

**Host-parasite interactions in the *Phengaris (Maculinea)***

***nausithous-Myrmica rubra* system**

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# Introduction

## Myrmecophily

Ant colonies provide a suitable microhabitat for numerous species, offering them reliable food resources and protection from weather and predation. About 10,000 arthropods species have evolved a tight life history relationship with ants (Thomas et al. 2005; Barbero et al. 2012) termed “myrmecophily”. These myrmecophiles can be mutualistic or parasitic and vary from facultative to obligate and are widespread because of the wide range of specific bio-ecological traits of the ants (Hinton 1951) which include:

- Large number of species and occurrence in a large variety of environments;
- Large number of individuals per nest;
- Ant nests are generally permanent, with presence of overwintering brood;
- Easy and frequent encounters with workers because of their patrolling and foraging behaviours.

More than 4000 Lepidoptera species (butterflies and moths) are myrmecophiles with Lycaenids representing the dominant group (Hinton 1951).

Many of these Lycaenids are obligatorily dependent on ants’ presence to complete their life cycle and interactions are typically highly species specific (Fiedler 1998; Pierce et al. 2002).

The myrmecophily of *Phengaris (Maculinea)* butterflies is arguably the most derived one. In taxonomy *Maculinea* is at present regarded as a subgenus of genus *Phengaris* (Fric et al. 2007), but in the context of the present thesis the reference to *Phengaris* is meant to only refer to species of this subgenus *Maculinea*. *Phengaris (Maculinea)* species have evolved particular

adaptations to avoid ant worker predation including a thicker cuticle compared to larvae of non-myrmecophilous butterflies. They can retract the head under a sclerotized plate as defence against the ant attacks (Malicky 1970; Sliwinska et al. 2006). Furthermore, *Phengaris* butterflies evolved special adaptations for communicating with ants including the use of chemical and acoustical signals (Akino et al. 1999; DeVries et al., 1993; Pierce et al. 2002; Barbero et al., 2009a; Barbero et al., 2009b; Sala et al., 2014).

Initial investigations on the host specificity of *Phengaris* butterflies suggested a strict association with single *Myrmica* host species (Thomas et al. 2005). Yet subsequent studies revealed much more complex host specificity patterns that can vary at the population level or at the regional scale (Elmes et al. 1994; Steiner et al. 2003; Sielezniew and Stankiewicz 2004; Tartally and Varga 2005; Witek et al. 2006; Tartally and Varga 2008; Tartally et al. 2008; Sielezniew and Stankiewicz-Fiedurek 2009; Sielezniew et al. 2010; Casacci et al. 2011; Barbero et al. 2012). The list of suitable *Myrmica* host species had to be expanded for nearly all *Maculinea* species. The only remaining exception is *P. nausithous* as the only species that apparently is associated with a specific single host species (*M. rubra*) all over its distributional range (Barbero et al., 2012). Only two isolated populations of *P. nausithous* in Transylvania and in Spain (Munguira and Martin 1999; Tartally et al., 2008) have been observed to exploit *M. scabrinodis* colonies. This unusual association may suggest that these *P. nausithous* populations are specific life form or cryptic species (Tartally et al., 2008; Rákosy et al. 2010).

## **Host-parasite co-evolution**

An adaptation in parasite to better exploit its host, may enhance the selective pressure on the host, selecting for a counter-adaptation and vice versa, resulting in co-evolutionary dynamics

termed “arms race” (Dawkins and Krebs 1979). The concept of an arms race in host-parasite evolution may help a lot to better understand the life history and specific adaptations of parasites and their hosts (Dawkins and Krebs 1979). Thus, it is not surprising to also see highly specific local adaptation in the *Myrmica-Phengaris (Maculinea)* system (Als et al. 2001, 2022; Nash et al. 2008; Solazzo et al. 2013, 2014).

Important preconditions for any local co-evolution between a host and a parasite are:

- i) Parasites have to be sufficiently abundant to select host resistance
- ii) restricted geneflow and
- iii) highly specific recognition mechanisms in hosts (Nash and Boomsma, 2008; Nash et al. 2008).

*Phengaris* parasitic butterfly larvae mainly use chemical mimicry to break into a *Myrmica* colony that is otherwise closed to all non-nestmates (Elmes et al. 1991). This mimicry involves the production of chemical compounds characteristic of its host, which also serves for a social parasite to disguise itself from its host (Nash and Boomsma, 2008). Thus, in a co-evolutionary arms race, parasites evolve better mimicry to increase their virulence and hosts improve their recognition of parasites (Foitzik et al. 2003; Nash et al. 2008).

## **Butterflies as social parasites: *Phengaris (Maculinea)***

### **Life cycle**

Butterflies of the subgenus *Maculinea* within the genus *Phengaris* are exclusively endemic in the Palaearctic Region and occur from Western Europe to Asia (Mongolia, China and Japan) (Munguria and Martin 1999): They have an obligate parasitic relationship with ant species of the genus *Myrmica*. The first records of this parasitic life cycle have been described for *Phengaris (M.) arion* and *P. (M.) alcon* (Hinton 1951). After mating, females lay single eggs on particular food-plants, which are species-specific. The foodplants of each species are well

known (Elmes and Thomas 1992): *Sanguisorba officinalis* (Rosaceae) for *P. (M.) nausithous* and *P. (M.) teleius*, *Gentiana pneumonanthe* (Gentianaceae) for *P. (M.)alcon*, *G. cruciata* (Gentianaceae) for *P. (M.) rebeli* and *Thymus* sp. and *Origanum vulgare* (Lamiaceae) for *P. (M.) arion*. The choice of the correct foodplant for oviposition is dependant on the flowering and fruiting phenology (Barbero et al. 2012). It is unknown if and how the butterfly females detect the presence of the host ants in the near surrounding of the food-plant (Schepper et al. 1995; Van Dyck et al. 2000; Wynhoff et al. 2008; Van Dyck and Regniers 2010; Patricelli et al. 2011), in some cases the foodplant and the *Myrmica* host species share a similar habitat (Thomas and Elmes 2001; Nowicki et al. 2005; Musche et al. 2006; Fürst and Nash 2010).

After 4-10 days of incubation the larvae leave the egg and enter flower buds and the seeds, where they live for the following 2-3 weeks. During this period, the larva completes 3 moults as a herbivorous feeder and acquires 1% of its body mass (Thomas et al. 1989). The fourth larval instar leaves the flowerheads and moves to the ground where it can encounter *Myrmica* host workers and can be carried into the host colony.

The adoption and integration of *Phengaris* caterpillars into *Myrmica* nests, passes through a series of ant behavioural filters (encounter, infection and exploitation; Nash et al., 2011) in which chemical mimicry is considered the main means of *Phengaris* larvae to be adopted and fully integrated into the host colony (Elmes et al. 1991). Nevertheless other signals like larval size, tactility, the secretion of dorsal nectary organ combined with behavioural “adoption” display and the emission of sounds to mimic the queen acoustical signals facilitate the adoption of *Phengaris* larvae (DeVries et al., 1993; Elmes et al., 2001; Barbero et al., 2009a; Barbero et al., 2009b; Sala et al. 2014).

Inside the colony the larva spends the following 9-10 months feeding and growing (Thomas 1984). Some larvae of *Phengaris* can spend one additional year inside the host colony to complete their development (Hovestadt et al. 2007; Witek et al. 2006; Tartally 2005; Elmes et al. 2001; Als et al. 2001; Schönrogge et al. 2000; Thomas et al. 1998).

*Phengaris* larvae exploit two different feeding strategies. The predatory feeders, *P. arion* and *P. teleius*, show an exceptional case of carnivorous nutrition among the Lepidoptera. They directly feed on larvae causing serious damage to the colony, which may lead to overexploitation (Thomas and Wardlaw 1992). The cuckoo feeders like *P. alcon* and *P. rebeli* cause nurse ants to feed through trophallaxis. This strategy allows several larvae to survive inside the same nest and never leads to overexploitation (Elmes et al. 1991).

The feeding strategy of *P. nausithous* is still under debate, some authors suggest the coexistence of both “cuckoo” and “predatory” (Thomas and Settele, 2004) lifestyles, while other authors regard *P. nausithous* a “cuckoo” feeder (Patricelli et al. 2010).

Inside the nests of host ants, the young larvae increase in weight acquiring about 99% of their final weight, and at the beginning of summer the larvae move into the upper part of the nest where they pupate and eclose after three weeks as an adult (Fiedler 1990; Thomas and Wardlaw 1992).

## **Origin and evolution**

The origin and evolution of *Phengaris* parasitism seems tightly linked to the adoption process (Pech et al. 2004). Since the predatory species of *Phengaris* use secretions of a dorsal nectary organ (DNO) during the adoption, and DNO secretions play an important role in mediating mutualistic association between lycaenid butterflies and ants, the predatory *Phengaris* species have been proposed as ancestral species (Fiedler 1998).

Pech et al. (2004) used a cladistics analysis based on morphological and ecological data, to show that the use of the DNO secretions during adoption is an ancestral state, which was subsequently reduced in *P. nausithous* and lost in cuckoo feeders *P. alcon* and *P. rebeli*. However they did not find an unequivocal solution for the origin of predatory and cuckoo feeding strategy. Treating *P. nausithous* as predatory or as polymorphic feeders, three scenarios are possible i) predatory and cuckoo strategies originated independently, ii) the predatory strategy evolved into the cuckoo strategy, iii) cuckoo is the ancestral habit that changed into predatory (Pech et al. 2004). Therefore the clarification of *P. nausithous* feeding habit might be helpful to understand the evolution of feeding strategies in these parasitic butterflies (Pech et al. 2004).

## Conservation Status

*Phengaris (Maculinea)* butterflies in Europe are declining because of their complex life cycle in combination with habitat loss and fragmentation (Wells et al. 1983). The extinction of *P. arion* in the United Kingdom in the 1970s (Thomas 1980) raised interest in the conservation of these butterflies (Elmes and Thomas 1992; Hochberg et al. 1994; Thomas 1994; Wynhoff 1998). During the past decades *Phengaris (Maculinea)* butterflies have become icons of European biodiversity conservation (Thomas and Settele 2004), and are perceived as umbrella species covering many grassland communities (Randle et al. 2005; Spitzer et al. 2009; Casacci et al. 2011).

Their conservational status in Europe has been classified as follows:

<i>Phengaris (M.)</i> spp.	Habitats Directive (Annex)*	Red list status Europe **	Red list status EU27 **	European Conservation Status***
<i>P. rebeli</i>				SPEC 1
<i>P. alcon</i>		LC	NT	SPEC 3
<i>P. arion</i>	II, IV	EN	EN	SPEC 3
<i>P. nausithous</i>	II, IV	NT	NT	SPEC 3
<i>P. teleius</i>	II, IV	VU	VU	SPEC 3

(\*Van Helsdingen et al. 1996 \*\*Van Swaay et al. 2010; \*\*\*Van Swaay and Warren 1999)

LC (Least Concern), EN (Endangered), VU (Vulnerable) NT (Near Threatened).

SPEC 1- threatened species at global level, restricted to Europe

SPEC 3 – Not restricted in Europe but threatened in Europe (unfavorable conservational status).

## **Ants as host: *Myrmica***

*Myrmica* ants are hosts of *Phengaris* butterflies. Their colonies have a monomorphic worker castes, and their workers show a reddish coloration with possibility of variation from the pale orange to the black-brown and a middle length between 5 and 10 mm.

Although the biology of many *Myrmica* species has not been studied in detail, it seems that a general life style is common to all species (Radchenko and Elmes 2010).

Queens lay eggs in early spring till late summer, parts of the larvae develop rapidly but others enter diapause and overwinter (Barbero et al. 2012). Larvae vary in size, the biggest will become queens, the others males or workers. The winged forms abandon the nest, and from the end of August until the beginning of September they complete their nuptial flight (Wynhoff 2001). Newly mated queens can either found a new nest (haplometrosis) or can be integrated into existing colonies (Thomas et al. 1993). Nests are built under stones, wood, leaves or accumulating musk (Elmes 1991). All *Myrmica* ants are saprophytic, generalist and predators. The number of workers per nest can vary from few hundreds to thousands of individuals, while the number of queens has been reported to vary between one to ten (Wardlaw and Elmes 1996). *Myrmica* colonies can be found in various kinds of habitat, such as meadows, woods, human altered landscapes and few xerophilous steppe forms (Arnoldi 1968; Radchenko and Elmes 2010). Most species are adapted to cool conditions and in the southern parts of their distribution, *Myrmica* are confined to high altitudes (Jansen et al. 2010).

*Myrmica* ants are widespread in the Holoartic region, with European, Asian and well separated North-American species (Czechowski et al. 2002). There are 150 species described (Czechowski et al. 2002) and 111 species are present in Eurasia (Elmes et al. 2002).

The genus is divided in four main taxonomic groups (Wardlaw et al. 1998):

1. “**lobicornis**” group: three species (*Myrmica lobicornis* Nylander, 1846; *Myrmica schencki* Emery, 1895; *Myrmica lobicornis* var. *lobulicornis* Nylander, 1857)
2. “**scabrinodis**” group: four species (*Myrmica sabuleti* Meinert, 1860, *Myrmica scabrinodis* Nylander, 1846; *Myrmica specioides* Bondroit, 1918; *Myrmica lonae* Finzi, 1926).
3. “**rugulosa**” group: three species (*Myrmica rugulosa* Nylander, 1849; *Myrmica gallieni* Bondroit, 1920 e *Myrmica sulcinodis* Nylander, 1846)
4. “**rubra**” group: two species (*Myrmica rubra* L., 1758 and *Myrmica ruginodis* Nylander, 1846) .

## Aims of research

The coevolutionary adaptations between *P. nausithous* and *M. rubra* are the core of this thesis.

Research on the interactions between *Phengaris* species and their hosts is not only relevant for understanding the co-evolutionary host-parasite dynamics but also for developing appropriate evidence based conservation strategies (Fiedler 1998; Als et al. 2004; Thomas and Settele 2004; Hovestadt et al. 2007; Nash et al. 2008; Barbero et al. 2012). For example *P. nausithous* can be more vulnerable to the habitat fragmentation and requires more efforts for conservation if a co-evolutionary arms race is going on, because on the contrary to other species like *P. alcon*, it can not shift into other *Myrmica* hosts when *M. rubra* resistance arises (Nash et al. 2008).

Until now most of the studies around coevolutionary adaptation between *Phengaris* butterflies and their hosts were focused on cuckoo species. *P. alcon* and *P. rebeli* are considered the most derived social parasite within the genus *Phengaris* because is shorter adoption time, more elaborate communication and a lower fitness cost imposed upon the ant hosts (Fiedler 1990, 1998; Elmes et al. 1991, 2002; Thomas and Elmes 1998). Why *P. nausithous* is less studied compared to other species is probably because it seems to be a “joining link” between cuckoo and predatory forms. Although it can exploit also ant brood as food source (Elfferich 1998), like cuckoo feeders it is highly specialized and achieves some social integration (Thomas and Settele 2004). However as earlier mentionated, there are any evidence to support the origin of cuckoo species from predatory forms (Pech et al. 2004).

*P. nausithous* like other *Phengaris* butterflies in Europe are declining and local extinctions have already been observed (Settele 1998), thus more efforts on this species are need. In any case, the high specificity for *M. rubra* across all of Europe (Thomas et al., 1989; Elmes et al., 1998; Tartally and Varga, 2005; Munguira and Martin, 1999; Stankiewicz and Sielezniew,

2002; Witek et al., 2008; Patricelli et al., 2010), its vulnerable status throughout its range (Van Helsdingen et al. 1996; Van Swaay and Warren 1999; Van Swaay et al. 2010), and its role as conservation icon together with the other *Phengaris* butterflies (Pyle et al. 1981; Settele et al. 2005) make *P. nausithous* a good model species for studying host-parasite co-adaptation and large blue butterfly conservation. Therefore *P. nausithous* was chosen as target species of this research.

Consequently, the basic research questions of the present thesis are:

Is the host-parasite interaction so tight to fuel a co-evolutionary arms race?

Was there an evolution of allomones in *P. nausithous* that elude *M. rubra* nest-mate recognition?

Do *M. rubra* infested populations respond by local adaptation and evolve a more careful nest-mate recognition as counter adaptation?

Does a long and intense parasitism of *P. nausithous* affect the population genetics of *M. rubra*?

To answer these questions a multidisciplinary approach had to be chosen included chemical-behavioural ecology and population genetics:

### *Chemical ecology*

The majority of social parasites gain access to host colonies by exploiting the recognition signals used by the social insects themselves (Nash and Boomsma 2008). However, little is known about the function of individual or classes of compounds within the mixture of cuticular hydrocarbons, while a positive interaction is evident during the retrieval of *Phengaris* larvae by workers (Schönrogge et al. 2004). Then the first question to address is: which are the chemical cues involved in the mimicry of *P. nausithous* caterpillars? The research of compounds that are common to both *M. rubra* and *P. nausithous* larvae may

represent the best candidates for *M. rubra* brood signature and *P. nausithous* mimicry. Subsequently, the second goal is to prove the behavioural response of shared compounds with their synthetic counterpart to find those compounds in *P. nausithous* larvae that might catch the attention of *M. rubra* workers.

#### *Behavioural ecology*

As *Myrmica* ants have evolved sophisticated nest-mate recognition abilities based on the complex signals of cuticular hydrocarbons, it is clear that any mimicry of a social parasite does not require to match the overall suite of signals produced by their hosts because not all compounds of the host are equally important. Most importantly a social parasite must mimic general signals involved in species recognition rather than those for intra-specific colony interaction. The parasite larvae need to be adopted into any colony of a host species not a specific one. Thus the attractivity of *P. nausithous* for *M. rubra* workers is expected to be lower than that of its own brood. Nevertheless there is strong selection on the parasitic larvae for optimizing their mimicry and reduce the avoidance by the local host workers.

In case of local adaptation to the parasite, sympatric and allopatric workers should show different behavioral responses towards *P. nausithous* depending on who is leading the arms race: host or parasite. Therefore the core questions are: i) do *Myrmica* workers recognize nest-mate larvae? ii) do workers prefer own brood over *P. nausithous* larvae? and iii) are there differences in *P. nausithous* larvae choice between *Myrmica* ants from sympatric or allopatric parasite populations?

#### *Population genetics*

The “Gestalt odour” hypothesis (Crozier and Dix 1979) predicts that nest-mates share a common colony specific label. Since a larger genetic variation will increase differences among genetically derived recognition labels, this may reduce the efficiency of nest-mate recognition. Thus, *P. nausithous* can take advantage in *M. rubra* colonies with high genetic variation because this increases the variability of odour among colony members and

consequently the workers' tolerance. Comparing the genetic variability of infested *M. rubra* populations with *M. rubra* populations with those that (at least for quite some time) are not infested by *P. nausithous*, is expected to find out host populations genetically more varied in association with the social parasite, because a higher genetic variation allows for an easier infiltration into the colony.

## References

- Akino T., Knapp J.J., Thomas J.A. and Elmes G.W. 1999. Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. Proc R Soc B 266: 1419-1426
- Als T.D., Nash D.R. and Boomsma J.J. 2001. Adoption of parasitic *Maculinea alcon* caterpillars (Lepidoptera: Lycaenidae) by three *Myrmica* ant species. Animal Behaviour 62: 99–106
- Als T.D., Nash D.R. and Boomsma J.J. 2002. Geographical variation in host-ant specificity of the parasitic butterfly *Maculinea alcon* in Denmark. Ecological Entomology 27: 403-414
- Als T.D., Villa R., Kandul N.P., Nash D.R., Yen S., Hsu Y., Mignault A.A., Boomsma J.J. and Pierce N. E. 2004. The evolution of alternative parasitic life histories in large blue butterflies. Nature 432: 386-390
- Arnoldi K.V. 1968. Zonal'nye zoogeografičeskie i ekologičeskie osobennosti mirmekofauny i naseleniâ murav'ev russkoj ravniny. Zoologičeskij Žurnal, 47: 1155–1178 [Zonal zoogeographic and ecologic features of myrmecofauna and the ant population of the Russian plain] (in Russian with English summary)
- Barbero F., Thomas J., Bonelli S., Balletto E. and Schönrogge K. 2009a. Queens ants make distinctive sounds that are mimicked by a butterfly social parasite. Science 323: 782-785
- Barbero F., Thomas J., Bonelli S., Balletto E. and Schönrogge K. 2009b. Acoustical mimicry in a predatory social parasite of ants. J Exp Biol 212: 4084-4090

- Barbero F., Patricelli D., Witek M., Balletto E., Casacci L.P., Sala M. and Bonelli S. 2012. *Myrmica* ants and their butterfly parasites with special focus on the acoustic communication. *Psyche* 11 pages
- Casacci L.P., Witek M., Barbero F., Patricelli D., Solazzo G., Balletto E. and Bonelli S. 2011. Habitat preference of *Maculinea arion* and its *Myrmica* host ants: implications for habitat management in Italian Alps. *J Insect Cons* 15: 103-110
- Crozier RH and Dix MX 1979. Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. *Behav Ecol Sociobol* 47: 217-224
- Czechowski W., Radchenko A. and Czechowska W. 2002. *The Ants (Hymenoptera, Formicidae) of Poland*. Museum and Institute of Zoology PAS, Warszawa pp. 200.
- Dawkins R. and Krebs J.R. 1979. Arms races between and within species. *Proc R Soc B* 205: 489-511
- De Vries P.J., Cocroft R.B. and Thomas J. 1993. Comparison of acoustical signals in *Maculinea* butterfly caterpillars and their obligate host *Myrmica* ants. *Biol J Linn Soc* 49: 229-238
- Elfferich N.W. 1998. New facts on the life history of the dusky large blue *Maculinea nausithous* (Lepidoptera: Lycaenidae) obtained by breeding with *Myrmica* ants in plaster nest. *Deinsea* 4: 97–102
- Elmes G.W., Thomas J.A. and Wardlaw J.C. 1991. Larvae of *Maculinea rebeli*, a large-blue butterfly, and their *Myrmica* host ants: wild adoption and behaviour in ant nests. *J Zool* 223: 447-460
- Elmes G.W. and Thomas J.A. 1992. Complexity of species conservation in managed habitats: interaction between *Maculinea* butterflies and their ant hosts. *Biodiversity and Conservation* 1: 155-169
- Elmes G.W., Thomas J.A., Hammarstedt O., Lopez Munguira M., Martin J. and Van der Made. 1994. Differences in host-ant specificity between Spanish, Dutch and Swedish

- populations of the endangered butterfly, *Maculinea alcon* (Denis et Schiff.) (Lepidoptera).  
*Memorabilia Zoologica* 48: 55-68
- Elmes G.W., Thomas J.A., Wardlaw J.C., Hochberg M.E., Clarke R.T. and Simcox D.J. 1998.  
The ecology of *Myrmica* ants in relation to the conservation of *Maculinea* butterflies. *J Insect Conserv* 2: 67-78
- Elmes G.W., Thomas J.A., Munguira M.L. and Fiedler K. 2001. Larvae of lycaenid butterflies that parasitize ant colonies provide exceptions to normal insect growth rules. *Biol J Linn Soc* 73: 259-278
- Elmes G.W., Akino T., Thomas J.A., Clarke R.T. and Knapp J.J. 2002. Interspecific differences in cuticular hydrocarbon profiles of *Myrmica* ants are sufficiently consistent to explain host specificity by *Maculinea* (large blue) butterflies. *Oecologia* 130: 525-535
- Fiedler K. 1990. New informations on the biology of *Maculinea nausithous* and *M. telius* (Lepidoptera, Licaenidae). *Nota lepid* 12: 246-256
- Fiedler K. 1998. Lycaenid-ant interaction of the *Maculinea* type: tracing their historical roots in a comparative framework. *J Insect Conserv* 2: 3-14
- Foitzik S., Fischer B. and Heinze J. 2003. Arms races between social parasites and their hosts: geographic patterns of manipulation and resistance. *Behav Ecol* 14: 80-88
- Fric Z., Wahlberg N., Pech P. and Zrzavy J. 2007. Phylogeny and classification of the *Phengaris-Maculinea* clade (Lepidoptera: Lycaenidae): total evidence and phylogenetic species concepts. *Systematic Entomology* 32: 558-567
- Fürst M.A. and Nash D.R. 2010. Host ant independent oviposition in the parasitic butterfly *Maculinea alcon*. *Biology letters* 6: 174-176
- Hinton H.E. 1951. Myrmecophilous Lycaenidae and other Lepidoptera - a summary. *Proc South Lond Ent Nat Hist Soc* 1949-50: 111-175

- Hochberg M.E., Clarke R.T., Elmes G. W. and Thomas J. A. 1994. Population dynamic consequences of direct and indirect interactions involving a large blue butterfly and its plant and red hosts. *J Anim Ecol* 63: 375-391
- Hovestadt T., Mitesser O., Elmes G. W., Thomas J. A. and Hochberg M.E. 2007. An Evolutionarily Stable Strategy Model for the Evolution of Dimorphic Development in the Butterfly *Maculinea rebeli*, a Social Parasite of *Myrmica* Ant Colonies. *The American Naturalist* 169: 466-480
- Jansen G., Savolainen R. and Vepäläinen. 2010. Phylogeny, divergence-time estimation, biogeography and social parasite-host relationships of the Holarctic ant genus *Myrmica* (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution* 56: 294-304
- Lukhatanov V.A. and Lukhtanov A.G. 1994. *Die Tagfalter Nordwestasiens*. *Herbipoliana* 3, Markt-leuthen pp 440
- Malicky H. 1970. New aspects of association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymenoptera). *Journal of the Lepidopterists Society* 24: 190-202
- Munguria M.J. and Martin J. 1999. *Action Plan for Maculinea Butterflies in Europe*. *Nature and Environment*, No. 97. Council of Europe Publishing, Strasbourg. pp 64
- Musche M., Anton C., Worgan A. and Settele J. 2006. No experimental evidence for host ant related oviposition in a parasitic butterfly. *Journal of insect Behaviour* 19: 631-643
- Nash D.R. and Boomsma J.J. 2008. Communication between hosts and social parasites. In: *Sociobiology of Communication: an interdisciplinary approach* (d'Ettoire P. and Hughes D.P., Eds), Oxford University Press, Oxford. pp 55-79
- Nash D.R., Als T.D., Maile R., Jones G.R. and Boomsma J.J. 2008. A mosaic of chemical co-evolution in a large blue butterfly. *Science* 319: 88-90
- Nash D.R., Als T.D. and Boomsma J.J. 2011. Survival and growth of parasitic *Maculinea alcon* caterpillars (Lepidoptera, Lycaenidae) in laboratory nests of three *Myrmica* ant species. *Insect Soc* 58: 391-401

- Nowicki P., Witek M., Skórka P. and Woyciechowsky M. 2005. Oviposition patterns in the myrmecophilous butterfly *Maculinea alcon* Denis & Schiffermüller (Lepidoptera: Lycaenidae) in relation to characteristics of foodplants and presence of ant hosts. *Polish Journal of Ecology* 53: 409-417
- Patricelli D., Witek M., Barbero F., Casacci L.P., Bonelli S. and Balletto E. 2010. Evidence of High Larval Host Ant (Hymenoptera: Formicidae) Specificity in the First Post-Adoption Phase for the Myrmecophilous Butterfly *Phengaris (Maculinea) nausithous* (Lepidoptera: Lycaenidae). *Sociobiology* 55: 861-869
- Patricelli D., Barbero F., La Morgia V., Casacci L.P., Witek M., Balletto E. and Bonelli S. 2011. To lay or not to lay: oviposition of *Maculinea arion* in relation to *Myrmica* ant presence and host plant phenology. *Animal Behaviour* 82: 791-799
- Pech P., Fric Z., Konvička M. and Zrzavý 2004. Phylogeny of *Maculinea* blues (Lepidoptera: Lycaenidae) based on morphological and ecological characters: evolution of parasitic myrmecophily. *Cladistic* 20: 362-375
- Pierce N.E., Brady M.F., Health A., Lohman D.J., Mathew J., Rand D.B. and Travassos M.A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology* 47: 733-771
- Pyle R., Bentzien M. and Opler P. 1981. Insect conservation. *Annual Review of Entomology*, 26: 233–258
- Radchenko A.G. and Elmes G.W. 2010. *Myrmica* ants (hymenoptera: Formicidae) of the Old World. *Natura Optima dux* Foundation, Warszawa Poland
- Rákosy L., Tartally A., Goia M., Mihali C. and Varga Z. 2010. The Dusky Large Blue – *Maculinea nausithous* ssp. *Kijevensis* (Sheljuzhko, 1928) in the Transylvanian basin: New data on taxonomy and ecology. *Nota lepid* 33: 23-28
- Randle Z., Simcox D.J., Schönrogge K., Wardlaw J.C. and Thomas J.A. 2005. *Myrmica* ants as keystone species and *Maculinea arion* as an indicator of rare niches in UK grasslands.

- In: *Studies on the Ecology and Conservation of Butterflies in Europe*. (Settele J., Kühn E. and Thomas J.A. Eds), vol 2: Species Ecology along a European Gradient: *Maculinea* butterflies as a model. Pensoft, Sofia/Moscow, pp. 230
- Sala M., Casacci L.P., Balletto E., Bonelli S. and Barbero F. 2014. Variation in Butterfly larval acoustics as a strategy to infiltrate and exploit host ant colony resources. PLoS ONE doi:10.1371/journal.pone.0094341
- Scheper M., Van der Made G. and Wynhoff I. 1995. *Maculinea alcon*: interactions between a myrmecophilous butterfly its larval foodplant and its host ant. Proceedings of Experimental and Applied Entomology NEVAmsterdam 6: 77-78
- Schönrogge K., Wardlaw J.C., Thomas J.A. and Elmes G.W. 2000. Polymorphic growth rates in myrmecophilous insects. Proc R Soc B 267: 771-777
- Schönrogge K., Wardlaw J.C., Peters A.J., Everett S., Thomas J.A. and Elmes G.W. (2004) Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. Journal of Chemical Ecology 30: 91-107
- Settele J (1998) *Metapopulationsanalyse auf Rasterdatenbasis - Modellgestützte Analyse von Metapopulationsparametern und naturschutzfachliche Umsetzung im Landschaftsmaßstab, erläutert am Beispiel von Tagfaltern*. Teubner, Leipzig/Stuttgart
- Settele J. 2005. How endangered is *Maculinea nausithous*? In: *Studies on the Ecology and Conservation of Butterflies in Europe*. (eds Settele J, Kühn E, Thomas JA), vol 2: Species Ecology along a European Gradient: *Maculinea* butterflies as a model. Pensoft, Sofia/Moscow, pp. 230
- Sibatani A., Saigusa T. and Hirowatari T. 1994. The genus *Maculinea* van Eecke, 1915 in the Eastern Palaearctic region. Tyo To Ga 44: 154-220
- Sielezniew M. and Stankiewicz A.M. 2004. Simultaneous exploitation of *Myrmica vandeli* and *M. scabrinodis* (Hymenoptera: Formicidae) colonies by the endangered

- myrmecophilous butterfly *Maculineaalcon* (Lepidoptera: Lycaenidae. Eur J Entomol 101: 693-696
- Sielezniew M. and Stankiewicz A.M. 2007. Differences in the development of the closely related myrmecophilous butterflies *Maculineaalcon* and *M. rebeli* (Lepidoptera: Lycaenidae). Eur J Entomol 104: 433-444
- Sielezniew M. and Stankiewicz-Fieddurek A.M. 2009. Host ant use by *Phengaris (=Maculinea)alcon* in Poland. Polish Journal of Entomology 78: 323-335
- Sielezniew M., Patricelli D., Dziekańska I., Barbero F., Bonelli S., Casacci L.P., Witek M. and Balletto E. 2010. The first record of *Myrmica lonae* (Hymenoptera: Formicidae) as a host of the socially parasitic Large Blue butterfly *Phengaris (Maculinea) arion* (Lepidoptera: Lycaenidae). Sociobiology 56: 465-475
- Sliwinska E.B., Nowicki P., Nash D.R., Witek M., Settele J. and Woyciechowski M. 2006. Morphology of caterpillars and pupae of European *Maculinea* species (Lepidoptera: Lycaenidae) with an identification table. Entomologica Fennica 17: 351-358
- Solazzo G., Moritz R.F.A. and Settele J. 2013. Choice behaviour of *Myrmica rubra* workers between ant larvae and larvae of their *Phengaris (Maculinea) nausithous* nest parasites. Insect Soc 60:57–64
- Solazzo G., Moritz R.F.A. and Settele J. 2014. The social parasite *Phengaris (Maculinea) nausithous* affects genetic diversity within *Myrmica rubra* host ant colonies. J Insect Conserv 18:69–75
- Spitzer L., Benes J., Dandova J., Jaskova V. and Konvicka M. 2009. The Large Blue butterfly, *Phengaris [Maculinea] arion*, as a conservation umbrella on a landscape scale: the case of the Czech Carpathians. Ecological Indicators 9: 1056-1063
- Stankiewicz A. and Sielezniew M. 2002. Host specificity of *Maculinea teleius* Bgstr. and *M. nausithous* Bgstr. (Lepidoptera:Lycaenidae). The new insight. Annals Zool 52: 403-408
- Steiner F.M., Sielezniew M., Schlick-Steiner, Höttinger H., Stankiewicz A. and Górnicki A.

2003. Host specificity revisited: new data on *Myrmica* host ants of the lycaenid butterfly *Maculinea rebeli*. *J Insect Conserv* 7: 1–6
- Tartally A. and Varga Z. 2005. Host-ant specificity of *Maculinea* species in Hungary, connection with parasitoids and host plants. In: *Studies on the Ecology and Conservation of Butterflies Along a European Gradient: Maculinea Butterflies as a Model* (Settele J., Kühn E., and Thomas J.A., Eds) vol. 2 Pensoft, Sofia, Moscow
- Tartally A. 2005. Accelerated development of *Maculinea rebeli* larvae under artificial conditions (Lycaenidae) *Nota lepid* 27: 303-308
- Tartally A. and Varga Z. 2008. Host ant use of *Maculinea teleius* in the Carpathian Basin (Lepidoptera: Lycaenidae). *Acta Zoologica Academiae Scientiarum Hungaricae* 54: 257-268
- Tartally A., Nash D.R., Lengyel S. and Varga Z. 2008. Patterns of host ant use by sympatric population of *Maculinea alcon* and *M. 'rebeli'* in the Carpathian Basin. *Insect Soc* 55: 370-381
- Tartally A., Rákosy L., Vizauer T.C., Goia M. and Varga Z. 2008. *Maculinea nausithous* exploit *Myrmica scabrinodis* in Transylvania: unusual host ant species of a myrmecophilous butterfly in an isolated region (Lepidoptera: Lycaenidae; Hymenoptera: Formicidae). *Sociobiology* 51: 373-380
- Thomas J.A. 1980. Why did the large blue become extinct in Britain? *Oryx* 15 243-247
- Thomas J.A. 1984. The behaviour and habitat requirements of *Maculinea nausithous* (the dusky large blue butterfly) and *M. teleius* (the scarce large blue) in France. *Biol Cons* 28: 325-347
- Thomas J.A., Elmes G.W., Wardlaw J.C., Woyciechowshi M., 1989 - Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. *Oecologia* 79: 452- 457
- Thomas J.A. and Wardlaw J.C. 1992. The capacity of a *Myrmica* ant nest to support a predacious species of *Maculinea* butterfly. *Oecologia* 91: 101–109

- Thomas J.A., Elmes G.W. and Wardlaw J.C. 1993. Contest competition among *Maculinea rebeli* butterfly larvae in ant nests. *Ecological Entomology* 18: 73-76
- Thomas J.A. 1994. The ecology and the conservation of *Maculinea arion* and other European species of large blue. In: *Ecology and Conservation of Butterflies* (Pullin A.S. Ed), Chapman & Hall, London pp 180-196
- Thomas J.A., Elmes G.W. and Wardlaw J.C. 1998. Polymorphic growth in larvae of the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc R Soc B* 265: 1895-1901
- Thomas J.A. and Elmes G.W. 2001 Food-plant niche selection rather than the presence of ant nests explain oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. *Proc R Soc B* 268: 471-477
- Thomas J.A. and Settele J. 2004. Evolutionary biology: Butterfly mimics of ants. *Nature* 432: 283-284
- Thomas J.A., Schönrogge K. and Elmes G.W. 2005. Specializations and host associations of social parasites. In *Insect Evolutionary Ecology* (Fellowes M., Holloway G. and Rolff J. Eds), CABI publishing, Wallingford pp. 479-518
- Tuzov V.K. (ed.) 1997. *Guide to the butterflies of Russia and adjacent territories*. Vol. 2 Pensoft Publishers, Sofia
- Van Dyck H., Oostermeijer G.B., Talloen W., Feenstra V., Van Der Hidde A. and Wynhoff I. 2000. Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? *Proceedings of the Royal Society B* 267: 861-866
- Van Dyck H. and Regniers S. 2010. Egg spreading in the ant-parasitic butterfly, *Maculinea alcon*: from individual behaviour to egg distribution pattern. *Animal Behaviour* 80: 621-627
- Van Helsdingen P.J., Willemse L. and Speight M.C.D. 1996. Background information on invertebrates of the Habitat Directive and the Bern Convention: Part 1: Crustacea,

- Coleoptera and Lepidoptera. Nature and Environment. Council of Europe Publishing, Strasbourg
- Van Swaay CAM, Warren MS (1999) *Red data book of European butterflies (Rhopalocera)*. Nature and environment No. 99. Council of Europe Publishing, Strasbourg
- Van Swaay C, Cuttelod A, Collins S, Maes D, López Munguira M, Šašić M, Settele J, Verovnik R, Verstrael T, Warren M, Wiemers M, Wynhoff I (2010) *European Red List of Butterflies*. Luxembourg. Publication Office of the European Union. pp. 47
- Wardlaw J.C. and Elmes G.W. 1996. Exceptional colony size in *Myrmica* species (*Hymenoptera: Formicidae*). *The Entomologist* 115: 191-196
- Wardlaw J.C., Elmes G.W. and Thomas J.A. 1998. Techniques for studying *Maculinea* butterflies: Identification guide to *Myrmica* ants found on *Maculinea* sites in Europe. *J Insect Conserv* 2: 119-127
- Wells S.M., Pyle R.M., Collins N.M., 1983 - The IUCN Invertebrate Red Data Book. IUCN Gland Switzerland p. 447
- Witek M., Sliwinska E. B., Skórka P., Nowicki P., Settele J. and Woyciechowsky M. 2006. Polymorphic growth in larvae of *Maculinea* butterflies, as an example of biennialism in myrmecophilous insects. *Oecologia* 148: 729-733
- Witek M., Sliwinska E.B., Skorka P., Nowicki P., Wantuch M., Vrabec V., Settele J. and Woyciechowski M. 2008. Host ant specificity of large blue butterflies *Phengaris (Maculinea)* (Lepidoptera: Lycaenidae) inhabiting humid grasslands in East-central Europe. *Eur J Entomol* 105: 871-877
- Wynhoff I. 1998. The recent distribution of the European *Maculinea* species. *J Insect Conserv* 2: 15-27
- Wynhoff I., 2001 – At home in foreign meadows: the reintroduction of two *Maculinea* Butterfly species. PhD thesis Wageningen Agricultural University, Wageningen Netherlands

Wynhoff I. Grutters M. and Van Langevelde F. 2008. Looking for the ants: selection of oviposition sites by two myrmecophilous butterfly species. *Animal Biology* 58: 371-388

**Tetracosane on parasitic butterflies *Phengaris (Maculinea) nausithous*  
cuticle triggers the first contact in the adoption process by *Myrmica rubra*  
foragers**

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**Abstract.** *Phengaris (Maculinea)* butterflies are social parasites of *Myrmica* ant colonies. Larvae of the parasite are adopted by the ant workers into the colonies. Apparently, chemical signals are used by *P. nausithous* larvae to mimic those of the host brood to be recognised by the ants. Here, chemical extracts of ant brood and butterfly larvae with four different solvents are tested in behavioural choice assays in search of compounds involved in the adoption process. Tetracosane is the main shared compound in all brood extracts of *M. rubra* and in all larvae of *P. nausithous*. The attractiveness of tetracosane for *M. rubra* workers is confirmed by testing synthetic tetracosane in behavioural choice assays, suggesting that the adoption ritual may be initiated by tetracosane.

**Keywords.** Chemical mimicry, social parasites, myrmecophily, co-evolution, brood recognition.

**Choice behaviour of *Myrmica rubra* workers between ant larvae and larvae of their *Phengaris (Maculinea) nausithous* nest parasites**

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Keywords: host-parasite co-evolution, brood recognition, adoption behaviour, local adaptation

Larvae of *Phengaris (Maculinea)* butterflies are adopted by *Myrmica* workers and are obligate myrmecophiles. Brood recognition by *M. rubra* workers was tested for concolonial larvae (*M. rubra*) vs allocolonial larvae (*M. rubra* and *P. nausithous*) to assay the mimetic efficiency of *P. nausithous*. In addition, we tested *M. rubra* ant colonies from different populations with and without the presence of *Phengaris*, to test for potential local adaptation in adoption behaviour. We show that *M. rubra* can distinguish between nest-mate and foreign

larvae as well as between *P. nausithous* and their own larvae. Workers from the allopatric population inspected and rejected more *P. nausithous* larvae than workers from the sympatric population. This might reflect a local host adaptation in which the social parasite more efficiently mimes its sympatric host ants than allopatric ones.

**The social parasite *Phengaris (Maculinea) nausithous* affects genetic diversity within *Myrmica rubra* host ant colonies**

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Keywords: social parasites, *Myrmica*, co-evolution, monogyny, polygyny, host resistance

Evolutionary theory predicts that high genetic variation maintains plasticity in a species' response to parasite pressure. However, higher genetic diversity might also cause easier

infiltration by social parasites, because odour diversity is high and nest-mate recognition poor. Here we test if the obligate myrmecophile Lycaenid butterfly *Phengaris nausithous*, a parasite of colonies of the highly polygynous ant *Myrmica rubra* causes local adaptation by enhancing genetic variance in parasitized vs. non parasitized ant populations. *M. rubra* colonies from six infested and three uninfested sites were assayed at five microsatellite loci to quantify genetic variation. Our results reveal isolation by distance and a significantly enhanced intracolony variance due to the parasite pressure.

# Synthesis

## *Phengaris nausithous* as model of study

The three studies of this thesis address the strategies that allow social parasites to break down colony barriers and the host counter adaptations against the parasitism.

For many years butterflies of the genus *Phengaris* (*Maculinea*) have been the subject of many studies on ecology, behavior and conservation biology. In particular *P. alcon* and *P. rebeli* became a model system for the study of the mechanisms used by social parasites to infiltrate colonies of social insects (Akino et al. 1999; Als et al., 2001, 2002; Schönrogge et al. 2004; Nash et al., 2008, 2011; Barbero et al., 2009a; Barbero et al., 2009b; Sala et al., 2014).

Although *P. nausithous* is a more specific social parasite, it has been rather understudied compared with its European congeners, for example none of the studies on the chemical ecology of *Phengaris* butterflies (Akino et al. 1999; Schlick-Steiner et al. 2004; Schönrogge et al. 2004; Nash et al. 2008; Witek et al. 2013) have taken *P. nausithous* into account, excluding the analysis of some post-adoption samples of *P. nausithous* in Witek et al. (2013).

In contrast this thesis enhances the validity of using *P. nausithous* and *M. rubra* as a model system to detect co-evolutionary adaptation between social parasites and their host. The absence of a competing good alternative host, like *M. ruginodis* that infrequently occurs in the wet meadows used by *P. nausithous*, might have led to this butterfly evolving a greater fidelity to its host over a very wide range (Elmes et al. 2002). The higher specificity of *Phengaris nausithous* suggests a monophyletic clade lacking cryptic species (Ritter et al. 2013), in contrast to other *Phengaris* species which show huge differences in host specificity suggesting the existence of cryptic species and/or speciation (Thomas et al. 2012; Bereczki et al. 2013). Several studies have obtained support for geographical variation in parasitism and host resistance (Thompson 1994, 2005; Van Baalen 1998; Gomulkiewicz et al. 2000; Brodie

et al. 2002; Stokke et al. 2008), hence *P. nausithous* and *M. rubra* may work as a general model to better understand the arms race, host-parasite co-evolution and their geographical variation without problems arising from cryptic and morpho-species, given that this social parasite only exploits just a single host over all of its distribution range (Lukhtanov and Lukhtanov 1994; Sibatani et al. 1994; Tuzov 1997; Wynhoff 1998; Munguira and Martin 1999). It is necessary to clarify that also some aspects of *P. nausithous* biology are till now not fully understood, mainly the feeding strategy. It is probably for this reason that *P. nausithous* is less studied compared to other species, clarification of *P. nausithous* feeding seems to be the only way to resolve the evolution of feeding strategies in these parasitic butterflies (Pech et al. 2004), which should encourage the study of *P. nausithous*.

### **Consequence for understanding host parasite co-evolution**

Combining all the results found for population genetics, behavioural and chemical ecology in *P. nausithous* and *M. rubra* interactions, this thesis suggests the existence of such evolutionary hot spots for *P. nausithous* in which the parasite evolves cuticular hydrocarbons that are attractive to *M. rubra* workers resulting in locally adapted actions between host and parasite. This thesis also suggests that after a long and intensive infestation period cold spots emerge, because currently uninfested host populations show strong differences in sociorganization and nestmate recognition as an adaptative resistance to parasitism. Hence, in host populations in which *M. rubra* is ahead of *P. nausithous*, the host developed a more efficient defence against the parasite, which may have caused local temporary extinctions of *P. nausithous*. On the other hand populations in which *P. nausithous* is ahead of *M. rubra*, the parasite larvae may better mimic the host brood, as already found for *P. alcon* (Als et al., 2001, 2002; Nash et al., 2008).

The dynamics between social parasites and their hosts are typically highly dynamic oscillating processes, often resulting in a host-parasite arms race. At regional scales such interactions

produce a geographic mosaic of co-evolutionary dynamics (Thompson 2005). To understand the geographical mosaic theory of co-evolution it has been suggested (Gomulkiewicz et al. 2007; Ruano et al. 2011) to detect: i) co-evolutionary hot and cold spots. Hot spots are defined as populations in which interacting species have reciprocal effects on each others fitness. These hot spots are often embedded within surrounding communities in which interspecific selection affects one, or neither species (cold spots) (Thompson 1994, 1997; Gomulkiewicz et al. 2000); ii) selection mosaic, populations that show different co-evolutionary outcomes, and iii) Trait remixing (Thompson 2005; Gomulkiewicz et al. 2007), transformations in co-evolutionary mosaic induced when the interacting populations change and when migration occurs.

The ability of a population to adapt to local conditions is determined by the strength of natural selection, effects of genetic drift and gene flow (Slatkin 1973; May et al. 1975; Endler 1977). In particular gene flow among populations is a fundamental evolutionary force that can determine the geographical spread of novel adaptations (Fisher 1930; Mayr 1942; Mayr 1963; Ehrlich and Raven 1969). Theoretical studies has revealed that, with gene flow, hot spots can affect the co-evolutionary dynamics in cold spots and vice versa (Gomulkiewicz et al. 2000). Hence, patterns of local adaptation can be strongly shaped by the mix of co-evolutionary hot and cold spots (Gomulkiewicz et al. 2000). However, migration may also interfere with this local co-evolution: Some authors suggested that gene flow may enable beneficial adaptations by acting as a source of beneficial mutations driving the dynamics of local adaptation (Forde et al. 2004; Morgan et al. 2005). Under this scenario the species with the higher migration rate is expected to become locally adapted (Gandon et al., 1996; Ruano et al. 2011). Other studies point out that the absence of (or restriction of) gene flow is essential for local coevolution, given that migration homogenizes the populations (Nuismer et al. 2003; Nash et al. 2008).

In the Upper Rhine valley, the study area where genetic variability has been tested for *M. rubra* (Solazzo et al. 2014) and *P. nausithous* (Anton et al. 2007) populations, it seems that

the genetic differentiation of host populations is an order of magnitude higher than *P. nausithous* (Seppä and Pamilo 1995; Nash et al. 2008; Solazzo et al. 2014), hence it should be interesting to test if this isolation facilitates host resistance and the cold spot allows *P. nausithous*, at least temporarily, to escape the arms race.

Thus: the high specificity of *P. nausithous* over a very wide range (Lukhtanov and Lukhtanov 1994; Sibatani et al. 1994; Tuzov 1997; Wynhoff 1998; Munguira and Martin 1999; Elmes et al. 2002), the presence of hot and cold spots (Solazzo et al. 2013, 2014), the asymmetry in migration between host and parasite, and the difference in breeding system (haplodiploid for *M. rubra* and diploid for *P. nausithous*) which erodes the genetic variation differently (Anton et al. 2007) suggest several future perspectives for the research on evolutionary dynamics between host-parasite co-adaptations that can be addressed using *P. nausithous* and *M. rubra* as a model system.

This direction for the research on co-evolution between *P. nausithous* and *M. rubra* can also provide an effective support for the conservation of this vulnerable parasitic butterfly.

### **Consequence for improving conservation**

As mentioned in the introduction, the coevolutionary adaptations between *P. nausithous* and *M. rubra* are the core of this thesis and the interactions between *Phengaris* species and their hosts may be relevant for developing appropriate conservation strategies (Fiedler 1998; Als et al. 2004; Thomas and Settele 2004; Hovestadt et al. 2007; Nash et al. 2008; Barbero et al. 2012).

The arms race between *P. nausithous* and *M. rubra* might be consistent with the intermittent arms race hypothesis (Soler et al. 1998). This suggests a scenario in which periods of frequent parasitism will alternate with periods of no or rare parasitism. During periods of intense parasitism, host defences are continuously improving and, as a consequence, the success rate of the parasites is decreasing. Once host defence has reached a high level, brood parasites will

benefit from emigration. After a period of parasites being absent, hosts will lose their defensive adaptations against parasites, and it will then be advantageous for the brood parasite to recolonize the area.

The Upper Rhine valley is an area where *P. nausithous* presence and absence has been studied since 1989 (Settele 1998; Anton et al. 2007; Solazzo et al. 2014). The risk of colony collapse is still unknown in this area but in some studies an annual death of *Myrmica* colonies has been estimated between 80% for nest infested by predator and 76% by cuckoo *Phengaris* (Thomas and Wardlaw 1992; Thomas et al. 1997; Thomas and Elmes 1998; Hovestadt et al. 2012).

Hence some meadows are intensively parasitized while on the other hand in meadows which are no longer infested, *M. rubra* populations are characterized by a higher monogyny (Solazzo et al. 2014) which consequently may promote a more carefully nestmate recognition and a lower acceptance for the parasites making more difficult *P. nausithous* infestations (Solazzo et al. 2013). Further, extinction and recolonization dynamics have already been observed for *P. nausithous* in the various meadows of the Upper Rhine valley (Settele 1998). All these traits are in agreement to the intermittent arms race hypothesis because in the Upper Rhine valley some *M. rubra* populations show highly resistance to be infested in which the parasite disappeared and other populations that seems more vulnerable in which *P. nausithous* survive.

Typically *Phengaris (Maculinea)* butterflies in Europe live in small and closed populations (Thomas 1995; Nowicki et al. 2005), indeed *P. nausithous* can be more vulnerable to habitat fragmentation and more efforts are required for its conservation than for other *Phengaris* species, because the latter can shift into other *Myrmica* hosts when the primary host enhances its resistance (Nash et al. 2008). In fact, as predicted by the intermittent arms race hypothesis, once host defence has reached a high level, parasite will benefit from emigration. Thus to help the survival of this butterfly it might be important to allow and facilitate *P. nausithous* to disperse into surrounding host populations. After a period of parasites being absent, hosts will

lose their defensive adaptations. Then it can be expected that polygynous colonies can be promoted again enabling *P. nausithous* to recolonize the area.

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#### References

- Akino T., Knapp J.J., Thomas J.A. and Elmes G.W. 1999. Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. Proc R Soc B 266: 1419-1426
- Als T.D., Nash D.R. and Boomsma J.J. 2001. Adoption of parasitic *Maculinea alcon* caterpillars (Lepidoptera: Lycaenidae) by three *Myrmica* ant species. Animal Behaviour 62: 99–106
- Als T.D., Nash D.R. and Boomsma J.J. 2002. Geographical variation in host-ant specificity of the parasitic butterfly *Maculinea alcon* in Denmark. Ecological Entomology 27: 403-414
- Als T.D., Villa R., Kandul N.P., Nash D.R., Yen S., Hsu Y., Mignault A.A., Boomsma J.J. and Pierce N. E. 2004. The evolution of alternative parasitic life histories in large blue butterflies. Nature 432: 386-390
- Anton C., Zeisset I., Musche M., Durka W., Boomsma J.J. and Settele J. 2007. Population structure of a large blue butterfly and its specialist parasitoid in a fragmented landscape.

Molecular Ecology 16: 3828-3838

- Barbero F., Thomas J., Bonelli S., Balletto E. and Schönrogge K. 2009a. Queens ants make distinctive sounds that are mimicked by a butterfly social parasite. *Science* 323: 782-785
- Barbero F., Thomas J., Bonelli S., Balletto E. and Schönrogge K. 2009b. Acoustical mimicry in a predatory social parasite of ants. *J Exp Biol* 212: 4084-4090
- Barbero F., Patricelli D., Witek M., Balletto E., Casacci L.P., Sala M. and Bonelli S. 2012. *Myrmica* ants and their butterfly parasites with special focus on the acoustic communication. *Psyche* 11 pages
- Bereczki J., Tóth J.P., Sramko G. and Varga Z. 2014. *Journal of Zoological Systematic and Evolutionary Research* 52: 32-43
- Brodie E.D. Jr, Ridenhour B.J., Brodie E.D. 3<sup>rd</sup>. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. *Evolution* 56: 2067-2082
- Elmes G.W., Akino T., Thomas J.A., Clarke R.T. and Knapp J.J. 2002. Interspecific differences in cuticular hydrocarbon profiles of *Myrmica* ants are sufficiently consistent to explain host specificity by *Maculinea* (large blue) butterflies. *Oecologia* 130: 525-535
- Endler J.A. 1977. *Geographic variation, speciation, and clines*. Princeton University Press, Princeton
- Ehrlich P.R. and Raven P.H. 1969. Differentiation of populations. *Science* 165: 1228-1232
- Fiedler K. 1998. Lycaenid-ant interaction of the *Maculinea* type: tracing their historical roots in a comparative framework. *J Insect Conserv* 2: 3-14
- Fisher R.A. 1930. *The genetical theory of natural selection*. Clarendon Press, Oxford
- Forde S.E., Thompson J.N. and Bohannan B.J.M. 2004. Adaptation varies through space and time in coevolving host-parasitoid interaction. *Nature* 431: 841-844
- Gandon S., Capowiez Y., Dubois Y., Michalakis Y. and Olivieri I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proc R Soc B* 263: 1003-1009

- Gomulkiewicz R., Thompson J.N., Holt R.D., Nuismer S.L. and Hochberg M.E. 2000. Hot spots, cold spots, and geographic mosaic theory of coevolution. *The American Naturalist* 156: 156-174
- Gomulkiewicz R., Drown D.M., Dybdahl M.F., Godsoe W., Nuismer S.L., Pepin K.M., Ridenhour B.J. Smith C.I. and Yoder B.J. 2007. Dos and don'ts of testing the geographic mosaic theory of coevolution. *Heredity* 98: 249-258
- Hovestadt T., Mitesser O., Elmes G. W., Thomas J.A. and Hochberg M.E. 2007. An Evolutionarily Stable Strategy Model for the Evolution of Dimorphic Development in the Butterfly *Maculinea rebeli*, a Social Parasite of *Myrmica* Ant Colonies. *The American Naturalist* 169: 466-480
- Hovestadt T., Thomas J. A., Mitesser O., Elmes G. W., and Schönrogge K. 2012. Unexpected benefit of a social parasite for a key fitness component of its ant host. *The American Naturalist* 179: 110-123
- Lukhatanov V.A. and Lukhtanov A.G. 1994. *Die Tagfalter Nordwestasiens*. *Herbipoliana* 3, Marktleuthen pp 440
- May R.M., Endler J.A., McMurtrie R.E. 1975. Gene frequency clines in the presence of selection opposed by gene flow. *The American Naturalist* 109: 659-676
- Mayr E. 1942. *Systematics and the origin of species*. Columbia University Press, New York
- Mayr E. 1963. *Animal species and evolution*. Harvard University Press, Cambridge
- Munguria M.J. and Martin J. 1999. *Action Plan for Maculinea Butterflies in Europe*. *Nature and Environment*, No. 97. Council of Europe Publishing, Strasbourg. pp 64
- Nash D.R., Als T.D., Maile R., Jones G.R. and Boomsma J.J. 2008. A mosaic of chemical coevolution in a large blue butterfly. *Science* 319: 88-90
- Nash D.R., Als T.D. and Boomsma J.J. 2011. Survival and growth of parasitic *Maculinea alcon* caterpillars (Lepidoptera, Lycaenidae) in laboratory nests of three *Myrmica* ant species. *Insect Soc* 58: 391-401

- Nowicki P., Witek M., Skórka P., Settele J. and Woyciechowski M. 2005. Population ecology of the endangered butterflies *Maculinea teleius* and *M. nausithous* and the implications for conservation. *Population Ecology* 47: 193-202
- Nuismer S.L., Thompson J.N. and Gomulkiewicz R. 2003. Coevolution between hosts and parasites with partially over-lapping geographic ranges. *J Evol Biol* 16: 1337-1345
- Morgan A.D., Gandon S. and Buckling A. 2005. The effect of migration on local adaptation in a coevolving host-parasite system. *Nature* 437: 253-256
- Pech P., Fric Z., Konvička M. and Zrzavý 2004. Phylogeny of *Maculinea* blues (Lepidoptera: Lycaenidae) based on morphological and ecological characters: evolution of parasitic myrmecophily. *Cladistic* 20: 362-375
- Ritter S., Michalski S.G., Settele J., Wiemers M., Fric Z.F., Sielezniew M., Šašić, Rozier Y. and Durka W. 2013. Wolbachia infections mimic cryptic speciation in two parasitic butterfly species, *Phengaris teleius* and *P. nausithous* (Lepidoptera: Lycaenidae). *PLoS ONE* doi:10.1371/journal.pone.0078107
- Ruano F., Devers S., Sanllorente O., Errard C., Tinaut A., and Lenoir A. 2011. A geographical mosaic of coevolution in a slave-making host-parasite system. *J Evol Biol* 24: 1071-1079
- Sala M., Casacci L.P., Balletto E., Bonelli S. and Barbero F. 2014. Variation in Butterfly larval acoustics as a strategy to infiltrate and exploit host ant colony resources. *PLoS ONE* doi:10.1371/journal.pone.0094341
- Schlick-Steiner B., Steiner F.M., Höttinger H., Nikiforov A., Mistrik R., Schafellner C., Baier P. and Christian E. 2004. A butterfly's chemical key to various ant forts: intersection-odour or aggregate-odour multi-host mimicry? *Naturwissenschaften* 91: 209-214
- Schönrogge K., Wardlaw J.C., Peters A.J., Everett S., Thomas J.A. and Elmes G.W. 2004. Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. *Journal of Chemical Ecology* 30: 91-107

- Seppä P. and Pamilo P. 1995. Gene flow and population viscosity in *Myrmica* ants. *Heredity* 74:200–209
- Settele J. 1998. *Metapopulationsanalyse auf Rasterdatenbasis - Modellgestützte Analyse von Metapopulationsparametern und naturschutzfachliche Umsetzung im Landschaftsmaßstab, erläutert am Beispiel von Tagfaltern*. Teubner, Leipzig/Stuttgart
- Sibatani A., Saigusa T. and Hirowatari T. 1994. The genus *Maculinea* van Eecke, 1915 in the Eastern Palaearctic region. *Tyo To Ga* 44: 154-220
- Slatkin M. 1973. Gene and selection in a cline. *Genetics* 75: 733-756
- Solazzo G., Moritz R.F.A. and Settele J. 2013. Choice behaviour of *Myrmica rubra* workers between ant larvae and larvae of their *Phengaris (Maculinea) nausithous* nest parasites. *Insect Soc* 60:57–64
- Solazzo G., Moritz R.F.A. and Settele J. 2014. The social parasite *Phengaris (Maculinea) nausithous* affects genetic diversity within *Myrmica rubra* host ant colonies. *J Insect Conserv* 18:69–75
- Soler M., Soler J.J., Martinez J.G., Pérez-Contreras and Møller A.P. 1998. Micro-evolutionary change and population dynamics of a brood parasite and its primary host: the intermittent arms race hypothesis. *Oecologia* 117: 381-390
- Stoke B.G., Hafstad I., Rudolfson G., Moksnes A., Møller A.P., Røskaft E. and Soler M. 2008. Predictors of resistance to brood parasitism within and among reed warbler populations. *Behavioural Ecology* 19: 612-620
- Thomas J.A. and Wardlaw J.C. 1992. The capacity of a *Myrmica* ant nest to support a predacious species of *Maculinea* butterfly. *Oecologia* 91: 101–109
- Thomas J.A. 1995. The ecology and conservation of *Maculinea arion* and other European species of large blue butterfly. In: *Ecology and conservation of butterflies* (Pullin A.S. ed). Chapman & Hall, London. pp 180-197

- Thomas J.A., Elmes G.W., Clarke R.T., Kim K.G., Munguira M.L. and Hochberg M.E. 1997. Field evidence and model predictions of butterfly-mediated apparent competition between gentian plants and red ants. *Acta Oecologica-International Journal of Ecology* 18: 671-684
- Thomas J.A. and Elmes G.W. 1998. Higher productivity at the cost of increased host-specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. *Ecological Entomology* 23: 457-464
- Thomas J.A. and Settele J. 2004. Evolutionary biology: Butterfly mimics of ants. *Nature* 432: 283-284
- Thomas J.A., Elmes G.W., Sielezniew M., Stankiewicz-Fiedurek A., Simcox D.J., Settele J. and Schönrogge K. 2012. Mimetic host shifts in an endangered social parasite of ants. *Proc R Soc B* 280: 2012-2336
- Thompson J.N. 1994. *The coevolutionary process*. University of Chicago Press, Chicago.
- Thompson J.N. 1997. Evaluating the dynamics of coevolution among geographically structured populations. *Ecology* 78: 1619-1623
- Thompson J.N. 2005. *The Geographic Mosaic of Coevolution*. The University of Chicago Press, Chicago
- Tuzov V.K. (ed.) 1997. *Guide to the butterflies of Russia and adjacent territories*. Vol. 2 Pensoft Publishers, Sofia
- Van Baalen M. 1998. Coevolution of recovery ability and virulence. *Proc R Soc B* 265: 317-325
- Witek M., Casacci L.P., Barbero F., Patricelli D., Sala M., Bossi S., Maffei M., Woyciechowski M., Balletto E. and Bonelli S. 2013. Interspecific relationships in co-occurring populations of social parasites and their host ants. *Biol J Linn Soc* 109: 699-709
- Wynhoff I. 1998. The recent distribution of the European *Maculinea* species. *J Insect Conserv* 2: 15-27

# Appendix

## Curriculum Vitae

### Personal Information

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### Work Experiences

February 2010 – : HIGRADE PhD in Martin-Luther-Universität Halle-Wittenberg and Helmholtz Zentrum für Umweltforschung- UFZ, Halle (Saale) Germany.

Department: Molecular Ecology Hoher Weg 4, 06099 Halle.

Thesis: “Parasite-host interactions in the *Phengaris (Maculinea) nausithous-Myrmica rubra* system”.

July 2013 – September 2013: Wissenschaftliche Mitarbeiter in Helmholtz Zentrum für Umweltforschung - UFZ, Halle (Saale) Germany.

Department: Community Ecology Theodor Lieser Straße 4, 06120 Halle.

Project: LEGATO “Land use and biodiversity”.

## Education

October 2007 - December 2009: Turin University (Italy) - Second Degree (Master)

Thesis “Dispersal in a butterfly metapopulation above the timberline”

Title: Master in Behavioural evolution - 10/12/2009.

October 2002 – April 2007: Palermo University (Italy) - First Degree (Bachelor)

Thesis “The Biology and Reproductive Behaviour of the Lanner Falcon, *Falco biarmicus*, in Sicily”

Title: Bachelor in Natural Science - 02/04/2007.

## Conferences

Oral contribution:

10<sup>th</sup>-11<sup>th</sup> December 2012: CLIMIT (Climate change impacts on Insects and their MITigation) in Halle (Saale) organized by UFZ: G. Solazzo, R.F.A. Moritz, J. Settele, “Can the Social Parasite *Phengaris (Maculinea) nausithous* Modify Genetic Diversity in *Myrmica rubra* Host Ant Populations?”.

2<sup>nd</sup> October 2012: Higrade Fall Conference 2012 in Leipzig (Germany): G. Solazzo, R.F.A. Moritz, J. Settele, “Can the social parasite *Phengaris (Maculinea) nausithous* modify genetic diversity in *Myrmica rubra* host ant populations?”.

25<sup>th</sup>-28<sup>th</sup> March 2011: IUSSE 2011 (International Union for the Study of Social Insects) 2<sup>nd</sup> Central European Meeting Osnabrück in Papenburg (Germany): G. Solazzo, R.F.A. Moritz, J. Settele, “Choice Behaviour of *Myrmica* Workers between *Maculinea* and *Myrmica* Larvae”.

25<sup>th</sup>-26<sup>th</sup> November 2010: CLIMIT (Climate change impacts on Insects and their MITigation) in Laufen (Salzach) organized by ANL and UFZ: G. Solazzo, R.F.A. Moritz, J. Settele, “Choice Behaviour of *Myrmica* Workers between *Maculinea* and *Myrmica* larvae”.

Poster presentations:

13<sup>th</sup>-18<sup>th</sup> July 2014: IUSSE 2014 (International Union for the Study of Social Insects) in

Cairns (Australia): G. Solazzo, K. Seidelmann, R. F. A. Moritz, J. Settele, “Co-evolutionary adaptation of *Phengaris nausithous* and its host *Myrmica rubra*”.

19<sup>th</sup>-22<sup>nd</sup> July 2012: E.C.B.B. 2012 (VIth European Conference on Behavioural Biology 2012) in Essen (Germany): G. Solazzo, R.F.A. Moritz, J. Settele, “*Myrmica* Workers Differentiate between Own Brood and *Phengaris (maculinea)* Nest Parasite”.

## List of publications

2014: Solazzo G., Seidelmann K., Moritz R. F. A. and Settele J. “Tetracosane on parasitic butterflies *Phengaris (Maculinea) nausithous* cuticle triggers the first contact in the adoption process by *Myrmica rubra* foragers” *Physiological Entomology* (Accepted).

2014: Solazzo G., Moritz R.F.A. and Settele J. “The Social Parasite *Phengaris (Maculinea) nausithous* Affects Genetic Diversity within *Myrmica rubra* Host Ant Colonies” *Journal of Insect Conservation* **18**: 69-75.

2013: Solazzo G., Moritz R.F.A. and Settele J. “Choice Behaviour of *Myrmica rubra* Workers between ant Larvae and Larvae of their *Phengaris (Maculinea) nausithous* Nest Parasites”. *Insectes Sociaux* **60**: 57-64.

2011: Casacci L.P., Witek M., Barbero F., Patricelli D., Solazzo G., Balletto E. and Bonelli S. “Habitat Preferences of *Maculinea arion* and its *Myrmica* Host Ants: Implications for Habitat Management in Italian Alps”. *Journal of Insect Conservation* **15**: 103-110.

## Declaration of own contributions to the original articles

Solazzo G., Seidelmann K., Moritz R. F. A. and Settele J. (Accepted). “Tetracosane on parasitic butterflies *Phengaris (Maculinea) nausithous* cuticle triggers the first contact in the adoption process by *Myrmica rubra* foragers” *Physiological Entomology*.

Design of the project:	80%
Sampling in the field:	90%
Experimental work:	80%
Data analysis:	100%
Wrote the paper:	70%

Solazzo G., Moritz R.F.A. and Settele J. 2014. “The Social Parasite *Phengaris (Maculinea) nausithous* Affects Genetic Diversity within *Myrmica rubra* Host Ant Colonies” *Journal of Insect Conservation* **18**: 69-75.

Design of the project:	80%
Sampling in the field:	100%
Experimental work:	100%
Data analysis:	100%
Wrote the paper:	70%

Solazzo G., Moritz R.F.A. and Settele J. 2013. “Choice Behaviour of *Myrmica rubra* Workers between ant Larvae and Larvae of their *Phengaris (Maculinea) nausithous* Nest Parasites”. *Insectes Sociaux* **60**: 57-64.

Design of the project:	80%
Sampling in the field:	100%
Experimental work:	100%
Data analysis:	100%
Wrote the paper:	70%

## **Declaration of self-contained work**

Halle (Saale), 06/10/2014

Hiermit erkläre ich an Eides statt, dass diese Arbeit von mir bisher weder an der Naturwissenschaftlichen Fakultät I - Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch an einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ich erkläre weiterhin, dass ich mich bisher noch nicht um den Doktorgrad beworben habe.

Weiterhin erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne fremde Hilfe verfasst sowie keine anderen als die im Text angegeben Hilfsmittel und Quellen verwendet habe.

Gaetano Solazzo