in detecting infochemical compounds that are attractive for *Cotesia* females during their host search. Improvements in the experimental design are suggested. Then, we discussed how parasitoids affect the growth of their caterpillar host, and how the condition of the host affects survival of the parasitoid larvae. Finally, genetic analyses were conducted to characterize the metapopulation structure of *C. eunomiae*. However, microsatellite loci failed to reveal inter-individual and inter-population polymorphism. Finally, we explored the possible role of the caterpillar parasitism as a regulatory factor of population dynamics...

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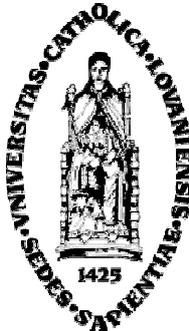
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**THE BUTTERFLY AND THE WASP:
HOST-PARASITOID RELATIONSHIP BETWEEN
BOLORIA EUNOMIA AND *COTESIA EUNOMIAE***



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GENERAL INTRODUCTION_____

THE CURRENT BIODIVERSITY CRISIS

Biodiversity crisis context

Worldwide, the Earth experiences a considerable wholesale degradation: all levels of life organization, genes, species, ecosystems, entire landscapes are injured (Mooney 2010). This is called the sixth biodiversity crisis. During the past, our planet has been shaken by drastic events that have profoundly affected it and demonstrates that life is vulnerable (the last event took place 65 million years ago and corresponds to the demise for the dinosaurs, Myers and Knoll 2001). But nowadays, the global environmental change is no more natural. Indeed, natural systems are affected by human activities, which deplete our collective natural capital (Ehrlich and Wilson 1991, Mooney 2010). Moreover, none of the previous crises has been so instantaneous and so important, with global biodiversity disappearing at an unprecedented rate (Sala et al. 2000). Among the almost 55000 plant and animal species assessed, nearly 17000 are known to be threatened with extinction, while more than 700 have disappeared over the past 500 years (<http://www.iucnredlist.org/>), and if present trends continue this could even be worse (Myers and Knoll 2001). These observations have lead scientists to draw, over the last century, the alarm regarding the loss of biodiversity. This has allowed the awareness of the Earth problem to develop at virtually all levels, from local to nation states and to international treaties. Therefore, at the international scale, several organizations (such as the Convention on Biological Diversity CBD, the Intergovernmental Panel on Climate Change IPCC, the International Union for Conservation of Nature IUCN) have created conventions giving precise goals concerning biodiversity conservation. For example, for 2010, the International Year of Biodiversity, the 190 Countries belonging to the Convention on Biological Diversity should have reduced the rate of biodiversity loss (Balmford et al. 2005,

Scholes et al. 2008) but at this date there is still a lack of substantive progress (<http://www.cbd.int/information/statements.shtml>).

Causes and consequences of the biodiversity crisis

The world and its ecosystems are threatened by several rapid changes: loss and fragmentation of natural habitats, increasing atmospheric CO₂ levels and associated climatic change, deposition of anthropogenically fixed nitrogen and biotic invasions (Tylianakis et al. 2008). All these causes have been grouped under a more general concept: global environmental change (GEC). The effects of GEC are perceived on populations and can alter the networks of interactions among species (Tylianakis et al. 2007). The final effect of these drastic changes results in the decline (or the extinction) of species and the modification of community composition.

Human activities have profoundly changed the landscape at an ever growing speed. Not only habitats are destroyed due to agricultural and urban development, road construction, deforestation, etc., but also the remaining habitat patches often become exceedingly fragmented. These two effects on natural habitats are the most important causes of population extinctions (Saunders et al. 1991a, Fahrig and Merriam 1994, Fahrig 1997, Sih et al. 2000, Ceballos and Ehrlich 2002). Destruction of natural habitats leads of course to local extinction of populations, while habitat fragmentation can have several effects on the local population dynamics of a species in the remaining patches which ultimately may affect the entire metapopulation of that species (Bull et al. 2007). Thus, a decrease in patch size usually leads to a reduction in the local population size of a species. Due to this reduction in size, populations are highly sensitive and more susceptible to extinction from stochastic perturbations (Shaffer 1981, Gilpin and Soulé 1986, Caughley 1994, Morris and Doak 2002): demographic stochasticity (random variation in demographic parameters due to the chance variation in individual birth

and death), genetic stochasticity (changes in allele frequencies due to founder effect, random drift or inbreeding), and environmental stochasticity (variation in the external environment, and both temporal and spatial, affecting demographic properties of an entire population) (Shaffer 1981, Morris and Doak 2002).

All species are linked in networks of biotic relationships (Ricklefs and Miller 2000). These interactions, occurring at various spatial and temporal scales, can differ in strength and in sign, either positive (facilitation), negative (inhibition) or neutral (Table i.1). These interactions involving all species (from the soil to the air) may be more susceptible to GEC, as they are sensitive to the phenology, behaviour, physiology and relative abundances of multiple species (Vidal and Tschardtke 2001, Tylianakis et al. 2008). Species interactions are among the most important forces structuring ecological communities (Gilman et al. 2010). Any change in interspecific interactions may, consequently, profoundly perturb community composition and functioning (Berg et al. 2010, Barbosa 1988, Thompson 1996). Such changes happen because, in a same community, species do not have the same potential in their ecological and evolutionary responses to deal with global changes. According to recent studies, climatic change has already caused phenological mismatches (Memmott et al. 2007, Both et al. 2009, Primack et al. 2009). Moreover, in small and isolated habitat fragments due to a pollinator deficit, it appears that plant population viability decreased through inbreeding depression and reduced seed production (Lennartsson 2002). The importance of conserving these interactions and associated processes, as well the component species, has been stressed repeatedly (van der Putten et al. 2004, Rayfield et al. 2009), particularly as humans rely directly or indirectly on ecosystem services associated with species interactions, such as pollination (through the yield of many crop and by the contribution of the healthy functioning of unmanaged terrestrial ecosystems Memmott et al. 2007) and biological control.

Preserving diversity is therefore dependent on preserving interactions (Tylianakis et al. 2008).

Table i.1. Categories of relationships between species (reprinted from chapter 20 in Ricklefs and Miller 2000).

Type of interaction	Effects of interaction on		Example of interaction
	Species 1	Species 2	
Competition	Negative	Negative	
Consumer-resource	Positive for consumer	Negative for resource	Predator-Prey Parasite (Parasitoid)-Host Herbivory Disease
Detritivore-detritus	Positive	Indifferent	
Mutualism	Positive	Positive	Pollinisation Symbiosis

The knowledge of multispecies interactions is fundamental to understand the regulation of biodiversity and the impact of environmental changes on communities (Berg et al. 2010) in order to better preserve them. The best way to describe community and population ecology might be to study all complex trophic interactions of multiple species. Besides, new frameworks and conceptual tools emerge to understand how species interactions are affected by global change and to predict the impact of such changes on species (Gilman et al. 2010). Nevertheless, trophic interactions are so complex and various that understanding simpler systems is often a useful prelude in order to understand more complex ones (Murdoch et al. 2003b). Among all interactions, the consumer-resource one plays an important role in regulating population dynamics, community structure, and diversity, and has been mentioned as being a fundamental unit of ecological communities (Rayfield et al. 2009). Indeed, virtually every species is part of such kind of relationship, as a consumer of living resources, as a resource for

another species, or as both. Such interactions are, in addition, fundamentally prone to being unstable. Therefore, understanding consumer-resource relations is required to study population dynamics needs (Murdoch et al. 2003a). Ecological theory has already produced a quantity of models for this interaction as Lotka-Volterra and Nicholson-Bailey models and later modifications of the original equations (for an overview of this classical theory, see Murdoch et al. 2003a).

Insects regroup the largest number of species at the Earth scale and are present in all landscape. They are also considered as being a major component of communities and ecosystem involved in many multi-species interactions, whether as prey, predator, parasite, pollinator or herbivore (Samways 1996). Therefore, they are included in many consumer-resource interactions either as the resource or the consumer. Insect herbivores are attacked by a wide range of natural enemies (Tanhuanpaa et al. 2001, Hooks et al. 2003). Determining and quantifying the impact of these enemies on the insect populations is a key question in ecology. Parasitoids, one of the insect consumers, have potentially a high influences on their insect resource population dynamics (Hawkins et al. 1997).

EVIDENCE OF HOST-PARASITOID INTERACTION IN NATURE

Host-parasitoid relationships are exceedingly common in nature and particularly in insect world. Parasitoids represent at least 10% of all metazoan species, they are included in almost all terrestrial insect communities and few insect species are exempt from attack of parasitoids (Godfray 1994). They also included some of the most specialized relationship and pose challenging research questions about the ecology and evolution of interactions at all levels from molecular to population, to community and to ecosystem (Ehrlich and Hanski 2004).

What is a parasitoid?

Parasitoids are insect species whose larvae develop to maturity by feeding on the still-living bodies of other organisms, usually insects, and eventually killing them (Godfray 1994). Parasitoids are abundant and diverse insects that are present in nearly all terrestrial ecosystems. Parasitoids could constitute 20-25% of all insect species (Godfray 1994), with the most important genera belonging to Hymenoptera wasps and Diptera flies (Godfray 1994).

The basic life history of such organisms has been described in details by several authors (e.g. “Parasitoids: behavioral and evolutionary ecology” by Godfray 1994; “The spatial and temporal dynamics of host-parasitoid interactions” by Hassell 2000; or “Parasitoid population biology”, by Hochberg and Ives 2000). The life cycle is split in two well distinguished phases: adults are free living, whereas larvae act as both predator and parasite (Fig. i.1 illustrates one example of parasitoid life cycle). For the second phase, depending on the host stage on which their larvae are feeding, one can group parasitoids into egg, larval, pupal, or adult parasitoids. Parasitoids that lay eggs in one stage and develop in the next stage are called

egg-larval or larval-pupal parasitoids. For the second phase, the development may be gregarious (gregarious parasitoids lay multiple eggs and many individuals can develop per host) or solitary (solitary parasitoids usually lay one egg in a host); moreover, larvae may be internal or external to the host as they develop (respectively, as endoparasitoids or ectoparasitoids) (Shaw 2006). According to the moment in which the host is killed as a result of parasitoid larval development, a distinction is made between koinobiont and idiobiont parasitoids. The first ones let their host continue to develop (usually continuing to feed, and being able of self-preserving behaviours) after the female parasitoid has oviposited into it; whereas the second ones kill or irreparably immobilize the host at the time of attack (Askew and Shaw 1986). It is suspected that koinobionts having an intricate physiological relationship with their host tend to have relatively narrow host ranges, while the host ranges of idiobionts tend to be potentially wide (though in practice resource security may allow them to evolve as specialists) (Askew and Shaw 1986, Shaw 2006). Depending on the number of host species that a parasitoid can attack, one can distinguish between generalist parasitoids that attack and develop on a wide range of host species and specialist parasitoid species that use only one or a limited number of host species. Table i.2 summarizes the main characteristics of parasitoids.

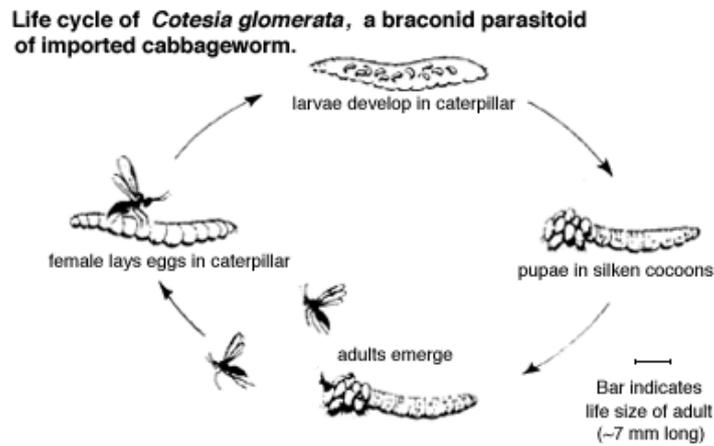


Figure i.1. Example of a caterpillar endoparasitoid life cycle. *Cotesia glomerata* is a Braconidae parasitoid attacking *Pieris rapae* caterpillars. (source: <http://www.nysaes.cornell.edu/ent/biocontrol/parasitoids/cotesia.html>).

Table i.2. Major distinguishing features of parasitoids.

2 types of parasitoids	endoparasitoids: develop in host body ectoparasitoids: develop on host body
Host survival after parasitoid oviposition	koinobiont: allow the host to develop until parasitoid egression idiobiont: the host is killed or irreparably immobilized at oviposition
Number of individual emerging from the host	solitary: one individual gregarious: more than one individual
Number of hosts	specialist: one host species generalist: more than one host species

This group of animals has long been neglected in conservation biology (Shaw and Hochberg 2001). However, the sensitivity of specialist parasitoids to habitat change is greater than that of their hosts (Kruess and Tscharntke 1994, Lei and Hanski 1997, Ryall and Fahrig 2005, Shaw 2006, Hilszczanski et al. 2005). Indeed, existing at higher trophic level, they are more likely to be eliminated by stochastic events, they are also highly vulnerable to periods of host scarcity (each host species being the keystone resource for the host specific parasite) (Thompson 1996). Some authors argue that specialist parasitoids of threatened hosts should be conserved not only for their own sake, but also for that of the host (Shaw 2006). Furthermore, parasitic wasps being dependent on taxonomically diverse groups of insects (e.g., Askew and Shaw 1986), a high diversity of parasitoids is generally accompanied with a high diversity of herbivores. Therefore, it appears that the monitoring of parasitic wasps could be useful to follow the recovery of habitat biodiversity, like forest in plantation stands (Maeto et al. 2009).

Parasitoid and their host relationship

The relationships between hosts and parasitoids within communities are frequently linked in complex food webs, with further potentially structuring influences arising from the presence of hyperparasitoids (parasitoids of parasitoids) (Muller et al. 1999). When we look at the pair-wise interaction -host and parasitoid-, much knowledge has been gained on the study of pest and their biological control, which provides key components of species interactions, but not necessarily addresses the processes involved in long-term stable interactions that occur in natural, unmanaged systems (Hawkins et al. 1999). These studies have shown among others that parasitoids can effectively reduce the size of their host population (see Hawkins et al. 1999, Hochberg and Ives 2000 for examples). As being a

consumer-resource interaction, the resource, which is here the host, is automatically killed. It is therefore clear that parasitoids can have an impact on the number of adult herbivores of that generation. This could lead to a reduction of herbivores in the next generation if the total number of (larval) offspring is also decreased. In a more “natural system”, studies realized on the ecology of Melitaeini butterflies (Wahlberg et al. 2001, Ehrlich and Hanski 2004) have brought several information on host-parasitoid interaction. According to these studies, it is obvious that parasitoids are an important part of their complex of natural enemies (Moore 1989, Lei and Hanski 1997) and it was suggested that they could play a predominant role in the population dynamics of butterflies (Ford and Ford 1930, Lei and Hanski 1997, van Nouhuys and Hanski 2002b). But in natural systems, the existence of such top-down control using parasitoids on host populations is still to confirm. It has been suggested that in natural situations, entire parasitoid complexes, rather than single species, control the number of herbivores or parasitoids associated with another source of fluctuations. Alternatively, many systems are likely to be controlled by 1) bottom-up processes, where the number of herbivores is dependent on the amount and quality of resources supplied through the plants, and 2) by top-down processes, where predators or parasitoids numbers depend on the number of herbivore hosts. Furthermore, in many instances the population dynamics of hosts and parasitoids are greatly influenced by variations in environmental conditions like weather regimes (Redfern and Hunter 2005, Stireman et al. 2005). Another possibility is that parasitoid populations are themselves regulated by a higher trophic level such as hyperparasitoids (van Nouhuys and Tay 2001). Currently, many authors agreed that in natural communities, both bottom-up and top-down regulations play a role simultaneously.

THE HOST BUTTERFLIES

Butterflies, as other insects, suffer of undergoing changes (Samways 1996, Fonseca 2009). Their populations, since several years now, are dramatically decreasing (Van Dyck et al. 2009). Studies with an interest for this ecological topic have been fulfilled during the past century and each year new ones abound in ecological journals. Butterflies have been the subject of various studies in population, metapopulation (e.g. Thomas and Hanski 1997, Baguette and Schtickzelle 2003), and community ecology (e.g. Steffan-Dewenter and Tschardtke 2002, Tschardtke et al. 2002b), to a large extent because they are conspicuous and are frequently the targets of either conservation effort or pest control (van Nouhuys and Hanski 2002b).

Butterflies interact with other species in their community, species at higher, lower and equal trophic levels. Understanding the link between a butterfly species and another organism is of great importance in ecology in order to better understand the studied system but also to better protect the two species involved. One important and obligate interaction (herbivory type) is the relation between caterpillars and their host plant. Another relationship, occurring mainly in the Lycaenidae family, is the one implying caterpillars and ants (myrmecophily) (Elmes et al. 2001, Thomas 2002). This well-studied relationship is usually a mutualism: the larvae secrete sugars and amino acids, which are harvested by the ants (Jordano and Thomas 1992). Butterflies represent also choice prey for predators (including birds, mammals, lizards and arthropods such as spiders and bugs). Moreover, all immature stages (eggs, larvae and pupae) of the butterfly life cycle can be attacked by parasitoids (Dempster 1984, Wahlberg 2000, van Nouhuys and Lei 2004, Stefanescu et al. 2009). The relationships between attacked butterfly host and parasitoid species have been invoked in several studies (Wahlberg et al. 2001, Anton et al. 2007, Stefanescu et al. 2009), as well as the tritrophic relation involving the first level of such interaction (the host

plant) (Nieminen et al. 2003, Fatouros et al. 2005). Indeed, natural enemies of herbivorous insects are influenced in various ways by the food plants of their host species; for example checkerspot larvae sequester iridoids from their food plants to defend themselves (Nieminen et al. 2003), and some volatile compounds released by herbivore infested plants attract parasitoid wasps (Havill and Raffa 2000). In the Pieridae family, parasitoids have been invoked to explain the change of host plant (Ohsaki and Sato 1994). Besides, parasitoids are one factor suspected to be responsible of the fluctuation of butterfly population size, some being able to push to disappear populations of their host (Hanski and Kuussaari 1995).

GENERAL OBJECTIVE OF THIS THESIS

As stressed before, a better knowledge of the interaction between two species is useful to understand and protect them under environmental changes. The host-parasitoid relationship studied in this thesis corresponds to the bog fritillary butterfly, *Boloria eunomia* (Lepidoptera: Nymphalidae), as host at the caterpillar stage of the parasitoid wasp *Cotesia eunomiae* (Hymenoptera: Braconidae: Microgastrinae). In Belgium, biological and ecological aspects (metapopulation, genetic, habitat, dispersal...) of this butterfly species were deeply studied, making *B. eunomia* a well-known species (e.g. Schtickzelle et al. 2002, Schtickzelle 2003, Baguette and Schtickzelle 2003, Schtickzelle et al. 2006, Turlure et al. 2009). However, its relation with parasitoids has never been profoundly investigated. In 1996, caterpillar parasitism was detected but without further studies (Waeyenbergh and Baguette 1996). Thus the objective of this thesis is twofold: 1) to improve knowledge of this specific relationship, mainly how the two species

interact and 2) to investigate the possible role of parasitoid in its host population regulation.

STUDY SYSTEM

The herbivore: The bog fritillary butterfly

- Description

B. eunomia is a small fritillary with average wingspan of 32 to 40 mm. The lesser fritillaries all tend to look very similar on the upper surface of the wings with black markings on an orange-brown to tan background. The outer margin of the wings is lined with silver chevrons. The bog fritillary is best characterized by the submarginal row of pearly spots surrounded by a black rim on the underside of the hindwing, which is orange with light non-metallic bands (Fig. i.2). Females differentiate from males through a darker upperside of the wings, a bigger abdomen, and as being taller than males.



Figure i.2. Underside of female (left, picture from C. Turlure) and male (right, picture from N. Schtickzelle) wings.

- Geographic range and habitat

B. eunomia is a glacial relict species. It presents a discontinuous boreo-alpine distribution (Fig. i.3). This specialist butterfly species inhabits, in Western Europe, peat bogs and unfertilized wet meadows where the Bistort (*Persicaria bistorta* L.; Polygonaceae) grows. It is the only host plant of caterpillars and food plant of imagoes in this part of its distribution area (Fig. i.4).

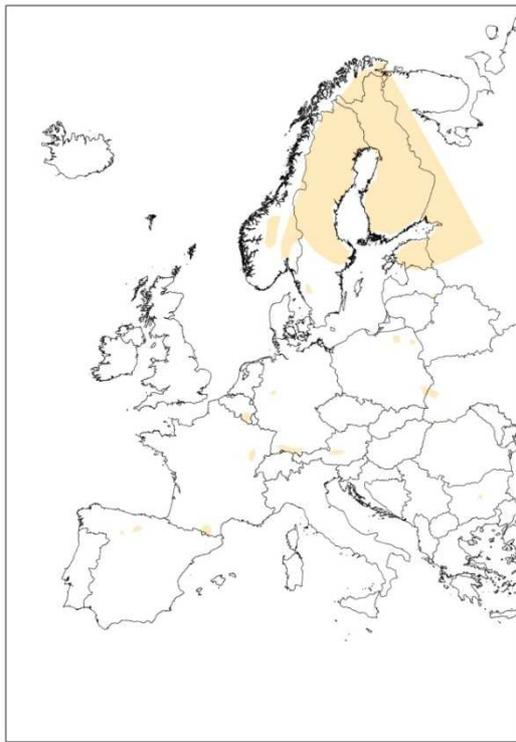


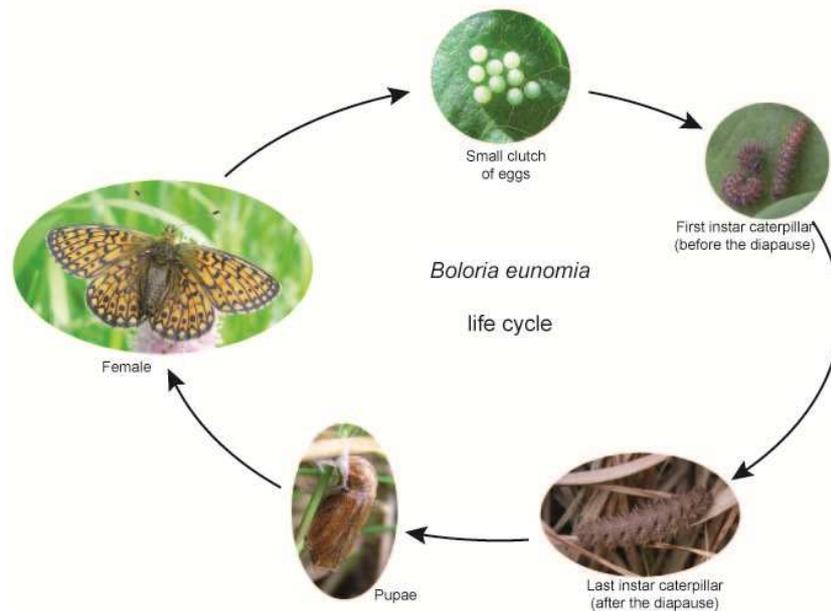
Figure i.3 European distribution of *B. eunomia* (reprinted from Fichet et al. 2008).



Figure i.4. *Persicaria bistorta* leaves (top: typical habitat of caterpillars) and flowers (below: adults food).

- Ecology

Adults are on the wing in one generation (univoltine species) from May to July, showing a marked protandry process (males appearing before females) to maximize their expected number of matings. During the flight period, males actively patrol in host plant patches looking for emerging females. Small, cream-colored eggs with longitudinal ribs, are laid in groups of 2-20 either under host plant leaves or on surrounding plants. The reddish-brown caterpillar has many branched spines. Third- and fourth- instars overwinter. The life cycle is represented in figure i.5.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Eggs												
Larva												
Pupa												
Adult												

Figure i.5. Life cycle of *B. eunomia* (pictures of adult, eggs and pupae from C. Turlure).

- Status

In Europe, according to the *IUCN Red List of Threatened Species* (Van Swaay et al. 2010), *B. eunomia* is considered as of least concern at both geographical Europe and the EU27 levels. In Wallonia, its current status is considered as rather rare and the species is in decline and protected by law (Fichefet et al. 2008).

The parasitoid

This butterfly is known to be parasitized at the larval stage by a *Cotesia* species (Waeyenbergh and Baguette 1996, Goffart and De Bast 2000) but this aspect has not yet been profoundly investigated. The parasitoid wasp species attacking *B. eunomia* larvae belongs to the Braconidae family, the second largest family of parasitic wasps (Shaw and Hochberg 2001). This species was in the past misidentified as *Cotesia vestalis* (HALIDAY 1834) (Shaw 2003). It is now recognized as a new species (see species description in Shaw 2009).

- The *Cotesia* genus

The current usage of the generic name *Cotesia* (Hymenoptera: Braconidae: Microgastrinae) is relatively recent (Mason 1981), and the previous literature pertaining to *Cotesia* species used the traditional name *Apanteles* Foerster (which now has a more restricted application: Mason 1981). *Cotesia* species are all koinobiont endoparasitoids, which can develop successive broods on a single host generation (Shaw et al. 2009).

Many *Cotesia* species are important natural enemies of agricultural and forestry pests, and a few have been manipulated as biocontrol agents. One, *C. glomerata* (Linnaeus), is a common parasitoid of the Eurasian cabbage white butterflies (species of *Pieris* Schrank) and has been studied in

considerable detail both in the laboratory and in the field, with the generation of a vast associated literature. *Cotesia* species are also known to parasitize Melitaeini butterflies, such as *Melitaea* and *Euphydryas* species which are parasitized by *Cotesia melitaeorum* and *Cotesia bignellii*. The potential impact of these parasitoids on their host population is suspected large (Ford and Ford 1930, Porter 1981, Lei and Hanski 1997, van Nouhuys and Lei 2004).

- The case of *Cotesia eunomiae*

A detailed description of this species has been realized recently by Mark Shaw (Shaw 2009). Except for sexual differences, males and females are morphologically identical. They length 2.4mm (Fig. i.6) and have a black body with slightly brown wing membrane.

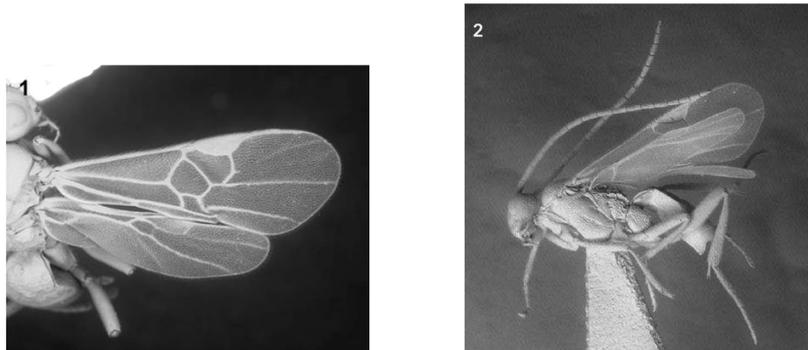


Figure i.6. *C. eunomiae* pictures (extracted from Shaw 2009). 1.: wing, 2.: female profile.

This *Cotesia* species is a gregarious koinobiont endoparasitoid. Its total host range is actually unknown but (Mark Shaw pers. comm.), *C. eunomiae* might be specialized on *B. eunomia* caterpillars. Many aspects of its life cycle are still unknown. However, we suspect that this species has, as the other *Cotesia* species, several generations in one host generation. Actually, we know that post diapause *B. eunomia* caterpillars are attacked

by this Braconidae, which emerges in concert from its host in June forming small yellow cocoons to pupate (Fig. i.7). Adults emerged on average 14 days after pupation (unpublished data). Furthermore, we also known (Annex III.1) that female wasps accept to lay eggs in *B. eunomia* first and second instar caterpillars.

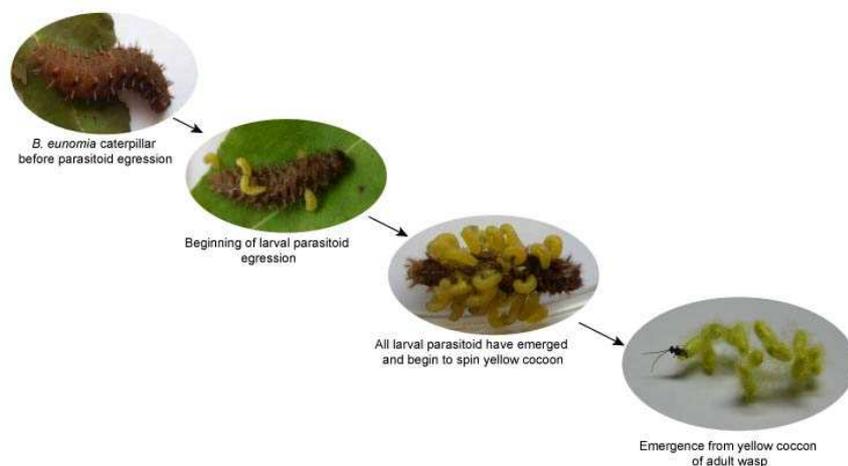


Figure i.7. Successive stages of parasitoid egression from a *B. eunomia* caterpillar. After parasitoid egression, caterpillars walk out the parasitoid cocoon muff and die a few days after.

During our field work, hyperparasitoid species attacking *C. eunomiae* have been collected. Until now, at least three species have been identified (Fig. i.8): *Gelis agilis* (Fabricius) (Ichneumonidae: Cryptinae), *G. proximus* (Foerster) and *Lysibia nanus* (Gravenhorst) (Ichneumonidae: Cryptinae) (MR Shaw, personal communication). They all are very common parasitoids of *Cotesia* (and other parasitoid) cocoons. *G. agilis*, a flightless generalist species, is also known to attack *C. melitaeorum* (parasitoid of *Melitaea cinxia* caterpillars) (Wahlberg et al. 2001, van Nouhuys and Hanski 2002b, Stefanescu et al. 2009). This species can even

cause local extinction of *C. melitaeorum* populations, which affects large-scale population dynamics of the host butterfly (van Nouhuys and Tay 2001, van Nouhuys and Hanski 2002b).

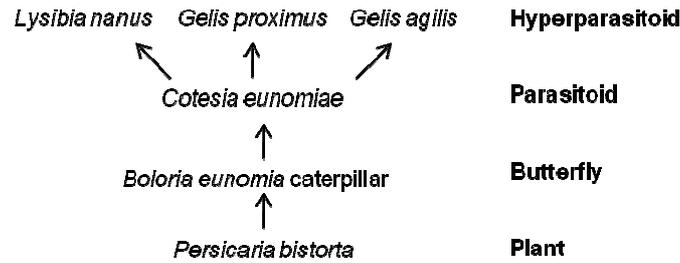


Figure i.8. The parasitoid food web associated with *Boloria eunomia* in Pisserotte.

The study area

This study was conducted on a 56 ha peat bog, the Fange de Pisserotte nature reserve (S-E, Belgium, 50°13'N, 5°47'E), located in the Plateau des Tailles landscape. This choice lies within the framework of researches realized by the UCL in the peat bogs of this landscape, where butterflies (Goffart and Waeyenbergh 1994, Goffart et al. 2001), plants, birds, mammals are studied since a long time but where parasitoids are still not well documented. Moreover, *B. eunomia* is still a long time studied in this Belgian region (e.g. Baguette and Nève 1994, Nève et al. 1994, Nève et al. 1996, Petit et al. 2001, Schtickzelle et al. 2007, Turlure et al. 2010b).

In the Fange de Pisserotte, *P. bistorta* occurred in 27 different patches (24053 m²) (Fig. i.9). Habitat type of each patch was previously determined according to the presence and abundance of plant species and characterized as being either wet meadows, in which species such as *Deschampsia cespitosa*, *Anemone nemorosa* occurred, short sedge fens where *M. caerulea* predominates, fen grasslands characterised by plants like

Valeriana reptans, *Angelica sylvestris*, *Cirsium palustre*, rushes represented mainly by *Juncus acutiflorus*, *Viola sylvestris* and *Rumex acetosa*, or heathlands, where Ericaceae species (*Vaccinium vitis-idea* and *Vaccinium uliginosum*), *Polytrichum sp.* and *Calluna vulgaris* are found (Turlure 2009).

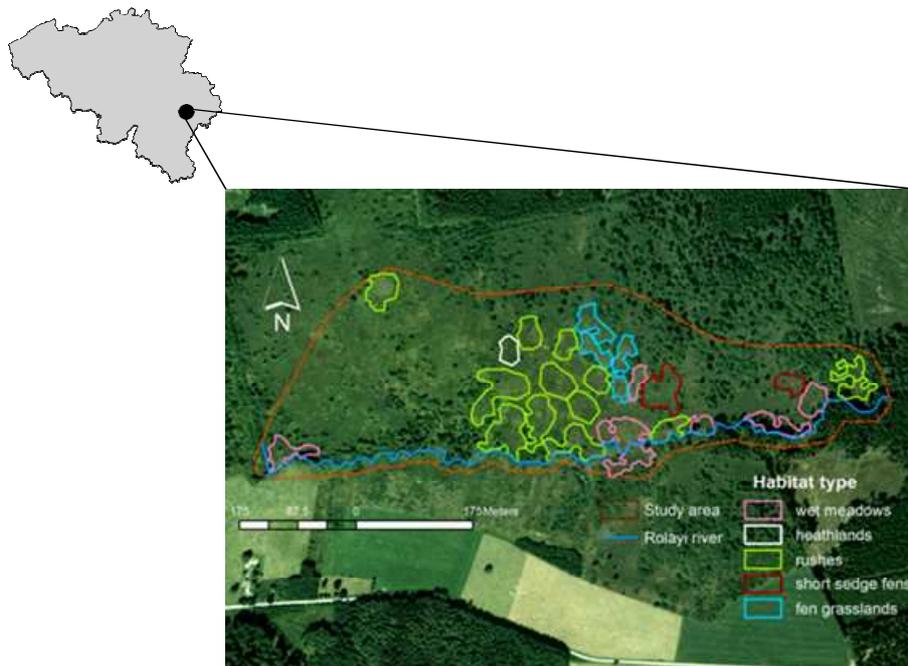


Figure i.9. Map of the study area: the Fange the Pisserotte with habitat type of the *P. bistorta* patches : wet meadows (pink), heathlands (white), rushes (green), short sedge fens (red), fen grasslands (blue).

OUTLINE OF THE THESIS

This thesis is divided in three parts. The first part is composed of the first two chapters and regroups information gathered on the host species, the bog fritillary butterfly. The second part, chapters 3, 4 and 5, considers the side of the parasitoid species. The last part focuses on the impact of the parasitism on its host population dynamics at a temporal scale.

In the first part, the point of view of the host was considered. In **Chapter I**, we addressed the question of how caterpillar micro-habitat and parasitoid prevalence interact to shape habitat selection in the bog fritillary butterfly *B. eunomia*. In this system, we focused on the importance of enemy-free space for the butterfly to reduce impact of parasitism. In **Chapter II**, we tried to understand why some Nymphalidae butterfly species are attacked by a higher number of Braconidae wasps than others. Thus, a literature-based database of host-parasitoid interactions was constructed. To find explanations to these observations, the importance of some ecological and morphological factors of butterfly hosts were evaluated. Results of this study was used to understand why our long term study of two peat bog Nymphalidae in Belgium (*B. eunomia* and *B. aquilonaris*) has revealed that only the first species, *B. eunomia*, was parasitized during its last larval instar.

In the second part, we brought more information concerning the parasitoid species. Firstly (**Chapter III**), a preliminary study investigated three categories of infochemicals proved, in studies of other host-parasitoid systems, as significant volatiles odors permitting to parasitoids to locate their host. Indeed, the survival of parasitoids is greatly dependent on their spatial and temporal presences with their hosts. To ensure such coincidence, parasitoids need not only to synchronize their development with their hosts but also to locate and to identify them. Infochemicals play an important role in the interactions between organisms; therefore a full understanding of the sensitivity with which a wasp responds to various volatiles associated with

the lower trophic levels would contribute to a better knowledge of the tri-trophic interaction between a parasitoid, its host and the host plant. Secondly (**Chapter IV**), we addressed the question of how *C. eunomia* alters the development of its host. Indeed, parasitoids are adapted to influence their host development so that their quantitative and qualitative nutritional requirements are met. And finally (**Chapter V**), genetic analyses have been carried out with a twofold aim: 1) to understand the metapopulation structure of *C. eunomia*, and 2) to see if superparasitism, a really common phenomenon in parasitic wasp and notably in other *Cotesia* species, is also present in our studied species. Specific microsatellite markers have been developed in this aim.

In the last part of the thesis (**Chapter VI**), we presented the interaction of the two species and more precisely if and how parasitoids could play a role in its host population dynamics. This question has been asked several times with different organisms. Several authors argue the importance of parasitoids in natural butterfly populations. Here, we explore the question using our studied system (*B. eunomia* and *C. eunomia*) for the Pisserotte population.

PART ONE

HOST PARASITOID RELATIONSHIP: FROM THE HOST SIDE

CHAPTER I _____

**PARASITISM COST OF LIVING IN A HIGH QUALITY HABITAT IN THE
BOG FRITILLARY BUTTERFLY.**

Chapter I is a submitted manuscript:
Julie Choutt, Camille Turlure, Michel Baguette & Nicolas Schtickzelle
in Plos One.

ABSTRACT

Habitat quality and the impact of natural enemies (such as parasitoids) might profoundly affect metapopulation dynamics and viability, mainly through effects on habitat carrying capacity and population size, respectively. However, their relative impact has usually been considered independently. Here we address the question of how caterpillar habitat quality and parasitoid prevalence interact to shape habitat selection in the bog fritillary butterfly *Boloria eunomia*. Caterpillars feed on a unique host plant and are parasitized by a specialist wasp, *Cotesia eunomiae*. We first classified caterpillar habitat quality by relating caterpillar density to descriptors of different microhabitat types. Secondly, we investigated parasitoid prevalence in those different micro-habitats. Our results show that caterpillars and parasitoids mapped onto the same microhabitat types, mainly patches with high abundance of the butterfly host plant within wet meadow type vegetation. Accordingly, we suggest that both egg-laying females and parasitoids use the same cues for habitat selection. As a consequence, there should be a fitness cost for *B. eunomia* females to lay their eggs in places where parasitoid prevalence is high. We indeed detected that *B. eunomia* females frequently laid eggs in habitat types that were suboptimal for caterpillars (such as fen grasslands). This suggests that the lower parasitoid prevalence in these suboptimal habitat types counterbalances lower caterpillar survival, leading to an overall similar survival in optimal (wet meadows) and suboptimal (fen grasslands) habitat types. Spreading eggs in both habitat types is thus expected to be a safe strategy to mitigate the adverse possible effects of environmental stochasticity and parasitism prevalence on offspring survival unequal among microhabitat types. In this system, the specialist parasitoid acts as a driver of suboptimal habitat use by its host, evidencing the importance of heterogeneity in the butterfly habitat as providing enemy-free space.

I.1. INTRODUCTION

Facing a considerable loss of natural habitats, as well as animal and plant species loss, conservation biologists have used several ecological theories and concepts to elaborate conservation guidelines (Fahrig 2003). Among these, the metapopulation theory (e.g. (Hanski 1999) and references therein) has already a long history in conservation ecology. However, evidence accumulates that patch size and connectivity (i.e. the two key parameters of the metapopulation paradigm) are not sufficient to describe the functioning of most metapopulations. Local aspects of population dynamics should also be included, especially habitat quality (Thomas et al. 2001, Dennis et al. 2006, Turlure et al. 2009), trophic interactions (Vidal and Tschardtke 2001, Tylianakis et al. 2007), and phenology that are often altered in these changing environments (Lei and Hanski 1997, Shaw et al. 2009). In particular, predation and parasitism are important trophic interactions, affecting individual habitat selection, metapopulation dynamics, community structure and ecosystem functioning (van der Putten et al. 2004, Haddad et al. 2009).

Several factors influencing butterfly metapopulation dynamics have already been studied (Hanski et al. 1995, Wahlberg et al. 2002, Schtickzelle and Baguette 2004), such as environmental stochasticity (Sutcliffe et al. 1996), variation of weather conditions (Roy and Thomas 2003), or site management (Schtickzelle et al. 2007). Nevertheless, few studies examined the role of caterpillar parasitism in these dynamics (but see van Nouhuys and Hanski 2002b), and its consequence(s) on the pattern of habitat use. However, parasitism impacts the metapopulation dynamics both through temporal changes in local population sizes and through changes in spatial pattern of habitat use. Indeed, according to the enemy-free space hypothesis introduced by Jeffries and Lawton (Jeffries and Lawton 1984), parasitoids can drive their hosts to use suboptimal habitats that are free of enemies. In

the case of butterflies, parasitoids play an important role as a major driver of temporal population dynamics and population size (Dempster 1984). In *Euphydryas aurinia*, cyclic trends of both parasitoid and butterfly population sizes have been observed since a long time (Ford and Ford 1930, Klapwijk et al. 2010). Studies on the parasitoid complex of *Melitae cinxia* brought much information about the dynamics of the host species and its primary parasitoids, but also of the higher trophic levels (i.e. hyperparasitoids) (Lei and Hanski 1997, van Nouhuys and Hanski 2002b). Parasitoids also induce modifications of realized ecological niche, mainly through changes in host plant use (Lill et al. 2002). Indeed, some species were observed to shift from their highest quality host plant to a plant with a lower quality value but (temporally) free of parasitoid attacks. Here we focus on spatial shift in habitat selection according to parasitoid prevalence.

The bog fritillary, *Boloria eunomia* (ESPER, 1799, Lepidoptera, Nymphalidae, formerly *Proclassiana eunomia*), is a vulnerable butterfly, whose caterpillars feed only on the bistort *Persicaria bistorta* ((L.) STAMP, 1753, Polygonaceae), and are parasitized by the specialist parasitoid wasp *Cotesia eunomiae* (CAMERON, Hymenoptera, Braconidae). While many studies focussed on the butterfly (e.g. Schtickzelle et al. 2006), the influence of parasitism on its habitat use and its metapopulation dynamics has not been investigated to date. In this paper, we address the following question: how does microhabitat quality influence the tri-trophic interaction “host plant - butterfly - parasitoid”? In order to do so, we assessed the importance of habitat quality descriptors in explaining the spatial variation in caterpillar density and survival, and their associated risk of being parasitized. Our working hypothesis is that caterpillar density and parasitoid prevalence should be highest where microhabitat quality is optimal. This means that the parasitoid prevalence should be lower in suboptimal microhabitats. If this hypothesis is true, parasitoid prevalence should weaken the differences in caterpillar fitness between optimal and suboptimal habitats. To investigate

this hypothesis, we related caterpillar density and parasitism rate to descriptors of habitat quality. Observed local higher caterpillar density could be the result of a better survival, a higher initial abundance of eggs due to oviposition choices made by females, or both; we thus individually tracked females of *B. eunomia* to address their egg-laying preferences according to habitat quality. We finally discuss 1) how caterpillar density and parasitism rate were related to microhabitat features, and 2) the importance of suboptimal habitat, providing enemy-free space, for (meta)population persistence.

I.2. MATERIAL AND METHODS

Study system

B. eunomia is a univoltine butterfly, flying from the end of May to the beginning of July in Belgium. It is strictly specialized on *P. bistorta*, used both as the host plant for caterpillars and the nectar plant for adults. Females deposit clutches of a few eggs on or near the host plant. Hatching occurs in June-July, and solitary larvae feed for about two months up to the diapause without building any nest. In the following spring, larvae resume feeding, and bask on old leaves of plants such as *Deschampsia cespitosa*, *Dryopteris cristata* or *Molinea caerulea*. They moult several times before the 15 days pupation period. In Belgium, the life cycle of *B. eunomia* is completed in one year. Recently, Turlure et al. (2009) defined high quality microhabitat for caterpillars as places with a high host plant abundance, grass tussocks and specific microclimatic conditions (temperature and humidity).

Cotesia eunomiae is a gregarious koinobiont endoparasitoid (i.e. the host development continues after being parasitized, and several wasps emerge from each host larvae) specialised on *B. eunomia* caterpillars (Shaw

2009). At the time of writing, it is the single primary parasitoid known of bog fritillary last instar caterpillars. Wasp larvae emerge from last instar caterpillars in June. Details of the life cycle of this species are still unknown. This study was conducted on a 56 ha peat bog, the Fange de Pisserotte nature reserve, located in the Plateau des Tailles landscape (S-E Belgium, 50°13'N, 5°47'E). *P. bistorta* covered 24053 m² (Fig. I.1) in 38 patches of different vegetation types (i.e. wet meadows, fen grasslands and rushes; see (Turlure 2009) for details).

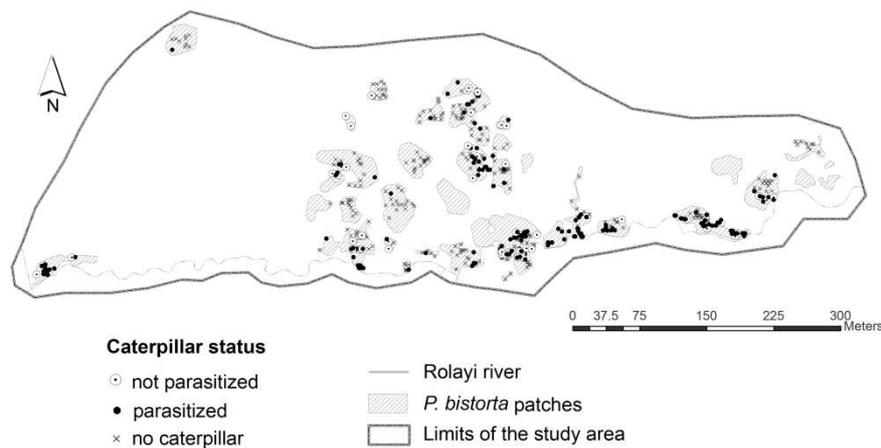


Figure I.1. Map of the study site, the Pisserotte peat bog nature reserve in S-Belgium. The map shows habitat patches for *B. eunomia* butterfly (patches of the host plant *P. bistorta*), and the locations of vegetation plots, with information about the presence (circle) or not (cross) of a caterpillar and its parasitism status (black circles = parasitized caterpillars; white circles = unparasitized caterpillars).

Sampling B. eunomia caterpillars

During spring 2005 (from 15 May to 5 June) and 2006 (from 11 May to 7 June), *bistorta* patches were sampled several times (between 2 and 7 times per patch, summing to 150 hours) by visual inspection to collect *B. eunomia*

caterpillars (all next to last and last instars caterpillars at that period of the year). The sampling effort in each host plant patch was proportional to its area (Pearson correlation tests: $R = 0.62$, $n = 26$, $P < 0.001$ for 2005; $R = 0.59$, $n = 23$, $P = 0.003$ for 2006).

Caterpillars found in the field were geolocalized by GPS, and brought to the lab to be reared individually until pupation or parasitoid egression (Chouff & Schtickzelle, unpub. ms). All parasitoid wasps and adult butterflies were then released in the field at the exact place where the caterpillar was collected.

Describing habitat quality

Descriptors of butterfly habitat quality were recorded in 1 m² plots for both the 216 locations of collected caterpillars (placed centrally over caterpillar position) and a series of 855 control plots, randomly chosen in places within the bistort patches where no caterpillar was found after repeated search (Fig. I.1). The number of control plots was higher than for caterpillar plots in order to cover the overall heterogeneity of the habitat. Four descriptors were measured for each 1 m² plot:

- 1) Abundance of the host plant *P. bistorta* (HOST), the single food source of both *B. eunomia* caterpillars and adults in the study area. Each plot was divided in 25 equal squares, and host plant abundance was estimated on the basis of its presence on each square, i.e. on a zero to 25 scale.

- 2) Microhabitat topography (TOPO). We counted in each plot the number of grass tussocks, essentially composed of *D. cespitosa* and *M. caerulea*.

- 3) Plant species composition (VEGE). To summarize plant composition at the scale of the study site, a Detrended Correspondance Analysis (DCA, Canoco Version 4.5; Ter Braak and Smilauer 2002) was performed on the abundance of the 68 plant species recorded at the scale of

the study site (1071 caterpillar and control plots) (Fig. I.2a). The combination of the two axes (VEGE1 and VEGE2) reflected specific plant associations in peat bogs: wet meadows characterized by a high density of *D. cespitosa* (positive values of VEGE1), rushes dominated by *Juncus sp.* (negative values of VEGE1), and fen grasslands with different flowering plant species (positive values of VEGE2).

4) Local microclimatic conditions. Temperature, moisture and luminosity indexes were computed as the weighted (by plant abundance) average of Ellenberg's indicator values of plant species for each parameter (Ellenberg 1974). This procedure took advantage of the integrative character of the plant presence over time, and hence was preferable than instantaneous, direct measures using data loggers. The three parameters being highly correlated (Pearson correlation tests: $n = 1071$, $p < 0.0001$ for all), they were combined into two independent variables using Principal Component Analysis. CLIM1 was positively correlated with all microclimatic variables and CLIM2 was positively correlated with moisture and negatively with temperature and luminosity.

Fig.I.2b provides a summary of the spatial combination/arrangement of these descriptors of habitat quality. Host plant abundance differed according to the vegetation type. Thus, higher host plant densities were found in wet meadows, and slightly decreased in fen grasslands and in rushes. Besides, humidity, temperature and light were negatively correlated with VEGE1 and positively with VEGE2, highlighting a microclimatic gradient from warmer, more opened and moister fen grasslands and rushes, to colder, darker and drier wet meadows.

Figure I.2.

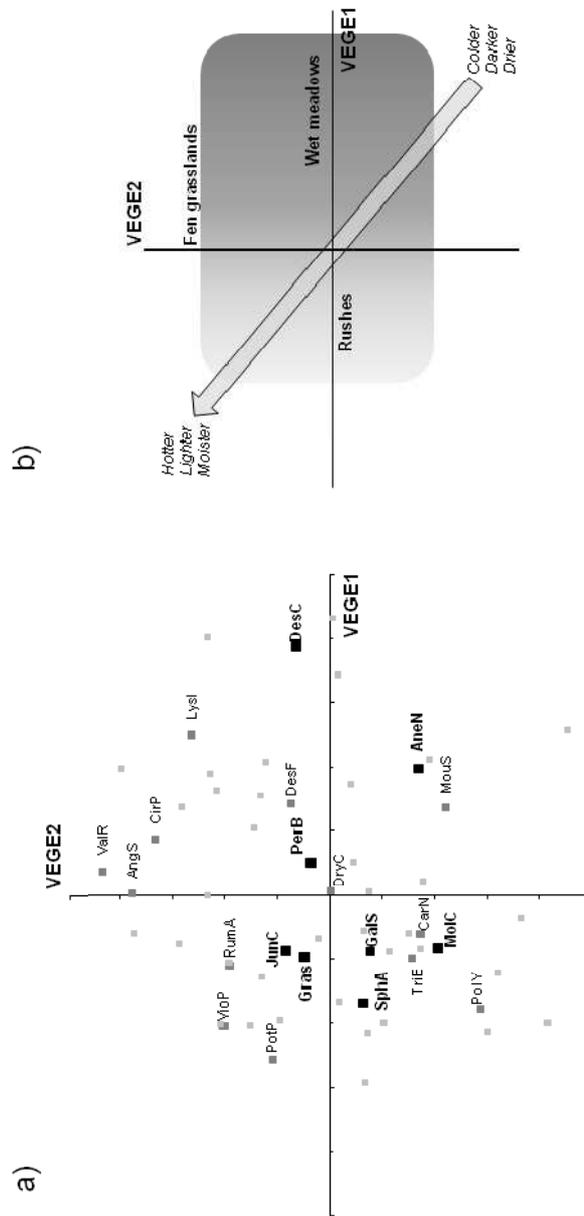


Figure I.2. Summary of study site vegetation. a) Graphical representation of Detrended Correspondence Analysis (DCA) on plant species abundance recorded on 1 m² plots (see text for details). Black and grey squares represented position of each plant species; the bigger and darker the square, the more the plant species contributed to the axis formation. Plant species with the highest influence were: PerB = *Persicaria bistorta*; JunC = *Juncus sp.*; SphA = *Sphagnum sp.*; DesC = *Deschampsia cespitosa*; MolC = *Molinea caerulea*; AneN = *Anemone nemorosa*; Gras = Grasses; GalS = *Galium saxatile*, ValR = *Valeriana repens*, AngS = *Angelica sylvestris*, CirP = *Cirsium palustre*, Lysi = *Lysimachia sp.*, DesF = *Deschampsia flexuosa*, CarN = *Carex nigra*, PolY = *Polytrichum sp.*, TriE = *Trientalis europaea*, PotP = *Potentilla palustre*, ViolP = *Viola palustris*, RumA = *Rumex acetosa*, DryC = *Dryopteris cristata*, MouS = Moss. b) Three major vegetation types (wet meadows, fen grasslands and rushes) are distributed along the two DCA axes. The existing microclimatic gradient is displayed, as well as the host plant gradient: a bigger density being found in wet meadows.

Relating caterpillar abundance and rate of parasitism to habitat features

Caterpillar density and parasitism prevalence were related to habitat descriptors using linear models. Due to multicollinearity between the biological variables investigated, a multimodel inference approach, based on corrected Akaike's information criterion (AICc), was chosen. This statistical method consists of several steps (for details see Anderson 2008 and references therein). (1) Generalized linear models corresponding to all possible combinations of explanatory variables are fitted to the data. (2) The power of each model to explain existing variations in the response variable is assessed through the AICc value. (3) The relative importance of each explanatory variable is quantified through its AICc weight (computed as the sum of AICc weight of all models where this explanatory variable appears). Those having the largest AICc weight predominantly affect the response variable. (4) A multimodel averaged estimate of each beta parameter and its standard error is computed as the mean of estimates given by each individual model, each individual estimate being weighted by the AICc weight of the model.

A multinomial distribution with cumlogit link function was used for modelling the caterpillar density, as it took five modalities in the dataset (0 for control plots, 1, 2, 3 and 4 caterpillars per plot were observed in the field). A binomial distribution with logit link function was used for modelling the caterpillar status (parasitized vs not parasitized).

Estimating potential sampling bias

In many host-parasitoid interactions, parasitoid larvae may lengthen the caterpillar stage, so that adult parasitoids emerge at the right time to encounter new hosts to oviposit (Vinson et al. 1998). In the case the caterpillar sampling occurred at a time when unparasitized caterpillars had already pupated, the observed parasitism rate could be overestimated,

biasing the results. A sensitivity analysis was therefore performed on three subsets of the parasitism data. We removed increasing fractions of the data collected during latest dates, where potential overestimation of parasitism rate might happen. The same statistical analyses (previous section) were performed on the observed data set and three subsets obtained by: (1) removing dates where all collected caterpillars were parasitized; removing dates where (2) 20% or (3) 10% of the unparasitized caterpillars should have already pupated. In this procedure, we derived the temporal distribution of caterpillar pupation from the temporal distribution of emergence of adults, obtained from Capture-Mark-Recapture (CMR) data of this *B. eunomia* population (Choult and Schtickzelle, unpub. data), using an average 15 days for pupation duration.

Mapping B. eunomia egg laying behaviour

B. eunomia females were individually tracked in the field from 9th June to 6th July 2004 to evaluate the female propensity to lay eggs in various vegetation types. Females were tracked during 20 min or until the female was lost, from a sufficient distance to prevent perturbing their behaviour. For each laying event, we recorded the number of eggs laid, their precise GPS location, and the vegetation type. We then computed an index of egg density for each vegetation type using the following formula, taking into account differential sampling effort and vegetation type availability:

$$\frac{\left[\left(\frac{\text{Eggs}}{\text{Effort}_{\text{track}}} \right) \times \left(\frac{\text{Females}}{\text{Effort}_{\text{CMR}}} \right) \right]}{\text{Area}}$$

with Eggs the total number of eggs laid, Effort_{track} the total duration of the tracks, Females the total number of females caught during CMR (Choult and

Schtickzelle, unpub. data), Effort_CMV the number of CMV sessions, and Area the total area of the considered vegetation type.

I.3. RESULTS

A total of 101 (in 87 plots) and 147 (in 129 plots) *B. eunomia* caterpillars were collected and reared, in 2005 and 2006 respectively, from which 76 (75%) and 112 (76%) were parasitized. Fig. I.1 illustrates the spatial distribution of caterpillars found in the field.

Caterpillar abundance and parasitism rate are related to the same habitat features

The same habitat descriptors (HOST, VEGE1 and VEGE2), furthermore acting in the same direction (Table I.1), had the highest effect on both caterpillar density and parasitism prevalence. Higher caterpillar density and parasitism prevalence were largely, but not perfectly, superposed (Fig. I.3), being found in microhabitat characterized by abundant host plant. The high importance of quadratic terms of VEGE1 and VEGE2 indicates the existence of an optimum for both caterpillar density and parasitism prevalence in one vegetation type, i.e. wet meadows. Additionally, more caterpillars were found where microclimatic conditions were cooler, darker and moister (i.e. under higher values of CLIM2). CLIM1 and TOPO had a slight effect on both caterpillar density and parasitism prevalence. Sensitivity analysis showed no indication of a bias due to potential lengthening of caterpillar stage by the parasitoid larvae. Results were indeed almost identical in terms of the relative impact of habitat descriptors on parasitism prevalence between analyses based on full and truncated data sets (Fig. I.4).

Table I.1. Multimodel inference on the relative influence of habitat descriptors on caterpillar density and parasitism prevalence (parasitized or unparasitized status). AICc weight represents the relative importance of the descriptor in explaining variation in the Y response variables (see text for details). The ranking is very similar for the two responses (descriptors with weight > 75% are shown in bold for easier reading). Furthermore, the most influential descriptors act on both responses in the same direction. CLIM1: positively correlated with all microclimatic variables; CLIM2: positively correlated with moisture and negatively with temperature and luminosity; HOST: host plant abundance; TOPO: number of grass tussocks; VEGE1: negatively correlated with humidity, temperature and light; VEGE2: positively correlated with humidity, temperature and light.

Variables	Variable weight		Parameter estimate (\pm SEM)	
	Density	Status	Density	Status
HOST	99%	79%	0.560 (\pm0.170)	0.265 (\pm0.133)
TOPO	37%	47%	0.036 (\pm 0.042)	-0.117 (\pm 0.105)
CLIM1	30%	34%	0.009 (\pm 0.070)	-0.105 (\pm 0.174)
CLIM1 ²	38%	37%	0.056 (\pm 0.064)	0.105 (\pm 0.135)
CLIM2	99%	30%	0.443 (\pm0.128)	0.032 (\pm 0.065)
CLIM2 ²	68%	27%	-0.244 (\pm 0.155)	0.017 (\pm 0.050)
VEGE1	100%	96%	1.044 (\pm0.183)	1.224 (\pm0.403)
VEGE1²	100%	98%	-0.670 (\pm0.167)	-1.366 (\pm0.437)
VEGE2	93%	35%	-0.306 (\pm0.113)	-0.060 (\pm 0.082)
VEGE2²	100%	87%	-0.609 (\pm0.130)	-0.370 (\pm0.157)

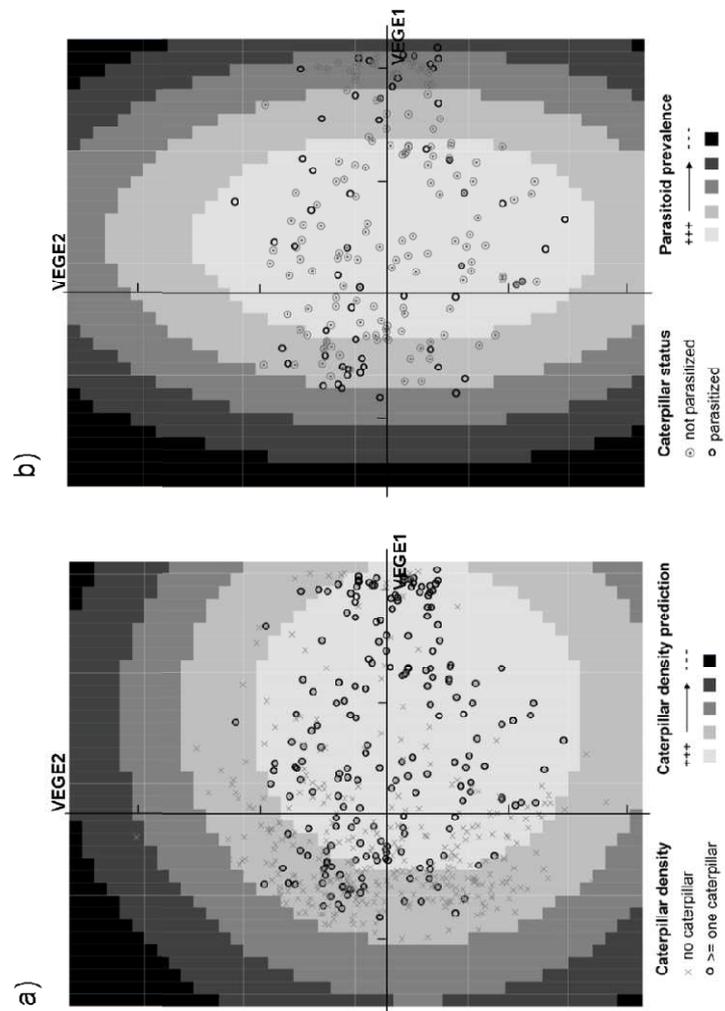


Figure I.3. Observed and predicted density of (a) *B. eunomia* caterpillars and (b) parasitoid prevalence within the two dimensional representation of vegetation (VEGE). The high importance of quadratic forms of VEGE1 and VEGE2 in explaining variation in the two responses variable led to the existence of an optimum. Predictions were computed using model-averaged value of parameter estimates (Table I.1).

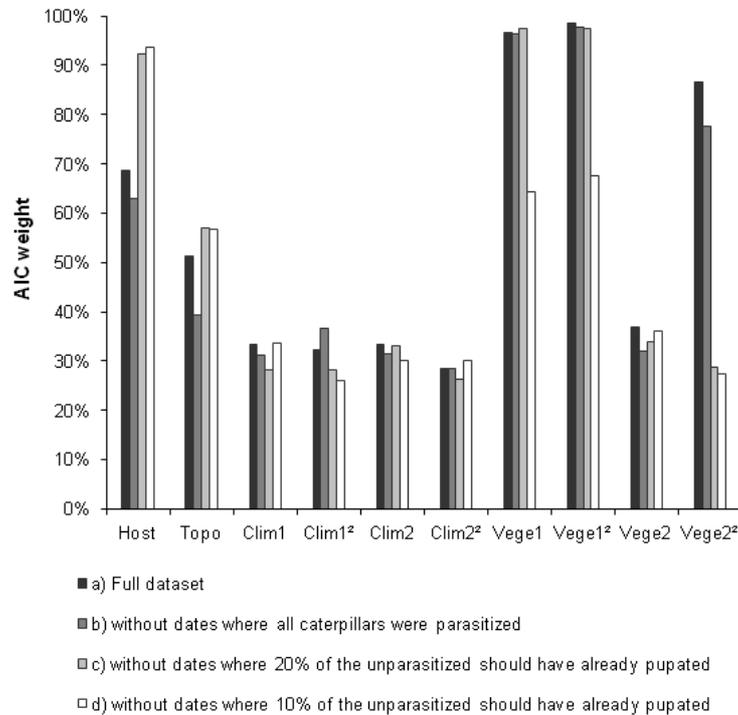


Figure I.4. Sensitivity analysis performed on caterpillar parasitism status data. Results of the sensitivity analysis performed on caterpillar parasitism status data to assess the possible sampling bias due to lengthening of the last instar caterpillar life by parasitoids.: a) complete data set, b) first sampling dates where all collected parasitized caterpillars were deleted, c) and d) the sampling dates above respectively the 10% and 20% pupae recruitment rate were removed of analyses.

Female egg laying behaviour

Females laid 226 eggs (in 42 batches, with 2 to 12 eggs per batch; no difference in batch size between habitats of different quality, one-way ANOVA: $F_{2, 37} = 2.30$, $P = 0.116$) during the 1337 minutes of tracking. Among the three types of vegetation (Fig. I.2), females laid eggs

preferentially in wet meadows, to a lesser extent in fen grasslands and rarely in rushes (Fig. I.5a). The density of caterpillars followed the same pattern (Fig. I.5b). Caterpillar survival until the last instar stage (estimated as the ratio between caterpillar density and egg density) was slightly higher in rushes and wet meadows compared to fen grasslands (Fig. I.5c). But survival until pupal stage (estimated as the ratio between unparasitized caterpillar density and egg density) was very similar in fen grasslands and wet meadows (Fig. I.5d), likely because parasitoid prevalence was more important in wet meadows compared to fen grasslands (Fig. I.5b); it was quite lower in rushes.

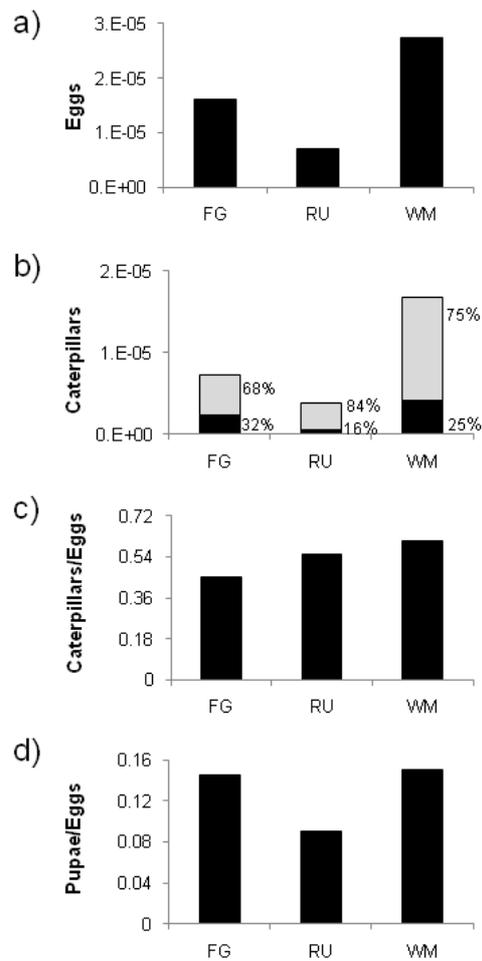


Figure I.5. Comparison of frequency and survival of *B. eunomia* life stages in the three main vegetation types. Relative indices of (a) density of eggs, (b) density of parasitized (grey) and unparasitized (black) caterpillars, (c) survival from egg to last instar caterpillar, and (d) survival from egg to pupae. Due to the method of computation (see text for details), these indices are relative values that can be compared between vegetation types (FG: fen grasslands; RU: rushes; WM: wet meadows) but their absolute values have limited meaning.

I.4. DISCUSSION

The density of *B. eunomia* caterpillars and the prevalence of parasitism by *C. eunomiae* covaried and were explained by the same three habitat features, being both higher (1) in places with high host plant abundance, (2) in wet meadow type vegetation, and (3) under cooler and more humid conditions. Areas with higher caterpillar density and parasitism prevalence were however not perfectly superposed, the former being centred on wet meadows, the latter on host plant abundance. According to this difference, female butterflies would have a higher probability of avoiding the parasitism of their offspring by laying their eggs in marginal parts of wet meadows, where the host plant is less abundant. This result therefore suggests that parasitism could induce the use of suboptimal habitat by the host, where expected lower offspring fitness is compensated by the lower impact of parasitism. Consequently, suboptimal habitats are likely to be important for the butterfly's population dynamics and persistence, as an enemy-free space where the mortality of caterpillars due to parasitoids is relaxed.

Given the high specialization of both butterflies and caterpillars on *P. bistorta*, the influence of *P. bistorta* abundance in predicting spatial variation of caterpillar density was expected, as shown for other species (Konvicka et al. 2003, Betzholtz et al. 2007). Newly hatched caterpillars are not so mobile and have to quickly find enough host plant material to feed. Therefore, patches with high *P. bistorta* densities are likely to provide better opportunities for females to both feed and lay their eggs, resulting in higher density of eggs and subsequently caterpillars. Vegetation type and microclimatic conditions also played an important role in explaining spatial variations of caterpillar density. They are intimately linked in the field in the way they define the suitable micro-environment for the species. Some vegetation features, such as vegetation height and structural complexity, are known to influence the incidence of caterpillars in some butterfly species

(Anthes et al. 2003, Betzholtz et al. 2007), while microclimate is important for caterpillar development (Alonso 1997, Kuhrt et al. 2005). *B. eunomia* is an ectothermic glacial relict species and accordingly depends upon precise thermal microenvironments to attain and/or maintain optimal body temperatures. It is therefore not surprising that at the microhabitat scale caterpillars are preferentially found in cooler and more humid conditions. Plant architecture, like grass tussocks, have been shown to offer a variety of temperature conditions (Gotthard 2008) that can be used by caterpillars through behavioural thermoregulation (Turlure et al. , Alonso 1997). Nevertheless, we did not detect any effect of the abundance (or presence *vs* absence) of tussocks on caterpillar density; this variable being probably included in the synthetic descriptors of vegetation composition.

Several, non-mutually exclusive, mechanisms may explain the higher parasitism prevalence in higher quality habitat. (1) Parasitoid wasps may be attracted during their search for host by olfactory or visual cues emitted by the caterpillars (Dicke et al. 2003, Steiner et al. 2007), by *P. bistorta* or by a combination of both, like the chemical released by *P. bistorta* leaves when eaten by caterpillars (Penuelas et al. 2005, Gols et al. 2008). Since both *P. bistorta* and *B. eunomia* were more abundant in higher quality habitat, attractive cues should be stronger there. (2) Besides the use of host density as a cue, parasitoid might increase their search efficiency when the host density is higher (Umbanhowar et al. 2003, Bezemer et al. 2010). (3) Alternatively, parasitoid search efficiency might be lower in suboptimal fen grassland habitat types because of specific features of the vegetation, such as taller or structurally more complex vegetation (Andow and Prokrym 1990, Randlkofer et al. 2007, Obermaier et al. 2008). (4) Higher habitat quality might also enhance caterpillar quality and survival, providing better prospects for parasitoid progeny (van Alpen et al. 2003), hence increasing their attractiveness for parasitoids if these latter are able to evaluate the quality of their hosts (Godfray 1994). However, this study was

not planned for discriminating the mechanisms accounting for the observed higher parasitism prevalence in higher quality habitats.

Whatever the mechanism(s) responsible for higher caterpillar density and higher parasitism prevalence, results of this study highlight their spatial matching within the habitat of the butterfly, indicating that a cost is paid *B. eunomia* caterpillars living in a high quality habitat, in terms of higher risk to be parasitized by *C. eunomiae* wasps. Escaping parasitoids is a challenge for potential hosts. Because limited movement ability does not give caterpillars much leeway, this challenge reverts to the adult females, mainly through the fine-tuning of their egg laying behaviour to the parasitoid behaviour. In some species, females use a lower quality host plant species to avoid parasitoids (Ohsaki and Sato 1994). Given the strict monophagy of *B. eunomia* caterpillars and adults in Western Europe, switching to another host plant seems to be no option. Females tended to preferentially lay their eggs in habitat type providing the locally optimal survival for caterpillars (i.e. wet meadows). But caterpillars living in these high quality habitat types were also more likely to be parasitized. Consequently, the higher caterpillar survival observed in wet meadows seemed nearly thwarted by an elevated mortality due to parasitism, leading to similar overall egg to pupae survival in fen grasslands and in wet meadows. Since similar survival rates for pupae have been observed in wet meadows and fen grasslands (Radchuck and Schtickzelle, unpub. data), this translates into similar fitness for females laying their eggs in both habitat types. Hence, these results suggest that egg-laying in a habitat suboptimal for caterpillar survival appears a working solution for females to relax caterpillar mortality due to parasitism. Laying eggs in wet meadows and in fen grasslands could then be a strategy of risk spreading, as mentioned for other butterflies (e.g. Albanese et al. 2008). CMR data provided some support for this hypothesis: 19% of females caught in fen grasslands and/or wet meadows at least on two different days were caught in both habitats. Further investigations on this system are necessary

to determine to what extent parasitism by *C. eunomia* affects *B. eunomia* metapopulation dynamics.

Variation in habitat quality and habitat selection may allow a given species to better cope with the natural or human-induced variations in resources or environment. Habitats that seem suboptimal may in fact be very useful, or even essential, to population persistence, offering temporary enemy-free space and hence, possible resistance to specific enemies. In a metapopulation context, such heterogeneity also participates in decoupling the dynamics of the local populations, because a common cause (e.g. climate change) may differently affect local populations differing in habitat quality (Liebhold et al. 2004). However, we expect that parasitoid search behaviour will also be under strong selection pressures, challenging the long-term advantage of the use of sub optimal habitats (Heard et al. 2006).

CHAPTER II _____

WHY SOME HOSTS ARE MORE HEAVILY PARASITIZED THAN OTHERS? A META-ANALYSIS OF THE RELATIONSHIP BETWEEN NYMPHALIDAE CATERPILLARS AND BRACONID PARASITIDS.

This article was written with
Nicolas Schtickzelle Michel Baguette & Camille Turlure.

ABSTRACT

Nymphalidae caterpillars have diverse defenses to protect against their predators and parasitoids attacks. Here, we tested if different ecological and morphological factors usually considered as putative defense mechanisms against parasitoid wasps were related to the number of Braconidae wasp species attacking Nymphalidae caterpillars. To tackle this issue, we performed a meta-analysis of literature data. Several ecological and morphological factors were used as predictor variables in a multimodel inference approach with the number of parasitoid attacking European Nymphalidae species as the response variable. The specialist *vs.* generalist character of the host and the morphological characteristics of the host were significantly related to the number of Braconidae parasitizing butterfly caterpillars. Life history traits of the host were not related to the response variable. A higher number of Braconidae wasps parasitized Nymphalidae caterpillars when the host was a generalist species (i.e. fed on a higher number of host plant families, more frequently on herbaceous host plants compared with woody hosts, and in different habitat types). Additionally, green caterpillars with spines had a higher parasitism pressure compared with other body colors and design. Despite the meta-analysis may be limited and/or distorted by possible biases, this study can provide some useful clues improving the knowledge of the evolutionary ecology of the parasitoid-host relationship.

II.1. INTRODUCTION

Caterpillars of butterflies and moths are among the most common herbivores found in a great diversity of terrestrial ecosystems, where they are involved in several food webs (Stamp and Casey 1993). Lepidoptera caterpillars interact with both the bottom (such as their host plant) and the top (such as their predators) levels of the trophic relationship. Besides, among the diversity of predators (such as birds, lizards, spiders) and pathogens attacking these organisms, parasitoids represent an important mortality factor for butterflies (Dempster 1984, Hawkins et al. 1997), killing not only caterpillars but also eggs and pupae (only rarely adults are parasitized) (Shaw et al. 2009). They are then believed to strongly impact on the butterfly population dynamics, because of the associated caterpillar mortality (van Nouhuys and Hanski 2002b). The most frequent parasitoids attacking Lepidoptera caterpillars belong to Diptera (mostly Tachinidae) (Sheehan 1994, Gentry and Dyer 2002) and Hymenoptera (mainly Ichneumonidae and Braconidae) orders (Weseloh 1995, Shaw et al. 2009).

To counteract parasitoid attacks, caterpillars invest in defenses, including chemical, behavioral, morphological, and physiological means, or a combination of these (Gross 1993, Veldtman et al. 2007, Barbosa and Caldas 2007a). These defenses against natural enemies occur at three levels that are both spatially and temporally separated. The primary level consists of morphological (like coloration), ecological (like egg-laying strategies, number of generations per year and food plant specialization) and behavioral defenses (like shelter building and group feeding), that prevent enemies from encountering the caterpillars. Once the caterpillar has been detected or attacked, the secondary level of protection can be provided by additional morphological and behavioral characteristics such as hairs, spines, regurgitating, thrashing, or dropping. Tertiary defenses, analogous to an immune system, act after enemies have overcome the first two lines of

defense and include cellular and endocrine mechanisms to resist not only to parasitoids, but also to parasites and pathogens (Gross 1993).

Some of these defenses have already been invoked to explain the parasitoid species assemblage of hosts or the incidence of parasitism. For example, host feeding niche and ecological characteristics of hosts are correlated with parasitism rate and the richness of parasitoid assemblages (Hawkins and Lawton 1987, Mills 1993). Studies on this topic concerned either herbivores living in plant structures such as galls or leaf mines (Bailey et al. 2009), tropical Lepidoptera species (Gentry and Dyer 2002), or studies of the entire parasitoid community (Hawkins and Lawton 1987, Barbosa and Caldas 2007b). Here we go a step further by controlling for the phylogenetic diversity in the host-parasitoid relationship. We selected the Nymphalid butterflies as representative host species, because this is a well-known and diversified family of European butterflies with contrasted morphological and ecological characteristics. Besides, we chose the Braconidae as representative parasitoids because this family of Hymenoptera is taxonomically well delimited in Europe, and its relations with Nymphalid species are well known (Shaw et al. 2009).

We focused here on the relationship between the number of Braconidae parasitoid species attacking a Nymphalidae given host species. Our working hypotheses are the following:

1. The diversity of parasitoid species is related to the habitat range of the host. More generalist hosts and species with a larger distribution are expected to be parasitized by a larger number of wasp species.
2. Host species with a complex life history cycle (several generations a year, diapause at the egg or adult stages,...) will be attacked by a lower number of parasitoid wasps.
3. Given the cost of developing defense mechanisms, host species with cryptic caterpillar and particular body design will be parasitized by a lower number of parasitoid species.

To test these hypotheses, we built a literature-based database of host-parasitoid interactions, focusing on European Nymphalidae and Braconidae species. We scanned the existing literature about (1) the distribution and the niche breadth of the butterflies, (2) their life cycle, and (3) the morphological characteristics of the caterpillars. We then related these three groups of factors to the number of parasitoid species attacking caterpillars using a multi-model inference approach. Finally, both the relevance of the selected factors explaining the number of parasitoid species attacking caterpillars and the potential bias of this kind of studies are discussed.

II.2. MATERIAL AND METHODS

In a first step, we recorded all known relations involving a Braconid species as a parasitoid of the caterpillar stage of a Nymphalid butterfly. This information was extracted from Shaw et al. 2009, which is the most comprehensive publication on the subject to date. As this paper compiled all the information known in the literature, other sources of information would not give any extra information. In a second step, the incidence of parasitism relationships was quantified for each Nymphalidae and Braconidae species by the number of relations recorded between this species and Braconidae parasitoids or Nymphalidae hosts, respectively. In other words, each species was characterized by the number of times it is a host or a parasitoid.

Several factors have been considered to explain the variation in parasitoid species number among host (see Table II.1 for a summary). Here are presented all the factors considered and their associated hypotheses with regards to parasitism pressure; factors 1-3 relate to the distribution and the specialist *vs.* generalist character of the host butterfly species, factors 4-6 to

its life cycle, and factors 7-8 to the morphological characteristics of its caterpillars. Ecological data for each butterfly species were collected in Carter et al. 1988, Bink 1992, Tolman and Lewington 1999) and the Appendix 1 of the European Red List of Butterflies (Van Swaay et al. 2010). These eight factors are detailed below.

1) *Number of habitat types used by the butterfly host in Europe.* We summed up the different habitat types used by each butterfly species. Hosts occupying wider habitat types (generalist species) should be exposed to, and hence attacked by, a higher number of parasitoid species (Askew and Shaw 1986).

2) *Number of host plant families used by the butterfly host in Europe.* Butterflies feeding on a larger number of host plants should also be exposed to, and hence attacked by, a higher number of parasitoid species (De Moraes et al. 1998, Dyer and Gentry 1999, Gentry and Dyer 2002). This is reinforced by the fact that generalist parasitoids, due to their relative lack of plant-derived chemical defenses (Bernays and Graham 1988), may use more polyphagous hosts than specialist parasitoids.

3) *Architecture of host plant.* Larger or structurally more complex host plant species, such as shrubs or trees compared to herbaceous plants, support more phytophagous species (Askew and Shaw 1986, Hawkins and Lawton 1987, Dyer and Gentry 1999). This might result in a higher number of parasitoids species attacking caterpillars feeding on such species (Askew and Shaw 1986). Host plants used by Nymphalidae caterpillars were then classified as woody species (i.e. trees and shrubs) or herbaceous species and the percentage of herbaceous species used was calculated.

4) *Host egg spreading.* Nymphalidae females have different laying strategies: eggs are laid singly, in small clutches, or in big batches up to hundreds of eggs (Stamp 1980, Dennis 1992). Since parasitoids may locate more easily hosts that are grouped (Vinson 1998), gregarious caterpillars should be characterized by a higher number of parasitoid species.

5) *Number of butterfly generations per year.* Host species having more than one generation per year are expected to be in contact with a larger number of parasitoid species (Hawkins 1988). On the other hand, species with more than one generation per year are more likely to uncouple their life cycle from parasitoid attacks.

6) *Overwintering stage.* Braconidae wasps attack mostly hosts at the caterpillar stage, with most species being koinobiont endoparasitoids. Species overwintering at the caterpillar stage may offer longer opportunity to a higher number of parasitoids species to be in contact / to find their host.

7) *Host morphology.* Morphological defenses of the host, such as spines, may require specific adaptations of parasitoids. Parasitism of spiny caterpillars should then be restricted to a fewer number of adapted specialist parasitoids than parasitism of hairy and smooth ones.

8) *Host color.* Cryptic species may be more difficult to find than colorful ones. Caterpillar coloration has been proved to be important to explain their susceptibility to parasitism in some species (Barbosa and Caldas 2007c): those caterpillars that were not brown were more often parasitized. Here we used a three level classification: dark (brown and black caterpillars), green and other colors.

Table II.1. List of factors used as potential explanation of the number of parasitoid species attacking Nymphalidae species in Europe.

Variables used	Hypothesized relationship with the number of Braconidae species	Values or levels	Sources
Factors relating to the specialist vs generalist character of the host			
Number of habitat types	positive relationship	total number of habitat profiles	4
Number of host plant family	positive relationship	total number of host plant family	1,2
% of herbaceous species in the diet breadth	positive relationship	%	1,2
Factors relating to the life history traits of the host			
Egg spreading	egg mass > single	clutch, solitary	1,2
Number of generations per year	positive relationship	one, more than one	1,2
Overwintering stage	caterpillar > other	caterpillar, other (adult, egg and pupae)	1,2
Factors relating to the morphological characteristics of the host			
Morphology	spiny > smooth	spiny, smooth	1,3
Color of caterpillar body	light < dark	green, others colours (light) dark (brown, black)	1,2

1. *Ecologische Atlas van de dagvlinders van Noordwest-Europa* (Bink, 1992)

2. *Guide des papillons d'Europe et d'Afrique du Sud* (Tolman and Lewington, 1999)

3. *Guide des chenilles d'Europe* (Carter and Hargreaves, 1988)

4. Appendix 1 of the European Red List of Butterflies

A linear model (multinomial error distribution and cumlogit link) was used to regress the number of Braconidae parasitoid species attacking caterpillars of each Nymphalidae species (simplified in three classes: 1, 2 or ≥ 3) with the previously described factors. Due to the possible multicollinearity between the biological variables investigated, a multimodel inference approach, based on corrected Akaike's information criterion (AICc), was chosen (Burnham and Anderson 2002). With this method, the AICc value assessed the power of each model to explain existing variations in the number of Braconid species attacking Nymphalid species, and the relative importance of each described factor is quantified through its AICc weight. Explanatory factors with a AICc weight above 60% were considered as having a significant effect the number of Braconid species attacking caterpillars of each Nymphalidae species. The analyses were realized with SAS software (www.sas.com).

II.3. RESULTS

We found that 36 Nymphalidae species (among the 211 species found in Europe, Van Swaay et al. 2010) were attacked by 23 Braconidae species, in a total of 54 relations (Annex II.1). Braconidae wasps attacking Nymphalidae caterpillars were grouped in 8 genera. The *Cotesia* genus was the most present; *Cotesia melitaearum*, the most generalist species parasitized 10 different butterfly species. The distribution of the number of parasitoid species per host is shown in Figure II.1. Most Nymphalidae species were parasitized by one parasitoid species; only one (*Maniola jurtina*) was parasitized by 5 wasp species. There is no relation between the number of parasitoid species that attacked a given host species and the

number of hosts that these parasitoid species are able to attack (Spearman rank correlation: $R = 0.003$, $n = 54$, $p = 0.99$).

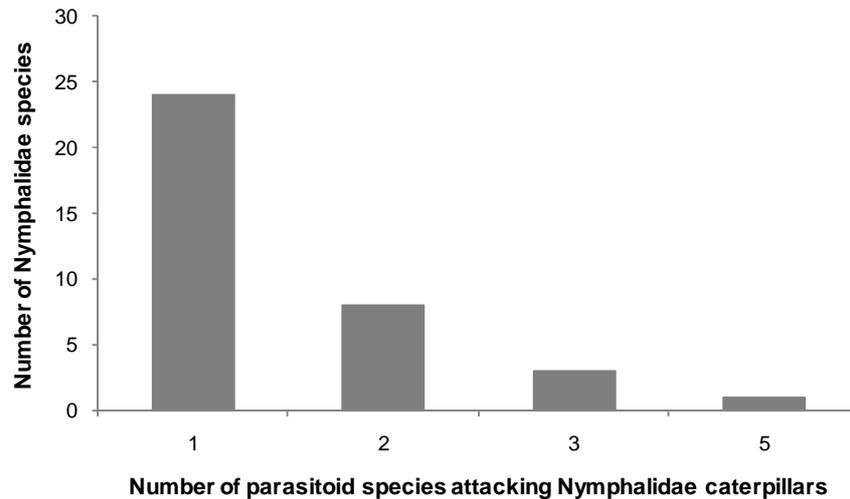


Figure II.1. Frequency distribution of the number of host-parasitoid relationships according to the number of Braconid species attacking each Nymphalidae caterpillar host.

Several factors explained why some Nymphalidae species were attacked by more Braconidae wasp species than others (Table II.2). Morphological characteristics of the caterpillars and factors relating to the specialist vs generalist character of the host were the most relevant to explain the number of Braconidae species attacking Nymphalid hosts. Factors relating to the life history traits of the host were weakly related to the parasitoid species number. Thus, a higher number of Braconidae wasps were found for (1) green caterpillars, (2) with spines on their body, (3) with a broader diet, (4) feeding preferentially on herbaceous compared to woody host plant, and (5) with a wider habitat profiles.

Table II.2. Results of the multimodel inference on the influence of caterpillar body characteristics and ecological factors) on the number of Braconidae wasps attacking Nymphalidae caterpillars. AICc weight represents the relative importance of the descriptor in explaining variation in the response variables (see text for details). Descriptors with weight > 60% are shown in bold for easier reading.

Variables	Modality	Variable weight	Parameter estimate (±SEM)
Number of habitat profiles		68.82%	0.992 (±0.6)
Number of host plant family		87.23%	1.408 (±0.696)
% of herbaceous species in the diet breadth		86.18%	7.793 (±4.341)
Morphology	spiny	92.61%	.
	smooth	.	-10.198 (±5.517)
Host color	dark	85.76%	-9.419 (±4.634)
	green	.	.
	other	.	-6.19 (±4.127)
Host cluchness	gregarious	15.87%	0.059 (±0.212)
	solitary	.	.
Number of host generation	one	40.89%	.
	more than one	.	-0.735 (±0.67)
Overwintering stage	caterpillar	18.82%	.
	other	.	-0.18 (±0.349)

II.4. DISCUSSION

The basic hypothesis that generalist hosts species are more parasitized than specialists is strongly supported in our analysis. Rather than segregating species in specialists *vs* generalists on a subjective basis, here we used three different criteria to relate the niche breadth to the number of parasitoid species. Firstly, host species living in many habitat types were parasitized by a larger number of parasitoid species. Secondly, host species that feed on a wide variety of plants were attacked by a larger number of Braconidae species. Thirdly, we found evidence that more Braconidae parasitoid species attacked hosts feeding on herbaceous plants than those feeding on woody plants. These three criteria are obviously not independent: oligo- or polyphagous butterflies are more prone to live in different habitat types simply because they are able to feed on different host plants, whereas butterflies feeding on woody host plants are restricted to forested habitats. However, these three criteria provide complementary insights on the specialist-generalist continuum, and their convergence as well as their strong support in the inference model selection approach clearly reinforces the conclusion that generalist hosts and species with a larger distribution (i.e. living in more habitat types) are expected to be parasitized by a larger number of wasp species. In a patchy landscape, generalist species living in different habitat types are clearly more abundant than specialists at the landscape scale. Accordingly, our finding corresponds to the general trend documented in the literature on host-parasite relationship, abundant hosts usually being attacked by richer parasite faunas (e.g. Vazquez et al. 2005).

Two different evolutionary processes acting on the host and on the parasitoid respectively could contribute to generate this pattern: according to the enemy-free space hypothesis, hosts could tend to exploit new habitats to escape the parasitism pressure, whereas according to the Red Queen hypothesis, parasites will preferentially specialize on the most abundant host

species (Poulin et al. 2000). Altogether, these two processes could also explain the lack of specificity of parasitoids attacking generalist host species: given the diversity and abundance of their potential hosts, these parasitoids have no advantage to evolve specialized strategies. This result contradicts the situation reported on real parasites (referring to organisms benefiting at the expense of their host), where parasite species attacking those host species that harbors a richer parasite fauna are more often species-specific (Vazquez et al. 2005). Competition between parasites sharing the same host at the same time, which is obviously ineffective among parasitoids, may explain this difference.

None of the factors relating to the life history traits of the host explained the number of Braconidae wasps attacking Nymphalidae caterpillars in our meta-analysis. Richness of the parasitoid fauna was not explained by the host egg spreading, solitary and gregarious caterpillars having the same risk to be parasitized by one or several wasps. Solitary caterpillars may be more difficult to detect than gregarious ones. However, even if colonial webs formed by caterpillars may enhance detection by parasitoids, it could also provide protection (Weseloh 1995). For example, parasitoids attacking *Euphydryas phaeton* caterpillars usually stay on the outside of the web, attacking caterpillars on the surface or those that can be reached by probing into the webbing (Stamp and Bowers 1988, Gross 1993). Aggregation may increase the efficacy of a defense by increasing its magnitude, such as more stinging hairs or a larger discharge of a chemical defense. Gregarious caterpillars may also increase their feeding efficiency reducing their development time and thus reducing the window of vulnerability to natural enemies (Clark and Faeth 1997, Stefanescu et al. 2009).

The lack of relation between host life cycles and the richness of their parasitoid fauna was unexpected. This might result from a long coevolution of the host-parasitoid relationship, which has selected for a perfect match

between the life cycles of both partners. The detailed study of the parasitism of *Apanteles bignellii* on *Euphydryas aurinia* indeed revealed that up to three regular generations of the parasitoids occur in one generation of the host (Porter 1983).

Spiny caterpillars support a larger richness of parasitoid fauna. It seems therefore that spines on caterpillars are not an efficient physical barrier to prevent parasitism, even if those of many checkerspot caterpillars (*Euphydryas sp.*) have been shown to be long enough to prevent oviposition by a common parasitoid (*Apanteles euphydryides*) (Gross 1993). As spiny caterpillars have a reduced susceptibility to predators (Dyer 1997), parasitoids can have a selective advantage to use these hosts with a lower probability of predator attack. Indeed, endoparasitoids use their host for a large portion of their life cycle; therefore, a host protected from predation may represent enemy-free space for parasitoids (Veldtman et al. 2007). Besides, spiny caterpillars are often associated with other characteristics like the production of conspicuous damage to host plant leaves that make caterpillars more apparent to generalist parasitoids, whereas smooth species tend to be solitary and produce inconspicuous damage (Gross 1993).

Insect color is more commonly associated with defense against parasitoids. Indeed, previous studies (Barbosa and Caldas 2007a) showed that caterpillar color had the greatest influence on susceptibility to parasitism; green caterpillars having the highest level of parasitism. We show here that green caterpillars face also a higher diversity of parasitoid species. Melanic caterpillars could be more resistant to both ecto- and endoparasitoids. Indeed, melanism is associated with elevated phenoloxidase activity, which in the haemolymph is associated with greater capacity to encapsulate and/or to melanize foreign objects, including parasites and parasitoids that enter the haemocoel (Wilson et al. 2001). In this study, the color classification of caterpillar species has been based on the human vision only (Higginson and Ruxton 2010). Further investigations are needed to

consider the whole visual spectrum of Hymenoptera wasps, such as ultra violet colors (Briscoe and Chittka 2001, Desouhant et al. 2010).

Several biases can distort and/or limit the significance of our analysis. First of all, the number of host-parasitoid relations is likely underestimated. All Nymphalidae species listed in the database have not been studied with the same intensity. As increasing the geographical range over which a study is conducted often directly increases the number of observed host-parasitoid relationships, the number of Braconidae wasps parasitizing Nymphalidae species may be underestimated. Such effects of sample size have been reported in other host-parasitoid community species (Memmott et al. 2000). Moreover, although it is well recognized that generalist host species are heavily parasitized, we should account for this result cautiously. Indeed, one host species can be parasitized on only one of its host plant or in one habitat type, while in this study we have considered its complete host plant range (Weseloh 1995). van Nouhuys and Hanski (1999) showed that *C. melitaeorum*, a specialist parasitoid of *M. cinxia* in Aland, successfully parasitizes those caterpillar groups that fed on *Veronica spicata* more often than those that fed on *Plantago lanceolata*. Moreover, interspecific competition between parasitoids frequently occurred (Stefanescu et al. 2009, van Nouhuys and Punju 2010), and can lead to the exclusion of one of the partners involved in the relation. Furthermore, the susceptibility of one species to parasitism can be influenced directly or indirectly by the environment such as the presence of other herbivore species, different interactions in the community, or the spatial structure of the habitat (Barbosa et al. 2007, Barbosa and Caldas 2007a, Bergerot et al. 2010). Finally, as we have no data on the parasitism rate inflicted by Braconid wasps on their host, species attacked by a smaller number of parasitoid species may in reality suffer from higher parasitism rate. However, despite this cautionary tale, we think that our study provides useful clues that can improve the understanding of the evolutionary ecology

of the parasitoid-host relationship. We had *a priori* decided to restrict our analysis to European Nymphalid butterflies and their parasitoids. However, it should be relatively simple to apply the same methodology to other study systems.

ANNEX II.1.

List of host-parasitoid relationships implying caterpillars of a Nymphalidae butterfly as host and a Braconidae wasp as parasitoid.

Butterfly genus	Butterfly species	Parasitoid genus	Parasitoid species
<i>Aglais</i>	<i>urticae</i>	<i>Cotesia</i>	<i>vanessae</i>
<i>Aglais</i>	<i>urticae</i>	<i>Cotesia</i>	<i>vestalis</i>
<i>Apatura</i>	<i>ilia</i>	<i>Psilomastrax</i>	<i>pyramidalis</i>
<i>Apatura</i>	<i>iris</i>	<i>Psilomastrax</i>	<i>pyramidalis</i>
<i>Argynnis</i>	<i>adippe</i>	<i>Cotesia</i>	<i>addippevora</i>
<i>Argynnis</i>	<i>aglaja</i>	<i>Cotesia</i>	<i>selenevora</i>
<i>Boloria</i>	<i>eunomia</i>	<i>Cotesia</i>	<i>eunomia</i>
<i>Boloria</i>	<i>selene</i>	<i>Cotesia</i>	<i>selenevora</i>
<i>Charaxes</i>	<i>jasius</i>	<i>Meteorus</i>	<i>pulchicornis</i>
<i>Coenonympha</i>	<i>oedippus</i>	<i>Diolcogaster</i>	<i>abdominalis</i>
<i>Coenonympha</i>	<i>pamphilus</i>	<i>Aleoides</i>	<i>coxalis</i>
<i>Coenonympha</i>	<i>tullia</i>	<i>Diolcogaster</i>	<i>abdominalis</i>
<i>Coenonympha</i>	<i>tullia</i>	<i>Aleoides</i>	<i>coxalis</i>
<i>Erebia</i>	<i>aethiops</i>	<i>Cotesia</i>	<i>tetrica</i>
<i>Euphydryas</i>	<i>aurinia</i>	<i>Cotesia</i>	<i>bignellii</i>
<i>Euphydryas</i>	<i>aurinia</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Euphydryas</i>	<i>desfontainii</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Euphydryas</i>	<i>matura</i>	<i>Cotesia</i>	<i>acuminata</i>
<i>Euphydryas</i>	<i>matura</i>	<i>Cotesia</i>	<i>bignellii</i>
<i>Euphydryas</i>	<i>matura</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Hipparchia</i>	<i>semele</i>	<i>Cotesia</i>	<i>vestalis</i>
<i>Lasiommata</i>	<i>maera</i>	<i>Cotesia</i>	<i>tetrica</i>
<i>Limnitis</i>	<i>camilla</i>	<i>Meteorus</i>	<i>colon</i>
<i>Limnitis</i>	<i>camilla</i>	<i>Cotesia</i>	<i>sibyllarum</i>
<i>Limnitis</i>	<i>populi</i>	<i>Glyptapanteles</i>	<i>vitripennis</i>
<i>Limnitis</i>	<i>populi</i>	<i>Protapanteles</i>	<i>sp.</i>
<i>Limnitis</i>	<i>reducta</i>	<i>Cotesia</i>	<i>sibyllarum</i>

Butterfly genus	Butterfly species	Parasitoid genus	Parasitoid species
<i>Maniola</i>	<i>jurtina</i>	<i>Meteorus</i>	<i>versicolor</i>
<i>Maniola</i>	<i>jurtina</i>	<i>Cotesia</i>	<i>tetrica</i>
<i>Maniola</i>	<i>jurtina</i>	<i>Cotesia</i>	<i>tibialis</i>
<i>Maniola</i>	<i>jurtina</i>	<i>Cotesia</i>	<i>vestalis</i>
<i>Maniola</i>	<i>jurtina</i>	<i>Aleoides</i>	<i>coxalis</i>
<i>Melanargia</i>	<i>lachesis</i>	<i>Aleoides</i>	<i>coxalis</i>
<i>Melitaea</i>	<i>athalia</i>	<i>Cotesia</i>	<i>acuminata</i>
<i>Melitaea</i>	<i>athalia</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Melitaea</i>	<i>cinxia</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Melitaea</i>	<i>deione</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Melitaea</i>	<i>diamina</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Melitaea</i>	<i>didyma</i>	<i>Cotesia</i>	<i>acuminata</i>
<i>Melitaea</i>	<i>didyma</i>	<i>Cotesia</i>	<i>lycophron</i>
<i>Melitaea</i>	<i>didyma</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Melitaea</i>	<i>parthenoides</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Melitaea</i>	<i>phoebe</i>	<i>Cotesia</i>	<i>acuminata</i>
<i>Melitaea</i>	<i>trivia</i>	<i>Cotesia</i>	<i>lycophron</i>
<i>Melitaea</i>	<i>trivia</i>	<i>Cotesia</i>	<i>melitaeorum</i>
<i>Nymphalis</i>	<i>polychloros</i>	<i>Cotesia</i>	<i>vestalis</i>
<i>Pararge</i>	<i>aegeria</i>	<i>Protapanteles</i>	<i>incertus</i>
<i>Polygonia</i>	<i>c-album</i>	<i>Microgaster</i>	<i>subcompletus</i>
<i>Pyronia</i>	<i>tithonus</i>	<i>Cotesia</i>	<i>tibialis</i>
<i>Vanessa</i>	<i>atalanta</i>	<i>Cotesia</i>	<i>vanessae</i>
<i>Vanessa</i>	<i>atalanta</i>	<i>Microgaster</i>	<i>nixalebion</i>
<i>Vanessa</i>	<i>atalanta</i>	<i>Microgaster</i>	<i>subcompletus</i>
<i>Vanessa</i>	<i>cardui</i>	<i>Cotesia</i>	<i>vanessae</i>
<i>Vanessa</i>	<i>cardui</i>	<i>Cotesia</i>	<i>vestalis</i>

A STUDY CASE: RELATING OUR FIELD OBSERVATION TO THE GENERAL PATTERN DESCRIBED USING THE META-ANALYSIS

Our long term study of two peat bog Nymphalidae in Belgium (*B. eunomia* and *B. aquilonaris*) (e.g. Schtickzelle and Baguette 2004, Turlure et al. 2009) has revealed that last instar caterpillars are parasitized only in *B. eunomia*. Such a finding raised the question as to why two closely related Nymphalidae species present such a divergence. Using results and conclusions brought by the analysis presented previously, we here focused on this question.

Caterpillars of the two species were sampled in peat bogs of two Belgian highlands: the Plateau des Tailles and the Plateau de Recogne (Fig. II.2). All habitat patches were sampled by visual inspection to collect caterpillars. In the Plateau des Tailles, *B. eunomia* was collected in the Pisserotte peat bog and *B. aquilonaris* in six different nature reserves (Fig. II.2). In the Plateau de Recogne, the two species were sampled in the same peat bog: The Troufferies de Libin (Fig. II.2). Table II.3 summarizes the number of collected caterpillars for the two Nymphalidae species as well as their status (parasitized vs pupated) according to the sampled site and the year.

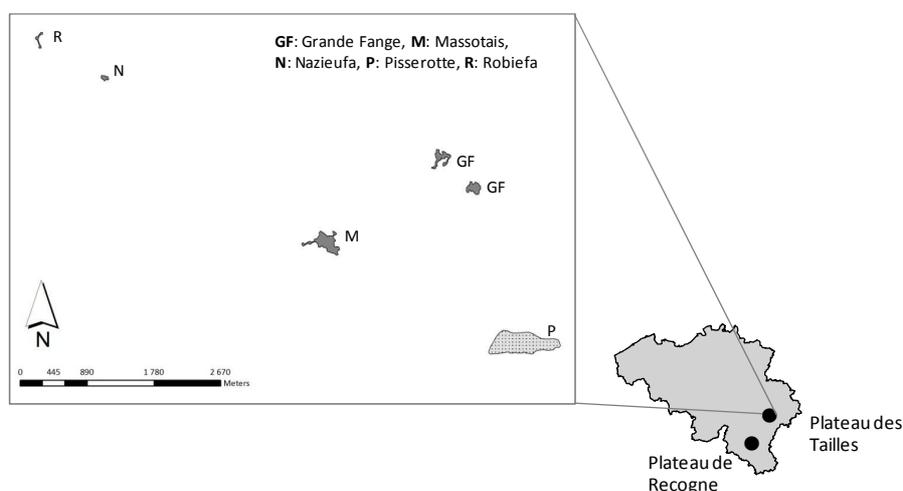


Figure II.2. Location in Belgium of the two sampled highlands: The Plateau des Tailles and the Plateau de Recogne. Inserts represents sampled sites in the Plateau des Tailles: *B. eunomia* caterpillars were sampled in one site (Pisserotte) while *B. aquilonaris* caterpillars were sampled in all the other sites. In the Troufferies de Libin, in the Plateau de Recogne, the two species were sampled.

Table II.3. Number of collected caterpillars for the two Nymphalidae butterfly species studied according to year and sample site. *B. eunomia* was the only species for which parasitism was observed.

Species	Sites	Caterpillars status	2005	2006	2008	2009
<i>B. eunomia</i>	Libin	parasitized	.	31	.	.
		pupated	.	82	.	.
		dead for unknown reasons	.	7	.	.
	Pisserotte	parasitized	76	112	9	60
		pupated	25	18	16	91
		dead for unknown reasons	1	17	1	19
<i>B. aquilonaris</i>	Libin		.	35	5	.
	Pisserotte		45	40	19	.
	Grande Fange		.	.	38	.
	Massotais		.	.	5	.
	Nazieufa		.	.	10	.
	Robiefa		.	.	2	.

Caterpillars collected in the field were brought to the lab and reared individually in Petri dishes (outdoor temperature and light fluctuations). Every two days, faeces and old plants were removed and new host plants added to ensure caterpillars were fed *ad libitum*. This was done until unparasitized caterpillars pupate, and parasitoid larvae emerge from parasitized caterpillars. When parasitoid larvae emerged from the caterpillar, they were placed in a laboratory chamber (temperature: 20°C, photoperiod L:D 16h:8h) for pupation. Some adult parasitoids were kept for identification, while the others were released in their site of origin, as were adult butterflies.

Whatever the study site and year (table II.3), larval parasitism has been observed in each case (all sites all years) for *B. eunomia*, but never for *B. aquilonaris*.

Our primary hypothesis was that *B. aquilonaris* caterpillars were not parasitized because of the female egg-laying strategy. Indeed, *B. aquilonaris* females lay eggs singly, contrary to *B. eunomia* ones which lay eggs in small clutch. As mentioned before by the database analysis results, it was unlikely that the host egg spreading can determine the number of Braconidae species attacking a Nymphalidae butterfly. Moreover, by the sample of 16 caterpillars (all years and sites considered) of *Boloria selene*, another related and sympatric species that lays eggs singly and whose caterpillars have a solitary life strategy, we observed that they were parasitized by *C. selenovera*, a Braconidae wasp. This example strengthens the database analysis results. Therefore, additional hypotheses need to be exposed to explain why *B. aquilonaris* caterpillars have until now never been observed to be unparasitized:

1. Since we collected only the last instar caterpillars, the species can be parasitized during the previous caterpillar stage. Moreover, all *B. aquilonaris* caterpillars were sampled in small and/or completely isolated populations. Since parasitoid species can be affected by the small size and/or the isolation of their host populations, this could explain why no parasitized *B. aquilonaris* caterpillars were detected. It is now necessary to collect caterpillars from larger populations in a meta-population complex to validate or refute this observation.

2. Caterpillars may minimize the risk of predation by avoiding encounter with parasitoids. *B. aquilonaris* caterpillars live in *Sphagnum* hummocks providing optimal cold temperature conditions (Turlure et al. 2010a). They might find both direct and indirect ways to escape parasitism by sheltering in this vegetation structure (i.e. through behavioral adaptations). Indeed, cold environment might prevent female parasitoids to reach *B. aquilonaris* caterpillars (Fink and Volkl 1995).

3. The insect immune system is also a key defense against parasitoid attack (Gross 1993). Incompatible hosts often eliminate parasitoids by encapsulation, a process in which hemocytes form a multilayered envelope around the invading organism (Smilanich et al. 2009). *B. aquilonaris* caterpillars could use encapsulation of larval parasitoid killing this parasitoid before its development.

4. A last hypothesis can be related to the spatial distribution of populations of this relict species in Western Europe. *B. aquilonaris* is restricted to active peat bogs with *Vaccinium oxycoccos*, the only host-plant of caterpillars. Following the natural distribution of peat bogs in the Tailles highlands, populations of this butterfly are fragmented. Such spatial configuration can impede negatively parasitoid species (Tschardt et al. 2002a).

Of course, these four hypotheses are not mutually exclusive. If several are true, our chance to find any parasitoid in this species will be

considerably reduced. Furthermore, in the rich and complex living world, it seems nearly impossible that a species is not parasitized at all.

PART TWO

**HOST PARASITOID RELATIONSHIP:
FROM THE PARASITOID SIDE**

CHAPTER III _____

**OLFACTORY RESPONSES OF *COTESIA EUNOMIAE* TO
VARIOUS HOST-ASSOCIATED VOLATILES.**

I am grateful to Victoria Radchuck for her help and her corrections.

ABSTRACT

Behavioral events leading to successful parasitism include host habitat location, host location, host acceptance and suitability. Infochemicals from lower trophic levels is considered essential in the foraging behavior of female wasps. By studying the system consisting in a parasitoid, *Cotesia eunomiae*, and its host, *Boloria eunomia* caterpillars, which fed exclusively on *Persicaria bistorta* leaves, we aim to determine which trophic level is more attractive for female wasps to locate host habitat and hosts. We tested three categories of infochemicals, previously proved as significant volatiles odors to allow parasitoids to locate their host: 1) infochemicals emanated from the host itself and from the host products (faeces), 2) infochemicals released from the damaged host plant and 3) both infochemicals enunciated previously together. With our experimental design (Y-tube olfactometer), none was significantly more attractive for *C. eunomiae* females. It seems however that females orientated toward infochemicals from the host itself or the host by-products more rapidly than when volatiles emanated from the damaged host plant leaves. However, the low number of experiments did not allow us to conclude definitively on the non detection of an effective infochemical. Thus, the no attractiveness of wasps towards mechanically damaged leaves could be explained by the capacity of *Cotesia* females to recognize infochemicals released by mechanically damaged vs eaten leaves. Moreover, frass quantity may be too low to activate reaction. Finally, since host habitat and host location arrived mainly during parasitoid flight, our experimental design may be inappropriate for such study. Other experiments are discussed.

III.1. INTRODUCTION

The survival of parasitoids is greatly dependent on the overlap with the ecological niche of their hosts in terms of both time and space. To insure such coincidence, parasitoids need not only to synchronize their development with their hosts but also to locate and to identify them (Lawrence 1981). Behavioral events leading to successful parasitism include host habitat location, host location, host acceptance and suitability (Vinson 1976). Parasitoid wasps that forage for herbivorous hosts evolved within multitrophic systems. In such context, information from lower trophic levels is considered essential in their foraging behavior (Vet et al. 1991). Hymenoptera parasitoids are known to use a variety of infochemicals to search for hosts but also to locate food or mates (Vinson 1976, Turlings and Tumlinson 1991, Turlings et al. 1991, Godfray 1994). Several odors have already been discovered and proved to be efficient for host finding and recognition by female parasitoids. Thus, female parasitoids can orientate toward chemicals released by their host (e.g. cuticular hydrocarbons, pheromones) or its products (faeces, silk, exuviae), by the host's food plant (volatiles induced by feeding or oviposition), or by organisms associated with the host presence (bacteria, fungi) (Vet and Dicke 1992, Cortesero et al. 1993, Steidle and van Loon 2003, Steiner et al. 2007). Two types of stimuli, involved in host habitat and host location, can be distinguished depending on the source and area of their impact on parasitoids (Vinson 1976). Volatile allelochemical substances emitted by the host plant are effective on long distances (larger area of impact). Indeed, due to the relatively large biomass of the host plant, such stimuli are usually readily available, allowing the parasitoid to move through the habitat of their host. Nevertheless, they are less reliable predictors of host presence and suitability (Vet et al. 1991). This is why at shorter distances, information from the host itself, often characterized by a low detectability but a high reliability on the presence,

identity, density and suitability of the host, becomes increasingly important (Vet et al. 1991).

Infochemicals play an important role in the interactions between organisms, therefore a full understanding of the sensitivity with which a wasp responds to various volatiles associated with the lower trophic levels would contribute to a better knowledge of the tri-trophic interaction between a parasitoid, its host and the host plant (van Nouhuys and Kaartinen 2007) and at a larger scale of the evolution and functioning of ecological food webs (Steidle and van Loon 2003). In a biological control context, this information proved to be crucial for the design of programs that use parasitoids and predators as biological control agents (NgiSong and Overholt 1997, Reddy et al. 2002, Steidle and van Loon 2003). Here, the studied system consists of a *Cotesia* wasp, *Cotesia eunomiae*, specialized on *Boloria eunomia* caterpillars, which feed exclusively, at least in the Belgium sites, on *Persicaria bistorta* leaves. In 1992, Vet and Dicke formulated the concept of dietary specialization and infochemical use in natural enemies. According to this classification, our system belongs to the highly specialized group (as many other relationships involving parasitoid species), in which the parasitoid species is specialist at the host level (according to MR Shaw) and the host is itself specialist at the host plant level. Such type of specialist species should use highly specific cues (Steidle and van Loon 2003) to be able to find a host.

In this preliminary study, we tested three categories of infochemicals, previously proved as significant volatiles odors, which allow parasitoids to locate their host. Firstly, infochemicals emanated from the host itself and from the host products (faeces) were tested. Secondly, parasitoid females were confronted with infochemicals released from the damaged host plant leaves. And finally, host plant with hosts were placed together to detect if this infochemical was relevant for *C. eunomiae* wasps. Since all of these odors are supposed to provoke a reaction in parasitoid females, this

experiment aims to determine that one of these odors is significantly attractive for host location.

III.2. MATERIAL AND METHODS

The wasp

- Natural history

The wasp *C. eunomia* (Hymenoptera: Braconidae) is a koinobiont gregarious endoparasitoid attacking at least the last caterpillar instar of the bog fritillary butterfly. Its life cycle is still not well known, but according to the description made by Shaw and colleagues (Shaw et al. 2009) the studied species could have successive generations on a single host generation, as other *Cotesia*. Preliminary experiments, conducted to test if parasitoid females accept young instar caterpillars (first or second) to lay eggs, revealed that it is, indeed, the case (Annex III.1.). Therefore, for the experimental design we hypothesized that the first caterpillar instars represent suitable hosts for *C. eunomia*.

- Wasp collection and rearing

A total of 166 *B. eunomia* caterpillars collected in a peat bog within the Fange de Pisserotte nature reserve (S-E Belgium, 50°13'N, 5°47'E) during spring 2009 were brought to the lab and reared until the parasitoid emergence or the pupation time. All parasitoid yellow cocoons were placed in cages in a laboratory room permitting their development (temperature: 20°C, photoperiod L:D 16h:8h). Adults of *C. eunomia* emerged 15 days after their pupation. Wasps were collected from the emergence cages for training and deposited in a climate room (temperature: 8°C, photoperiod L:D

16h:8h) to prevent premature mortality events due to an excess of activity. All wasps which emerged the same day from one cage were placed together to allow for mating. They were supplied with a 20% honey-water solution. Females used for experiments were assumed to be mated (since mating was allowed) (Gross 1993) and without previous oviposition experience.

Olfactometric device

Experiments were conducted in a dual-choice Y-tube olfactometer made of transparent glass (0.5cm diameter) with each arm connected to a glass reservoir holding the odor source (Fig. III.1). Air stream was generated by a pure air connected to a flowmeter, the flow rate was fixed to 5ml/min. Air was passing through a filter before entering the olfactometer. The olfactometer consisted of a glass tube divided into 5 zones (Fig. III.1): zone 1 (Z1), 3cm long, consists of the female entrance area; zones 2 and 4 (Z2, Z4), 10.5cm long, are two arms leading to reservoirs (Z3 and Z5) 3.5cm diameter, where the odor source was placed. The device was illuminated from beneath with uniform light. After each run, the olfactometer was disconnected from the air flux and thoroughly washed with water, rinsed with 70% ethanol and dried.

The female was placed individually in zone 1 of the olfactometer and a flow of pure air entered through the two opposite ends. Each insect was allowed to make a choice during 10min and each was used only once for each treatment. The choice (reservoir with odor *vs* reservoir with no odor) and the time that females spent in each zone were recorded. A choice was considered to be made when the female wasp was entering in a reservoir.

The position of the odor-emitting substance in container was inverted between each test to avoid effects of any directional bias, in case wasps would preferentially reject the right or left arm for external reasons.

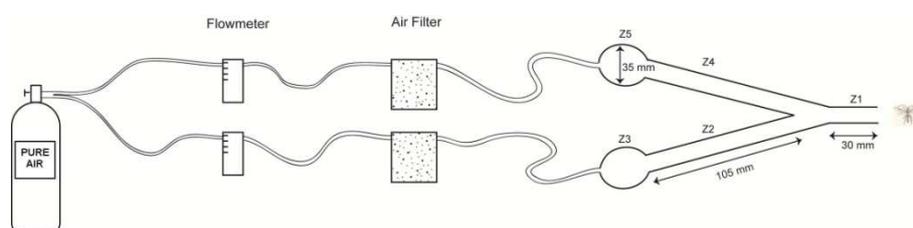


Figure III.1. Olfactometric device. Z1, Z2, Z3, Z4 and Z5: delimited zones. Z3 and Z5: two reservoirs.

Sources of odors

To examine choice preference, four odor sources were tested in a dual choice experiment. We always tested one odor source put in one reservoir *vs* nothing in the second. The first two treatments corresponded to infochemicals emanated from the host itself:

- Treatment A: 5 *B. eunomia* caterpillars of the first or second instar *vs* nothing,
- Treatment B: caterpillar frass (150mg) *vs* nothing.

Infochemicals from damaged host plant were tested in the third treatment:

- Treatment C: mechanically damaged *P. bistorta* leaves *vs* nothing.

And finally, the last treatment combined infochemicals coming from both the host and the host plant:

- Treatment D: caterpillars and not mechanically damaged *P. bistorta* leaves *vs* nothing.

Odors obtention

In June 2009, 11 butterfly females were captured in the field and allowed to lay eggs under controlled conditions (females were placed individually in

cages, which were put outside at the field station in similar weather conditions than the field ones). Eggs were collected daily, and placed in Petri dishes. We noted the hatching time and followed caterpillar development. Young caterpillars were reared with *ad libitum* access to fresh and young host plant leaves, in natural light and temperature conditions. Frass produced by caterpillars fed on *P. bistorta* were collected the same day when the experiment using this odor source was performed.

Host plant leaves (young leaves as required by caterpillars) were collected in the field. We experimentally mimicked the feeding activities of caterpillar on bistort leaves by scratching the leaf surface with a scalpel to emanate the volatiles from the host plant. A piece of 1cm² was used for each replicate of C and D treatments.

Statistical analysis

For each treatment, the choice made by females (i.e. going into odor or no odor reservoir), the time spent to make the choice (in sec.), the time spent in each arm of the olfactometer (in sec.) and the number of roundtrips (comings and goings of females in olfactometer arms) were recorded. Results from the dual choice tests were compared using χ^2 test. The effect of odor source on the time spent to make the choice was analyzed with one way ANOVA, with experiment as our explanatory variable and time as the dependent one. The percent of time spent in the arm of choice was analyzed with one way ANOVA. Analyses were realized using SAS software (www.sas.com).

The choice made by parasitoid females was regressed with the treatment, the time spent to make the choice, the number of roundtrips and the percent of time spent in the arm of choice, to assess which factors explained this choice. Due to multicollinearity between the variables investigated, a multimodel inference approach, based on corrected Akaike's information criterion (AICc), was chosen (Burnham and Anderson 2002). A

binomial distribution with logit link function was used to model the choice made by parasitoid females (odor source vs no odor source). This approach was also used to analyse the time spent to make the choice with the treatment, the number of roundtrips and the choice made by parasitoid females as explanatory variables. A normal distribution with identity link function was used for modelling this variable.

III.3. RESULTS

Among the 166 collected *B. eunomia* caterpillars, 60 were parasitized. On average 18.6 ± 4.7 (min = 2, max = 56) parasitoid larvae successfully emerged from their host and spinned a yellow cocoon. Due to a low emergence rate (5.6%), only 35 parasitoid females were tested. Moreover, due to some technical problems and time delay between emergence of parasitoid adults and young host caterpillars, in total only 62 replicates were performed (Table III.1) in total. Overall, 24 females were directed toward infochemicals, 25 toward nothing and 13 made no choice (i.e. after 10min they did not reached a reservoir).

Table III.1. Results of dual test choice, for the four treatments, realized with *Cotesia eunomiae* females.

	Choice made by <i>C. eunomiae</i> females		
	odor source	no odor source	no choice
A. <i>B. eunomia</i> caterpillars vs Nothing	10	11	5
B. Frass vs Nothing	5	2	4
C. Damaged <i>P. bistorta</i> leaves vs Nothing	6	9	3
D. <i>P. bistorta</i> leaves + <i>B.</i> <i>eunomia</i> caterpillars vs Nothing	3	3	1

For all tested combinations, there was no difference between the effect of odor source on the female choice (χ^2 test, $p > 0.05$).

Female choice was best explained by 1) the percentage of time spent in the arm of choice (AICc weight = 99.9%) and 2) the number of roundtrips (AICc weight = 96.1%). Thus, females went more frequently towards tested odor when they spent longer time in the arm of choice and when they made few roundtrips.

The final time to make a choice was not statistically different according to the treatment (One-Way ANOVA, $F_{3, 45} = 0.09$, $p = 0.9618$). It was only influenced by the number of roundtrips (AICc weight = 98.2%), not surprisingly: the more females made back and forth movements, the longer the decision time is. No difference in the final time to make a choice towards odor source (One-Way ANOVA, $F_{3, 20} = 0.81$, $p = 0.5018$) or towards reservoir with nothing (One-Way ANOVA, $F_{3, 21} = 1.09$, $p = 0.373$) were observed between experiments (Fig. III.2).

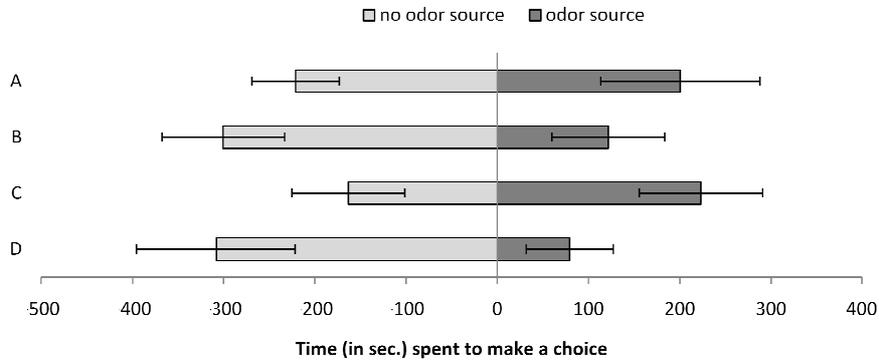


Figure III.2. Response of *Cotesia eunomiae* in a Y-tube olfactometer to four different treatment. The X axis indicates the mean time (in seconds) (\pm SEM) for female to make a choice (either toward odor source or no odor source).

Moreover, for all treatments, there was no statistical difference in the percent of time spent in the arm of choice (One-Way ANOVA, $F_{3, 45} = 2.41$, $p = 0.0792$). Finally, even not statistically significant, we observed that parasitoid females tend to spend less time in the arm leading to the odor tested when the infochemicals emanated from the mechanically damaged leaves (Fig. III.3).

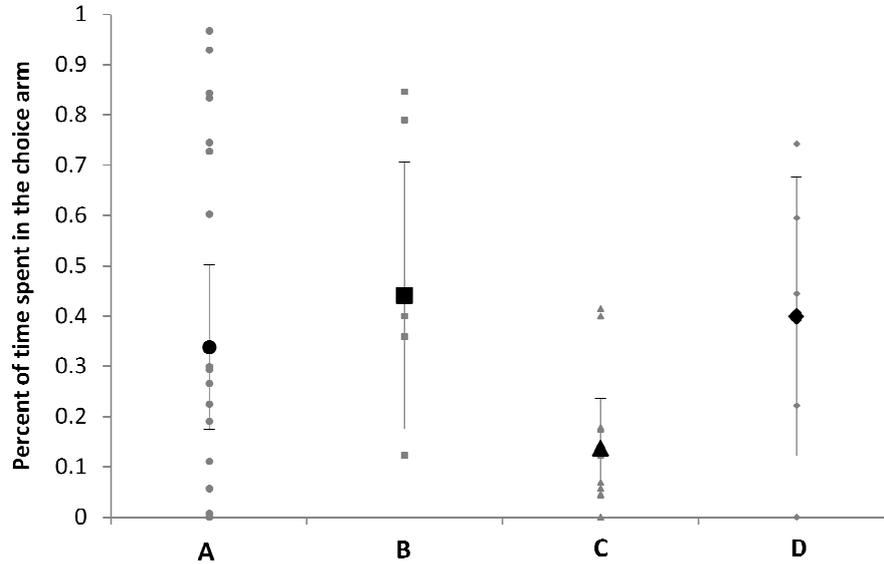


Figure III.3. Mean percent of time spent in the arm of the olfactometer with at its end the odor source (the arm of choice) for each treatment tested. Treatment A (circle): *B. eunomia* caterpillars odor; treatment B (square): caterpillar frass; treatment C (triangle): damaged *P. bistorta* leaves; treatment D (diamond): both caterpillars with host plant leaves. Grey and small symbols correspond for each treatment to the observed time. Bigger and darker symbols correspond to the mean time for each treatment with standard deviation.

III.4. DISCUSSION

Among the four infochemicals tested, none was significantly attractive for *C. eunomia* females. But the low number of replicates did not allow us to conclude definitively on the non detection of an effective infochemical. As both plants and herbivores produce odors and thus potential information, which may be use by natural enemies to locate their hosts it will be very surprising that no tested odor attracted parasitoid females (Vinson 1998). Thus, more replicates, more treatments and improvements in the experimental design will be necessary. In the following paragraphs, some explanations and improvements will be suggested.

The non attractiveness of *P. bistorta* leaves could be explained by the fact that artificially damaged plants released different volatiles, which do not attract parasitic wasps (Baldwin et al. 2002). Indeed, parasitoids, such as *Cotesia* species, are able to discriminate between herbivore-infested vs uninfested or mechanically damaged plants (Turlings et al. 1991, Bleeker et al. 2006). Indeed, when herbivores feed on, and somehow attack, their host plant, they not only cause damage but also introduce saliva derived compounds to the wound sites; this process activates some volatile emissions that differ in their compositions from those released after a manual destruction (Baldwin et al. 2002). This could also explain why during the experience realized with both host plant and *B. eunomia* caterpillars, female wasps showed an equal absence of preference for this odor. Indeed, caterpillars were moving all the time without feeding and there was no host plant leaves attacked. To improve this experiment and to know exactly if *P. bistorta* plays a role in host location by *C. eunomia* females, it could be interesting to compare plant volatile compounds emitted by correspondingly uninfested and infested leaves by *B. eunomia* caterpillars. Moreover, the whole food plant could also be tested. Several chemical analyses (e.g. extraction of leaf volatile compounds - a solid phase micro extraction

technique could be used -, analysis of these compounds by a gas chromatograph for example, and analysis of volatiles by a mass spectrometer and finally their identification) might be used (Obonyo et al. 2008).

Concerning the host by-products, the quantity of frass used to attract female wasps could have been too low to induce the female reaction (NgiSong and Overholt 1997). Furthermore, source of attractive volatiles in frass can be either the host itself or the processed host plant, or the combination of both. But the link between parasitoids preference during their host search and frass of their host can be more complicated. Indeed, it has been shown that some parasitoids used, for host and mate finding, active compounds originating not only from the host frass, but also from the host-associated micro-organisms living in the frass (Steiner et al. 2007). Caterpillars being reared in laboratory, such organisms may be not present in their frass (used for experiments). Moreover to broaden the study, it seems that some specialist parasitoid species are able to distinguish volatiles from frass of host caterpillars and of non host caterpillars (Alborn et al. 1995, Afsheen et al. 2008a). Since *P. bistorta* leaves are the host plant of several peat bog species (such as *Lycaena helle* caterpillars), it could be interesting to test if *C. eunomiae* female are able to distinguish between frass coming from different herbivores feeding on *P. bistorta* in order to make the most appropriate orientation toward habitat.

Improved olfactometry tests should also be realized, with a better control of factors that could affect the results (such as atmospheric pressure or stable water balance, Martinez and Hardie 2009). Moreover, in our olfactometer device, female wasps were not allowed to fly, only walking was possible; the observed responses may therefore due to the physical design of the bioassay which disabled the wasps from performing flights (Steinberg et al. 1992). Consequently, before to go deeper in this kind of experiments, an effective bioassay in terms of high responsiveness (e.g. wind tunnel, glasshouse set-up or another olfactometer design) need to be constructed and

tested for *C. eunomia*. Finally, field experiments will be required to validate the conclusions from laboratory tests.

In this study, we focus on chemical cues, which are more persistently released than sound and more traceable over larger distance. But we might also be interested in nonchemical cues (Steidle and van Loon 2003); for example van Nouhuys and Kaartinen (2007) have revealed that *Hyposoter horticola* use visual landmarks to locate their host eggs; alternatively, two species of *Coeloides*, parasitoids of concealed hosts, were reported to use host vibrations to locate larvae of bark beetles (Lawrence 1981).

ANNEX III.1

The host acceptance behavior of *C. eunomiae* parasitoid was studied using first instar of *B. eunomia* caterpillars (3mm size) known as being the host of the parasitoid species during its last caterpillar instars (after the diapause). Six caterpillars were introduced in an arena consisting of a glass Petri dish, 8.5 cm in diameter and 1.5 cm in height, together with a female parasitoid. Behavior of the wasp was recorded during 30 minutes. Eleven females were in contact with *B. eunomia* caterpillars. All larvae were reared on *P. bistorta* leaves until the diapause time. For the diapause, they were placed in a climate room (temperature: 8°C, photoperiod L:D 16h:8h) mimicking the temperature of peat bog during this period (cool during the night and low temperature during the day). However, the rearing of parasitized first instar caterpillars failed to detect a second parasitoid generation (mainly due to the gaps on knowledge on development requirements, more specifically, during the diapause of the *B. eunomia* young caterpillars and, consequently, inability to fulfill those requirements).

C. eunomiae female behavior was similar to the one of other parasitoid females when they encounter a host. There was a hierarchy of behavioral steps. Thus, when approaching a host caterpillar, the wasp walked drumming the surface with its antennae until it located the caterpillar. Then, it jumped on the caterpillar, briefly drummed the caterpillar body with its antennae and then inserted its ovipositor. 21 sting events were observed. The average length of a sting was 14 second (min = 8 s, max = 21 s, SEM = 3.87 s). According to literature, this length of sting could correspond to effective egg laying. Thus in *C. sesamiae* and *C. flavipes* oviposition occurred very rapidly (5-6 s) (Obonyo et al. 2010), and in other braconids, such as *C. glomerata*, oviposition lasted 16-20 s (Tagawa et al. 1987). The difference in the length of oviposition time can be explained by different host behaviors. Indeed, Lepidopteran larvae that feed on plants, whether externally or

internally, defend themselves against parasitoids either directly by hiding during times when vulnerable to attack or by biting, spitting or flicking the parasitoid off (*B. eunomia* caterpillars adopt a curl behavior). Thus, due to this aggressive behavior of their hosts, some parasitoid species are under pressure to oviposit quickly once they contact a larva. Therefore, first instar of *B. eunomia* seems to be accepted by *C. eunomiae* female as hosts for its offspring even if more observations are needed.

CHAPTER IV _____

**THE CATERPILLAR AND THE PARASITOID: INTERPLAY
BETWEEN HOST SIZE, HOST GROWTH AND PARASITOID
SUCCESS IN THE BOG FRITILLARY BUTTERFLY.**

This article is submitted:
Julie Choutt and Nicolas Schtickzelle
in The Open Ecology Journal.

ABSTRACT

Growth of holometabolous insects is affected by both abiotic and biotic factors. Parasitoids are biotic factors perturbing the development of their host so that their quantitative and qualitative nutritional requirements are met, until they inevitably kill it. In this paper, we address the question of the interplay between host (bog fritillary *Boloria eunomia* caterpillars) and parasitoid (*Cotesia eunomiae* wasp, a koinobiont larval endoparasitoid) development: (1) how the development of the host is altered when parasitized, and (2) how host condition influences success or failure of parasitoid larvae. Our results show that this parasitoid species clearly perturb the growth of *B. eunomia* caterpillars: parasitized caterpillars exhibited a delayed growth rate (smaller weight and lower growth rate). Moreover, while bigger caterpillars contain on average more parasitoid larvae than smaller one, the weight of each of these parasitoid larvae decreased when their number increased. Besides, parasitoid larvae fail to egress from a number of parasitized caterpillars, especially when numerous parasitoids developed in a smaller host. A change in host condition during parasitoid larvae development, a suboptimal choice of the female at the oviposition time, or superparasitism are three hypotheses advanced to explain the unsuccessful parasitism.

IV.1. INTRODUCTION

“With respect to natural selection, an efficient growth trajectory is the combination of growth rate, survival, and timing of adult emergence that maximizes lifetime reproductive success” (Gotthard 2008). In holometabolous insects such as butterflies, beetles, flies, and wasps, most if not all growth occur at the larval stage, which may then be seen as an adaptation for efficient growth with crucial implications for the adult life (Speight et al. 2008). The size that a larva attains at the time of metamorphosis indeed defines the adult body size (Tammaru 1998, D'Amico et al. 2001), which is itself often linked to their fitness (Haukioja and Neuvonen 1985, Honek 1993). During this larval growth period, individuals go through a varying number of instars, during which they are always confronted to a trade-off between growth benefits and costs (risk of mortality, declining environmental conditions) (Speight et al. 2008).

Juvenile growth is affected by both abiotic and biotic factors, e.g. temperature (Kaitaniemi and Ruohomaki 1999, McMillan et al. 2005), food supply and quality (Stamp and Bowers 1988), presence of natural enemies such as predators or parasitoids (Alleyne and Beckage 1997). Insect parasitoids are known to influence their host development, either immediately (idiobiont parasitoids) or with a certain delay (hosts parasitized by koinobiont continue to grow as the parasitoid's offspring matures) (Askew and Shaw 1986). Moreover, many parasitoids divert their host physiology and behavior to meet their own requirements (Wani et al. 1994, Gelman et al. 1998, Harvey et al. 1999, Beckage and Gelman 2004, Lauro et al. 2005, Thompson and Redak 2008), and consequently to their survival (Godfray 1994, Grosman et al. 2008). Thus, by manipulating their host behavior, parasitoids can decrease host predation (Tanaka and Ohsaki 2009) and by altering their host metabolism (Alleyne and Beckage 1997, Gelman et al. 1998, Salvador and Consoli 2008) they create a favorable environment

for their development. Besides, at the same time host development is manipulated by primary parasitoids, the latter can in turn be affected by their conspecifics through superparasitism (referring to a female parasitoid laying in a host already parasitized by itself or a conspecific female: (Godfray 1994) or by a higher trophic level represented by hyperparasitoids (parasitizing the primary parasitoids, which inevitably leads to their dead) (van Nouhuys and Hanski 2002b, Shaw et al. 2009).

In this study, we investigated the impact of parasitism in the caterpillar stage of the bog fritillary butterfly *Boloria eunomia* (Lepidoptera: Nymphalidae), a vulnerable specialist species of peat bogs and unfertilized wet meadows. While the biology and ecology of this butterfly is well-known (e.g. Baguette et al. 1998, Schtickzelle et al. 2002, Schtickzelle and Baguette 2004, Schtickzelle et al. 2006, Turlure 2009), its relations with its specialist koinobiont and gregarious endoparasitoid, the *Cotesia eunomiae* wasp (Hymenoptera: Braconidae: Microgastrinae) (Shaw 2009), have barely been sketched despite the expected important impact on butterfly population dynamics. We quantified the impact of parasitoids on caterpillar development by comparing parasitized and healthy hosts in terms of weight and growth rate. We also studied why some parasitoid larvae failed to emerge and died together with their host caterpillar.

IV.2. MATERIAL AND METHODS

During spring 2005 (from 15 May to 5 June), *B. eunomia* caterpillars were collected in the Fange de Pisserotte nature reserve, located in the Plateau des Tailles landscape (S-E Belgium, 50°13'N, 5°47'E) (Choutt). Patches of the bistort *Persicaria bistorta* (Polygonaceae), their unique host plant in Belgium, were scrutinized and all caterpillars found were collected. They

were all next to last and last instar caterpillars after hibernation. They were brought to the lab to be individually reared in Petri dishes under outdoor natural temperature and light fluctuations. Every two days, frass and unused plant material were removed and fresh pieces of bistort leaf added to ensure caterpillars were fed *ad libitum*. All plant material was removed from the Petri dish at the beginning of caterpillar pupation.

All caterpillars were weighed every two days on a balance (Ohaus, 0.1 mg). Unparasitized individuals were monitored until the pupation time, and parasitized ones until the day after the egression of parasitoids (synchronous for all larvae from a given caterpillar). Caterpillars that died prematurely were dissected to assess whether they were parasitized or not. For analyses, caterpillars were classified into two groups according to their status: (1) unparasitized and (2) parasitized, this last group being itself divided according to the fate of the parasitism: (2.a) success or (2.b) failure. For the success group, the number of emerged *C. eunomiae* wasps was counted after emergence ceased, representing the parasitoid load. The total weight of the cocoons was also measured. For failure group, parasitoid larvae counted during dissection represented the parasitoid load.

We first tested for an effect of parasitoid presence on the weight and growth rate of the host caterpillar. We compared unparasitized and parasitized groups for (1) the initial caterpillar weight (at collection date) by a two way crossed ANOVA with parasitism status and collection date (random block) factors; (2) the maximum weight attained; (3) the difference of the last two weight measures (before pupation for unparasitized group, before parasitoid egression for success group, and before the dead of caterpillars for failure group), and (4) the total growth rate (weight difference between pupation/parasitoid egression and collection time, divided by number days between these two events) by two sample t-tests. For these analyses, caterpillars with only one weight measure were discarded.

For success group, the effect of parasitoid load on caterpillar growth (initial weight, maximum weight, total growth rate, weight loss before parasitoid egression, and total weight of parasitoid cocoons) was quantified using Pearson correlation. So was also the existence of a correlation between brood size and total and individual weight of the cocoons.

We finally compared success and failure groups for (1) the initial weight of caterpillars collected the same day by a two-way crossed ANOVA with parasitism fate (success vs failure) and collection date (random block) factors, and (2) the parasitoid load by a two sample t-test.

IV.3. RESULTS

In 2005, a total of 101 caterpillars were found in the Pisserotte peat bog, among which 24 pupated, 76 were parasitized and one died for unknown reasons (diseases or virus could be involved). Among the 76 parasitized caterpillars, parasitoids succeeded in egressing in 54 cases. Parasitoid load (number of parasitoid larvae per caterpillar) ranged from 11 to 69 with a median of 32 and a mean of 34.5 (Fig. IV.1).

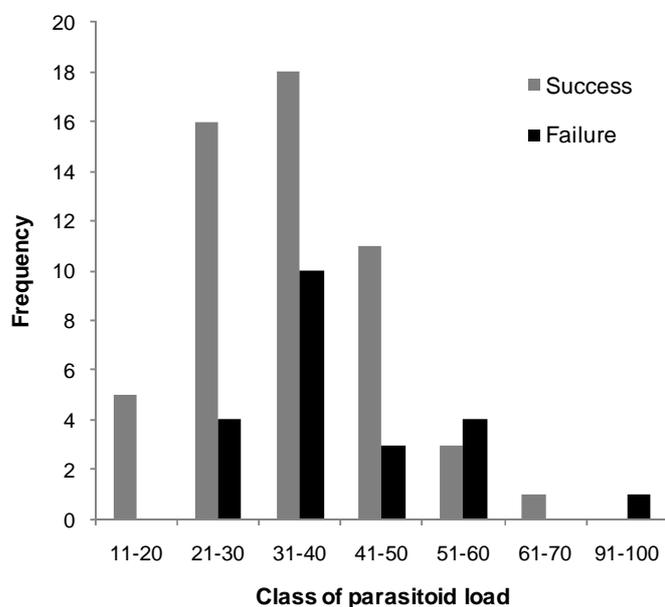


Figure IV.1. Distribution of *Cotesia eunomiae* parasitoid load of *Boloria eunomia* caterpillars. Grey bars represent success group (parasitoid larvae succeeded to egress from their host) and black bars failure group (parasitoid larvae failed to emerge from their host).

The initial weight (at caterpillar collection) of unparasitized caterpillars was two times higher than parasitized ones (Table IV.1). The maximum weight reached by the two caterpillar groups were not significantly different (Table IV.2). Besides, parasitized caterpillars showed a different growth curve: they had a lower growth rate on average and lost some weight just before the parasitoid egression, while unparasitized caterpillars continued growing until the pupation time (Table IV.2).

Table IV.1. Initial weight of unparasitized caterpillars was two times higher than parasitized ones (two way crossed ANOVA with collection date as a random factor): 0.204 ± 0.087 vs 0.112 ± 0.059 (mean \pm SEM). Statistical significance of the test is indicated with bold p value.

Variables	dF	Error terms	Sum of squares	Mean square	F ratio	p-value
Status	1	Date * Status	0.1324	0.132	45.66	< 0.0001
Date	6	Residuals	0.0556	0.009	2.158	0.058
Date * Caterpillar	6	Residuals	0.0174	0.003	0.675	0.67

Table IV.2. Mean (and SEM, in g) difference between parasitized versus unparasitized caterpillars for: (1) maximum weight reached, (2) weight loss before the end of larval stage and (3) total growth rate. Statistical significance of the two sample t-test is indicated with bold p value.

	Unparasitized	Parasitized	t	dF	p-value
Maximal weight	0.258 (\pm 0.0306)	0.232 (\pm 0.0708)	1.3	64	0.21
Weight lost before the end of larval stage	0.00927 (\pm 0.0352)	-0.0174 (\pm 0.0228)	3.5	64	0.0009
Total growth rate	0.00965 (\pm 0.0099)	0.00365 (\pm 0.0096)	2.1	64	0.0394

The initial weight of parasitized caterpillars and maximum weight they reached were positively correlated with parasitoid load, but the total growth rate and the weight lost before parasitoid egression were not (Table IV.3). When parasitoid larvae were more numerous, the individual weight of one parasitoid cocoon was lower ($n = 51$, $R = -0.274$, $p = 0.0515$) but the total weight of all cocoons higher ($n = 51$, $R = 0.818$, $p < 0.0001$), indicating that reduction in larvae size was not large enough to prevent larger broods to be heavier in total.

Table IV.3. Correlation of parasitoid load observed in parasitized caterpillars with weight variables. Statistical significance of the test is indicated with bold p value.

	N	Pearson correlation coefficient	p-value
Initial weight of the host	51	0.411	0.0027
Maximal weight	51	0.457	0.0007
Total growth rate	51	0.104	0.464
Weight lose before parasitoid egression	51	0.137	0.337

Whatever the collection date on the field, caterpillars from success group were significantly heavier than those from failure group (Table IV.4). Moreover, failure parasitized caterpillars had a significantly higher parasitoid load than success ones (two sample t-test: $t = -2.45$, $dF = 74$, $p = 0.0167$) (Fig. IV.2).

Table IV.4. Whatever the collection date on the field, caterpillars from success group were significantly heavier than those from Failure group: 0.14 ± 0.064 vs 0.076 ± 0.028 (mean \pm SEM). Statistical significance of the test is indicated with bold p value.

Variables	dF	Error terms	Sum of squares	Mean square	F ratio	p-value
Parasitized status	1	Date * Parasitized status	0.0379	0.0379	19.56	< 0.0001
Date	5	Residuals	0.0255	0.0051	1.13	0.4471
Date * Parasitized status	5	Residuals	0.0225	0.0045	2.32	0.0608

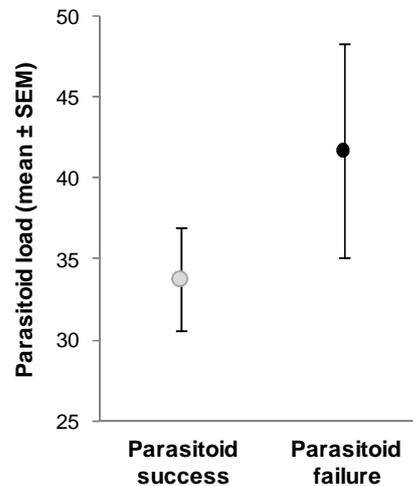


Figure IV.2. Caterpillars from which parasitoids failed to emerge had a significantly higher parasitoid load.

IV.4. DISCUSSION

Koinobiont larval endoparasitoids, such as *C. eunomia*, depend entirely on their host for shelter and food, acquiring their nutrients directly from the host haemolymph during all or most of their immature development, finally consuming virtually all host tissues until the pupation (Harvey et al. 1999). Such a haemolymph feeding habit (adopted by a lot of Microgasterinae species) allows parasitoids to match their requirements to available resources, by consuming variable amounts of host resources in response to differences in parasitoid burden or host condition (Whitfield 1998). This kind of parasitoids are, therefore, adapted to influence the host physiology so that their quantitative and qualitative nutritional requirements are met (Mironidis and Savopoulou-Soultani 2009). Obviously, such life strategy interferes with different events of host development. As shown in this study

and others (Tanaka et al. 1992, Wani et al. 1994, Harvey et al. 1999, Elzinga et al. 2003, Mironidis and Savopoulou-Soultani 2009), the host growth is one of the various aspects which may be altered. Thus, caterpillars parasitized by *C. eunomiae* exhibited a delayed growth, presenting, in this case, a smaller weight at any given date and an overall lower growth rate. Such disruption of development is mainly attributed to abnormalities in various aspects of the host endocrine system (Cole et al. 2002) as the result of the suite of parasitoid products delivered in the host (Vinson and Iwantsch 1980, Thompson and Redak 2008). Moreover, just before parasitoid egression, parasitized caterpillars lost weight because they stopped feeding, as the distorted leaves still intact in Petri dishes indicated. Braconid wasps of the genus *Cotesia* are known to stop development of their hosts in the larval stage, prior to its metamorphosis (Beckage and Gelman 2004). This decrease in body weight could reflect the end of host actions, which corresponds to the preparation of parasitoid egression (Alleyne et al. 1997).

Heavily parasitized individuals, which contained on average more parasitoid larvae, attained a larger body mass compared to lightly parasitized ones, as observed in other host-parasitoid systems (Gu et al. 2003, Bezemer and Mills 2003). This suggests that host growth may be manipulated by parasitoids to compensate for competition and thereby optimize nutrient transfer to the wasps, through some changes in the rate of consumption and the efficiency of utilization of food by their hosts (Alleyne and Beckage 1997). However, the weight of each parasitoid larvae tended to decrease when their number increased, indicating that some competition for food was still on play. Further studies are required to assess whether this impacts parasitoid fitness.

The premature death of the host together with its parasitoid larvae occurred when more numerous parasitoids developed in smaller hosts. Two main reasons can be advanced for the occurrence of such overpopulation of larvae within a host: either a decrease in host condition during its

development, or a number of deposited parasitoid eggs excessive according to the host condition.

For gregarious parasitoid species, host growth may have to be dynamically regulated based on the number of developing parasitoid larvae to ensure optimal resource allocation to all larvae (Nakamatsu et al. 2006). However, the mode of interaction in the host corresponds to a scramble competition for resources (Godfray 1994). If resources are lacking due to a poor host condition (e.g. host starvation), adjustment capacities may be overtaken, and parasitoid growth, development and survival affected (Harvey et al. 1999, Nakamatsu et al. 2006).

Parasitoid females need to make a series of choices for reproduction (Vinson and Iwantsch 1980). They should of course preferentially parasitize the most suitable hosts, i.e. those in which the probability of successful development is highest for the highest number of larvae (Godfray 1994). Suboptimal hosts may be chosen if high quality hosts are lacking (Harvey et al. 1999, Li et al. 2006, Mironidis and Savopoulou-Soultani 2009), but clearly, female wasps must adapt the number of eggs laid to the host quality to ensure optimal larvae development. However, an excessive number of eggs deposited within a host might happen as a consequence of a female error, but also and probably more importantly as a strategy. Indeed, when more eggs must physiologically be laid than the available hosts will support, increasing their number in each host might be a better strategy than simply losing them. A specific case of this strategy might be the superparasitism, observed widespread phenomenon in parasitoid species (Hamelin et al. 2007), referring to a parasitoid female depositing eggs in a host already parasitized by a conspecific female (van Alphen and Visser 1990, Godfray 1994). While often supernumerary larvae are eliminated (Mayhew and Hardy 1998), there may be conditions under which superparasitism may be adaptive (i.e. increase parasitoid fitness): when host availability is limited due to a low host population size, or when a high number of parasitoid females competing

for hosts. Superparasitism could represent an adaptive strategy at the individual level especially when the lifetime expectancy of females is reduced due to environmental conditions or senescence (Godfray 1994, Bezemer and Mills 2003, Dorn and Beckage 2007, Hamelin et al. 2007). Although superparasitism has been demonstrated in other *Cotesia* species (Tenhumberg et al. 2001, Gu et al. 2003), it remains to determine whether it occurs in *C. eunomia* and whether it is the cause of the parasitoid failure to egress in our results. The development of microsatellites markers for this species has been unsuccessful so far (Choutt, unpub. data), preventing the use of genetic analyses to determine whether all parasitoid larvae in a host are the progeny of a single or several females. However, with relatively high parasitism rate of *B. eunomia* caterpillars (75% in 2005:Choutt et al. submitted), the probability for a *C. eunomia* female parasitoid wasp to encounter a healthy host was very low, making the existence of superparasitism not unlikely.

This first study on the interplay between *B. eunomia* caterpillars their *C. eunomia* parasitoid wasp brings a descriptive situation of this host-parasitoid relationship concerning the impact of parasitoid on host development and the influence of host condition on the fate of parasitoid larvae. Further researches are needed to go deeper in the knowledge of this relation, e.g. optimal clutch size according to host condition, reasons for failure of parasitoid larvae to egress (e.g. superparasitism), sex-ratio allocation in *C. eunomia*.

CHAPTER V _____

**DEVELOPMENT OF MICROSATELLITE MARKERS IN A
SPECIALIZED BUTTERFLY PARASITOID: *COTESIA EUNOMIAE*
(HYMENOPTERA, BRACONIDAE).**

I am grateful to Sofie Vandewoestjine for her help and corrections.

ABSTRACT

Estimating the population structure of the higher trophic level in relation with lower levels is of prime importance to understand how species occupying higher trophic level persist. Host-parasitoid interactions are ideal within this perspective. Parasitoids are highly sensitive to habitat fragmentation and may be influenced by both the spatial structure and the population dynamics of their host population. Here, we investigated the population structure of the primary caterpillar parasitoid, *Cotesia eunomiae*, of the bog fritillary butterfly, *Boloria eunomia*. By using microsatellite markers, we had four aims: 1) to characterize the genetic spatial population structure of this parasitoid, 2) to compare host and parasitoid population structures to check whether *C. eunomiae* displays a more distinct population structure than its host, 3) to test the effect of fragmentation and isolation on parasitoid population structure and 4) to test for superparasitism in *C. eunomiae*. Since no species-specific microsatellite markers exist for *C. eunomiae* and cross-amplification of microsatellite markers from closely related species was not successful, we developed *C. eunomiae* specific microsatellite markers. However, neither inter-individual nor inter-population polymorphism was detected. To explain our observation of multi-locus monomorphism in microsatellites both technical and biological explanations specific to this species will be discussed. To conclude, we believe that the low amount of genetic variation and the lack of genetic differentiation observed in *C. eunomiae* is likely due to small population size and the reproductive system of the species.

V.1. INTRODUCTION

General context

The impact of habitat fragmentation on single species abundance and persistence, and on species diversity has received considerable attention (Saunders et al. 1991b, Fahrig and Merriam 1994). Habitat fragmentation can also influence inter-specific interactions (Tscharntke 1992, Didham et al. 1996), such as among interspecific competitors, predators and their prey (Ryall and Fahrig 2005), parasitoids and their hosts (Cronin and Abrahamson 2001, Cronin 2003, Cronin 2004) or plants and their pollinators (Lennartsson 2002, Kolb 2008). In a multitrophic systems context, the higher trophic levels are thought to be more susceptible to extinction or population decline than are the lower ones (Kruess and Tscharntke 1994, Holt et al. 1999, Thies et al. 2003). Estimating the population structure of the higher trophic level in relation with lower levels is therefore of prime importance to understand how species occupying higher trophic level persist. Host-parasitoid interactions are ideal within this perspective. Parasitoids are highly sensitive to habitat fragmentation especially if they are specialist of their host (Shaw et al. 2009). Indeed, their larval stage depends entirely on the presence of their host. Moreover, the population ecology of parasitoids may be influenced by both the spatial structure and the population dynamics of the host population (van Nouhuys and Lei 2004).

The majority of community studies suggest that parasitoids have smaller dispersal ability than their hosts (Kruess and Tscharntke 1994, Zabel and Tscharntke 1998, for examples relating to other *Cotesia* species see Lei and Hanski 1998, Kankare et al. 2005). Thus, more distinct spatial structure for parasitoid species than host species is expected, and has been observed, especially in parasitoid species with a low dispersal range and a small

population size (Johannesen and Seitz 2003, Kankare et al. 2005, Bergerot et al. 2010).

A wide taxonomic array of insect species are parasitized and immature stages of butterflies are no exception (Dempster 1984). Mortality following parasitism is a factor often invoked in inducing population fluctuations (Lei and Hanski 1997). Taking advantage of the vast knowledge on the natural history and metapopulation biology of the bog fritillary butterfly *Boloria eunomia* (Nymphalidae) (e.g. Schtickzelle and Baguette 2004, Vandewoestijne and Baguette 2004, Turlure et al. 2009), we investigated the population structure of its primary parasitoid, *Cotesia eunomiae* (Hymenoptera: Braconidae: Microgastrinae) (Shaw 2009). *C. eunomiae* is a specialist wasp parasitizing *B. eunomia* caterpillars. This Braconidae, with a haplodiploid sex-determining system (fertilized eggs develop into females and unfertilized ones into males), is a gregarious koinobiont endoparasitoid (i.e. host development continues after having been parasitized, and several wasps emerge from each host larva). Many *Cotesia* species are well known as being either important natural enemies of agricultural and forestry pests or primary parasitoids of almost every Melitaeini species that has been studied in detail (Lei et al. 1997, van Nouhuys and Hanski 2002b, Stefanescu et al. 2009).

We wish to characterize the genetic spatial population structure of this parasitoid for several reasons. First of all, we aim to compare host and parasitoid population structures to check whether *C. eunomiae* also displays a more distinct population structure than its host, as observed in other parasitoid-host comparisons. Its host, the bog fritillary butterfly, is a specialist species with a highly fragmented distribution in the southern part of its distribution range and a strong colonisation capability at the local scale (Nève et al. 1996, Petit et al. 2001). Two genetic population studies of the host butterfly show that as fragmentation increases, populations are more genetically differentiated (Vandewoestijne and Baguette 2004, Nève et al.

2008). Second, we aim to test the effect of fragmentation and isolation on parasitoid population structure. For this, we compare the genetic population structure for two wasp populations found in two distinct host populations. The two host populations differ in their host plant patch configuration: one having connected patches while patches of the other are more isolated from each other. We expect an increased genetic differentiation between parasitoid patches within the population characterized by more isolated host patches (Kankare et al. 2004). Thus, in the host population with connected host plant patches, we expect to find a low spatial population structure. Finally, using genetic markers, we aim to test for superparasitism in *C. eunomiae*. Foraging decisions and fitness are directly linked and can influence parasitoid population processes (Godfray 1994). One important foraging decision of parasitoids is whether or not to oviposit in hosts already parasitized by a conspecific (i.e. superparasitism) or another species (i.e. multiparasitism). After being a subject of strong controversy, superparasitism is now recognized as adaptive in a number of situations (van Alphen and Visser 1990). Superparasitism may be adaptive at the population level when host availability is limited due to a low host population size, a reduced proportion of unparasitized hosts in good body condition, or a high number of parasitoid females competing for hosts (Dorn and Beckage 2007). At the individual level, superparasitism may be adaptive when the lifetime expectancy of females is reduced due to environmental conditions or near the end of its life. Therefore, identifying and quantifying superparasitism in *C. eunomiae*, in relation to host population size, can improve our understanding of the host-parasitoid dynamics.

Choice of molecular marker

Several molecular markers exist and have been used to answer ecology related hypotheses. Microsatellite markers (regions of DNA composed of

short (≤ 6 bp) sequences repeated in tandem (Queller et al. 1993, Meglecz et al. 2007), currently the most frequently used in population biology (Meglecz et al. 2007), is the genetic marker of choice for many population biology studies (Jarne and Lagoda 1996). Several characteristics of microsatellites make them appropriate population genetic markers. They are ubiquitous throughout eukaryotic genomes and have been detected in the genomes of every organism analysed so far (Li et al. 2002); although the number of available markers varies strongly among taxa (such as between Lepidoptera and Hymenoptera) (Nève and Meglecz 2000, Meglecz et al. 2007). Moreover, many of them have high-mutation rates (on average 5×10^{-4} mutations per locus per generation) that generate the high levels of allelic diversity necessary to detect evolutionary processes acting on ecological time scales (Schlotterer 2000). Additionally, microsatellites are abundant across genomes in both coding (Li et al. 2002, Li et al. 2004) and especially noncoding regions of the genome. They are consequently powerful markers used in population genetics (e.g. Jarne and Lagoda 1996), determination of kinship (e.g. Queller et al. 1993) behavioural studies (e.g. Burton-Chellew et al. 2008). No species-specific microsatellite markers exist for *C. eunomiae*. Consequently, we begin by cross-amplifying microsatellite markers from closely related species. Subsequently, we develop *C. eunomiae* specific microsatellite markers.

V.2. STUDY REGION, SAMPLING SITES AND PARASITOID REARING

Parasitoids for this study were obtained by sampling post-diapause host larvae in the spring of 2006 for two populations. In the laboratory, immature wasps emerging from the parasitized hosts were kept in ventilated Petri dishes at room temperature until the adult wasps emerged. To avoid

perturbing both the host and parasitoid populations, only some emerging adults were kept to other studies, the remaining parasitoids and adult butterflies, were released in the field. Adult wasps, which had not emerged from the cocoon, were extracted and frozen.

Populations were sampled in two peat bogs distant by 46 km (Fig. V.1). The Fange de Pisserotte nature reserve consists of a 56 ha peat bog (S-E, Belgium, 50°13'N, 5°47'E, altitude between 550 and 605 m), located on the Tailles highland. Host plant patches, *Persicaria bistorta*, were sampled several times by visual inspection to collect *B. eunomia* caterpillars. *P. bistorta* occurred in 27 different patches (24053 m²), the maximal distance between bistort patches is 862 m (Fig. V.1). The Troufferies de Libin (49°57'N, 5°19'E, altitude: 430 m) is a 52 ha peat bog, located on the Recogne highland. In this site, *P. bistorta* occurred in 14 different patches (10805 m²), with a maximal distance between two patches of 385 m (Fig. V.1). The two host populations situated in two different subregions differ by their host plant patch configuration: the Libin population having more connected patches than the Pisserotte one (Fig. V.1).

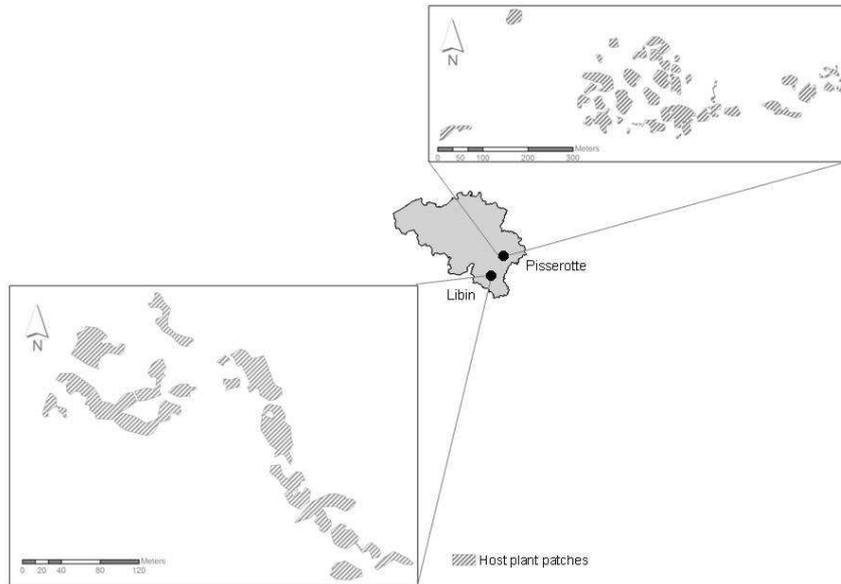


Figure V.1. Map of the two study sites. Location in Belgium of the two sampled populations (Pisserotte and Libin). For each site, box corresponds to the host plant patch configuration: Pisserotte patches are more distant than Libin ones.

V.3. MOLECULAR ANALYSES

Total genomic DNA was extracted from adult samples of *C. eunomiae* using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle 1990). For the following analyses DNA of individuals coming from different patches were used (to maximize chances of discovering polymorphism in the tested markers).

Test of existing primers

We cross amplified microsatellite markers developed for *Cotesia congregata* (Jensen et al. 2002) on *C. eunomiae*. Indeed, flanking regions can be highly

conserved across taxa, allowing successful cross-species amplification of microsatellite loci using primers developed from other closely related species, i.e. within the same genus but sometimes within the same taxonomic family. This is especially true for vertebrates such as fishes, reptiles and mammals (Selkoe and Toonen 2006). Therefore, we tested 6 primer pairs developed for *C. congregata* (Jensen et al. 2002). Unfortunately, none of these primers revealed polymorphism for *C. eunomiae* individuals.

Two of the six *C. congregata* primer pairs successfully cross amplified DNA from *C. eunomiae*. However, no polymorphism was detected at these loci for the tested individuals (Table V.1).

Table V.1. Results of the cross species amplification using 6 primers pairs developed for *C. congregata*.

Locus	GenBank accession no.	n	No. of alleles	Allele size
Cco-1A	AF453312	20	1	124
Cco-5A	AF453313	6	NA	NA
Cco-27	AF453314	6	NA	NA
Cco-42	AF453315	20	1	91
Cco-65A	AF453317	6	NA	NA
Cco-65B	AF453317	6	NA	NA

Development of species specific primers

Due to a lack of polymorphism in the cross-amplification experiment, a microsatellite-enriched library was constructed using the protocol of Billotte *et al.* (1999). Genomic DNA was first restricted with *RsaI* and fragments were ligated to selfcomplementary adaptors Rsa 21 and phosphorylated Rsa 25. Ligated DNA was pre-amplified by polymerase chain reaction (PCR).

Purified products were allowed to hybridize to $I_5(CT)_8$ and $I_5(CA)_8$ 5' biotin-labelled microsatellite oligoprobes. Fragments containing microsatellite sequences were captured using streptavidin-coated magnetic particles. Selected fragments were PCR amplified, cloned into a pGEM-T vector (Promega) and transformed into XL1-Blue electroporation-competent cells (Stratagene). A total of 192 white transformant clones were PCR amplified with Rsa 21 primer and transferred onto Hybond N+ nylon membranes which were hybridized at 56 °C with [$\gamma^{32}P$] dATP end-labelled (GA) $_{15}$ and (GT) $_{15}$ probes. The enrichment success was about 70%. 47 clones were sent for sequencing (Genome Express). 35 primer pairs were designed using the primer 3 program (Rozen & Skaletsky 2000).

15 primer pairs were tested on few *C. eunomiae* individuals to select those primer pairs which successfully amplified single loci. PCR amplifications were performed in 15 μ L reaction mixture containing 20 ng template DNA, 1.5 μ M of each primer, 1.2 mM of each dNTP, 1.5 mM Tris-HCl, 0.2 U *Taq* polymerase (Roche) and 1 \times PCR buffer using 2400 or 9700 thermocyclers (PerkinElmer). The PCR conditions were: preheating for 1 min at 94 °C, then 38 cycles of 30 s at 94 °C, 30 s at annealing temperature (55 °C for all the primer pairs), and 15 s at 72 °C, and finally one elongation step of 5 min at 72 °C. The PCR products were electrophoretically separated on 1.5% agarose gels and stained by ethidium bromide.

The PCR products were detected in an ABI PRISM 3100 sequencer and sized with genemapper software (Applied Biosystems version 3.5) using 400HD ROX (Applied Biosystems) as an internal size standard.

We succeeded in amplifying 15 different loci using the species-specific primers (Table V.2). With the exception of one locus, all *C. eunomiae* specific markers presented a dinucleotide repeat motif of at least 6 repetitions.

Table V.2. Characterization of 15 species-specific microsatellite loci in *Cotesia eunomia*: repeat motif, size of the cloned allele and primer sequences.

Locus	Repeat motif	size (pb)	Primer sequences (5'-3') (F= forward, R=reverse)	forward Ta (annealing T°C)	reverse Ta (annealing T°C)
A01	(ac) ₁₇	212	F=CTATTGGGCTAAATCGTTTC R=GTTTGTGGTCTTTAGAAGCTG	54.22	54.37
A08	(ca) ₈	81	F=ACACAAAATTACTCCTACAAAAA R=ACGTGTATGTGCTGATGTT	52.81	52.03
B06	(ag) ₁₇	128	F=GGCCAACTATTTTACGGTCT R=TTGTCTCCCTACACCTTCTC	55.91	54.28
B07	(ca) ₇ (ac) ₁₁	84	F=CTTCTGAAAAGAGGTTCA R=TTTTTA ACTTCTCGCGTAT	48.16	50.52
C01	(ac) ₉	124	F=ACAAGCATACACACACTCA R=GTATTCGTGCGAGAGAGA	55.04	51.99
cl7	(tg) ₆	162	F=GTATTTACCCGCGTTTCTAA R=ACATACTCCAAACCCAGAAT	54.73	53.53
cl2	(ac) ₁₃	209	F=ATCGGCTCAAATTCTCATGG R=TCGACGTTTGAAGGTCACAG	60.04	59.87
cl3	(ca) ₁₉	172	F=CGAATCGGTCCAAATCGTAT R=GCGATGGTGTCTGTATGTGTG	59.78	60.05
cl6	(tg) ₇	177	F=TTGGTTTTACCCGTTTTTC R=GGTTGAGCCGTTCAAAGTT	58.83	59.22
A02	(tg) ₂₄	230	F=TTTCCCGATGATGTCCGTAT R=CTCAAGCTATGCATCCAACG	60.16	59.44
c02	(ca) ₈	165	F=AAAAGGTCAGTTTCGCCAAG R=CGCGTGGACTAGTTTTACCC	59.35	59.63
c12	(ca) ₁₁	246	F=CTCTTTGAGAAGCCGTTTGG R=ATATGTTTCCGTCGCTCCTG	59.99	60.1
D03	(ca) ₂₅	163	F=CGGTCAAACCGTTCAAAGT R=ACGATTTGCTTTGATTGCT	60.01	59.85
D06	(gt) ₆	161	F=GCGGGTAATCGATTCTCTTG R=GGATCCGCTCAACGTAAGAA	59.67	60.21
D09	(ac) ₇	169	F=CGGTCAAACCGTTCAAAGT R=CGCGTGGACTATGGCTTTAT	60.01	60.12

Polymorphism of the 15 microsatellite loci developed specifically for *C. eunomiae* was investigated in two distinct populations (Libin and Pisserotte). Although some within individual diversity was observed, i.e. heterozygote state, no differences were observed between individuals. Indeed, all 24 tested individuals were characterized by identical genotypes for all primers tested (Table V.3). Consequently, no inter-population variation was detected. Because all tested parasitoids, originating from different patches and geographically distant populations, had identical genotypes, we decided not to extend the genetic analyses any further with the available samples.

Table V.3. Number of tested individuals per site for each microsatellite locus, with the number of heterozygote individuals and size range.

Locus	Site	n	n of heterozygote	Allele size
A01	Libin	8	0	210
	Pisserotte	16	2	208, 210
A08	Libin	8	2	74, 75
	Pisserotte	16	0	74
B06	Libin	4	0	128
	Pisserotte	8	0	128
B07	Libin	8	0	77
	Pisserotte	16	0	77
C01	Libin	4	0	121
	Pisserotte	8	0	121
C17	Libin	4	0	NA
	Pisserotte	8	0	162
C12	Pisserotte	8	0	NA
C13	Pisserotte	8	8	151, 164
C16	Pisserotte	8	0	178
A02	Pisserotte	8	0	NA
C02	Pisserotte	8	0	NA
C12	Pisserotte	8	0	NA
D03	Pisserotte	8	8	178, 196
D06	Pisserotte	8	0	269
D09	Pisserotte	8	0	96

V.4. DISCUSSION

Lack of cross-species polymorphism

Four *C. congregata* markers failed to amplify DNA of *C. eunomiae*. This lack of cross-species amplification has been observed in other studies. For example, one Cco-locus (Cco42), isolated from *C. congregata*, and four Cme-loci (Cme1, 3, 15, 17), isolated from *C. melitaeorum*, failed to amplify for almost all of the *C. acuminata* or *C. bignellii* individuals (Kankare and Shaw 2004). Another example is found for *Lysiphlebus fabarum*, a specialist primary parasitoid (Hymenoptera, Braconidae) attacking the specialist tansy aphid. The majority of microsatellites developed from *L. fabarum* and from *L. testaceipes* did not succeed to amplify DNA of *L. hirticornis* (Nyabuga et al. 2009). It seems therefore that in Hymenoptera, and more precisely for Braconidae, primer-binding sites are not always conserved between closely species leading to a lack of cross-species amplification success (Nyabuga et al. 2009). However, cross species amplification has been proved useful in many other studies. Therefore, as it has been already highlighted, the success with which species specific microsatellite loci can be used on other species must be determined on a case-by-case basis (Nève and Meglec 2000). Moreover, success rate of amplifications decreases proportionally within the genetic distance between the focal species and the species of origin (Primmer et al. 1996). As this distance between *C. eunomiae* and *C. glomerata* is unknown, this might indicate that these two species are not so close genetically.

Success in microsatellite development

We succeeded in developing 15 microsatellite loci for *C. eunomiae*. Unfortunately, neither inter-individual nor inter-population polymorphism

was detected. Indeed, samples from two populations of *C. eunomia*, distant by almost 50 kilometers, displayed identical alleles for all 15 microsatellite loci. When studying host-parasitoid system, the genetic structure of parasitoid species needs to be compared to the host one. However until now, we cannot rule on the population structure of the host butterfly. Indeed, two genetic analyses, realized at the regional scale, had different conclusions about this. A study using RAPD markers shown that the Pisserotte population was closed to other populations and characterized by a high genetic diversity compared to a more fragmented population (Vandewoestijne and Baguette 2004). According to this study, the Libin population, not situated in a population network such as the Pisserotte one, should be characterized by a lower diversity genetic. However, the genetic structure of *B. eunomia* populations in an earlier study using allozymes, revealed that subregional differentiation has not yet occurred (Nève et al. 2000). About ten years separated the sampling between these two studies. Additionally, the mutation rate of RAPD's is faster than for allozymes. These two facts may explain the differences observed between the studies, and suggest that subregional differentiation now exists in the host population. Hence, we expected to find significant differences between the two parasitoid populations sampled as has been found elsewhere (Kankare et al. 2005).

Lack of polymorphism of species specific primers

Publications of multi-locus monomorphism in microsatellites are rare. This may be because results are not “acceptable” for publication. Indeed, in both *Molecular Ecology Notes* and *Conservation Genetics Resources* journals for example, it is clearly mentioned that to submit a manuscript “at least eight novel polymorphic loci” are required. Beside publication bias, other

possibilities exist. These include both technical and biological explanations specific to this species. The different hypotheses are discussed below.

The presence of null alleles may be a technical explanation for the few heterozygote individuals detected in this study. Variation in the nucleotide sequences of flanking regions (Callen et al. 1993), preferential amplification of short alleles (due to inconsistent DNA template quality or quantity) or slippage during PCR amplification (Gagneux et al. 1997, Shinde et al. 2003) are all possible causes of microsatellite null alleles (Chapuis and Estoup 2007). However, it is unlikely that null alleles fully explain monomorphism at 15 loci (Dakin and Avise 2004), especially since heterozygotes were detected. This leads us to consider *C. eunomiae* as being effectively monomorphic in the two sampled populations.

To our knowledge, two others cases of multi-locus monomorphism in microsatellites have been found: one corresponds to the island fox (*Urocyon littoralis*) population on San Nicolas island off the southern coast of California (Aguilar et al. 2004), and the other is the highly endangered *Parnassius Apollo* butterfly from four sites of the Mosel valley (Habel et al. 2009). Monomorphism of the island fox population was explained by the small effective population size and a recent colonization history. For the butterfly species, authors explained monomorphism by very low long-term effective population size and/or a strong historic bottleneck.

- Monomorphic due to small effective population size

Firstly, monomorphism could be explained by a small effective population size. Parasitoid populations are subject to fluctuations and their size can be limited by both the bottom-up level (availability of hosts) and the top-down level (natural enemies). Indeed, a large fraction of individuals in a parasitoid population can be killed by natural enemies such as predators and secondary parasitoids (hyperparasitoids) (van Nouhuys and Tay 2001). Moreover, wide

temporal fluctuations in host abundance, frequently observed in butterfly populations (McLaughlin et al. 2002, Baguette and Schtickzelle 2003), are a potential source of instability and stochasticity in the population dynamics of associated parasitoid species (Vindstad et al. 2010), with small host populations resulting in small parasitoid populations (Lei and Hanski 1997, Anton et al. 2007). The sampled year corresponds to a small *B. eunomia* population (between 2004 and 2006, the Pisserotte population has declined by 22.5%), which could *a fortiori* lead to a decrease in parasitoid effective population size. Small population size increases the frequency of sib mating, not mating at all and / or mating late in life, leading to low genetic diversity or inbreeding depression. Furthermore, genetic diversity in this parasitoid could be eroded by the haplodiploid breeding system (Anton et al. 2007). Consequently, small effective population size due to a small number of individuals and a haplodiploid reproductive system could result in the observed lack of genetic variation.

- Monomorphism following population bottlenecks

Demographic events, such as species bottlenecks commonly hypothesized as causes of reduced genetic variation, can also be evoked. As mentioned before, the species host, *B. eunomia*, is characterized by highly fluctuating population sizes (Schtickzelle et al. 2002) which could lead to numerous population bottlenecks.

- Monomorphic because sex-bias in sampling?

In haplodiploid species, usually males develop from unfertilized haploid eggs and females from diploid fertilized eggs. If the analyzed samples consisted mostly of males, this could explain the low frequency of heterozygotes. However, diploid males tend to be produced in species characterized by a haplodiploid reproductive system when inbreeding occurs

(Godfray 1994). The mechanism behind this, polymorphism at a single-locus complementary sex determination (sl-CSD) (Cook and Crozier 1995), has been found in the closely related *Cotesia glomerata* parasitoid (Zhou et al. 2006). With this system, if the CSD is diploid but homozygous, a diploid male will result. If this is also the case in *C. eunomiae*, and if samples were mostly males, this would suggest that the effective population size is small. Since sexing of parasitoids was not possible before analyses, it is difficult to discuss this hypothesis further.

- Monomorphism due to small sample size and restricted geographic sampling range.

Another hypothesis explaining the observed monomorphism may be small sample size at a restricted geographical scale (only two sampled populations in Belgium) and the absence of an outgroup. Similarly, Kankare et al. (2004) noted that two microsatellites developed for *C. melitaearum* were monomorphic in their study region (Scandinavia) but polymorphic at a larger geographic scale (Europe and Asia). Hence, *C. eunomiae* could be monomorphic at small spatial scales but may be more polymorphic at larger geographic scales.

- Monomorphism due to high dispersal capacity

Finally, contrary to other *Cotesia* species showing a low dispersal capacity (Lei and Hanski 1998, Kankare et al. 2005), *C. eunomiae* could have a high dispersal capacity. Indeed, since the two parasitoid populations sampled revealed the same alleles, the species may be a good disperser and emigration/immigration have occur within the whole area up to a recent time resulting in the absence of genetic differentiation between populations. No information concerning the dispersal capacity of *C. eunomiae* is available to help us infer or confirm this hypothesis.

Conclusion

To conclude, we believe that the low amount of genetic variation and the lack of genetic differentiation observed in *C. eunomia* is likely due to small population size and the reproductive system of the species. It is plausible that *C. eunomia* compensates lack of genetic variability with increased plasticity to respond to varying biotic and abiotic conditions.

Knowing exactly how the parasitoid populations are structured according to their host populations is very important and it will be interesting to pursue this study at a larger geographic scale. Of course, more studies are needed to determine precisely the mating system of *C. eunomia* and its dispersal capacity. A larger number of individuals and populations from a wider geographic range should also be used. Temporal sampling would enable us to estimate effective parasitoid sample size. Finally, the species-specific primers developed for *C. eunomia* may also be useful in braconid species if cross amplification is successful.

PART THREE

**HOST PARASITOID RELATIONSHIP:
PARASITIDS A POSSIBLE CAUSE OF
POPULATION REGULATION?**

CHAPTER VI_____

**PARASITIDS AS A POSSIBLE CAUSE OF
POPULATION REGULATION IN *B. EUNOMIA*.**

ABSTRACT

Identifying and understanding factors that drive fluctuations of insect population is a central issue in ecology. Butterfly populations are known to be affected by both density-dependent (negative effect of abundance) and density-independent factors (environmental stochasticity, mainly weather conditions). Natural enemies, such as parasitoids, are also clearly important sources of mortality for many insect herbivores and butterfly caterpillars are not free of their attacks. However, their impact on their host population dynamic remains unknown while suspected. With this study, we aim to quantify this impact, by assessing whether temporal variation in *B. eunomia* population growth rate is correlated to temporal variation in the parasitism rate of caterpillars by a specialist parasitoid wasp. Both the studied butterfly population and the parasitism rate fluctuated over the studied period. High correlation between growth rate (corrected for density dependence and weather effects) and parasitism rate has been revealed, with a high parasitism rate (above 75%) affecting negatively the *B. eunomia* population growth rate. Due to the small sample size, this observed tendency was not statistically significant and needs to be confirmed. We will discuss the impact parasitism by *C. eunomiae* wasp may have on its host population growth rate.

VI.1. INTRODUCTION

Populations of insect herbivores are well known to fluctuate through time (Hanski 1990). Identifying and understanding factors that drive these fluctuations is a central question of population ecology (Nowicki et al. 2009). The relative importance of density-dependent vs density-independent factors in determining population dynamics was the subject of several debates during the last century (Turchin 1995). Now, it is accepted that most population dynamics results from the influence of factors of both kinds (Turchin 1995, Benton et al. 2006). Moreover, as multiple factors affect population dynamics, it is more and more assumed that their interaction (for example density-independent factors influencing density-dependent ones) can greatly complicate the situation (Hunter and Elkinton 1999). In populations of insect species, both environmental stochasticity (mainly weather conditions) (Stiling 1987), and the strength of density-dependent factors need to be considered to understand the fluctuations of their population size (Sinclair and Pech 1996), for example for the conservation of endangered species (Pickens 2007).

Butterflies are organisms of choice to study the effect of both density-dependent and density-independent factors (Thomas 2005). Population dynamics of some butterfly species have already been carried out. From these studies, weather patterns seem to be the crucial agents to explain variation from one generation to the next of their population size (Ehrlich et al. 1980, Pollard 1988, Roy et al. 2001, Nowicki et al. 2009). Besides, evidence for density-dependent regulation in butterfly populations has been shown (e.g. Schtickzelle and Baguette 2004, Baguette and Schtickzelle 2006). However, specific causes of density dependence are still unknown even if larval nutrition (decrease in quality and/or quantity) and impact of natural enemies are the most likely (Hanski 1990).

Through adaptations of insects against attacks by natural enemies, the successful cases of biological control, and population models, natural enemies can be viewed as having a major role in the dynamics of insect herbivores population (Cornell and Hawkins 1995). Parasitoids are one kind of enemy commonly evoked in butterfly populations (Lei and Hanski 1998, van Nouhuys and Hanski 2002b). Insect parasitoids are ubiquitous in terrestrial ecosystems and they are important in influencing the abundance and population dynamics of their hosts (Godfray 1994). Besides, in butterfly literature, several studies showed that their dynamics can be greatly influenced by parasitoids. Huge fluctuations in population size of *Euphydryas aurinia* over 30 years coincided with great changes in the rate of parasitism by *Cotesia bignellii* (Ford and Ford 1930, Porter 1981). Local extinction of *Melitaea cinxia* populations was associated to high rates of parasitism by *Cotesia melitaeorum* (Hanski and Kuussaari 1995, Lei and Hanski 1997). Consequently, the role of parasitoids as mortality factors for butterflies appears clearly. It remains however to determine whether parasitoids could be a regulatory factor of their host population dynamics (Stiling 1987) and if it is the case to quantify its effect.

The bog fritillary butterfly, *Boloria eunomia*, is a well known species with a vast knowledge gathered on its natural history, specific habitat requirements (Turlure et al. 2009) and metapopulation dynamics (e.g. Schtickzelle et al. 2002, Schtickzelle and Baguette 2004, Schtickzelle et al. 2007). While density-dependent processes (negative effect of abundance) and the impact of weather conditions explained a major part of its population fluctuations (Schtickzelle and Baguette 2004), the impact of natural enemies, such as caterpillar parasitoids remains unknown (Schtickzelle and Baguette 2004). The aim of this study is then to try and quantify this impact, by assessing whether temporal variation in *B. eunomia* population growth rate is correlated to temporal variation in the parasitism rate of caterpillars.

One *B. eunomia* population was monitored during seven years and both population size and parasitism rate were estimated. To remove the effect of other factors known to influence population growth in this species (abundance and weather conditions), we first computed the expected growth rate for each year according to observed abundance and weather, and computed the difference with the observed population growth rate. We then correlated this difference to the magnitude of parasitism.

VI.2. MATERIAL AND METHODS

The species

B. eunomia is a univoltine species, with adults flying in one generation between the end of May and the beginning of July. This specialist butterfly inhabits, in Western Europe, peat bogs and unfertilized wet meadows where the bistort grows (*Persicaria bistorta* L.; Polygonaceae). *P. bistorta* is the only host plant of caterpillars and food plant of adults in this part of its distribution area (Goffart and De Bast 2000). Females deposit clutches of a few eggs on or near the host plant. After hatching (June-July), caterpillars feed on *P. bistorta* leaves for about two months up to the diapause. In the following spring, caterpillars resume feeding, and bask on old leaves of vegetation. They moult several times before the pupation period. Last caterpillar stages (post hibernation ones) are known to be parasitized by a *Cotesia* wasp (Hymenoptera: Braconidae: Microgastrinae) (Waeyenbergh and Baguette 1996, Shaw 2009). *C. eunomiae* species is a gregarious koinobiont endoparasitoid, specialist of *B. eunomia* larvae (M. Shaw personal communication). Braconidae larvae emerge from their host in June, forming small yellow cocoons to pupate.

Study site

The Pisserotte peat bog, located on the Tailles highland (S-E, Belgium, 50°13'N, 5°47'E; altitude between 550 and 605 m), consists of a network of suitable habitat patches spread along the Rodayi River (27 patches totaling 24053 m²).

Capture-Mark-Recapture data collection

The Pisserotte population of *B. eunomia* was studied yearly by Capture-Mark-Recapture (CMR) from 2002 to 2009 (with the exception of 2003). During the entire flight period, all patches of the study site were visited daily when suitable weather conditions allowed butterfly activity. For each (re)capture, the following data were recorded: marking code, sex, date and patch.

Demographic parameters (i.e. survival and recapture rates, daily and total population size) were inferred from CMR data using Mark program (White and Burnham 1999). We followed the procedure as described in Schtickzelle, et al. (2002) (with one difference: the POPAN procedure now built in the MARK software was used in place of the POPAN-5 stand alone version).

Parasitism data collection

From spring 2004 to spring 2009, bistort patches were sampled by visual inspection to collect *B. eunomia* next to last and last instars caterpillars. Caterpillars found in the field were geolocalized by GPS, and brought to the lab to be reared individually in Petri dishes (outdoor temperature fluctuations; photoperiod L:D 12h:12h), until they pupate (when they are not parasitized) or the parasitoid larvae egress from the caterpillar. Every two days, frass and unused plant material were removed and fresh plants added to ensure caterpillars were fed *ad libitum*. All plant material was removed from the Petri dish at the beginning of caterpillar pupation. Caterpillars that

died prematurely were dissected to assess whether they were parasitized or not; for some of them the reasons of death remained anyway unknown.

The density dependence function

The total population size (N_t) and its variance have been estimated during a 7-yr period (2004–2010) for the Pisserotte population. From these values, five estimates of population growth rates ($R_t = N_t/N_{t-1}$) were computed. Since, these data were too few to fit a density-dependent function and such information was available for a nearby *B. eunomia* population (the Prés de la Lienne, PL), we used the density-dependence function of this PL population for the Pisserotte one. The effect of both density dependence (total population size of *B. eunomia* adults at year t-1) and weather conditions on the population growth rate was assessed for the PL population from a 19 year (1992-2010) population size time series, giving a function describing the population growth rate according to butterfly abundance and weather conditions (unpublished data, but see Schtickzelle and Baguette 2004 for first results based on a 10 yr time series). At the regional scale, it is reasonable to assume that monthly weather conditions were similar for all butterfly populations (Sutcliffe et al. 1996); the weather part of the function estimated in PL could therefore be transferred as it to the present case of the Pisserotte *B. eunomia* population. However, the transfer of the effect of butterfly abundance had to take into account that the Pisserotte habitat quality and quantity were higher than in the PL; hence a higher carrying capacity was expected (3 times higher, according to resource-based functional area: Schtickzelle et al. unpublished data). The transfer of the density-dependent effect of population abundance has then been done by dividing the slope of this effect by a factor 3.

Using this function, the expected growth rate in Pisserotte was computed according to the population size the year before and weather

conditions during the full life cycle of the species. Then, we computed the difference between the observed and the expected population growth rate, and related this residual to the magnitude of parasitism observed the same year (parasitized caterpillars collected the year t affecting the adult population size of the same year t).

VI.3. RESULTS

Seven years of CMR data totaled to 3194 marked individuals (1169 females, 2025 males) and 4268 recaptures of adults (877 females, 339 males) (Table VI.1).

Table VI.1. Summary of CMR data sets for the seven studied generations of the Pisserotte population.

Year	Number of CMR sessions	Number of captured individuals			Number of recaptures		
		Females	Males	Total	Females	Males	Total
2004	23	322	594	916	540	1705	2245
2005	17	164	216	380	106	482	588
2006	12	65	77	142	27	51	78
2007	6	32	59	91	7	35	42
2008	8	82	170	252	30	165	195
2009	12	172	363	535	44	380	424
2010	10	332	546	878	123	573	696

Both the daily abundance of individuals (peak ranging from 804 to 123 for males and from 761 to 81 for females) and the total population size varied greatly between years (Table VI.2 and Fig. VI.1). The tendency was a decrease from 2004 to 2007 and an increase for the three last studied years, as quantified by the estimate of the population growth rate R_t (Table VI.2).

Table VI.2. Estimated population size and population growth rate (with 95 % confidence interval) of *B. eunomia* population in Pisserotte over 8 generations.

year	Females	Males	Total	Population growth rate
2002	593 (± 57.1)	663 (± 44.64)	1256 (± 72.48)	
2003				
2004	632 (± 85.51)	804 (± 46.47)	1436 (± 98.29)	0.423
2005	345 (± 79.08)	262 (± 17.31)	607 (± 80.68)	0.532
2006	166 (± 45.78)	157 (± 42.17)	323 (± 73.01)	0.632
2007	81 (± 29.07)	123 (± 33.49)	204 (± 55.52)	2.074
2008	182 (± 63.8)	241 (± 26.91)	423 (± 71.83)	2.366
2009	499 (± 114.3)	502 (± 40.92)	1001 (± 123.03)	1.550
2010	761 (± 157.4)	791 (± 58.5)	1552 (± 174.42)	

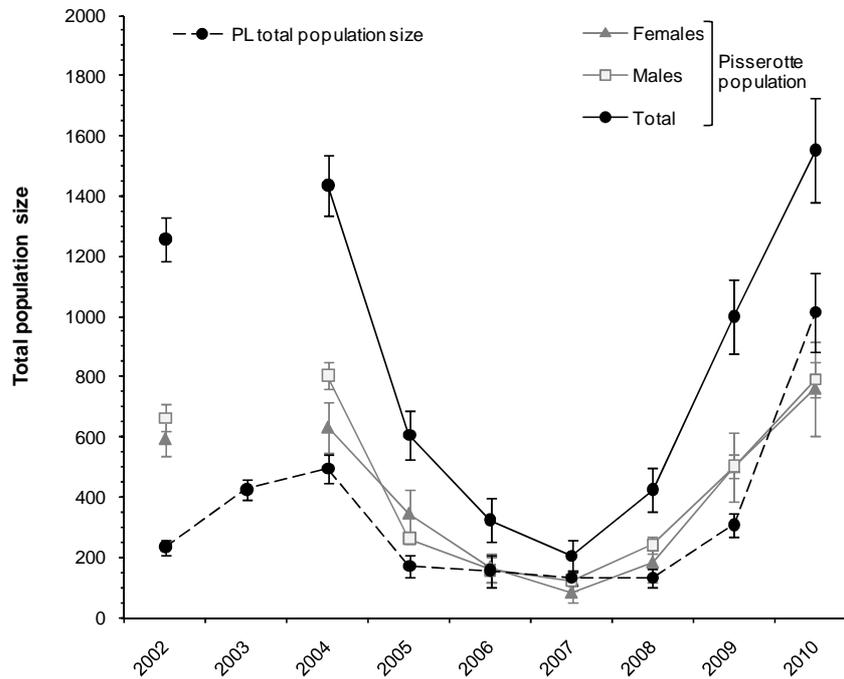


Figure VI.1. Temporal change in the Pisserotte population size from 2002 to 2010, and the PL population to illustrate their temporal synchrony.

The comparison for the same time lag (Fig. VI.1) of the population size evolution in the two studied sites, PL (Schtickzelle unpublished data) and Pisserotte, showed synchronous fluctuations.

Parasitism rate varied from 76% in 2006 to 36% in 2009 (Table VI.3).

Table VI.3. Number of caterpillars collected in the Pisserotte *B. eunomia* population, together with their parasitism status, and yearly estimate of parasitism rate.

	Parasitized	Not parasitized	Parasitism rate
2004	4	3	57%
2005	76	25	75%
2006	112	35	76%
2007	.	.	.
2008	16	10	62%
2009	60	106	36%

Difference between observed growth rate and predicted one ranked from -0.47 to 0.85 (Fig. VI.2). A strong negative correlation was detected between this difference and the parasitism rate, though only the Spearman rank correlation was statistically significant due to the very small sample size (Spearman rank correlation $R = -1.00$, $p < 0.0001$; Pearson correlation $R = -0.80$, $p = 0.20$; $n = 4$) (Fig. VI.3).

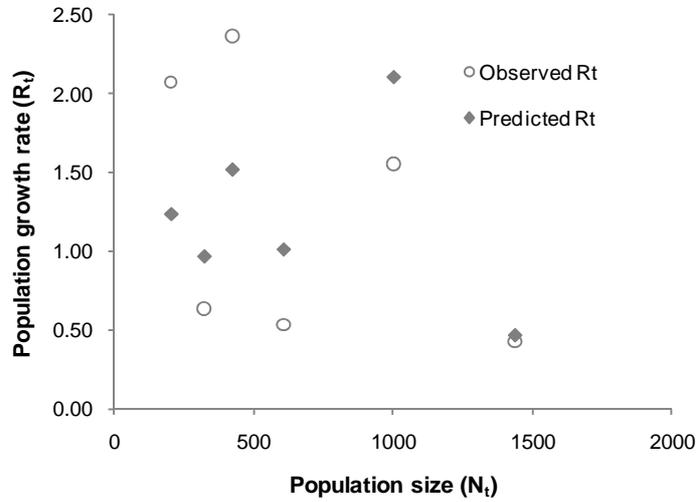


Figure VI.2. Observed and predicted growth rate of the *B. eunomia* population in Pisserotte. The predicted growth rate was estimated on the basis of parameters obtained from the PL population which were subsequently transferred to the Pisserotte one.

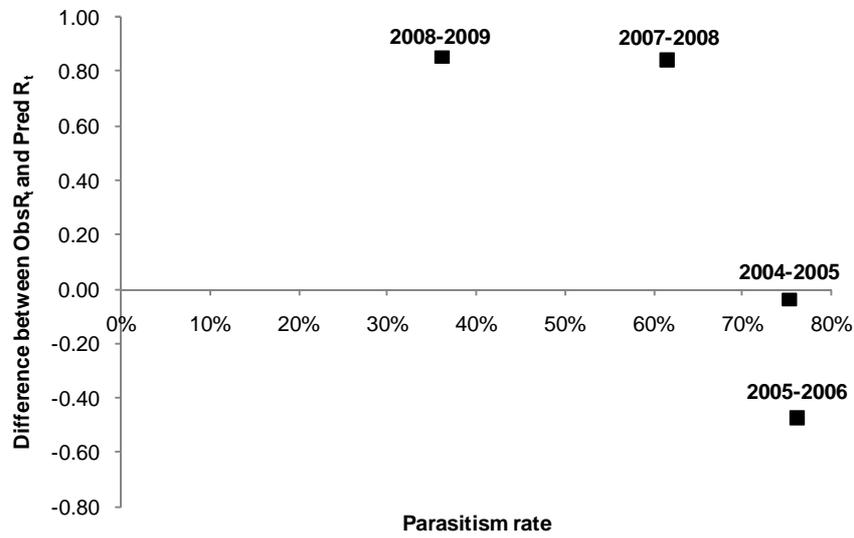


Figure VI.3. Correlation between residual growth rate and parasitism rate.

VI.4. DISCUSSION

At the temporal scale, the *B. eunomia* population size showed variations from one generation to the next, not only in the Pisserotte site but also in the PL one (Schtickzelle unpublished data). Such spatial synchrony between two populations distant by around 15 kilometers is mainly explained by weather conditions (Sutcliffe et al. 1996, Jones et al. 2003, Koenig 2006). These temporal fluctuations are principally explained by density-dependent processes (negative effect of abundance) and environmental stochasticity as previously demonstrated for other species (Hochberg et al. 1992, Steffan-Dewenter and Tschardt 2002, Pickens 2007, Nowicki et al. 2009), including *B. eunomia* (Schtickzelle and Baguette 2004; Schtickzelle et al. unpublished results).

To explain the existence of a residual difference between observed and predicted population growth rates, two main factors can be proposed: food plant shortage (Hanski and Kuussaari 1995, Schtickzelle and Baguette 2004) and caterpillar parasitism (Wilson and Roy 2009, Porter 1983, Lei and Hanski 1997). The first factor was not retained. Indeed, according to the high host plant quantity and quality in Pisserotte (Turlure et al. 2009), *B. eunomia* caterpillars were never numerous enough to consume more than a small fraction of the food available. Moreover, it was unlikely that females found no suitable egg-laying site. That is why we focus on the parasitism agent.

Our results revealed that a high parasitism rate (above 75%) tended to negatively affect the growth rate of the bog fritillary population. This tendency suggests that caterpillar parasitism can have a direct negative effect on their host population size. Besides, this direct parasitism effect might be underestimated by our analysis. Indeed, when we removed the impact of weather factors on the butterfly population dynamics, we removed not only its direct impact (on larval mortality for example) but maybe also some indirect effects implying the parasitoids, because weather conditions affect

interactions between butterflies and other species (Wilson and Roy 2009). For example, as host-parasitoid relationships are influenced by the coincidence of the adult stage of the parasitoid with the larval stage of the host (van Nouhuys and Lei 2004, Tylianakis et al. 2008), any phenological mismatch, due to different time development between *C. eunomiae* and *B. eunomia*, can lead to variable incidence of the parasitoid species on its host (Porter 1983, Cobbold et al. 2009). Or a more direct weather effect on parasitism: *C. eunomiae* population size is also likely to be influenced by weather. In such a case, removed weather effects on *B. eunomia* growth rate may have been partly due to parasitism, leading to an underestimation of the impact of parasitism.

Anyway, it remains difficult to demonstrate that such mortality factor can regulate their host population (Tschardtke 1992, Anton et al. 2007). Another hypothesis can also be advanced to explain the relationship between the parasitism and the butterfly population dynamics: an indirect effect. Thus, other studies shown that parasitism can suppress densities of its host population sufficiently to allow regulation by other density dependent factor(s) (Hochberg et al. 1996, Teder et al. 2000).

To conclude, we are aware that this study is embedded of several biases. First, due to the small sample size, we cannot definitely reject the hypothesis that parasitism has no effect on the population dynamics and the residual difference between the two estimated growth rates will be due to some environmental noise. Extending the time series should allow resolving this problem. Second, our estimates of both the growth rate and the parasitism rate may be biased. Even if we can reasonably assume that long-term weather effects were very similar at the regional scale and that the strength of density-dependent regulation is a species-specific parameter (at least within the same part of the distribution area where the ecological features of the species are identical), it would definitely be better to estimate the density dependence function directly in the Pisserotte population.

Besides, we cannot forget that a precise measure of the parasitism rate from post-hibernation instar hosts is difficult to obtain. Indeed, it is often assumed that parasitized larvae of this instar have a prolonged instar of about few days. Therefore, if field sampling is carried out late in the season, very high parasitism rates may be documented, conversely early sampling may result in a significant underestimate of parasitism rates (Choutt et al. submitted, Klapwijk et al. 2010). Finally and more importantly, only the estimation of the parasitism rate along the entire *B. eunomia* larval development (from egg to pupae) will give a correct assessment of the impact of parasitism to relate to the population growth rate, and then confirm or not the observed tendency of a negative impact of parasitism on the butterfly population dynamics.

GENERAL DISCUSSION

AND

PERSPECTIVES_____

GENERAL DISCUSSION

Threatened and endangered species do not exist in a vacuum; on the contrary they depend on more or less intimate interactions with other species (Ricklefs and Miller 2000). Understanding how species interact with the others is important to better understand their population dynamics and how they persist and evolve. Moreover, throughout the last decade, models to predict viability of populations have emerged as a tool to provide practical conservation guidelines for threatened species (Akçakaya and Sjögren-Gulve 2000, Morris and Doak 2002, Beissinger and McCullough 2002). Integrating species interactions in such models could lead to more realistic predictions (Sabo 2008). This task represents however a huge challenge, since each species is integrated in a complex network, specific of each ecosystem (Sabo 2008). In order to perform such kind of work, firstly pair-wise interactions must to be identified and understood.

Host-parasitoid interactions are highly specific and intimate associations that are linking the two species involved in the relation. Throughout this thesis, we gathered information on one host-parasitoid relationship, i.e. caterpillars of the bog fritillary butterfly, *B. eunomia*, and its primary specialist parasitoid, *C. eunomiae*. This study focused on knowledge acquisition on both species involved in the interaction (how the parasitoid species influences the host one and the reverse) and on the possible role of the parasitoid species in the regulation of its host population. In this discussion, we first of all compile the obtained results and discuss their role and usefulness for further researches, as well as their possible application for other studies. Finally based on the results obtained, we develop some research perspectives.

*New insights on the host-parasitoid relationship implying *B. eunomia* butterfly and its primary parasitoid *C. eunomiae**

- Host parasitoid relationship from the host side

Firstly, we studied the ability of *B. eunomia* to escape parasitoid attack at the population scale by investigating the impact of habitat quality on the parasitism prevalence. Chapter I added some rather convincing evidence of the importance of enemy-free space (EFS) to reduce the impact of parasitism. Thus, our results suggest that *B. eunomia* caterpillars living in a high quality habitat face with costs in terms of higher risk to be parasitized. The consistency of these results at both spatial and temporal scales still need to be assessed (Heard et al. 2006). At the spatial scale, one way to address the issue of suboptimal habitat quality as an “enemy-free space” is to compare this kind of EFS in different, spatially separated, sites. If habitat quality plays a role in parasitism avoidance in other sites, we could conclude on the effectiveness of this type of EFS. We might also evaluate EFS at the temporal scale. Indeed, the evolution of parasitoid searching behaviors may be shifted, and suboptimal habitats in Pisserotte then would consist of EFS only because they represent a transitory phase. If this is the case, this type of habitat will not present any benefit in terms of escape from parasitism attack. To conclude on the importance of habitat quality heterogeneity as an enemy-free space, understanding both how the parasitoids orientate towards habitat to find hosts and how butterfly females lay their eggs depending on habitat quality could be useful. Moreover, knowing if habitat quality heterogeneity indeed plays an important role in parasitism avoidance could be useful for the generalization of this notion and for better understanding of the system, and, consequently, its protection. Besides that, this poses some questions from the viewpoint of the parasitoid as following: why does the parasitism prevalence differ at the population scale according to habitat quality? Is it

because individuals fail to locate their host (due to presence of a low signal or a low dispersal capacity)? Is this because the hosts are of lower quality? *C. eunomia* may also prefer to search a high habitat quality in which the host performs the best (differences in host quality according to habitat quality).

As a second step (Chapter II), we investigated both ecological and morphological factors of host caterpillars that can explain the number of Braconid parasitoids attacking them. The main result (generalist species were more parasitized than specialist ones) was in concordance with other studies. This study brings out an assumption that *C. eunomia* is not the only primary parasitoid of *B. eunomia* caterpillars. Indeed, they possess characteristics which make us to suppose that they can be parasitized by other wasp species. A sampling covering a larger study area (Europe, for example) could be useful in order to determine the entire caterpillar parasitoid complex of *B. eunomia*. Apart from that, a larger sampling and more research would be required to answer the question about the reasons why *B. aquilonaris* caterpillars are not parasitized. Moreover, this study has only focused on the caterpillar stage attacked by Braconidae, but it could be interesting to enlarge the study to other parasitoid orders attacking Lepidoptera caterpillars, such as Ichneumonidae. Knowledge of why some Lepidoptera species are more parasitized than others and which morphological, ecological or behavioral factors could explain such differences is an important factor for the improvement of our understanding of species interactions. However, we think that the impact of all kinds of predators has to be considered to have a more realistic and complete picture.

- Host parasitoid relationship from the parasitoid side

Identification of the parasitoid species as a new one, specialized on *B. eunomia* caterpillar (Shaw 2009), allowed to establish the real relation

between the two species. However, information obtained on the parasitoid species still remains far from complete.

Due to habitat complexity, *C. eunomiae* females need to disperse to find available hosts. To insure this first task (finding hosts), as host encounter rate is directly correlated with the production of offspring (Godfray 1994), they have developed complex and highly successful strategies (Vinson 1976, Vinson et al. 1998). Unfortunately, our work (Chapter III) failed to give exhaustive conclusions about the most relevant infochemicals involved in this behavior. Since chemical cues associated with the host or the host microhabitat have already been shown to play an important role in host location (Steinberg et al. 1993, Afsheen et al. 2008b), we think that improvement of the experimental design could bring more information on host search by *C. eunomiae* females. Firstly, more dual choice experiments can be performed: entire plant instead of leaves, live eating caterpillars instead of mechanically damaged leaves, caterpillar exuvia, etc. Secondly, we should improve the experimental design to allow female flying.

In Chapter IV, we showed that, as other endoparasitoids, *C. eunomiae* affected its host development: unparasitized caterpillars were heavier than parasitized ones. Moreover, in order to insure a complete development of the parasitoid larvae (at least until their host egression), the host-parasitoid complex seems to play a role: the heavier this complex, the bigger number of parasitoid larvae succeeds to emerge from the host. Two main hypotheses have been proposed to explain the parasitoid egression failure: 1) a decrease in host condition during its development and 2) an excessive number of deposited parasitoid eggs according to the host condition. To answer which of the hypotheses takes place in the reality, further studies are required.

According to our genetic analyses (chapter V), *C. eunomiae* could be a monomorph species at the Belgium scale. However, what stays

unknown is: why is it so? More details and deeper knowledge could be gained with a larger population sampling. Moreover, if *C. eunomia* is indeed a good disperser allowing a constant gene flow between its host populations, it could be interesting and useful to determine its dispersal capacity and how it orientates towards habitat (this links directly to the questions raised in the chapter III: the individual level, through the host search by female wasp have implications at the parasitoid population level). Besides that, knowledge on the dispersal capacity of the wasps is important not only for characterization of its population genetic structure but also for understanding the dynamics of the host-parasitoid interaction at the spatial scale (van Nouhuys and Hanski 2002b), especially because the bog fritillary population dynamics is already well-known.

To complete our understanding of the parasitoid species, more studies and enhancement of the conducted experiments are required. Physiological, chemical and behavioral studies could be helpful to determine how the host and parasitoid species interact. For example, physiological studies should be undertaken to explore if *B. eunomia* caterpillars are able to encapsulate the parasitoid or if *C. eunomia* females insert polydnviruses during oviposition. As the host immune response is viewed as the most effective defense of caterpillars against parasitism (Smilanich et al. 2009), such information would improve our understanding of the relation between two species and of their evolution. Behavioral studies would be helpful and indispensable to answer questions such as 1) is caterpillar behavior helpful to prevent parasitoid attacks? 2) can female wasps discriminate between healthy hosts and already parasitized ones? or/and 3) what is the dispersal capacity of parasitoid individuals? Chemical studies might be needed for understanding which clues and in which way female wasps use while moving within their habitat. Such kind of research can contribute to deepen the knowledge on the natural history of the parasitoid species and to better understand its population dynamics. Finally, these results could be integrated

in models of host-parasitoid dynamics (for example optimal foraging strategies) (Hochberg et al. 1996, Tenhumberg et al. 2006). We would like also to emphasize that for understanding multitrophic interactions in natural systems a field approach is a must, as the laboratory experiments will never be able to simulate the complexity of the interacting environmental factors (Gols et al. 2005). Indeed, if we take the example of host search behavior by female wasp, infochemicals of lower trophic level are of great importance to localize their hosts; however in nature, this information can be perturbed by wind or rain conditions. In a similar way, the efficiency of the parasitoid to locate a host-infested plant can be affected by the vegetation composition around the host food plant (Gols et al. 2005).

- Parasitoids as a possible cause of *B. eunomia* population regulation

In the last chapter (VI), we demonstrated that in the study site, both the bog fritillary butterfly population size and the parasitism rate fluctuated greatly during the studied time period. While density dependent processes and weather conditions explained in a great part the butterfly population fluctuations, the observed tendency of a negative impact of parasitism on its host growth rate needs to be confirmed. Furthermore, some hypotheses were raised to explain parasitism rate fluctuations, but more studies are required to fully understand parasitoid population fluctuations. Thus, the role of environmental and demographic stochasticities on parasitoid species needs to be explored more deeply. For this, answering the following questions would be of help: 1) how parasitoids (at both the individual and population levels) are affected by weather conditions? 2) how do they respond to host density in patches? and 3) does host density in patch affect foraging time of female wasps? Moreover, the impact of parasitism on its host population growth rate remains unknown. The combination of these studies performed

using experimental approach could provide an answer to this ecological issue (Hunter et al. 1997).

We studied only one population, but it could be interesting to detect both the host and parasitoid population dynamics in other sites at a larger landscape scale. Can we establish at the landscape scale a couple of host-parasitoid population dynamics? An answer to this question could be useful in a conservation context. Moreover, the stability of the entire system would clearly depend on the spatial population structures of each species. A comprehensive understanding about the habitat and the biology of each species is needed in order to draw conclusions about the relative contributions of large-scale and small-scale factors to the stability of populations of interacting species (van Nouhuys and Hanski 2002b).

PERSPECTIVES

As stressed before, a deeper understanding of the studied host-parasitoid complex would gain from improving and enlarging the preliminary experiments conducted here and from new experiments. Accordingly, we propose some future research objectives leading to a better understanding of the interaction between *B. eunomia* and *C. eunomiae*.

First of all, the lack of knowledge about the parasitoid life cycle is a major limitation for any better understanding of the studied relationship. We still do not know if *C. eunomiae* as other *Cotesia* species has several generations in its host. To answer this question, the knowledge on rearing of *B. eunomia* caterpillars, especially during their diapause period, will be essential. Another important question is: does parasitoid enter in diapause and if so, when?

Moreover, identifying the parasitism impact on all butterfly stages is required to better understand the influence of this factor on the host population dynamics. Thus, recently, during one of our field seasons (2009) we learned that *B. eunomia* pupae are parasitized by at least one generalist Ichneumonidae species. *Ichneumon gracilicornis* Gravenhorst (Ichneumonidae: Ichneumoninae), pupal parasitoid of *B. eunomia*, is a generalist species known to attack pupae of a wide range of Nymphalidae (Heliconiinae, Nymphalinae and Satyrinae) (Shaw et al. 2009), like pupae of *Melitaea cinxia* (Lep: Nymphalidae) (Lei et al. 1997), or *Euphydryas aurinia* (Komonen 1997). Concerning egg parasitism, *Trichogramma* wasps are well known to be egg parasitoids of Lepidopteran species but it has never been recorded in *B. eunomia*. More investigations in this field could be the subject for future researches.

Some future research topics can be formulated as the following questions.

1. How may the dynamics of host-parasitoid interactions be influenced by interactions with other species?

Based on the studied literature, we conclude that even though studies of pair-wise interactions are important for a better understanding of species relations, to have a more realistic view, depicting the natural situation, the integration of the other multi-trophic levels will be necessary. The relationships between hosts and parasitoids within communities are frequently linked in complex food webs with among others the presence of hyperparasitoids (Muller et al. 1999, Lewis et al. 2002). Population dynamics of primary parasitoids can be influenced by this higher order enemy, which may weaken their impact on the herbivore level. As *C. eunomia* is attacked by at least three hyperparasitoid species, it could be interesting to go further in studying the relation between *C. eunomia* and its parasitoids in order to make the dynamics of the entire system, implying *B. eunomia*, more realistic, even if such studies are much more complicated (Tschardtke and Hawkins 2002).

2. How the dynamics of host-parasitoid interactions may be influenced by spatial landscape structure?

The persistence of functional relationships in prey/parasitoid system across a fragmented landscape is a crucial conservation topic (Holt 2002). Spatial distribution of habitat plays a role in any species interaction. Thus, both the isolation of habitat patches and the quality of matrix have been proved to affect parasitoid species (Kruess 2003); an increase in habitat isolation negatively impacted the parasitoid presence (Tschardtke et al. 2002a). The knowledge collected so far on *B. eunomia* provides a solid background for further research on the impact of landscape structure on parasitoid species and on their population dynamics. Indeed, it has been shown (by both CMR and genetic studies) that in the Plateau des Tailles population network

(which includes the Pisserotte population) are linked together due to the dispersal events (Vandewoestijne and Baguette 2004). But, what about parasitoids, especially keeping in mind that their dispersal ability is often assumed to be more restricted than that of herbivores (van Nouhuys and Hanski 2002a, Esch et al. 2005, Kankare et al. 2005). Therefore, it could be interesting to integrate the parasitoid population dynamics into the dynamics of its host in this Belgian landscape in order to have a more realistic (despite being also more complex) view of the population dynamics of the bog fritillary butterfly.

3. How the host-parasitoid interactions may be influenced by the global change context? How the relationship between *B. eunomia* and *C. eunomiae* will evolve?

Several studies showed that climate change, for example, affected this kind of relation through direct or indirect effects (effect of CO₂ increase: Stiling et al. 1999, effect of climate change: Voigt et al. 2003). Thus, species may respond to climate in tandem, with no net change in the indirect effect, or species may respond differently, leading to enhancement or weakening of the indirect effect (Tylianakis et al. 2008). Understanding impact of climate change on the studied interaction would be extremely useful for the creation of more efficient conservation plan (Barton et al. 2009).

To conclude, the consideration of higher trophic level, the dimension of the landscape structure (composition and isolation) and the global change impact may render the host-parasitoid dynamics more realistic and can be useful to better understand each species involved in the relationship and the population fluctuations at a landscape level (Diez et al. 2006).

Importance of a diversity of studies to better understand host parasitoid relationships

Finally, I would like to highlight the importance of interdisciplinary knowledge for a thorough understanding of host-parasitoid interactions, something which is also true for the ecological sciences in general. To fully understand the functioning of interactions over variable spatio-temporal scales, it becomes an imperative to address and link processes operating at levels from the gene to the community (Vet and Godfray 2008). Indeed, this kind of relationships is extremely complex and can be studied only by using different approaches. Through this thesis, we have explored some of them, and some have been more successful than others.

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