

**"Genetic diversity and plant fitness in *Sanguisorba officinalis* (Rosaceae)
populations supporting an endangered large blue butterfly"**

D i s s e r t a t i o n

zur Erlangung des akademischen Grades

Dr. rer. nat.

vorgelegt der

Naturwissenschaftlichen Fakultät I
Biowissenschaften

der Martin-Luther-Universität Halle-Wittenberg

von

Herrn Martin Musche

geb. am: 03. 09. 1974 in: Bernburg

Gutachter /in

1. PD Dr. Josef Settele
2. Prof. Dr. Isabell Hensen
3. Prof. Dr. Martin Diekmann

Datum der Verteidigung: 10. 06. 2008

urn:nbn:de:gbv:3-000013966

[<http://nbn-resolving.de/urn/resolver.pl?urn=nbn%3Ade%3Agbv%3A3-000013966>]

Contents

Abstract (3-4)

1 Introduction (5-21)

- 1.1 Consequences of landscape change for the host plants of specialized herbivorous insects
- 1.2 The *Maculinea* system
- 1.3 Host plant selection behavior in herbivorous insects
- 1.4 Genetic structure, genetic diversity, and fitness in plant populations
- 1.5 Selection by agricultural practice and succession
- 1.6 References

2 No experimental evidence for host ant related oviposition in a parasitic butterfly (22)

3 Genetic population structure and reproductive fitness in the plant *Sanguisorba officinalis* in populations supporting colonies of an endangered *Maculinea* butterfly (23)

4 Performance and response to defoliation of *Sanguisorba officinalis* (Rosaceae) seedlings from mown and successional habitats (24)

5 Synthesis (25-32)

- 5.1 Interactions between *Maculinea* butterflies and their two essential resources
- 5.2. Genetic diversity, genetic population structure and reproductive fitness of *Sanguisorba officinalis*
- 5.3 The response of *S. officinalis* from meadows and successional fallows to mowing
- 5.4 Conclusions
- 5.5 References

6 Acknowledgements (33)

7 Appendixes (34-39)

- 7.1 Curriculum vitae
- 7.2 List of publications
- 7.3 Declaration of own contributions to the original articles
- 7.4 Declaration of self-contained work

Abstract

Changes in human land use have caused significant losses, as well as fragmentation and degradation of suitable habitats for many plant species. Hence, a growing number of species are restricted to small and more isolated populations. In such populations plant fitness may be reduced due to harsh environmental conditions and due to a loss of genetic variation. Reduced fitness, in turn, may increase the extinction risk of populations, thereby endangering the persistence of associated organisms, for example specialized herbivorous insects.

Sanguisorba officinalis represents the sole host plant of two endangered large blue butterfly species, *Maculinea nausithous* and *M. teleius*. Caterpillars of *Maculinea* butterflies initially feed on the inflorescences of their host plants before they leave the plant and complete their life cycle as social parasites of *Myrmica* red ants. In the present thesis I investigated patterns of interactions between the dusky large blue, *M. nausithous*, its host plant *S. officinalis*, and its host ant *Myrmica rubra*. The question whether adult butterflies are able to locate their host ants prior to oviposition was in the focus of a field experiment. Due to intensive land use and abandonment, many butterfly populations are restricted to small habitat patches which are exposed to secondary succession, and which carry small populations of the host plant *S. officinalis*. I examined the genetic structure of 24 *S. officinalis* populations which support the butterfly *M. nausithous* and measured traits related to sexual reproduction to find out whether these populations may be threatened by a loss of genetic variation. To investigate whether selection pressures associated with mowing and succession may create genetic differentiation between plant populations I grew seedlings originating from regularly mown meadows and successional fallows in a common environment and exposed them to a defoliation treatment. The experiment was also designed to examine whether plant performance and the ability to compensate for biomass loss caused by mowing may differ between populations of different size, density, and level of genetic variation.

There was no experimental evidence for host ant related oviposition in *M. nausithous* despite the close obligate association between butterflies and ants. Rather, eggs were deposited according to host plant traits which indicate a sufficient availability of resources. This pattern was consistent across time and independent from butterfly densities. The results indicate that adult females maximize offspring fitness by avoiding intra-specific competition between

caterpillars within ant nests, and by preferentially selecting plants which support the early larval development best.

Analysis of AFLP profiles revealed only marginal genetic differentiation between 24 populations of *S. officinalis* and the absence of differentiation between populations located in managed meadows and successional fallows. Further, populations did not follow a pattern of isolation by distance. Intra-population genetic diversity was variable but not related to population size, plant density, and the habitat of origin. The results suggest that considerable gene flow compensates the effects of genetic drift. The commonness of the plant, its pollination by generalist and highly mobile insects, and the outcrossing breeding system are likely to promote gene flow among populations. Seed mass and the percentage of germination strongly declined in small and sparse populations. However, this decline was not associated with decreasing genetic diversity. Thus, environmental factors, for example inter-specific competition, are likely to account for the fitness loss.

Seedlings of *S. officinalis* originating from mown meadows and successional fallows differed neither in performance nor in their ability to compensate for the loss of above-ground biomass. However, independently from the habitat of origin populations differed in leaf development and also exhibited variation in their response to defoliation. This variation was not related to population size, plant density, or the level of genetic variation. Thus, unknown selection pressures rather than genetic drift and inbreeding may explain the observed population differentiation. The absence of any differences between habitats may be explained by the perennial life form of *S. officinalis* and gene flow which both may have prevented effective selection.

The present thesis revealed that populations of *S. officinalis* supporting the butterfly *M. nausithous* are currently not threatened by genetic erosion. As intra-population genetic variation is not reduced in small populations and gene flow from surrounding sites seems sufficient, conservation efforts should focus on the improvement of habitat quality. As plant offspring from successional fallows retain the potential to cope with defoliation, mowing at low frequencies seems to be an appropriate management strategy to conserve these populations and their associated butterfly populations in the long term.

1 Introduction

1.1 Consequences of landscape change for the host plants of specialized herbivorous insects

Vascular plants serve as food for approximately half of the insect species in the world (Schoonhoven et al., 2005). Most herbivorous insects are restricted to the use of one or a few plant species whereas a smaller proportion of them feed on a wider range of taxa (Strong et al., 1984). The host range of a species may be limited by a number of factors, such as trade-offs in feeding efficiency (Joshi and Thompson, 1995), or neuronal constraints on host plant selection behavior (Bernays, 1998). Generalist herbivores may benefit from higher resource availability (Bernays and Minkenberg, 1997) and may shift to alternative plants in the course of environmental change (Braschler and Hill, 2007). However, food specialists as monophagous butterflies are expected to respond more sensitively to resource limitation (Steffan-Dewenter and Tscharrntke, 2000).

In most contemporary landscapes, human activity represents a major source of variation in resource availability. There has been a significant loss, fragmentation and degradation of habitat for many plant species, caused by intensive agriculture, settlement, and the abandonment of traditional land use practices (WallisDeVries et al., 2002). Hence, many plant species are restricted to small remnant populations, which often grow under unfavorable environmental conditions. Such changes are expected to have major consequences for those herbivores which rely on a single host plant and which are very unlikely to shift to alternatives.

On one hand, small and isolated habitat patches are less likely occupied by a species due to a higher extinction risk and a lower colonization probability (Hanski and Gilpin, 1997). Thus, specialized herbivores may show lower incidences in small plant populations than in large populations (e.g. Kéry et al., 2001; Zeipel et al., 2006). On the other hand, changes in landscape structure and land use practice may endanger the persistence of insect populations by affecting their host plant populations.

Small plant populations may face an increased risk of extinction because they are more susceptible to environmental and demographic stochasticity (Lande, 1988; Boyce, 1992). Further, plants growing in small populations may suffer from reduced fitness due to harsh environmental conditions (e.g. Schmidt and Jensen, 2000; Vergeer et al., 2003a), and due to a loss of genetic variation (Ellstrand and Elam, 1993; Reed and Frankham 2003). Specialized

herbivores may be adversely affected if such processes affect the quality and quantity of the target plant tissue, for example seed set (Colling and Matthies, 2004). In addition, the interacting effects of genetic erosion, poor habitat quality, and environmental stress may endanger the long term persistence of plant populations in agriculturally used landscapes, and therewith the long term persistence of their associated herbivorous insects. Thus, gaining knowledge on factors which may affect plant quality and the viability of plant populations is essential for the effective conservation of higher trophic levels, such as herbivores, and their parasites or predators.

The present thesis focuses on the plant *Sanguisorba officinalis* which represents the single host plant of two endangered *Maculinea* butterfly species. One part investigates patterns of interaction between the butterfly *Maculinea nausithous*, and its plant and ant host. However, the main emphasize is put on the genetic population structure of host plant populations and its relationship to plant fitness. The question whether plant populations have adapted to agricultural management represents a further aspect of this work.

1.2 The *Maculinea* system

Lycaenid butterflies comprise approximately 6000 species, and therewith represent one of the most species rich families within the Lepidoptera. From those species whose life histories are known, about 75 percent have established facultative or obligate associations with ants (Pierce et al. 2002). Most of these associations are mutualistic, i.e. phytophagous or aphytophagous caterpillars provide additional food supplies to ants, which in turn protect caterpillars against natural enemies (e.g. Pierce et al., 1987; Seufert and Fiedler, 1996). In a few lycaenid species caterpillars enter the ant nests to feed on ant brood, resources of the ant colonies, or to be fed by worker ants. Only 37 lycaenid species are known to exhibit such social parasitism, among them all currently known species of the genus *Maculinea* (Fiedler, 1998).

Adult *Maculinea* butterflies lay their eggs on the inflorescences of specific host plants (Table 1) where the newly hatched larvae feed on flowers and developing seeds until they reach the 4th instar. At this stage caterpillars leave the plant and, if discovered by foraging workers of specific *Myrmica* ants (Table 1), they are picked up and carried into the ant nests. Once adopted, caterpillars live as social parasites within ant nests until pupation. *Maculinea* species have evolved two different strategies to exploit their host ants. So called cuckoo feeders receive food particles from the worker ants, whereas predatory species prey on the ant brood (Table 1). Adults

emerge from pupae after one or two years of development inside the ant nests (Thomas et al., 1998; Witek et al., 2006). The chemical mimicry of the hydrocarbon surface of the ant brood enables both, caterpillar adoption and the integration into the colonies (Elmes et al., 2002). Caterpillars are parasitized by specialized ichneumonid wasps, either on the host plant (Anton et al., 2007), or within ant nests (Thomas and Elmes, 1993), depending on the species. From the currently described *Maculinea* species which all show a palaeartic distribution, there are five species known from Europe (Wynhoff, 1998, Table 1). However, recent analyses based on genetic markers consider *Maculinea alcon* and *Maculinea rebeli* as one, ecologically differentiated species (Als et al., 2004). All European *Maculinea* species are named in the Red Data Book of European Butterflies (Van Swaay and Warren, 1999) as well as in many national and regional red lists (Wynhoff, 1998). They are considered as vulnerable or endangered by the World Conservation Union (IUCN, 2000) and three of them are listed in the EC Habitats' Directive. Because all *Maculinea* species depend on two resources during their life cycle they are considered to respond particularly sensitive to human influence (Thomas, 1995; Johst et al., 2006). As they represent typical inhabitants of endangered habitats, and due to their complex interactions with different trophic levels, *Maculinea* butterflies have been proposed as suitable indicator organisms for habitat quality and biodiversity (Thomas et al., 2005). To get a deeper knowledge on the inter- and intra-specific variation in their functional ecology across Europe, the EC-funded research project "MacMan – *Maculinea* butterflies of the habitats directive and European red list as indicators and tools for habitat conservation and management" (EVK2-CT-2001-00126) was initiated, in which the present PhD thesis is embedded.

Table1 Host plant, host ant, and habitat use of the five European *Maculinea* species.

<i>Maculinea</i> species	Host plants	<i>Myrmica</i> host ants	Feeding style	Habitat ^a
<i>M. arion</i>	<i>Origanum vulgare</i> ^a	<i>M. sabuleti</i> ^a	predatory	warm, dry grassland
	<i>Thymus spec.</i> ^a	<i>M. lobicornis</i> ^f		
<i>M. rebeli</i>	<i>Gentiana cruciata</i> ^a	<i>M. schencki</i> ^a	cuckoo	Very dry grassland
<i>M. alcon</i>	<i>Gentiana pneumonanthe</i> ^a	<i>M. scabrinodis</i> ^a	cuckoo	moist grassland
	<i>Gentiana asclepiadea</i> ^c	<i>M. ruginodis</i> ^a		
		<i>M. rubra</i> ^a		
		<i>M. salina</i> ^b		
		<i>M. vandeli</i> ^c		
<i>M. nausithous</i>	<i>Sanguisorba officinalis</i> ^a	<i>M. rubra</i> ^a	predatory	moist grassland, fen
<i>M. teleius</i>	<i>Sanguisorba officinalis</i> ^a	<i>M. scabrinodis</i> ^a	predatory	moist grassland, fen
		<i>M. gallienii</i> ^d		
		<i>M. rubra</i> ^d		
		<i>M. ruginodis</i> ^d		

^aThomas (1995)^bTartally (2005)^cSielezniew and Stankiewicz (2004)^dStankiewicz and Sielezniew (2002)^eEbert and Rennwald (1991)^fSielezniew et al. (2003)

1.3 Host plant selection behavior in herbivorous insects

Plants that fall into the host range of an herbivorous insect may vary substantially in their nutritional and anti-herbivore components (Schoonhoven et al., 2005) and thus, may vary in their suitability to serve as food for the herbivore. Variation in plant characteristics may occur in space and across time, among species, populations, and individuals. Thus, insects need to distinguish between plants of variable quality. Many herbivorous insects have evolved sophisticated mechanisms which enable them to locate and to evaluate potential host plants efficiently by using visual, chemical, and mechanical cues (Bernays and Chapman, 1994).

The immature stages of many herbivorous insect taxa, e.g. the caterpillars of most Lepidoptera, show a low mobility and thus, a restricted ability to choose their diet. Their development and survival largely depend on the host choice of their parents. Based on this assumption, evolutionary theory predicts a correlation between adult oviposition preference and

offspring performance (Jaenike, 1978; Thompson and Pellmyr, 1991). However, there exist only few examples for such relationship (e.g. Via, 1986; Singer et al., 1988; Janz et al., 1994). Contrariwise, oviposition on alternative plants rather than those supporting offspring development best appears to be common (Thompson, 1988; Mayhew, 1997). Among the factors which have been discussed to be responsible for such alternative behavior, the environmental context of a plant seems to be the most important one. The spatial arrangement of host plants (Moravie et al., 2006), the presence of predators and parasites (Yamaga and Ogushi, 1999), or the availability of nectar sources (Janz et al., 2005) may drive the decision of an insect towards a low-quality plant.

For lycaenid butterflies which have established facultative or obligate associations with ants the suitability of a plant may be determined by the presence of ants rather than by characters of the host plant. Indeed, some mutualistic lycaenids have been shown to lay their eggs in the vicinity of their associated ants (e.g. Pierce and Elgar, 1985; Jordano et al., 1992). In contrast to mutualistic lycaenids, caterpillars of *Maculinea* butterflies spend only a short time on their host plants before they finish their life cycle as social parasites within host ant colonies. Regarding the dependence of *Maculinea* caterpillars on their host ants it has been suggested that females may enhance the chance of their offspring to be detected and adopted by ovipositing on plants growing within the feeding range of their host ants (Van Dyck et al., 2000; Wynhoff 2001). However, the limited capacity of an ant colony to support a certain number of caterpillars may select against ant dependent oviposition (Thomas and Elmes, 2001). While Van Dyck et al. (2000) found temporally constricted oviposition patterns which may indicate adult host ant recognition in one *Maculinea* species other work suggests random oviposition regarding the presence of ants (Thomas and Elmes, 2001; Nowicki et al., 2005). Current models describing the dynamics of *Maculinea* populations assume random distribution of eggs (Hochberg et al., 1994; Thomas et al., 1998; Griebeler and Seitz, 2002). A change of this assumption may alter the outcome of these models and therewith, the recommendations for species conservation (Thomas and Elmes, 2001).

Recent studies investigating the oviposition behavior in the genus *Maculinea* have been largely descriptive. However, to assess whether adult butterflies actively use ant cues for host plant selection or whether egg-laying is mediated by habitat parameters and host plant characters, experimental manipulation is necessary.

Chapter 2 of the present thesis examines the role of host ant odors and host plant characteristics for the selection of oviposition sites by *M. nausithous* butterflies. It also investigates whether butterflies change their behavior across time to encounter intra-specific competition for suitable plants. The present work represents the first applying an experimental approach under field conditions.

1.4 Genetic structure, genetic diversity, and fitness in plant populations

Genetic diversity which represents one of the three fundamental levels of biodiversity determines the evolutionary potential of a species or population to adapt to changing environmental conditions. Genetic diversity arises from mutation or may be added to a population by gene flow, whereas genetic drift and directional selection may eliminate it. The relative impact of each factor largely depends on population size and varies among characters, as coding and non-coding DNA, protein polymorphism, or quantitative characters (Frankham et al., 2002). Human induced habitat fragmentation which divides large and continuous populations into smaller and more isolated remnants has a major impact on the strength of genetic drift and the magnitude of gene flow.

Genetic drift refers to the random change of allele frequencies across generations (Ridley, 1996). The probability of an allele to get lost through genetic drift depends on its initial frequency and on the size of the population (Wright, 1931; Kimura, 1983). Rare alleles are predicted to disappear from small populations more rapidly than from large populations. Thus, small populations are more likely to lose genetic variation by random genetic processes. Inbreeding, i.e. mating among relatives represents a further consequence arising from small population sizes. Inbreeding increases homozygosity and it may promote the expression of deleterious alleles in the next generation. Thus, offspring emerging from small populations is more likely to suffer from inbreeding depression (Ellstrand and Elam, 1993; Reed and Frankham, 2003). In plants, survival (e.g. Oostermeijer et al., 1994) and traits related to growth (e.g. Luijten et al., 2000), reproduction (e.g. Ågren, 1996; Hensen and Oberprieler, 2005), and stress tolerance (e.g. Vergeer et al., 2003b; Pluess and Stöcklin, 2004) may be particularly affected.

Gene flow may compensate for the loss of genetic diversity. Its magnitude may be influenced by various environmental factors such as the spatial separation of populations

(Wright, 1943) and the presence of physical barriers (Keller and Largiadère, 2003), or by species traits as dispersal ability (Peterson and Denno, 1997) and breeding system (Loveless and Hamrick, 1984). The restriction of gene flow, as resulting from fragmentation processes, may facilitate genetic erosion within populations and genetic differentiation between populations. Under the assumption of the island model (Wright, 1943) most gene flow in dispersal limited organisms is likely to occur among neighboring populations and should decline as geographic distances increase. Thus, populations should become genetically more isolated over distance. Isolation by distance is predicted to occur if gene flow and genetic drift are at equilibrium (Hutchison and Templeton, 1999). The absence of such relationship combined with strong population differentiation indicates that genetic drift has exceeded the impact of gene flow. However, low genetic structure and the lack of isolation by distance point at strong recent or historical gene flow (Hutchison and Templeton, 1999).

Recent meta-analyses confirm the commonness of reduced genetic variation and fitness in small populations of many plant species (Leimu et al., 2006; Honnay and Jacquemyn, 2007). While most studies have concentrated on rare species new results suggests that common species may be likewise or even stronger affected (Honnay and Jacquemyn, 2007). As common species provide food for a larger number of organisms than rare species (Strong et al., 1984) any adverse effects of habitat fragmentation may have strong implications for the maintenance of trophic interactions within ecosystems and thus, for species diversity.

Sanguisorba officinalis which represents the sole host plant of two endangered large blue butterfly species, *Maculinea nausithous* and *Maculinea teleius*, is a common plant species in the Upper Rhine Valley (Germany). Despite its commonness the majority of plant populations are not suitable for the butterflies as they are exposed to intensive agricultural use. Mowing up to three times per year prevents the development of caterpillars which feed on the inflorescences of the plant. Thus, many butterfly populations are restricted to small habitat patches which carry small host plant populations and which are exposed to ongoing succession (Geißler-Strobel, 1999; Loritz and Settele, 2005a). In these populations genetic variation might be eliminated by genetic drift having negative consequences for plant fitness. Additionally, mowing and succession differently influence the flowering phenology of *S. officinalis* (Musche, personal observation) so that gene flow between managed and unmanaged populations might be restricted.

Recently, restricted gene flow between (Hatcher et al., 2004), and the loss of genetic diversity and fitness within host plant populations (Severns, 2003) have emerged as factors which may put populations of specialized herbivorous insects at risk, including *Maculinea* butterflies (Kéry et al., 2001). Knowing the genetic structure of *S. officinalis* may help to assess the suitability of the currently occupied plant populations for the conservation of the butterflies *M. nausithous* and *M. teleius* in an intensively used landscape.

Chapter 3 investigates the genetic structure of 24 *S. officinalis* populations, growing in mown and successional habitats, which all support colonies of the butterfly *M. nausithous*. Additionally, it is examined whether measures of reproductive fitness can be related to population size, plant density, genetic diversity, or habitat of origin.

1.5 Selection by agricultural practice and succession

Environmental heterogeneity is often associated with genetic heterogeneity as different environments produce different selection pressures favoring those characters which maximize fitness under the prevailing conditions (Antonovics, 1971; Hedrick et al., 1976). Further, different environments may establish barriers against gene flow which enhance genetic differentiation (Silvertown et al., 2005). Thus, many plant species which grow across a variety of habitats show patterns of local adaptation and small-scale genetic differentiation (Linhart and Grant, 1996). Agricultural practice represents a main force shaping the environmental conditions in human-used landscapes. For example, the application of herbicides (Warwick, 1991), grazing (Carman and Briske, 1985), or trampling (Warwick, 1980) have been shown to cause strong selection favoring locally adapted genotypes.

Mowing represents the most common land use practice applied on meadows inhabited by the study plant *S. officinalis*. Selection by mowing has also been shown to favor traits which enable plants to avoid foliage loss (Warwick and Briggs, 1978) or which facilitate regrowth (e.g. McNeilly, 1981; Painter et al., 1989). Due to the abandonment of meadows many populations of *S. officinalis* have become subject to secondary succession. Successional change however, may alter selection pressures, resulting in populations dominated by highly competitive genotypes (Ronce and Olivieri, 1997). Consequently, adaptation to contrasting selection pressures associated with mowing and succession may cause population differentiation in both, morphological characters and response to defoliation.

The response of host plants to stress caused by agricultural practice may be relevant for the conservation of *Maculinea* butterflies because all five European species exist in habitats which have been created by human land use and whose maintenance depends on certain management schemes (Thomas et al., 1998; Johst et al., 2006; Loritz and Settele, 2005b). Although the habitat requirements among *Maculinea* species differ in many ways, intensive land use and abandonment have been considered as common threats which affect all of them likewise (e.g. Thomas, 1995; Höttinger et al., 2003; Sielezniew et al., 2005). Populations of the dusky large blue *Maculinea nausithous* can be found in both, mown meadows and successional fallows. The latter often carry large populations and higher densities of the butterfly (Settele and Geißler, 1988; Skórka et al., 2007) and thus, represent important sources for the colonization of empty habitat patches. Fallow habitats support large densities of the host ant *Myrmica rubra* (Seifert, 1996), a circumstance which is likely to explain the high population densities of *M. nausithous* (Anton et al., in press). However ongoing succession suppresses the host plant *S. officinalis* due to increasing interspecific competition. Thus, for the maintenance of plant and butterfly populations habitat management is essential (Stettmer et al., 2001; Grill et al., in press).

Chapter 4 examines whether *S. officinalis* seedlings of currently unmanaged sites retain their potential to cope with defoliation, or whether mowing and succession may create population differentiation in plant performance and response. Further, the question whether population size, plant density, or the level of genetic diversity may affect the ability of plants to compensate for biomass loss is addressed.

1.6. References

- Als, T. D., Vila, R., Kandul, N. P., Nash, D. R., Yen, S. H., Hsu, Y. F., 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., Musche, M., and Settele, J. (2007) Spatial patterns of host exploitation in a larval parasitoid of the predatory dusky large blue *Maculinea nausithous*. *Basic and Applied Ecology* 8, 66-74.
- Anton, C., Musche, M., Hula, V., and Settele, J. (in press) *Myrmica* host-ants limit the density of the ant-predatory large blue *Maculinea nausithous*. *Journal of Insect Conservation*.
- Antonovics, J. (1971) Effects of a heterogeneous environment on genetics of natural populations. *American Scientist* 59, 593-599.

- Ågren, J. (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77, 1779-1790.
- Bernays, E. A. and Chapman, R. F. (1994) Host-plant selection by phytophagous insects. New York, London: Chapman & Hall.
- Bernays, E. A. and Minkenberg, O. P. J. M. (1997) Insect herbivores: different reasons for being a generalist. *Ecology* 78, 1157-1169.
- Bernays, E. A. (1998) The value of being a resource specialist: behavioral support for a neural hypothesis. *American Naturalist* 151, 451-464.
- Boyce, M. S. (1992) Population viability analysis. *Annual Review of Ecology and Systematics* 23, 481-506.
- Braschler, B. and Hill, J. K. (2007) Role of larval host plants in the climate-driven range expansion of the butterfly *Polygonia c-album*. *Journal of Animal Ecology* 76, 415-423.
- Carman, J. G. and Briske, D. D. (1985) Morphologic and allozymic variation between long-term grazed and non-grazed populations of the bunchgrass *Schizachyrium-Scoparium Var Frequens*. *Oecologia* 66, 332-337.
- Colling, G. and Matthies, D. (2004) The effects of plant population size on the interactions between the endangered plant *Scorzonera humilis*, a specialised herbivore, and a phytopathogenic fungus. *Oikos* 105, 71-78.
- Ebert, G. and Rennwald, E. (1991) Die Schmetterlinge Baden-Württembergs. Stuttgart: Eugen Ulmer GmbH & Co.
- Ellstrand, N. C. and Elam, D. R. (1993) Population genetic consequences of small population-size - implications for plant conservation. *Annual Review of Ecology and Systematics* 24, 217-242.
- 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. (1998) Lycaenid-ant interactions of the *Maculinea* type: tracing their historical roots in a comparative framework. *Journal of Insect Conservation* 2, 3-14.
- Frankham, R., Ballou, J. D., and Briscoe, D. A. (2002) 'Introduction to conservation genetics.' (Cambridge University Press: Cambridge.)
- Geißler-Strobel, S. (1999) Landschaftsorientierte Studien zu Ökologie, Verbreitung, Gefährdung und Schutz der Wiesenknopf-Ameisenbläulinge *Glaucopsyche* (*Maculinea*) *nausithous* und *Glaucopsyche* (*Maculinea*) *teleius*. *Neue Entomologische Nachrichten* 44, 1-105.

- Griebeler, E. M. and Seitz, A. (2002) An individual based model for the conservation of the endangered Large Blue Butterfly, *Maculinea arion* (Lepidoptera : Lycaenidae). *Ecological Modelling* 156, 43-60.
- Grill, A., Cleary, F. R., Stettmer, C., Bräu, M., and Settele, J. (2007) A mowing experiment to evaluate the influence of management on the activity of host ants of *Maculinea* butterflies. *Journal of Insect Conservation*.
- Hanski, I. and Gilpin, M. E. (1997) *Metapopulation biology: ecology, genetics and evolution*. Toronto: Academic Press.
- Hatcher, P. E., Wilkinson, M. J., Albani, M. C., and Hebborn, C. A. (2004) Conserving marginal populations of the food plant (*Impatiens noli-tangere*) of an endangered moth (*Eustroma reticulatum*) in a changing climate. *Biological Conservation* 116, 305-317.
- Hedrick, P. W., Ginevan, M. E., and Ewing, E. P. (1976) Genetic polymorphism in heterogeneous environments. *Annual Review of Ecology and Systematics* 7, 1-32.
- Hensen, I. and Oberprieler, C. (2005) Effects of population size on genetic diversity and seed production in the rare *Dictamnus albus* (Rutaceae) in central Germany. *Conservation Genetics* 6, 63-73.
- 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 ant hosts. *Journal of Animal Ecology* 63, 375-391.
- Honnay, O. and Jacquemyn, H. (2007) Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* 21, 823-831.
- Höttinger, H., Schlick-Steiner, B. C., and Steiner, F. M. (2003) The Alcon blue *Maculinea alcon* (Lepidoptera : Lycaenidae) in eastern Austria: Status and conservation measures. *Ekologia-Bratislava* 22, 107-118.
- Hutchison, D. W. and Templeton, A. R. (1999) Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* 53, 1898-1914.
- IUCN (2000) *IUCN red list of threatened species TM*. IUCN-International Union for the Conservation of Nature.
- Jaenike, J. (1978) On optimal oviposition behaviour in phytophagous insects. *Theoretical Population Biology* 96, 350-356.
- Janz, N., Nylin, S., and Wedell, N. (1994) Host plant utilization in the comma butterfly: sources of variation and evolutionary implications. *Oecologia* 99, 132-140.
- Janz, N., Bergstrom, A., and Sjogren, A. (2005) The role of nectar sources for oviposition decisions of the common blue butterfly *Polyommatus icarus*. *Oikos* 109, 535-538.

- Johst, K., Drechsler, M., Thomas, J., and Settele, J. (2006) Influence of mowing on the persistence of two endangered large blue butterfly species. *Journal of Applied Ecology* 43, 333-342.
- Jordano, D., Rodriguez, J., Thomas, C. D., and Fernandez Haeger, J. (1992) The distribution and density of a lycaenid butterfly in relation to *Lasius* ants. *Oecologia* 91, 439-446.
- Joshi, A. and Thompson, J. N. (1995) Trade-Offs and the Evolution of Host Specialization. *Evolutionary Ecology* 9, 82-92.
- Keller, I. and Largiader, C. R. (2003) Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. *Proceedings of the Royal Society of London Series B* 270, 417-423.
- Kéry, M., Matthies, D., and Fischer, M. (2001) The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. *Journal of Ecology* 89, 418-427.
- Kimura, M. (1983) *The neutral theory of molecular evolution*. Cambridge, UK: Cambridge University Press.
- Lande, R. (1988) Genetics and demography in biological conservation. *Science* 241, 1455-1460.
- Leimu, R., Mutikainen, P., Koricheva, J., and Fischer, M. (2006) How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* 94, 942-952.
- Linhart, Y. B. and Grant, M. C. (1996) Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27, 237-277.
- Loritz, H. and Settele, J. (2005a) Changing a butterfly's landscape-persistence of the Dusky Large Blue in managed grasslands. In: Settele, J., Kühn, E., and Thomas, J. A.: *Studies on the Ecology and Conservation of Butterflies in Europe Vol. 2: Species Ecology along a European Gradient: Maculinea butterflies as a model*, 221-224. Sofia-Moscow: Pensoft Publishers.
- Loritz, H. and Settele, J. (2005b) Effects of human land-use on availability and quality of habitats of the Dusky Large Blue butterfly. In: Settele, J., Kühn, E., and Thomas, J. A.: *Studies on the Ecology and Conservation of Butterflies in Europe Vol. 2: Species Ecology along a European Gradient: Maculinea butterflies as a model*, 225-227. Sofia-Moscow: Pensoft Publishers.
- Loveless, M. D. and Hamrick, J. L. (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15, 65-95.
- Luijten, S. H., Dierick, A., Oostermeijer, J. G. B., Raijmann, L. E. L., and Den Nijs, H. C. M. (2000) Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in The Netherlands. *Conservation Biology* 14, 1776-1787.

- Mayhew, P. J. (1997) Adaptive patterns of host-plant selection by phytophagous insects. *Oikos* 79, 417-428.
- McNeilly, T. (1981) Ecotypic differentiation in *Poa annua* - inter-population differences in response to competition and cutting. *New Phytologist* 88, 539-547.
- Moravie, M. A., Borer, M., and Bacher, S. (2006) Neighbourhood of host plants influences oviposition decisions of a stem-boring weevil. *Basic and Applied Ecology* 7, 545-554.
- Nowicki, P., Witek, M., Skorka, P., and Woyciechowski, M. (2005) Oviposition patterns in the myrmecophilous butterfly *Maculinea alcon* Denis & Schiffermuller (Lepidoptera : Lycaenidae) in relation to characteristics of foodplants and presence of ant hosts. *Polish Journal of Ecology* 53, 409-417.
- Oostermeijer, G. B., Eijck, M. W., and den Nijs, J. (1994) Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae) *Oecologia* 97, 289-296.
- Painter, E. L., Detling, J. K., and Steingraeber, D. A. (1989) Grazing history, defoliation, and frequency dependent competition - effects on 2 North-American grasses. *American Journal of Botany* 76, 1368-1379.
- Peterson, M. A. and Denno, R. F. (1997) The influence of intraspecific variation in dispersal strategies on the genetic structure of planthopper populations. *Evolution* 51, 1189-1206.
- Pierce, N. E. and Elgar, M. A. (1985) The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. *Behavioral Ecology and Sociobiology* 16, 209-222.
- Pierce, N. E., Kitching, R. L., Buckley, R. C., Taylor, M. F. J., and Benbow, K. F. (1987) The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. *Behavioral Ecology and Sociobiology* 21, 237-248.
- Pierce, N. E., Braby, M. F., Heath, 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.
- Pluess, A. R. and Stocklin, J. (2004) Genetic diversity and fitness in *Scabiosa columbaria* in the Swiss Jura in relation to population size. *Conservation Genetics* 5, 145-156.
- Reed, D. H. and Frankham, R. (2003) Correlation between fitness and genetic diversity. *Conservation Biology* 17, 230-237.
- Ridley, M. (1996) *Evolution*. Oxford: Blackwell.
- Ronce, O. and Olivieri, I. (1997) Evolution of reproductive effort in a metapopulation with local extinctions and ecological succession. *American Naturalist* 150, 220-249.

- Schmidt, K. and Jensen, K. (2000) Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (Scrophulariaceae) and its relation to population size and reproductive components. *American Journal of Botany* 87, 678-689.
- Schoonhoven, L. M., Van Loon, J. J. A., and Dicke, M. (2005) *Insect-plant biology*. Oxford: Oxford University Press.
- Seifert, B. (1996) *Ameisen beobachten, bestimmen*. Augsburg: Naturbuchverlag.
- Settele, J. and Geißler, S. (1988) Schutz des vom Aussterben bedrohten Blauschwarzen Moorbläulings durch Brachenerhalt, Grabenpflege und Biotopverbund im Filderraum. *Natur und Landschaft* 63, 467-470.
- Seufert, P. and Fiedler, K. (1996) Life-history diversity and local co-existence of three closely related lycaenid butterflies (Lepidoptera: Lycaenidae) in Malaysian rainforests. *Zoologischer Anzeiger* 234, 229-239.
- Severns, P. (2003) Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus ssp kincaidii* (Fabaceae). *Biological Conservation* 110, 221-229.
- Sielezniew, M., Stankiewicz, A., and Bystrowski, C. (2003) First observation of one *Maculinea arion* pupa in a *Myrmica lobicornis* nest in Poland. *Nota Lepidopterologica* 25, 249-250.
- Sielezniew, M. and Stankiewicz, A. M. (2004) Simultaneous exploitation of *Myrmica vandeli* and *M-scabrinodis* (Hymenoptera : Formicidae) colonies by the endangered myrmecophilous butterfly *Maculinea alcon* (Lepidoptera : Lycaenidae) *European Journal of Entomology* 101, 693-696.
- Sielezniew, M., Buszko, J., and Stankiewicz, A. *Maculinea arion* in Poland: distribution, ecology and conservation prospects. In: Settele, J., Kühn, E., and Thomas, J, A.: *Studies on the Ecology and Conservation of Butterflies in Europe Vol. 2: Species Ecology along a European Gradient: Maculinea butterflies as a model*, 231-233. Sofia-Moscow: Pensoft Publishers.
- Silvertown, J., Servaes, C., Biss, P., and Macleod, D. (2005) Reinforcement of reproductive isolation between adjacent populations in the Park Grass Experiment. *Heredity* 95, 198-205.
- Singer, M. C., Ng, D., and Thomas, C. D. (1988) Heritability of oviposition preference and its relationship to offspring performance within a single insect population. *Evolution* 42, 977-985.
- Skorka, P., Settele, J., and Woyciechowski, M. (2007) Effects of management cessation on grassland butterflies in southern Poland. *Agriculture, Ecosystems and Environment* 121, 319-324.
- Stankiewicz, A. and Sielezniew, M. (2002) Host specificity of *Maculinea teleius* Bgstr. and *M-nausithous* Bgstr. (Lepidoptera : Lycaenidae) the new insight. *Annales Zoologici* 52, 403-408.
- Steffan-Dewenter, I. and Tscharrntke, T. (2000) Butterfly community structure in fragmented habitats. *Ecology Letters* 3, 449-456.

- Stettmer, C., Binzenhöfer, B., Gros, P., and Hartmann, P. (2001) Habitatmanagement und Schutzmaßnahmen für die Ameisenbläulinge *Glaucopsyche teleius* und *Glaucopsyche nausithous*. Teil 2: Habitatansprüche, Gefährdung und Pflege. *Natur und Landschaft* 76, 366-376.
- Strong, D. R., Lawton, J. H., and Southwood, T. R. E. (1984) *Insects on plants. Community patterns and mechanisms*. Oxford: Blackwell.
- Tartally, A. (2005) *Myrmica salina* (Hymenoptera : Formicidae) as a host of *Maculinea alcon* (Lepidoptera : Lycaenidae). *Sociobiology* 46, 39-43.
- 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. and Elmes, G. W. (1993) Specialized searching and the hostile use of allomones by a parasitoid whose host, the butterfly *Maculinea rebeli*, inhabits ant nests. *Animal Behaviour* 45, 593-602.
- Thomas, J. A. (1995) The ecology and conservation of *Maculinea arion* and other European species of large blue butterfly. In: Pullin, A. S. (1995) *Ecology and conservation of butterflies*, 180-197. London: Chapman & Hall.
- 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. *Proceedings of the Royal Society London B* 265, 1895-1901.
- Thomas, J. A., Simcox, D. J., Wardlaw, J. C., Elmes, G. W., Hochberg, M. E., and Clarke, R. T. (1998) Effects of latitude, altitude and climate on the habitat and conservation of the endangered butterfly *Maculinea arion* and its *Myrmica* ant hosts. *Journal of Insect Conservation* 2, 39-46.
- Thomas, J. A., Clarke, R. T., Elmes, G. W., and Hochberg, M. E. (1998) Population dynamics in the genus *Maculinea* (Lepidoptera: Lycaenidae). In: Dempster, J. P. and McLean, I. F. G. (1998) *Insect Populations*, 263-290 Dordrecht: Kluwer Academic Publishers.
- Thomas, J. A. and Elmes, G. W. (2001) Food-plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmicophilous butterfly genus *Maculinea*. *Proceedings of the Royal Society London B* 268, 471-477.
- Thomas, J. A., Clarke, R. T., Randle, Z., Simcox, D. J., Schönrogge, K., Elmes, G. W., Wardlaw, J. C., and Settele, J. (2005) *Maculinea* and myrmecophiles as sensitive indicators of grassland butterflies (umbrella species), ants (keystone species) and other invertebrates. In: Settele, J., Kühn, E., and Thomas, J. A.: *Studies on the Ecology and Conservation of Butterflies in Europe Vol. 2: Species Ecology along a European Gradient: Maculinea butterflies as a model*, 28-31. Sofia-Moscow: Pensoft Publishers.
- Thompson, J. N. (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata* 47, 3-14.

- Thompson, J. N. and Pellmyr, O. (1991) Evolution of oviposition behaviour and host preference in Lepidoptera. *Annual Review of Entomology* 36, 65-89.
- 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 London B* 267, 861-866.
- Van Swaay, C. and Warren, M. (1999) Red data book of European butterflies (Ropalocera). *Nature and Environment* 99. Strasbourg: Council of Europe Publishing.
- Vergeer, P., Rengelink, R., Copal, A., and Ouborg, N. J. (2003a) The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. *Journal of Ecology* 91, 18-26.
- Vergeer, P., Rengelink, R., Ouborg, N. J., and Roelofs, J. G. M. (2003b) Effects of population size and genetic variation on the response of *Succisa pratensis* to eutrophication and acidification. *Journal of Ecology* 91, 600-609.
- Via, S. (1986) Genetic covariance between oviposition preference and larval performance in an insect herbivore. *Evolution* 40, 778-785.
- Von Zeipel, H., Eriksson, O., and Ehrlen, J. (2006) Host plant population size determines cascading effects in a plant-herbivore-parasitoid system. *Basic and Applied Ecology* 7, 191-200.
- WallisDeVries, M. F., Poschlod, P., and Willems, J. H. (2002) Challenges for the conservation of calcareous grasslands in northwestern Europe: integrating the requirements of flora and fauna. *Biological Conservation* 104, 265-273.
- Warwick, S. I. and Briggs, D. (1978) The genecology of lawn weeds. II. Evidence for disruptive selection in *Poa annua* L. in a mosaic environment of bowling green lawns and flower beds. *New Phytologist* 81, 725-737.
- Warwick, S. I. (1980) The genecology of lawn weeds. VII. The response of different growth forms of *Plantago major* L. and *Poa annua* L. to simulated trampling. *New Phytologist* 85, 461-469.
- Warwick, S. I. (1991) Herbicide resistance in weedy plants - physiology and population biology. *Annual Review of Ecology and Systematics* 22, 95-114.
- Witek, M., Sliwiska, E. B., Skorka, P., Nowicki, P., Settele, J., and Woyciechowski, M. (2006) Polymorphic growth in larvae of *Maculinea* butterflies, as an example of biennialism in myrmecophilous insects. *Oecologia* 148, 729-733.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics* 16, 97-159.
- Wright, S. (1943) Isolation by distance. *Genetics* 28, 114-138.

Wynhoff, I.(1998) The recent distribution of the European *Maculinea* species. *Journal of Insect Conservation* 2, 15-27.

Wynhoff, I. (2001) At home on foreign meadows: the reintroduction of two *Maculinea* butterfly species. PhD Thesis, Wageningen Agricultural University.

Yamaga, Y. and Ohgushi, T. (1999) Preference-performance linkage in a herbivorous lady beetle: consequences of variability of natural enemies. *Oecologia* 119, 183-190.

2 No experimental evidence for host ant related oviposition in a parasitic butterfly

Martin Musche, Christian Anton, Andrew Worgan, and Josef Settele (Journal of Insect Behavior 19, 631-643)

Abstract

The ability of adult butterflies of the genus *Maculinea* to locate their host ants prior to oviposition has been the subject of much discussion. We studied the egg laying behavior of the dusky large blue *Maculinea nausithous* whose larvae parasitize colonies of the ant *Myrmica rubra*. Flowerheads of the initial food plant were sprinkled with soil from ant nests, which contain chemicals involved in the nest recognition behavior of ants. The experiment was conducted to determine whether ant-released chemicals may act as oviposition cues and whether intraspecific competition for suitable plants may force female butterflies to alternative decisions. Host plant choice was not influenced by the presence of nest-derived host-ant cues. Density dependent shifts to less suitable host plants could not be ascertained nor changes in egg laying behavior across the flight period. The observed egg distribution could be primarily explained by host plant characteristics and environmental variability among sites. The result confirms the theory that host ant dependent oviposition appears to be a disadvantageous strategy in the face of resource limitation within ant colonies and the immobility of caterpillars.

3 Genetic population structure and reproductive fitness in the plant *Sanguisorba officinalis* in populations supporting colonies of an endangered *Maculinea* butterfly

Martin Musche, Josef Settele, and Walter Durka (International Journal of Plant Sciences, in press)

Abstract

The loss of genetic variation in small populations through drift and inbreeding is thought to decrease fitness and population viability. In order to evaluate the suitability of small *S. officinalis* populations for the long-term conservation of an endangered *Maculinea* butterfly species we investigated the plants' genetic population structure using amplified fragment length polymorphism (AFLP) and measured life history traits related to reproduction. Genetic distances among populations were low (mean $F_{ST} = 0.008$) and not correlated with geographic distances indicating that substantial gene flow compensates for the effects of genetic drift. Analysis of molecular variance indicated the absence of genetic differentiation among different habitat types and low differentiation among populations. High outcrossing rates ($t_m = 0.856$ and $t_m = 0.972$) obtained in two populations suggest that gene flow is promoted by the mating system. Populations differed in the level of intra-population genetic variation. These differences were not related to habitat type, population size, or plant density. Mean seed mass and the percentage of germination decreased in small and low-density populations. However, reduced fitness was not related to lower levels of genetic variation. Thus, the observed fitness decline was presumably due to lower habitat quality associated with small populations and low plant densities. The relevance of the results for the conservation of *Maculinea* butterflies is discussed.

4 Performance and response to defoliation of *Sanguisorba officinalis* (Rosaceae) seedlings from mown and successional habitats

Martin Musche, Josef Settele, and Walter Durka (Annals of Botany, submitted)

Abstract

In agricultural habitats selection may favor plants that show a pronounced ability to tolerate stress induced by specific management methods. However, genetic erosion which may be associated with habitat fragmentation may diminish this ability. To assess the role of mowing as selection pressure and the impact of fragmentation processes on the ability to tolerate foliage loss we grew 215 plants of the perennial herb *Sanguisorba officinalis* L. originating from 14 differently sized populations, located in mown meadows and successional fallows, in a common environment and measured their performance and response to defoliation. Plants from meadows and fallows neither differed in performance characters nor in their ability to compensate foliage loss. However, independently from the habitat of origin populations differed in both, performance and response to defoliation. This variation was not due to differences in population size, plant density, or level of genetic variation, indicating its independence from genetic drift and inbreeding which may go along with habitat fragmentation. Thus, differences in performance and response between populations appear to be the outcome of unknown selection pressures. Plants from successional fallows retain their potential to cope with mowing presumably due to the low generation turnover of the perennial species. Selection by mowing may act over time scales larger than those reflected by the developmental stage of the current habitats.

5 Synthesis

5.1 Interactions between *Maculinea* butterflies and their two essential resources

Host plant choice of female *Maculinea nausithous* butterflies was not influenced by the presence of nest-derived host ant cues. The behavioral pattern did not change across the flight season and was independent from host plant and butterfly densities. The results suggest that oviposition in this species is independent from the presence of its host ant *Myrmica rubra*. Further, there are no indications for a trade-off between oviposition in the vicinity of ant nests and the avoidance of intra-specific competition on the host plants, as suggested by Van Dyck et al. (2000). Although ant-dependent oviposition may enhance the probability of adoption, subsequent overcrowding of the resource limited ant colonies may lead to scramble competition between the predatory caterpillars. By distributing eggs randomly across the habitat, butterflies are considered to avoid intra-specific competition and to enhance overall caterpillar survival (Thomas and Elmes, 2001), despite the fact that a large percentage of eggs end up in the foraging range of non-host ant species (Thomas and Elmes, 1998). Therefore, random oviposition with regard to the presence of host ants seems to be an appropriate and cost effective strategy to maximize offspring fitness.

Instead of host ant cues, host plant traits and habitat specific factors determined the distribution of eggs among plants. Butterflies avoided ovipositing on small inflorescences and they preferred a distinct medium flowering stage, as already demonstrated in previous work (Figurny and Woichiechowski, 1998). Large inflorescences contain more flowers and seeds and therefore, provide more resources for the early development of caterpillars. However, increased parasitism by the wasp *Neotypus melanocephalus* that likewise prefers large inflorescences (Anton et al., 2007) might challenge the benefits resulting from the observed oviposition behavior.

While host plant traits shape the interaction between plants and butterflies at the individual level, there are no indications that host plant characters may influence butterflies at the population level. A related study on *M. nausithous* (Anton et al., in press) showed that the density of adults and eggs at a given site is clearly limited by the density of host ants, whereas plant density, the number of inflorescences per plant, and the size of inflorescences seemingly do not influence these life cycle stages. However, reduced plant availability is likely to lead to intra-specific competition among caterpillars on the inflorescences (Anton et al. in press).

The absence of ant-dependent oviposition and the strong host ant limitation at the population level are likely the result of the predatory feeding style of *M. nausithous*. In contrast to predatory *Maculinea* species, the cuckoo species *M.alcon* and *M. rebeli* use their diet more efficiently because feeding on the resources of the ant colony causes less damage than feeding on the ants themselves (Thomas and Elmes, 1998). Hence, one host ant colony supports a much larger number of caterpillars (e.g. Als et al., 2002), and overcrowding of ant colonies leads to contest competition instead of scramble competition (Thomas et al., 1993). Hence, the need to avoid the concentration of caterpillars within single ant colonies might not be as essential as for predatory species. Whether cuckoo species might even benefit from ant-dependent oviposition cannot be answered at that time. The results that were presented for the cuckoo feeder *M.alcon* (Van Dyck et al., 2000) might indicate such behavior. However, more detailed experimental work is needed in the future to investigate oviposition in non-predatory *Maculinea* species more detailed.

5.2. Genetic diversity, genetic population structure and reproductive fitness of *Sanguisorba officinalis*

Populations of *S. officinalis* showed an extremely low genetic structure across the study region. While some populations were slightly differentiated, there was no differentiation among habitats. Further, genetic population structure did not follow a pattern of isolation by distance. These results suggest that gene flow among populations is not restricted and compensates any effects of genetic drift. Although present gene flow cannot be directly estimated from patterns of genetic structure, the commonness of the species and its pollination by generalist and highly mobile pollinators suggest that gene flow at a sufficient level is still maintained. The outcrossing breeding system which has been confirmed in this work is also likely to promote gene flow. The absence of genetic differentiation among meadows and successional fallows may indicate that gene flow is not restricted between both habitats. However, the pattern may also be explained by the possibility that populations from successional fallows have preserved the initial genetic composition of meadow populations.

All populations retained a high level of intra-population genetic variation, regardless of their size, density, and habitat of origin. Thus, genetic drift and inbreeding have not eliminated genetic variation from small and sparse populations. Additionally, there are no indications that different selective forces which may be associated with different habitats have reduced the level

of genetic variation in meadows or fallows. The maintenance of genetic variation may be either due to the above mentioned compensatory effects of gene flow, or due to the perennial life form of the species. Genetic drift is expected to act slowly in species with long generation turnover (Loveless and Hamrick, 1984), so that recent fragmentation processes do not lead to an immediate loss of genetic variation.

Plants from small and sparse populations of *S. officinalis* developed significantly lighter and less fertile seeds. However, the decline in reproductive fitness was not accompanied by a loss of genetic variation. Thus, environmental factors associated with population size and plant density are more likely to be responsible for the observed fitness loss than inbreeding depression. Most populations of small size and low density were situated in successional fallows where inter-specific competition may reduce maternal investment into sexual reproduction, for example seed mass (Platenkamp and Shaw, 1993). Nonetheless, it cannot be excluded that genetic factors might have contributed to the observed patterns. In particular, the density dependence of germination which could not entirely be explained by seed mass might be an indication for increased selfing or biparental inbreeding caused by altered pollinator behavior.

Although plant density often correlates with population size it is considered as the more accurate measurement in continuously distributed plant species (Gram and Sork, 1999). Plant density may also be of greater biological relevance because it shapes the interaction between plants and their pollinators (Charnov, 1976; Klinkhamer et al., 1989; Kunin, 1997). Nonetheless, plant density has been included in few population genetic studies only (e.g. Tarayre and Thompson, 1997; Van Rossum et al., 2004). Future work on *S. officinalis* should focus on the relationship between plant density and mating system by incorporating a larger number of populations. The calculation of outcrossing rates based on seeds and seedlings may help to assess whether genetic load is expressed at the time of germination. Finally, common garden experiments investigating the relationship between pollinator behavior and reproductive success (see Kunin, 1993; Bosch and Waser, 2001) may uncover the mechanisms underlying the correlation between plant density and germination in *S. officinalis*.

5.3 The response of *S. officinalis* from meadows and successional fallows to mowing

Selection pressures associated with mowing and succession may generate population differentiation in morphological characters and in response to defoliation (e.g. Carman and

Briske, 1985; Houssard and Escarré, 1995). Seedlings of *Sanguisorba officinalis* originating from frequently mown meadows and successional fallows developed similar amounts of above-ground biomass and did not differ in leaf traits. Moreover, plants from both habitats responded in a similar manner to artificial defoliation. The absence of differentiation is most likely due to the perennial life form of *S. officinalis*. Perennial species with low generation turnover are thought to respond to selection with a certain time lag (Linhart and Grant, 1996). Plants from successional fallows are likely the survivors of former meadow populations which have retained the ability to cope with defoliation. However, selection for phenotypic plasticity or the homogenizing effects of gene flow may also explain the observed patterns in part.

Independently from their habitat of origin populations were differentiated in both, performance traits and response to defoliation. These differences could not be attributed to population size, plant density, and the level of intra-population genetic diversity. Thus, it is unlikely that genetic drift and inbreeding which may go along with habitat fragmentation account for the observed population differentiation. Plants from small or genetically less diverse population may be particularly vulnerable to environmental stress (e.g. Heschel and Paige, 1995; Pluess and Stoecklin, 2004) as caused by agricultural practice. *S. officinalis* plants from such populations did not show reduced performance, even though they had been exposed to defoliation. Therefore, habitat fragmentation does not seem to influence plant response to mowing in the study area. Rather, the observed population differentiation is likely due to unknown selection pressures which may be associated with environmental heterogeneity.

However, to assess whether population differentiation was caused by genetic drift or selection future work should focus on a comparison of population differences at neutral marker loci and quantitative traits (F_{st} vs. Q_{st} matrices, Merilä and Crnokrak, 2001; Willi et al., 2007). Such comparison was not done within the present study because it was primarily designed to search for differences between habitats. In the present work a similar ability of seedlings from meadows and fallows to perform and to respond to mowing was revealed. However, selection by mowing and succession might affect later stages of the life cycle, for example clonal propagation (Houssard and Escarré, 1995) or sexual reproduction (McKinney and Fowler, 1991; Ronce et al., 2005). Therefore, future work should consider the response of plants in the long term. Additionally, the inclusion of a competition treatment (e.g. Painter et al., 1989; Pluess and Stoecklin, 2005) may uncover whether more competitive genotypes dominate successional

fallows. The extension of the experiment to established plants from the original habitats may help to assess whether selective events acting between juvenile and adult stage may shape both, plant performance and response to defoliation.

5.4 Conclusions

The persistence of the dusky large blue, *Maculinea nausithous*, largely depends on the availability of its two essential resources, the host plant *Sanguisorba officinalis* and the host ant *Myrmica rubra*. Despite the fact that host ants represent the limiting resource on most sites (Anton et al., 2007) adult butterflies oviposit randomly with respect to the presence of ants. Therefore, population models describing the spatial and temporal dynamics of *Maculinea* populations should be based on a random distribution of eggs rather than on ant-dependent oviposition.

Plant populations in the study area, all of them supporting populations of the butterfly *M. nausithous*, are currently not threatened by genetic erosion. Gene flow from surrounding populations seems sufficient to prevent the loss of genetic diversity which may be caused by genetic drift and inbreeding. In order to increase reproductive fitness of small populations and to maintain them as refuges for the threatened butterflies, conservation efforts should concentrate on the improvement of habitat quality. However, this recommendation does not necessarily apply to other regions. Plant populations at the range margin of the distribution which are commonly smaller and more isolated may be more severely affected by genetic erosion. In such regions, alternative conservation measures should consider the maintenance of gene flow and large population sizes.

Although successional fallows support high population densities of the host ant *M. rubra*, strong interspecific competition from other plant species endangers the persistence of the host plant for *S. officinalis*. Thus, habitat management is essential to preserve these sites for the conservation of the butterfly *M. nausithous*. The present work revealed that *S. officinalis* seedlings from successional fallows retain the potential to establish under mowing conditions. Moreover, the ability to withdraw defoliation is not affected by genetic erosion which may be associated with small population sizes and low plant densities. Thus mowing represents an appropriate conservation measure for all investigated plant populations. However, the time and

frequency of mowing should take the requirements of the host ants and the phenology of butterflies into account (Johst et al., 2006).

Apart from mowing, many habitats supporting *M. nausithous* are grazed by cattle and horses (Loritz 2003). However, *S. officinalis* has been described as vulnerable to trampling (Stammel, 2003). Future experiments should be designed to evaluate the role of grazing and trampling on the establishment, performance and persistence of *S. officinalis* and to investigate whether trampling by livestock may cause evolutionary changes in this plant species.

5.5. References

- 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.
- Anton, C., Musche, M., and Settele, J. (2007) Spatial patterns of host exploitation in a larval parasitoid of the predatory dusky large blue *Maculinea nausithous*. *Basic and Applied Ecology* 8, 66-74.
- Anton, C., Musche, M., Hula, V., and Settele, J. (in press) *Myrmica* host-ants limit the density of the ant-predatory large blue *Maculinea nausithous*. *Journal of Insect Conservation*.
- Bosch, M. and Waser, N. M. (2001) Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia* 126, 76-83.
- Carman, J. G. and Briske, D. D. (1985) Morphologic and allozymic variation between long-term grazed and non-grazed populations of the bunchgrass *Schizachyrium-Scoparium Var Frequens*. *Oecologia* 66, 332-337.
- Charnov, E. L. (1976) Optimal foraging, the marginal value theorem. *Theoretical Population Biology* 9, 129-136.
- Figurny, E. and Woyciechowski, M. (1998) Flowerhead selection for oviposition by females of the sympatric butterfly species *Maculinea teleius* and *M. nausithous* (Lepidoptera : Lycaenidae). *Entomologia Generalis* 23, 215-222.
- Gram, W. K. and Sork, V. L. (1999) Population density as a predictor of genetic variation for woody plant species. *Conservation Biology* 13, 1079-1087.
- Heschel, M. S. and Paige, K. N. (1995) Inbreeding Depression, Environmental-Stress, and Population-Size Variation in Scarlet-Gilia (*Ipomopsis-Aggregata*). *Conservation Biology* 9, 126-133.
- Houssard, C. and Escarre, J. (1995) Variation and covariation among life history traits in *Rumex acetosella* from a successional old field gradient. *Oecologia* 102, 70-80.

- Johst, K., Drechsler, M., Thomas, J., and Settele, J. (2006) Influence of mowing on the persistence of two endangered large blue butterfly species. *Journal of Applied Ecology* 43, 333-342.
- Klinkhamer, P. G. L., Dejong, T. J., and Debruyn, G. J. (1989) Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos* 54, 201-204.
- Kunin, W. E. (1993) Sex and the single mustard – population density and pollinator behavior effects on seed set. *Ecology* 74, 2145-2160.
- Kunin, W. E. (1997) Population size and density effects in pollination: Pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology* 85, 225-234.
- Linhart, Y. B. and Grant, M. C. (1996) Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27, 237-277.
- Loritz, H. (2003) Habitatqualität und Landnutzungsdynamik am Beispiel des Dunklen Wiesenknopf-Ameisenbläulings im Queichtal bei Landau (Pfalz). Diplomarbeit, Westfälische Wilhelms-Universität Münster.
- Loveless, M. D. and Hamrick, J. L. (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15, 65-95.
- McKinney, K. K. and Fowler, N. L. (1991) Genetic adaptations to grazing and mowing in the unpalatable grass *Cenchrus incertus*. *Oecologia* 88, 238-242.
- Merilä, J. and Crnokrak, P. (2001) Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology* 14, 892-903.
- Painter, E. L., Detling, J. K., and Steingraeber, D. A. (1989) Grazing history, defoliation, and frequency dependent competition - effects on two North American grasses. *American Journal of Botany* 76, 1368-1379.
- Platenkamp, G. A. J. and Shaw, R. G. (1993) Environmental and genetic maternal effects on seed characters in *Nemophila menziesii*. *Evolution* 47, 540-555.
- Pluess, A. R. and Stöcklin, J. (2004) Genetic diversity and fitness in *Scabiosa columbaria* in the Swiss Jura in relation to population size. *Conservation Genetics* 5, 145-156.
- Pluess, A. R. and Stöcklin, J. (2005) The importance of population origin and environment on clonal and sexual reproduction in the alpine plant *Geum reptans*. *Functional Ecology* 19, 228-237.
- Ronce, O., Brachet, S., Olivieri, I., Gouyon, P. H., and Clobert, J. (2005) Plastic changes in seed dispersal along ecological succession: theoretical predictions from an evolutionary model. *Journal of Ecology* 93, 431-440.

- Stammel, B. (2003) Impact of grazing on vegetation and on selected plant species of calcareous fens. PhD Thesis. Technische Universität München.
- Tarayre, M. and Thompson, J. D. (1997) Population genetic structure of the gynodioecious *Thymus vulgaris* L. (Labiatae) in southern France. *Journal of Evolutionary Biology* 10, 157-174.
- 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. 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 Elmes, G. W. (2001) Food-plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. *Proceedings of the Royal Society London B* 268, 471-477.
- 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 London B* 267, 861-866.
- Van Rossum, F., De Sousa, S. C., and Triest, L. (2004) Genetic consequences of habitat fragmentation in an agricultural landscape on the common *Primula veris*, and comparison with its rare congener, *P. vulgaris*. *Conservation Genetics* 5, 231-245.
- Willi, Y., Van Buskirk, J., Schmid, B., and Fischer, M. (2007) Genetic isolation of fragmented populations is exacerbated by drift and selection. *Journal of Evolutionary Biology* 20, 534-542.

6 Acknowledgements

First of all, I would like to thank Josef Settele for his support during all stages of this thesis. He gave me the opportunity to study *Maculinea* butterflies, their host plants and host ants within the great international research project “MacMan”. I would like to thank Walter Durka for the stimulating discussions on plant population genetics. He always exercised great patience if things in the laboratory did not work. I also would like to thank Isabell Hensen for her willingness to supervise my work at Halle University.

This work had not been done without support from many other people. I would like to thank Christian Anton, Ellen Selent, Maria Filowa, Ina Geier, Sarah Gwilym, Martina Hermann, Vladimir Hula, Stefan Klotz, Elisabeth Kühn, Holger Loritz, Christof Manhart, Daniel Prati, Verena Schmidt, Ellen Selent, Christian Stettmer, Dana Weinhold and Andrew Worgan.

I was very lucky to work in a pleasant and supportive environment in the Department of Community Ecology at the Helmholtz Centre for Environmental Research in Halle.

This work was funded by the EC within the RTD project project “MacMan” (EVK2-CT-2001-00126).

Finally, I would like to thank Anke and my children for their support during all difficult stages of my work.

7 Appendixes

7.1 Curriculum vitae

Name: Martin Musche
Date of birth: 03.09.1974
Place of birth: Bernburg (Germany)
Family status: unmarried, 3 children
Degree: Diplom-Biologe

Scientific employments

Since 09/2007 Scientist (Helmholtz Centre for Environmental Research, Halle, Germany)

03/2002-09/2005 PhD student (Helmholtz Centre for Environmental Research, Halle, Germany)
Research topics: Population genetics of *Sanguisorba officinalis*, trophic interactions in the *Maculinea* system, biodiversity of insects in agriculturally used landscapes

06/2001-01/2002 Scientist (The Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, Germany)
Research topic: Impact of genetically modified plants on non-target organisms

04/2000-10/2000 Guest researcher (University of Leeds, UK)
Research topic: Insect-plant relationships

07/1998-02/2001 Scientific assistant (Helmholtz Centre for Environmental Research, Leipzig, Germany)
Research topic: Biodiversity of insects on flood plains

Education

10/1995-04/2001 Studies of biology (Martin Luther-Universität Halle-Wittenberg, Germany)
Focus: zoology, botany, limnology, biochemistry

09/1990-07/1993 Gymnasium Carolinum Bernburg, Abitur

Further employments

09/1993-11/1994 Civil service (Klinikum Bernburg, Germany)

08/2006-09/2007 Parental leave

Halle, 17 December 2007

Martin Musche

7.2 List of publications

Musche, M., Settele, W., and Durka, W. (in press) Genetic population structure and reproductive fitness in the plant *Sanguisorba officinalis* in populations supporting colonies of an endangered *Maculinea* butterfly. *International Journal of Plant Sciences*.

Anton, C., **Musche, M.**, Hula, V., and Settele, J. (in press) *Myrmica* host-ants limit the density of the ant-predatory large blue *Maculinea nausithous*. *Journal of Insect Conservation*.

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.

Schweiger, O., **Musche, M.**, Bailey, D., Billeter, R., Diekötter, T., Hendrickx, F., Herzog, F., Liira, J., Maelfait, J. P., Speelmans, M., and Dziock, F. (2007) Functional richness of local hoverfly communities (Diptera, Syrphidae) in response to land use across temperate Europe. *Oikos* 116, 461-472.

Anton, C., **Musche, M.**, and Settele, J. (2007) Spatial patterns of host exploitation in a larval parasitoid of the predatory dusky large blue *Maculinea nausithous*. *Basic and Applied Ecology* 8, 66-74.

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 Behavior* 19, 631-643.

Dziock, F., Jentzsch, M., Stolle, E., **Musche, M.**, and Pellmann, H. (2004) Rote Liste der Schwebfliegen (Diptera: Syrphidae) des Landes Sachsen-Anhalt. *Berichte des Landesamtes für Umweltschutz Sachsen-Anhalt* 39, 403-409.

Thomas, C. D., Bodsworth, E. J., Wilson, R. J., Simmons, A. D., Davies, Z. G., **Musche, M.**, and Conradt, L. (2001) Ecological and evolutionary processes at expanding range margins. *Nature* 411, 577-581.

7.3 Declaration of own contributions to the original articles

Because several co-authors contributed to the original articles in the following the percentage of own work is displayed.

Chapter 2: 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 Behavior 19, 631-643.

- Data collection in the field: 80%
- Data collection in the lab: 100%
- Statistical analysis: 95%
- Manuscript: 90%

Chapter 3: Musche, M., Settele, W., and Durka, W. (in press) Genetic population structure and reproductive fitness in the plant *Sanguisorba officinalis* in populations supporting colonies of an endangered *Maculinea* butterfly. International Journal of Plant Sciences.

- Collection of plant materials and field data: 100%
- Data gathering fitness traits: 100%
- AFLP analysis: 100%
- Statistical analysis: 100%
- Manuscript: 90%

Chapter 4: Musche, M., Settele, J., and Durka, W. (submitted to Annals of Botany) Performance and response to defoliation of *Sanguisorba officinalis* (Rosaceae) seedlings from mown and successional habitats

- Experimental setup in the green house: 100%
- Plant harvest and data collection in the lab: 100%
- Statistical analysis: 100%
- Manuscript: 95%

Status of co-authors:

Christian Anton: Helmholtz Centre for Environmental Research, Scientist

Walter Durka: Helmholtz Centre for Environmental Research, Scientist

Josef Settele: Helmholtz Centre for Environmental Research, Scientist, Supervisor

Andrew Worgan: Centre for Ecology and Hydrology (CEH), Scientist

Halle (Saale), 17 December 2007

PD Dr. Josef Settele

Martin Musche

(Supervisor)

7.4 Declaration of self-contained work

Hiermit erkläre ich, dass ich diese Arbeit bisher weder der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zur Promotion vorgelegt habe.

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

Im Übrigen erkläre ich, dass ich mich noch nie um einen Doktorgrad beworben habe.

Halle (Saale), den 17. 12. 2007

Unterschrift:

Martin Musche