At Home on Foreign Meadows:

the Reintroduction of two Maculinea Butterfly Species

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Proefschrift

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Abstract

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Maculinea butterflies live as obligate parasites of specific *Myrmica* host ants in meadow and heathland habitat maintained by low intensity landuse. Changes in agriculture caused the decline and extinction of many populations. In The Netherlands, *Maculinea nausithous* and *M. teleius* disappeared in the 1970s. In 1990, they were reintroduced following the recommendations of the IUCN. This study focuses on the evaluation of this reintroduction into the nature reserve Moerputten in the province of Northern Brabant. Population establishment and dispersal were monitored and accompanied by research on the impact of the reintroduction on species-specific genetic composition and behaviour. *Maculinea teleius* immediately established itself on one meadow, where the population still occurs today. *Maculinea nausithous*, being the more mobile species, colonized habitat patches with the specific host ant at higher distances. Three subpopulations were founded.

At the small spatial scale the females of both species were able to select habitat patches with host plants and host ants to deposit their eggs, rather then loosing many offspring by random oviposition. At the ladscape scale the occurence of the populations was also strongly related to the presence of the specific host ant species. However, through the limited dispersal capacity of the adults high quality patches at greater distance remain uncolonized. Genetic analysis showed that the new populations have not experienced a bottleneck in numbers following the translocation. However, the populations experienced selection due to changed ecological conditions at the founder site, but ecologically relevant traits were apparently not affected.

The conservation of the reintroduced population should concentrate on keeping a high habitat quality on the colonized sites and enabling the development of metapopulations by improving the management of potential sites within colonization distance. Ultimately, the persistence of the populations depends on the availability of an extensive network of suitable habitat patches. This requires conservation management at a landscape scale. This study emphasizes that a carefully planned and implemented reintroduction can be a successful tool for species conservation if both small and large spatial scales are taken into account.

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Stellingen

behorende bij het proefschrift:
At home on foreign meadows
the reintroduction of two *Maculinea* butterfly species

1 Poolse en Nederlandse *Myrmica* mieren spreken dezelfde taal (dit proefschrift).

2 Een combinatie van een hoge dichtheid aan waardplanten en een hoge dichtheid aan waardmieren levert een slechte leefomgeving voor mierenblauwtjes op (dit proefschrift).

3 Metapopulaties van zeldzame en bedreigde soorten in het Nederlandse landschap hebben niet zozeer last van onvoorspelbare tegenslagen veroorzaakt door demografische, omgevings- en genetische factoren, maar veel meer van gebrek aan ondersteuning op beleids- en uitvoeringsniveau (dit proefschrift).

4 Restoration ecology heeft in Nederland nog wel eens negatieve gevolgen voor de natuur, omdat over het hoofd wordt gezien dat men werkt aan relatief weinig verstoorde eilandjes in een sterk verstoorde omgeving, en niet aan sterk verstoorde eilandjes in een weinig verstoorde omgeving.

5 Het kan niet anders of Tijmblauwtjes uit open, montane populaties zijn in staat om hun waardmieren te detecteren.

6 De grootste bijdrage aan Flamenco in de afgelopen eeuw, wat de tekst betreft, is gedaan door Frederico García Lorca (1898-1936).

7 Pecunia non olet sed non habet dulcem odorem aeternae laetae vitae (met dank aan Marian Picker).

8 Het is noodzakelijk om de anonimiteit van zaaddonorschap te verbieden zodat kinderen het recht hebben hun vader te kennen.

9 Mobieltjes maken de mens minder mobiel.

10 Een afzuigkap is een belediging voor een goede kok.

11 Bei Niederländern kommt der Wink mit dem Zaunpfahl nur dann an, wenn man den Adressaten mit dem Zaunpfahl erschlägt.

Irma Wynhoff
Wageningen, oktober 2001

Flügelt ein kleiner blauer
Falter vom Winde geweht,
Ein perlmutterner Schauer,
Glitzert, flimmert, vergeht.

So mit Augenblicksblinken,
So im Vorüberwehn
Sah ich das Glück mir winken,
Glitzern, flimmern, vergehn.

Hermann Hesse

für meine Mutter

Content

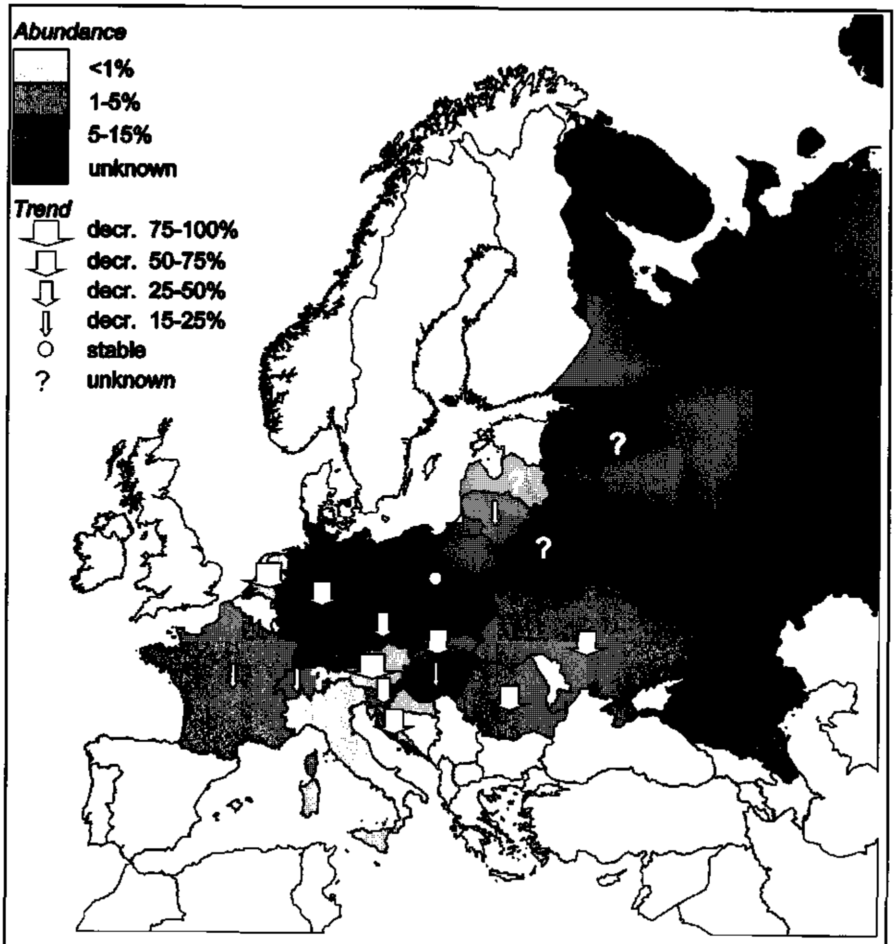
General introduction

General introduction

Nature pays a high prize for the increase in numbers of human beings all over the world. At least a thousand plant and animal species have been driven to extinction by people just since 1600 AD (Jenkins 1992), and this is probably only a part of what is lost forever. Many more species still survive but are facing the same fate, soon. Why do so many species go extinct? Most were dwellers of small islands and were simply harvested until no individuals were left. Other small island species were unable to withstand introduced predators. The mainland situation is different. Here many species are threatened with extinction because, apparently, they have too narrow a tolerance for environmental change as well as needing a very specific combination of ecological conditions. Yet, few of these species are extinct already, and with enduring efforts these species may still be preserved. *Maculinea* butterflies are typical for the latter group of species: they have highly specific ecological requirements combined with little tolerance for change. *Maculinea* butterflies, of which five species occur in Europe, show a typical adaptation of their biology: during a part of their life cycle they parasitise the nests of *Myrmica* ants (Thomas 1984). This study concentrates on the ecology and conservation of two species, *Maculinea teleius*, the scarce large blue butterfly, and *Maculinea nausithous*, the dusky large blue. Both species frequently live sympatrically on the same meadows. They use the same hostplant, *Sanguisorba officinalis*, and during their parasitic life stages they behave in a similar way in the ant nests (Thomas 1984, Thomas & Elmes 1998).

Habitat fragmentation, environmental deterioration and the decline of species

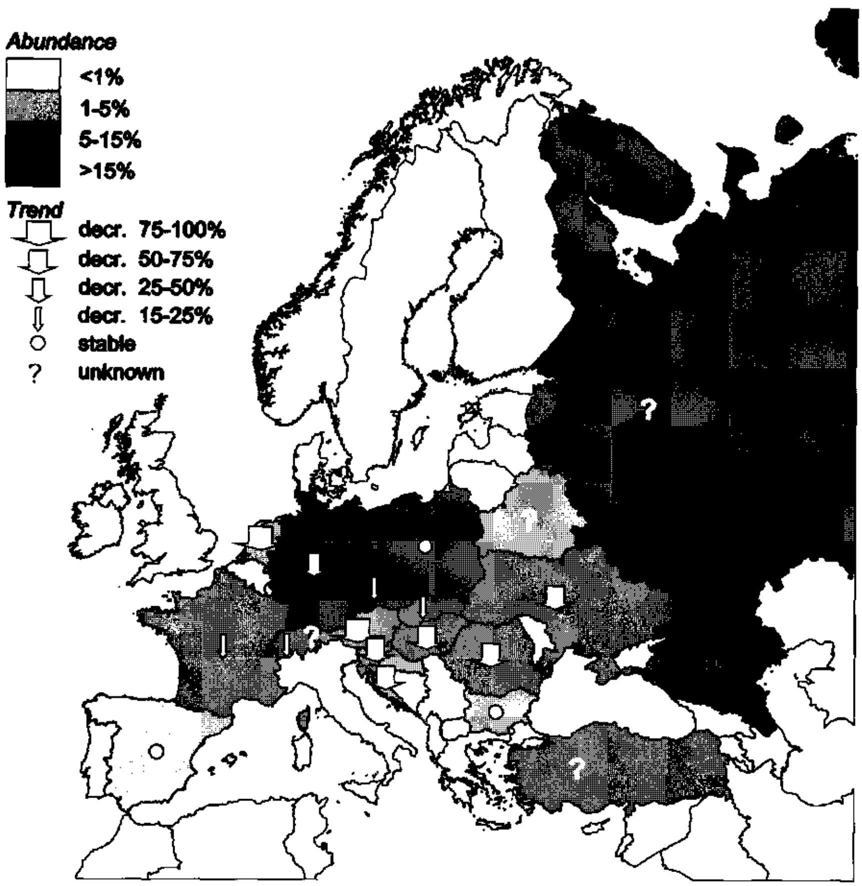
Although there are indications that Lepidoptera started to decrease in numbers and distribution early in the twentieth century, they have declined throughout Europe most markedly during the past few decades. Many regional and national reviews as well as case studies are presented in Pavlicek-van Beek *et al.* (1992) and Pullin (1995). According to national Red Data Lists, about 40-50% of all Lepidoptera species occurring in Germany and Austria are endangered and 2-5% of the species occurring in these countries have become nationally extinct (Erhardt 1995). In The Netherlands the situation is even worse: 24% of the sedentary species have gone extinct and 43% are threatened (Wynhoff & van Swaay 1995). Especially in the



Figuur 1.1: Abundance and status of *Maculinea teleius* in Europe (after Van Swaay & Warren 1999). Abundance is given in percentage of occupation of country surface, in white countries the species is missing. Trend is given in development during the last 25 years.

Central and West European lowlands many butterfly habitats are endangered and species are threatened. Only in the Alpine and Mediterranean region does this decline seem to be less severe (Erhardt 1995, Munguira 1995).

The five *Maculinea*-species (*M. arion, M. teleius, M. nausithous, M. alcon* and *M. rebeli*) represent a small group of highly specialised and sensitive butterflies that are currently being severely endangered. In the majority of countries within their range the abundance is less than 15% and within countries often even less than 5% of the area. The comparison between the abundance 25 years ago and the present abundance shows a decrease of



Figuur 1.2: Abundance and status of *Maculinea nausithous* in Europe (after Van Swaay & Warren 1999). Abundance is given in percentage of occupation of country surface, in white countries the species is missing. Trend is given in development during the last 25 years.

20 to 50% for four of these species, with *Maculinea arion* experiencing a decrease of 50 to 80% in Europe (Van Swaay & Warren 1999). The present trend and status of *Maculinea teleius* is given in figure 1.1, for *Maculinea nausithous* see figure 1.2. *Maculinea arion*, *M. teleius* and *M. nausithous* are already mentioned in the Appendix II of the Bern Convention, and it is recommended to include also *M. alcon* and *M. rebeli* (Van Swaay & Warren 1999). It is obvious that conservation measurements must be taken to ensure long term survival of these species (Munguira & Martín 1999).

Decline and extinction of Maculinea teleius and M. nausithous in the Netherlands

Both *Maculinea* species have most likely had a restricted distribution in Western and Central Europe since scientific research began, but locally they could be very abundant. The wet meadows in the valleys of the riversystems, which represented their habitat, used to form a continuous landscape where refuges were always present enabling survival of catastrophic events such as inundations or mowing during the flight period. Most likely the primary habitat in the beginning of the Holocene also consisted of long bands of rough vegetation in the gradient between riverine vegetation and forest edges with locally wet and dry patches and quite dynamic characteristics. Metapopulations must also have existed in systems of open patches in forests that dominated the landscape some 10,000 years ago (Prins 1997). Extended open grasslands were almost absent, therefore *Maculinea* butterflies must have been quite localised. It is likely that they existed in dynamic metapopulations on constantly changing networks of patches. Probably the increase in human population density and the development of slash and burn agriculture around 5,000 B.P. resulted in more patches with open vegetation to be colonised by the butterflies. In the Middle Ages (700 B.P.) many forest areas were cleared and used for fields and meadows. Furthermore, haymaking became increasingly common (Prins 1997). These early improvements in agricultural activities created an open but patchy landscape with many suitable places for various butterfly species. Amongst others, *Maculinea nausithous* and *M. teleius* must have increased in numbers of populations and in numbers of butterflies per population. *Maculinea teleius* also occurred at borders of peat bogs, which were to a lesser extent influenced by human activities.

However, with the later "agricultural improvements", that started in the 1960s, the beneficial effect of low intensity farming came to an end (Ebert & Rennwald 1991). Intensification of management by fertilisation and increasing of the mowing frequency resulted in the loss of many plant species and levelling of the vegetation structure. Cessation of traditional agriculture and abandoning of meadows favours a rough vegetation, which in many cases is not favourable for butterflies. Meadows were destroyed by ploughing and growing crops. Others were mown frequently during the flight season which resulted in removal of eggs and caterpillars. The surviving females were not able to deposit eggs anymore because hostplants were missing. Another reason was the increase of grazing intensity which had a disastrous effect on the *Myrmica* host ant populations. *Maculinea* populations were also lost through house building and new infrastructure. In nature reserves, populations were destroyed by mowing the whole area in the beginning of August because mowing at that time of the year is considered most beneficial for the conservation and restoration of a species-rich vegetation. Thus butterflies even became the victim of nature restoration projects. Anyhow, this caused the disappearance of both *Maculinea* species from the nature reserve Moerputten in the centre of The Netherlands, where they were later reintroduced.

The last indigenous Dutch populations of *Maculinea teleius* and *M. nausithous* used to thrive in an area called "Elfenmeertje" close to the small village of Herkenbosch in Central Limburg. Even though Lepidopterists had warned nature conservation organisations, this area was not bought and preserved. In the beginning of the 1970s, the development of a camping place started, which resulted in the extinction of the last populations (Geraedts 1986, Tax 1989).

Maculinea teleius used to occur in the central parts of the province of

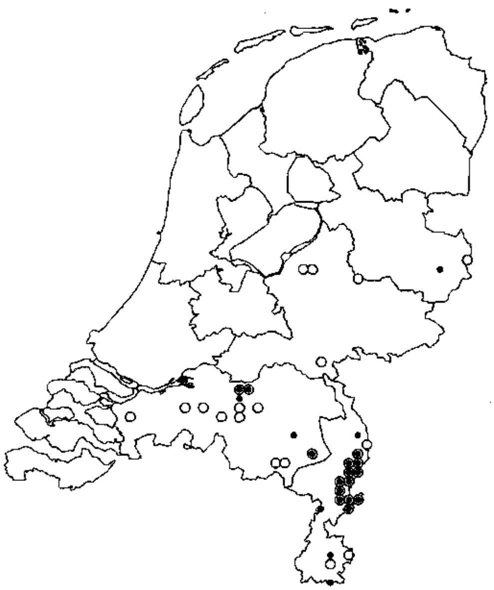


Figure 1.3: Distribution of *Maculinea teleius* and *Maculinea nausithous* in the Netherlands from 1870 to 1976 (*from: Database Dutch Butterfly Conservation*).
Circle: *Maculinea teleius*, dot: *Maculinea nausithous*

Limburg around the cities of Roermond and Venlo (figure 1.3). In this area the species was found in the riverine grasslands along the River Maas (Meuse) and the streams Roer, Swalm and Schandelosche Beek. The populations in the province of Northern Brabant were located in the stream valleys around Oisterwijk and in the region between Oisterwijk and Breda and Valkenswaard. The few populations in Overijssel were isolated from these (Tax 1989).

The distribution of *Maculinea nausithous* was to a large extent the same as the one of *Maculinea teleius* (figure 1.3). However, there were no populations in the north except for the one in the Hasseler Beek close to the city of Hengelo (Tax 1989).

Ecological restoration and reintroduction

In West Europe, most modern day *Maculinea* habitats are of secondary nature, such as different types of meadows or heathland in agricultural landscapes. Such habitat patches easily loose their quality for butterflies if the habitat management changes. Under such changes, effects on the delicate interplay of *Maculinea* butterflies, *Myrmica* ants and the vegetation are to be expected. Some kind of (agricultural) activity is therefore needed to keep the habitats suitable for the species. In the management of nature reserves, the same aims as achieved by traditional land use should be aimed for. However, there are often conflicting management scenario's for different species sharing the same location.

Butterfly populations have often disappeared due to changes in management of sites, as has happened with the *Maculineas* in the Moerputten nature reserve. It seems very attractive to owners and wardens of nature reserves to restore the habitat sites and reintroduce the lost species again, particularly if the concerned species are attractive to the general public.

Reintroduction or translocation of animal species is by no means new. It has been done for many species, since many years ago and almost all over the world. In America, amongst others, the grey wolf has been reintroduced in a number of States (Bangs *et al.* 1998, Noss *et al.* 1999), the wolf was brought back to the Yellowstone National Park (*Fritts* et al. 1997) and black bears were released in Arkansas (Clark & Smith 1994, Smith & Clark 1994). Large herbivores were reintroduced (Bison and elk: Truett 1996, caribou: Pitt & Jordan 1994)) as well as small species (otter: Swimley *et al.* 1999). In Africa, many large and medium-sized mammal species have been reintroduced into nature reserve after their local extinction due to overhunting. In many of these cases, resource competition between the introduced species and a resident species of approximately the same body weight may have played a role whether a reintroduction succeeded or failed (Prins & Olff 1998). Several fish and bird species have also been subject to a reintroduction project (Byrd *et al.* 1997, Foin *et al.* 1997, Winnemiller & Anderson 1997). In Europe, after severe habitat destruction, the beaver has been reintroduced in many countries, for instance in Sweden (Ellegren *et al.* 1993), The Netherlands (Nolet & Baveco 1996), Scotland (Macdonald *et al.* 2000, South *et al.* 2000), Germany (Rieder & Rohrer 1982), Switzerland (Stocker 1984, 1985), Czechia (Kostkan *et al.* 1997) and Croatia (Grubesic & Mayer 1996). Much attention has been given to reintroduction of the lynx as top predator in a number of regions (Breitenmoser & Haller 1993, Scott *et al.* 1999, Stahl & Vandel 1999). In the Netherlands, bringing back the lynx to the nature reserve "Hoge Veluwe" appeared to be not feasible (De Jong *et al.* 1997). The aim of these reintroductions is generally the restoration of ecological processes. At least within the published papers, this seems to be possible after good feasibility studies have been conducted and no potential problems for the founder populations could be discovered. However, even when applied according to the recommendations of the IUCN, success is not guaranteed. Publications mainly evaluate successful reintroductions, while only a few failures of reintroductions or translocations were published.

In butterflies, reintroduction seems to be quite difficult. Oates & Warren (1990) showed, that newly founded populations mostly become extinct within five years after release. The failure is often due to ecological requirements of the species which are not met in the reintroduction area, or by translocating only a very low number of founders (e.g. Dempster & Hall 1980). The number of successful (re)introductions is therefore limited.

A famous example for a successful reintroduction of a butterfly species is the case of *Maculinea arion* in England. Very large populations of the ant species *Myrmica sabuleti* are needed to support the parasitic butterfly whose caterpillars feed on ant grubs and hibernate in these ants' nests. Adequate densities of the host ant species only occur on warm sites with a very short sward kept by high levels of domestic grazing. Through cessation of grazing and decline of rabbit densities the vegetation sward grew up. *Myrmica sabuleti* decreased in nest densities and *Maculinea arion* went extinct. Following successful research into the complicated relationship between butterfly and host ant and the impact of management, nature reserves were restored. Under the old grazing level, the ant population were able to recover and the butterfly was successfully reintroduced (Thomas 1989, 1991, 1995, Thomas & Elmes 1992).

In 1989 the Ministry of Agriculture and Fisheries decided to reintroduce both *Maculinea* species in the Netherlands. A feasibility study showed that in the Moerputten nature reserve the only host plant *Sanguisorba officinalis* was very abundant and both host ant species were present. It was possible to prevent mowing of occupied meadows between the beginning of June and the middle of September. Then the lost species were brought back to their former sites. On the warm evening of July 30th, 1990, 33 males and 53 females of *M. teleius*, and 22 males and 48 females of *M. nausithous*, captured from a metapopulation in Poland, were released on the meadows of the Moerputten (Wynhoff 1991, 1992, Wynhoff & Van der Made 1995). Populations of both species are now present again.

For these founders, the reintroduction is comparable to a population bottleneck: they are physically isolated from any other metapopulation and there will be no exchange of individuals from any source. Such isolated populations have a higher chance of stochastic extinction compared to populations embedded in a metapopulation system. Due to the geographically isolated position of the reintroduction site, the introduced populations have to survive without any possibility of exchange of individuals and gene flow. After having settled, numbers have to increase in the nature reserve. Then by means of local dispersal, other ecologically suitable sites have to be colonised. The aim of any reintroduction of species within a natural system must be to establish a metapopulation system consisting of several subpopulations of each of the *Maculinea* species or a very big single core-population. Only then there is a chance of long-term persistence.

Population genetics of endangered species with small population size

The reintroduction of a population to an empty habitat patch leads to conditions under which the exchange of individuals between sites is reduced or impossible. Consequently, gene pools are then separated and, under pressure of differences in selection, may start to evolve in different directions. In any population, individuals with a high fitness at the new site contribute more progeny to the next generation compared to individuals with low fitness. The processes leading to differences in reproductive success of individuals within a population involve random effects (such as of weather) and natural selection, which is brought about by components of the environment. Evolution occurs when there is a change in the characteristics of a population and in gene frequencies over a number of generations due to selection or random effects (Hartl & Clarke 1989, Brakefield & Shreeve 1992).

Furthermore, founder populations involved in reintroductions are usually low in numbers of individuals. Populations being reduced to a very small size experience loss of genetic variation through loss of variants of genes, or alleles (Gilpin 1991, Maynard Smith1998). Such events, involving chance rather than selection, are described as genetic drift. In the meta-population situation such losses may be compensated for by immigration of individuals from other subpopulations.

Reintroduction creates a situation in which losses through chance are expected for the progeny of the founders: there will most likely be a decrease of genetic variation and loss of alleles compared to the original source population (Hartl & Clarke 1989, Maynard Smith 1998). There is no counterbalance through immigration and gains through mutation happen so rarely that they can be neglected. The amount of loss is also related to the duration of the bottleneck. There will be fewer problems, when the founder population can rapidly increase in size after having passed a bottleneck (Saccheri *et al.* 1999).

Another effect of the decrease in numbers of reproductive individuals by means of reintroduction could be that there is a higher chance of inbreeding (Hartl & Clarke 1989, Maynard Smith 1998). When sibs are not able to recognise each other and mate randomly, then the chance of sib-mating decreases with an increase in population size. Within any butterfly population no two individuals are identical, but sibs resemble each other more than two randomly chosen individuals. Therefore inbreeding increases homozygosity, and thereby may lower the fitness of individuals (Saccheri *et al.* 1998). The loss of fitness at individual level may lead to an increased risk of extinction of the population concerned. This effect is undesirable in a reintroduced population. Changes in genetic variation can be studied at the genotypic or phenotypic levels, or on any set of individual characteristics resulting from interactions between genes and the environment during development (Brakefield & Shreeve 1992). Even in a species such as *Maculinea nausithous*, which is comparably constant in its phenotype and has not evolved any subspecies, differences in wing size, spot number and spot size between individuals and between populations are easily detectable. Furthermore, studying the phenotype is often less destructive to a population compared to studying the genotype.

Myrmecophily

Butterflies of the family Lycaenidae engage in a rich and fascinating diversity of interactions with other species. Although as adults they behave as typical butterflies, as larvae many lycaenids inhabit a sequence of very different niches. Several species are carnivorous as larvae; some feed on homopterans, while retationships between others form essentially parasitic relations with ants, living in their nests and feeding on the ant grubs. Lycaenids and ants span the continuum of positive and negative interactions: from predation or parasitism (plus/minus) through commensalism (plus/zero) to true mutualism (plus/plus) (Bristow 1984).

The evolutionary success of myrmecophily is based on the appeasement of the aggressiveness of ants and the possibility to use it for own protection. Butterfly species with a myrmecophilic life style may gain selective advantages over non-myrmecophilic species (Fiedler & Maschwitz 1988) which can be of great importance in ecosystems with high species numbers and nest densities of ants, such as tropical rain forests. In colder climates, myrmecophily is less frequent (Fiedler 1991).

Myrmecophilous organs

During the evolution of myrmecophily, several specific larval organs have developed. Three types of larval organs play major roles in the ant-lycaenid interactions:

- pore cupola organs (=lenticles)
  They are scattered over the whole body surface. The (volatile?) secretions of the pore cupola organs are supposed to appease the ants that encounter lycaenid caterpillars or pupae (Malicky 1969, Pierce 1983, 1984, Cottrell 1984).
- dorsal nectary organ (=Newcomer's gland, =honey gland)
  The dorsal nectary organ is located on the seventh abdominal segment. It produces watery secretions containing mainly dissolved carbo-hydrates in concentrations of about 15% (Maschwitz *et al.* 1975, Pierce 1983, 1984). In a mutualistic symbiosis with the ants protecting the larvae against predators or parasitoids, the ants may gain important nutritional benefits from the sugary secretion (Fiedler & Maschwitz 1988). Furthermore, it induces recruitment of nestmates and establishes more or less permanent trophobiontic associations with the myrmecophilous lycaenid larvae.
- retractile tentacular organs (=retractile tubercles, =lateral organs)
  The tentacular organs are a pair of extrudable epidermal tubes located on the eighth abdominal segment, covered with numerous branched hairs.

The tentacular organs occur in myrmecophilous lycaenid larvae, which also possess a dorsal nectary organ but in some species either dorsal nectary organ or tentacular organs are missing (Kitching & Luke 1985, DeVries *et al.* 1986). They are extruded and retracted repeatedly when the attending ants palpate a larva intensively (Malicky 1969). The species-specific reaction and behaviour of the ant species concerned is caused by a chemical stimulus, possibly an alarm pheromone or an analogous substance (Henning 1983, Kitching & Luke 1985). The function is probably allomonal. In certain taxa of the Polyommatinae, the tentacular organs are thought to produce volatile compounds that appear to mimic some of the alarm pheromone signals of the ants (Cottrell 1984).

Types of myrmecophily

Within the European Lycaenidae, many types of relationships with ants occur. Myrmecoxenous species, such as most of the genus *Lycaena*, do not show any relationship with ants at all, but thanks to their pore cupola organs they are also not attacked. From the weakly myrmecophilous species (hairstreaks), very few ant associations have been reported and stable ant-associations are formed only exceptionally. The larvae of the common species, for example *Celastrina argiolus*, have often been observed attended by ants, but they may also live solitary. They are classified as being moderately myrmecophilous. All *Plebejus* and *Polyommatus* species are steadily myrmecophilous: most, if not all, mature larvae live in association with ants. Many Lycaenids belong to this type (Fiedler 1991, Fiedler & Maschwitz 1989). The most extreme kind of myrmecophily is found in the obligately myrmecophilous butterflies. Their larvae are completely dependent on ants. The *Cigaritis* species live as commensales in the ant nests (Rojo de la Paz 1992), species of *Maculinea* actually complete their lifecycle as parasites.

The relationship between ants and butterflies is based on the caterpillar's provision of food secretion with the abovementioned specialised glands to ants in exchange for protection against enemies and parasites (DeVries *et al.* 1993). In addition, the caterpillars may also possess other specialised behavioural and morphological features that play a role in maintaining symbiotic associations with ants, and it is likely that these characters have all been selected for directly by the ants. Ants live in high numbers together in organised colonies and have highly developed communication systems. Thus, the main problem for the caterpillars is to integrate into the communication system that is specific to the ants. The close relationship with the ants, after having entered the nest, gives rise to the idea that next to other adaptations, some kind of communication takes place. As much of the social behaviour of ants is released and controlled by pheromones, chemical communication is likely to take place (Henning 1983).

Adaptations to myrmecophily in Maculinea

In *Maculinea*-species, the relationship with ants is very intense. Most of the lifetime of an individual is spent in the ant nests, being dependent on food and/or care delivered by the host ants. Thus in the morphology and behaviour of the caterpillars, differences to non-myrmecophilous and non-parasitic species can be found.

The caterpillars of all lycaenids have a thick and tough skin to protect them against ant bites (Malicky, 1969). The body shape is different from the general shape of caterpillars: vital organs are protected behind protruding ridges containing non-vital organs (Cottrell 1984). When feeding on ant grubs the *Maculinea*-caterpillars pull the front ridges over the head, thereby hiding their prey. Worker ants don't even seem to notice that the caterpillars are feeding.

Myrmecophilous caterpillars have different types of glands which produce various kinds of secretions to interest and attract ants and at the same time suppress low levels of aggressiveness (Cottrell 1984, Akino *et al.* 1999). This is probably the most important means by which the caterpillars deceive their hosts. Secretion may also be important as a food source for the ants. All *Maculinea* species possess only pore cupola organs and a dorsal nectary organ while tentacular organs are missing. Kitching & Luke (1985) describe the myrmecophilous organs of *Maculinea arion.*

Caterpillars having a myrmecophilous life style are very slow. They also lack the beat-reflex. Hence any increase in aggressiveness in the ants by rapid movements is avoided (Cottrell 1984). The ultimate larval instar (L4) of several *Maculinea* species imitate the behaviour of ant grubs to be taken to the ant nests. Furthermore, caterpillars and pupae of *Maculinea* make species-specific sounds, resembling the sounds of the host ants (DeVries *et al.* 1993, Elfferich 1998).

Specificity of lycaenid - ant relationship

Within Europe most lycaenid larvae are unspecific in their relationship with ant taxa and facultative in the sense that the larvae can develop well in the absence of ants. Their relationships show little specificity within the limits of the ant taxa (Malicky 1969, 1970). The associations formed by ants with most lycaenid species are controlled far more by the extent to which the habitat preferences of the lycaenids overlap the foraging territories of the ants than by any specificity inherent to the partners. In such relationships the caterpillars benefit from protection from parasites and predators by the ants. They also experience lower levels of ant aggression and escape predation, as ants are important predators. With such aims, it is better to be able to appease as many ant species as possible instead of being dependent on only a few numbers of ant species (Fiedler 1991). The *Maculinea* species contrast with this in their specificity to only a few species within the genus *Myrmica*. By also being restricted to only one hostplant or a few hostplant species within a single genus, their niches become even smaller.

There must be an evolutionary advantage for *Maculinea*-species, to simultaneously be dependent on only a few hostplant species and a few ant species. The longest time span during the life cycle is the larval stage. Advantages of a comparatively enemy-free space during the larval instars are evident. It is unknown, however, why they remain on the hostplant until the L4 instar and do not leave it earlier. Advantages could be related to the winterperiod. Changes in microclimate, especially during the winter, are buffered in the ant nest. However, there is still a high mortality of caterpillars in the ant nests (Thomas & Elmes 1998). They can starve as a consequence of overexploitation or can be detected as parasites and fed upon by the ants. It is a question whether the advantages compensate for the risks of ant predation or starvation after the food source available in the ant nest is exhausted.

The intense specialisation of *Maculinea* butterflies and their association with specific *Myrmica spp.* means an extra risk to any reintroduction project. For *Maculinea alcon* the host specificity differs between regions (Elmes *et al.* 1997) but for *Maculinea teleius* and *M. nausithous* we have no knowledge of such spatial variation. If host specificity has evolved to extreme specialisation, it could mean that the caterpillars from the founders are not able to break the communication code of the host ants at the reintroduction site. The success of the reintroduction project could then be influenced by the relatedness between the founder population and the vanished population at the reintroduction site.

Ecology of Maculinea teleius and M. nausithous

Life cycle of Maculinea teleius and M. nausithous

Maculinea butterflies typically live in closed populations with little or no dispersal. All species are univoltine, with the adults flying for 2-4 weeks, depending on the geographical location and the size of the population. *Maculinea teleius* and *Maculinea nausithous* are on the wing from mid-June to the first week of September. Populations are known to have sizes from less than 50 to more than 5000 individuals in one summer. On sites with both species, *Maculinea teleius* is almost always the rare one.

Maculinea teleius deposits the eggs on very young green inflorescence of *Sanguisorba officinalis*. Each flowerhead is suitable for oviposition for no more than 5 to 7 days. The females normally lay only one egg per flowerhead (Thomas 1984).

Maculinea nausithous uses the flowerheads of *Sanguisorba officinalis* when they just have begun to develop red coloration. Large flowerheads are preferred upon smaller ones. Usually two to three eggs at a time are being laid. The egg load can increase up to 33, but only few caterpillars will survive (Thomas 1984).

The eggs are deposited on the only host plant *Sanguisorba officinalis* (figure 1.4) (Thomas 1984). However there are marked differences in the phenological state when the flowerheads are used as an oviposition site by either species or as a nectar source (Figurny & Woyciechowski 1998). The eggs are hidden between the buds of the host plant. They lack a thick eggshell to protect themselves against parasitoids (Munguira 1986). Almost all eggs will hatch into tiny caterpillars. During their first instars they will stay on the flowerheads and feed as phytophagous insects on the developing seeds. There is no difference in the behaviour between the two species. The caterpillar hollows out one or more seeds. The last moulting takes place in the tunnel thus created. On opening a flowerhead the traces of a successful survival until the early L4 larval instar will be found. The tunnel in the seed last consumed is filled with the skin of the penultimate L3-caterpillar, with on one side the head shield and on the other side the faeces. The overall survival on the flowerhead from the egg to the L4-caterpillar is for *Maculinea teleius* 50% and for *Maculinea nausithous* 47% (Wynhoff, unpublished data). From mid July to mid September the caterpillars live on their host plant.

For ***Maculinea teleius*** the main host ant species is ***Myrmica scabrinodis***, with in addition *Myrmica ruginodis* in France (Lhonoré, pers. comm.), *Myrmica rubra* in Poland (Figurny, pers. comm.) and in Central Germany (Ebert & Rennwald 1991) and occasionally *Myrmica vandeli* and *Myrmica sabuleti* (SBN 1987, Thomas *et al.* 1989).

Maculinea nausithous is probably most specialised to one host ant species. Nearly all over Europe, the host ant is ***Myrmica rubra*** L. (Bink 1992, Ebert & Rennwald 1991, SBN 1987, Thomas *et al.* 1989). Recently on a Polish site, survival in nests of *Myrmica scabrinodis* has been observed (Figurny, pers. comm.). Also one of the Spanish populations is a parasite of *Myrmica scabrinodis*.

The behaviour of the caterpillars of *Maculinea teleius* is very similar to that of *Maculinea arion* (Thomas & Elmes 1998). On the flowerhead of *Sanguisorba officinalis*, normally only one egg is deposited. This reduces food competition in the first to third larval instar. Occasionally, up to three caterpillars can be found on one flowerhead. However, later on eggs of *Maculinea nausithous* can also be deposited on the same flowerhead. As a consequence, food competition between larvae of two species can occur. The caterpillars of *Maculinea nausithous* almost always experience food competition as a consequence of the high egg load. They reach the L4-instar in two to three weeks, while *Maculinea teleius* caterpillars need three to four weeks. The caterpillars of *Maculinea teleius* are also bigger when they leave the flowerhead.

Having moulted to the fourth instar, the caterpillars drop off their hostplants onto the ground. They have to be found by workers of *Myrmica*-species, "adopted" and taken to the ant nests. The first contact between caterpillar and ant determines largely whether it has a chance to finally develop into a butterfly. The adoption procedure involves specific rituals and is different depending on *Maculinea* and *Myrmica* species. In the case of *Maculinea teleius* it can last for 30-90 minutes or even longer (Thomas 1984, Fiedler 1990). The caterpillar is palpated and in response offers droplets of secretion from the dorsal nectary organ. Finally, after showing "humping" behaviour, it is carried to the ant nest. The adoption ritual of *Maculinea nausithous* is much shorter.

One important aspect of parasitism is that only certain, species-specific *Myrmica* ants can act as host. Both *Maculinea* species can only survive the winter when they are adopted by the correct ant species. *Maculinea teleius* survives best in the nests of *Myrmica scabrinodis*, but *Maculinea nausithous* prefers *Myrmica rubra* (Thomas *et al.* 1989).

Once in the ant nest, the caterpillars are ignored completely. From now on vegetarian food is not accepted anymore: they change their diet to feeding on ant grubs. Even while feeding on the ant grubs, the worker ants do not react. In rearing experiments, the caterpillars do not stay among the brood but prefer to be apart. Only occasionally have they been observed being fed with ant regurgitations. They receive only little attention and will not be defended or carried away in case of disturbances. Therefore, both species are classified as primitive parasites (Thomas & Wardlaw 1992). The number of caterpillars of *Maculinea teleius* per ant nest is always low, rarely exceeding one individual (Ebert & Rennwald 1991, Thomas *et al.* 1998). Furthermore they are able to starve for a period. This may be very important because the nests of *Myrmica scabrinodis* are quite small. The larvae are able to decrease the number of ant grubs so heavily that the colony moves away. The caterpillars are able to wait for recolonisation of the empty colony site and then deplete the next ant nest. *Maculinea nausithous* is able to survive with several caterpillars in one host ant nest; these ants' colonies, however, are several times bigger than those of *Myrmica scabrinodis* (Wardlaw & Elmes 1996, Thomas & Elmes 1987, 1998).

The caterpillar will stay in the nest for approximately ten months, feeding from the ant grubs and hibernating. More than 90% of its final body mass is gained in the ant colony. Finally pupation takes place in the upper chambers of the ants' nests.

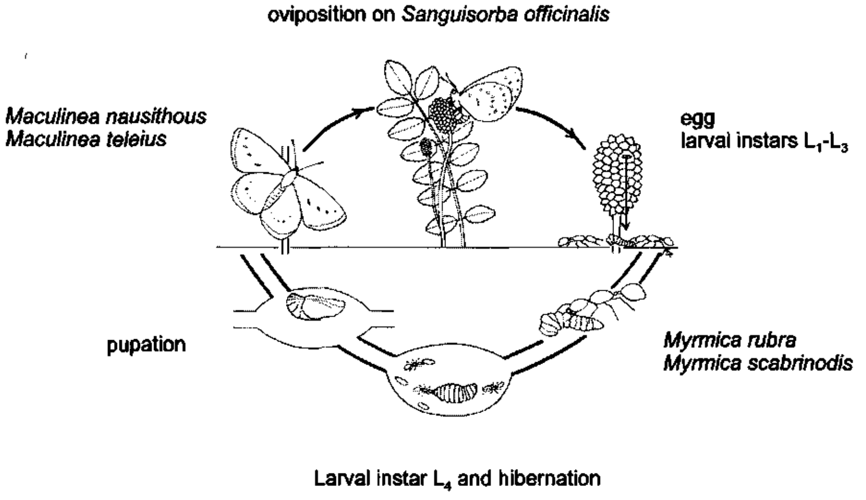


Figure 1.4: The life cycle of *Maculinea nausithous* and *M. teleius.*

The *Maculinea* caterpillars can themselves be parasitised by *Ichneumon* and *Neotypus* hymenopteran wasps. These species are very rare and with the exception of *Ichneumon eumerus* parasitising on *Maculinea rebeli*, their ecology is not yet unravelled (Hochberg *et al.* 1996, Hochberg *et al.* 1998, Thomas & Elmes 1993).

Habitat of *Maculinea teleius* and *M. nausithous*

At first sight, both *Maculinea* species seem to share closely similar habitat types. However, after a closer look, there are differences explaining the coexistence on many sites. The habitat of both *Maculinea* species can best be described as marshy lowland. Sites are often situated in stream valleys or besides lakes. Most populations thrive on vegetations, which can be characterised as *Molinion* or humid *Arrhenaterion* (Ebert & Rennwald 1991, Rozier 1999, Wynhoff 1992). In these humid meadows the vegetation is mown once to twice a year. *Maculinea teleius* is more restricted to open vegetation, while *Maculinea nausithous* can also be found on rough vegetation, such as meadows which have been abandoned for several years (Bergmann 1952, Bink 1992, Ebert 1991, Geißler 1990, Richard 1997, Sonnenburg 1996, Thomas 1984, Wynhoff 1992, 1996).

In general, meadows with low-intensity agricultural use or periodically abandoned grasslands are the best habitats for both species. In Switzerland, they also occur on slopes with bushes (Gonseth 1987).

Maculinea teleius occurs in open *Junco-Molinion* vegetation's in the North via humid *Arrhenaterion* to closed *Phragmites* or *Carex* vegetations in the South of its distribution range. It cannot be found in low quality habitat types.

Maculinea nausithous occurs on rougher types of *Molinion* and *Arrhenaterion* vegetations up to *Agropyro-Rumicion crispi*. On wet location and in a mosaic of dry and wet types of vegetation, *Maculinea nausithous* can be found in *Filipendulion*, *Calthion palustris* and *Phragmition* vegetations. Road verges, embankments and canal borders can be very important as habitat.

Habitat patch size and habitat use

Habitat patches for *Maculinea teleius* and *M. nausithous* can be very small and can have a low density of *Sanguisorba officinalis*. The latter butterfly species can even be found on sites with sometimes not more than two to three large, branched plants (Gonseth 1987, Geißler 1990, Wynhoff

1992). Many sites are part of agricultural land and consist of a mosaic of abandoned and mown parts (Ebert & Rennwald 1991). In such vegetation, *Maculinea teleius* uses regrowth of *Sanguisorba* in the mown parts for oviposition, whereas the females go to the abandoned parts for nectar. *Maculinea nausithous* tends to stay in the abandoned part for as well ovipositioning as nectar feeding. *Maculinea teleius* uses many flower species as nectar sources whereas *M. nausithous* is nearly totally restricted to *Sanguisorba* for feeding (Thomas 1984, Wynhoff 1992, 1994).

The butterflies have a small home range and do not readily leave their habitat patch (Settele 1998, Wynhoff 1995, 1996). To a certain extent *M. nausithous* makes use of corridors along roads, forest edges, streams and canals. While flying over road verges, they use high plants emerging from the vegetation canopy, such as flowering *Plantago* and *Centaurea jacea* for their orientation. The vegetation structure along corridors seems to be important, whereas the presence of *Sanguisorba* probably helps in dispersal but is not really needed. *Maculinea teleius* has a lower dispersal capacity and uses such corridors only very rarely.

In their normal daily behaviour, *Maculinea teleius* seems to be much more active than *M. nausithous*. The percentage of captures of flying individuals in mark-release-recapture studies in the reintroduced Dutch population was in all years remarkably higher than in *Maculinea nausithous*. Furthermore, in individual behaviour observations, these results are supported. Yet it would be wrong to conclude from these data that they also disperse more readily. On the contrary, the species with more dispersal is the somewhat "lazy" *Maculinea nausithous* that spends most of its time basking and resting (Wynhoff 1994, 1996).

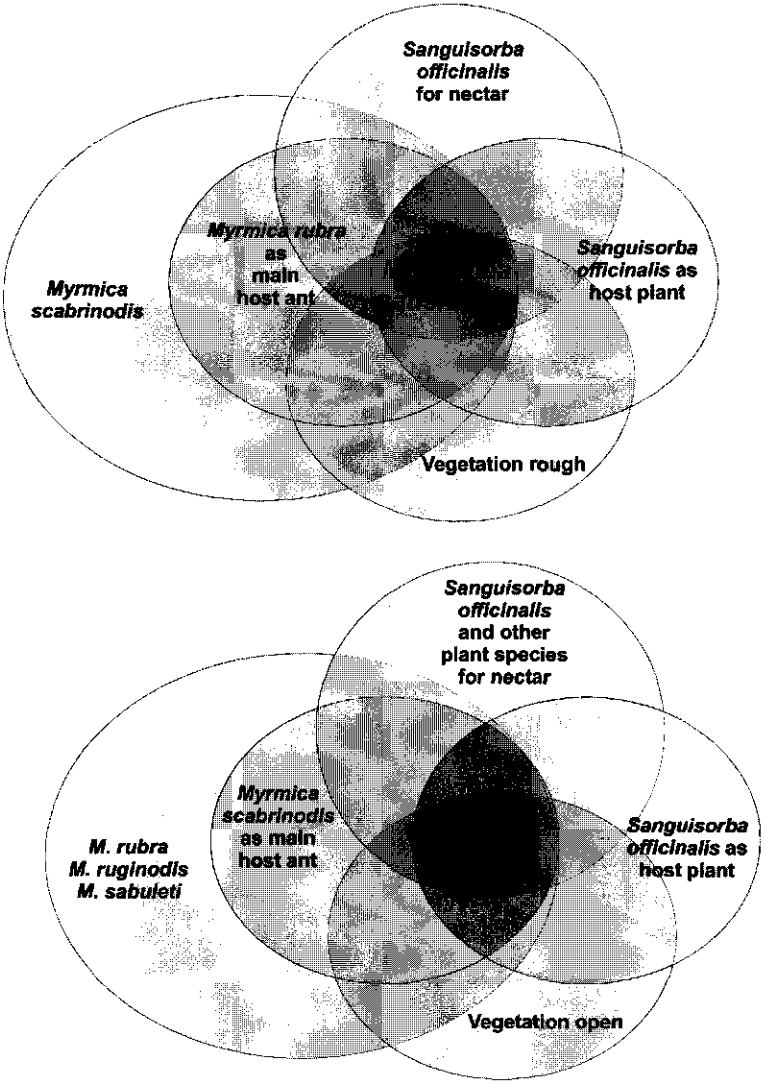


Figure 1.5: Venn-diagram illustrating the complexity of interspecific relationships and the narrow niches in the ecology of *Maculinea nausithous* and *M. teleius*.

Ecology of Sanguisorba officinalis

Wet meadows with *Sanguisorba officinalis* are very conspicuous during the summer: already from a distance one sees the millions of deeply red flowerheads moving in the wind. At a closer look these meadows belong to the plant communities *Molinion* and *Calthion* on the wet locations where *Sanguisorba officinalis* shares its habitat with *Molinia caerulea*, *Succisa pratensis*, *Cirsium officinale* and *Danthonea decumbens*. On drier sites it can be found in *Arrhenaterion* communities with *Filipendula ulmaria* and *Thalictrum flavum* on moist nutrient rich locations. Furthermore, it occurs together with very common plant species such as grasses alongside roads, rivers and canals. Sites with *Sanguisorba* have a moderate soil acidity and productivity. During the winter, locations might be inundated, but in the summer they are dry (Oberdorfer 1979, Weeda *et al.* 1987).

The distribution of *Sanguisorba officinalis* is circumpolar but disjunct. The species does not occur in the Scandinavian countries. In Western Europe it occurs mainly in river valley systems. In The Netherlands these are Overijsselse Vecht and Maas with associated tributaries (figure 1.6) (Mennema *et al.* 1985, FLORBASE-2F Floron). Hence in The Netherlands its distribution is separated into three regions: Northern Overijssel, Central Brabant and Central Limburg. The species used to be much more common, but has decreased due to land drainage and fertilisation of agricultural land.

Sanguisorba officinalis is an important nectar source for small butterfly species and Syrphids. It is pollinated by Syrphids, but sexual reproduction occurs only rarely because the seeds germinate and establish themselves only in open places. Usually the occurrence of such places is caused by damage to the vegetation sod, for instance during mowing in rainy years when the soil is wet. The plants can persist for long periods even under unfavourable conditions through their strong roots (Weeda *et al.* 1987, own unpublished data).

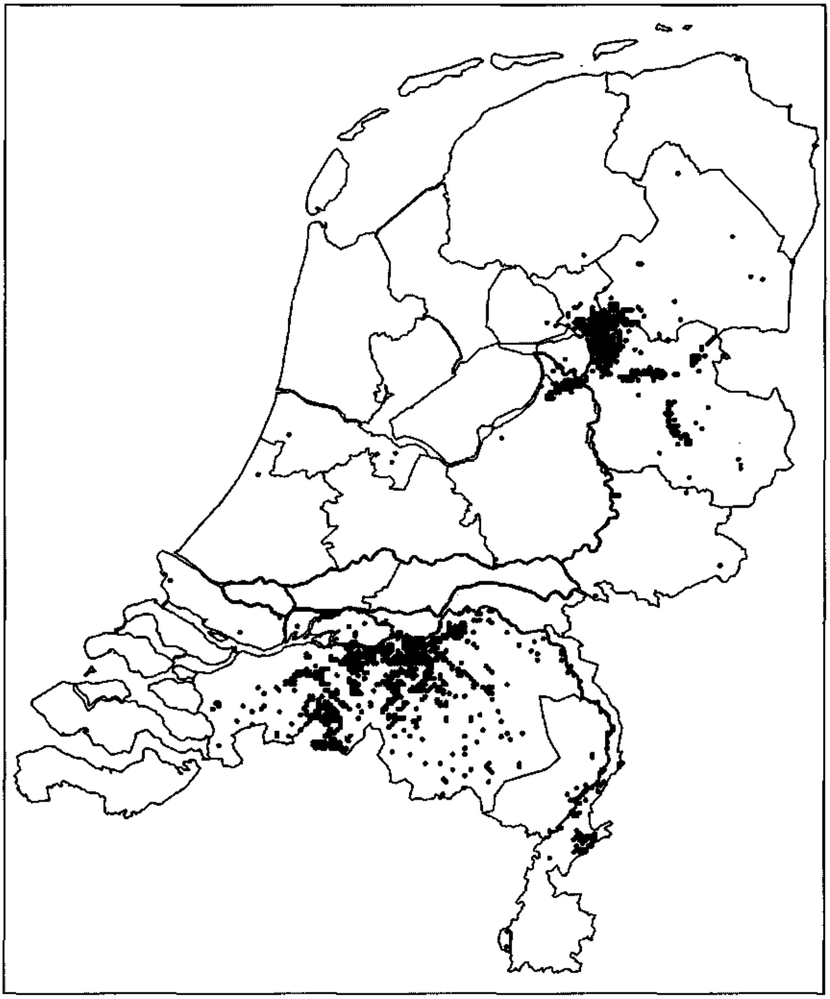


Figure 1.6: Distribution of *Sanguisorba officinalis* in The Netherlands, 1975-1999 (From: Florbase-2F, Floron Dutch Flora Conservation, Leiden).

Ecology of Myrmica ants

Within Europe, the genus *Myrmica* is common and widespread (Seifert 1988, 1993). Each species lives in ecologically distinct habitats. However, there is always a degree of overlap between species and in many sites, two or three *Myrmica* species occur in competition with each other (Elmes 1982). In table 1.1, an overview of the common *Myrmica* species and their main habitat is presented. *Myrmica*'s can be found from cool and wet moorlands up to hot and dry chalk grasslands and from dunes to mountainsides. Only a limited number of species of this genus are associated with *Maculinea* parasites.

Life cycle of Myrmica ants

All *Myrmica* species are polygynous; in each nest multiple queens can be found, from 1 to about 100 depending on the species. *Myrmica sabuleti* and *M. scabrinodis* have quite small nests with 1 to 3 queens. The nests of *M. rubra* normally contain about 10 queens. In July and August the queens lay many eggs. During the summer, most of them will develop into workers, but later when the temperature decreases, the development will slow down. At the end of September, a nest contains about as many hibernating larvae as workers.

The larvae show some variation in size. The bigger and heavier ones will develop into young queens after hibernation in the next spring, while the smaller ones will be workers or males. Finally in June, when the queens start to lay eggs again, all larvae have reached the pupal phase. About a month later, new workers, males and virgin queens will accompany her. In general, unfertilised eggs will develop into males, while fertilised give queens or workers, depending on the care and food given to them. Only the workers will leave the nest to search and bring in food, the sexual ants will stay inside until the nuptial flights in the last weeks of August and beginning of September. *Myrmica* ants are rather primitive, there is no soldier caste (Hölldobler & Wilson 1990).

In the contact between ants and caterpillars, an integrated set of life-history characteristics is needed, and has evolved, to enlarge the chance of a caterpillar to be found by the right ant species and later on to optimise the use of ant larvae as important food source. The periods of highest activity in *Myrmica* ants are in the early morning hours and from the afternoon to the early evening. At that time many workers leave the nest to search for food. The fourth instar caterpillars leave their host plant in the late activity period. It seems to be important to them to be found as soon as possible after they have left the hostplant. If the caterpillars have to wait long before being found, their chance to be adopted and taken to the ant nest decreases. Furthermore, the other common ant species from the genus *Lasius* and *Formica*, which normally also occur in the habitats of the different *Maculinea* species, have passed the period of maximum activity in the late afternoon. Thereafter, the chance for the caterpillar to be found by a predator worker ant declines.

After hibernation and pupation, the young butterflies are confronted with the presence of their hosts. Rearing experiments have shown that the more butterflies eclose on the same day, the smaller the chance of survival is for the later ones. The ants become increasingly aggressive and will kill the butterflies before they have left the ant nest. However, most butterflies eclose and leave the nest in the morning, before the ants become active. Besides, there seems to be some spreading in eclosion. This prevents more than one or two butterflies leaving the ant nest on the same day. How this spreading in eclosion comes about is not known.

Table 1.1: The *Maculinea* parasites and habitat preferences of the common European *Myrmica* species in northern Europe (after Elmes & Thomas 1992). In bold the two species that are subject of the present study.

Myrmica species	*Maculinea* parasite	Main habitat
COOL AND MOIST LOVING SPP.		
M. sulcinodis	none	Mountains and moorlands
M. ruginodis	*M. alcon* *M. teleius*	Woods, wet heathlands and bogs
M. lobicornis	none	Mountains and moorlands
WARM AND MOIST LOVING SPP.		
M. rubra	*M. nausithous* *M. teleius* *M. alcon*	Edges of bogs, rivers and woods
M. limanica	none	Marshlands near lakes
M. vandeli	none	Bogs in the Alps
M. scabrinodis	*M. teleius* *M. nausithous* *M. alcon* *M. rebeli* *M. arion*	Grasslands, bogs and moors
WARM AND XEROPHILIC SPP.		
M. rugulosa	none	Exposed grasslands
M. sabuleti	*M. arion*	Warm, dry grasslands
HOT AND XEROPHILIC SPP.		
M. schencki	*M. rebeli*	Hot mountainsides, heathlands and sand dunes
M. specioides	none	Hot grasslands in the north

A meadow is only suitable as a reintroduction site for *Maculinea* butterflies, when there are many *Myrmica* ant nests of the good host ant species. These nests have to be large to prevent food shortage for the caterpillars during their period of parasitism (figure 1.5). A proportion of the ant nests needs to be close to the host plant *Sanguisorba officinalis* to ensure that many caterpillars will be found. However, a large part must be located at greater distance to the host plant to bud and recolonise empty patches after a caterpillar has depleted a host ants' nest (Hochberg *et al.* 1994, Clarke *et al.* 1998).

The study site

The only possible site for reintroduction of *Maculinea teleius* and *M. nausithous* butterflies in The Netherlands was the nature reserve "Moerputten", located in a central position of the country. Other nature reserves within the former distribution area of *M. teleius* and *M. nausithous* were not acceptable for the reintroduction, either because the number and density of *S. officinalis* was too low, or the specific *Myrmica* host ant species could not be found (Wynhoff 1992).

The nature reserve "Moerputten" (115 hectares) is situated at the lowest place in its surroundings (figure 1.7). The lowest parts are lower than 2.00 m -NAP (below sea level: NAP is reference level in the Netherlands), the meadows where this study has been done, are situated at the outside borders at an altitude of about 2.20 m + NAP (Van Gerven *et al.* 1994). The soil is a loamy sand, covered by a peat layer, which is locally overlain again by sandy sediments deposited by former inundations. Villages were situated on higher parts in the landscape, but from about the year 1000 AD the construction of dykes started to protect the area from flooding. From the beginning of the 13th century the economic importance and hence the political power of the city of 's Hertogenbosch increased. Therefore plans for extended and better embankments and public rules for dyke maintenance were implemented. However, as the area formed a part of the fortifications around the city, inundations for military purposes used to occur quite regularly during the 16th and 17th century. In the 18th century problems caused by high waterlevels increased. In 1740 the whole area between 'sHertogenbosch and Werkendam was flooded. It resulted in the realisation of a plan to control the water by means of retention areas that could act as basins to store water during high water levels so as to decrease the chance

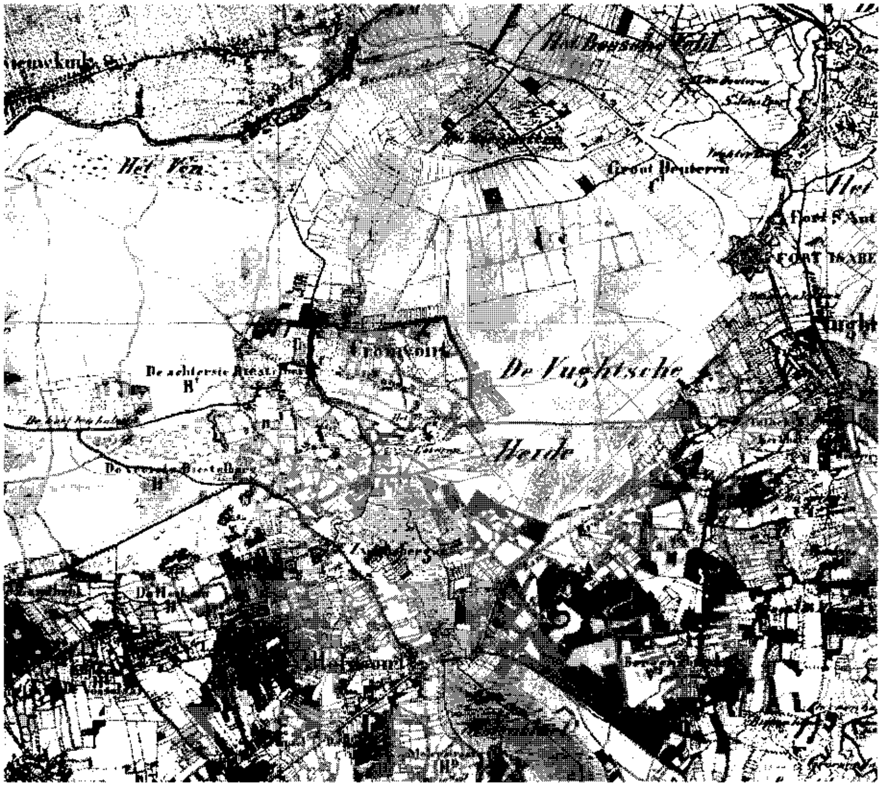


Figure 1.7: Historical map from Moerputten and its surrounding (1:50.000). From: Grote Historische Atlas van Nederland.

of dyke breakage. Since 1766 the Moerputten is part of the Beerze/ Baardwijkse Overlaat (=spillway) (figure 1.8), which means that in periods of high water levels of the rivers and streams within the landscape the Moerputten and the agricultural fields and meadows in its surroundings were purposely flooded (Van der Aalst 1989). This happened in many winters (Stuurman & Foppen 1993). Yet, often dykes broke and villages were still inundated. These floods reached a mean level of 4.00 m +NAP, with a record of more than 6.00 m +NAP in 1880 (Van der Aalst 1989), when in the night of 29 December the dyke next to Nieuwkuijk gave way and many people died (Van den Oord 1992, figure 1.9). In 1904 high floods were finally prevented by replacing the main river bed of the river Maas by opening the artificial canal Bergse Maas.

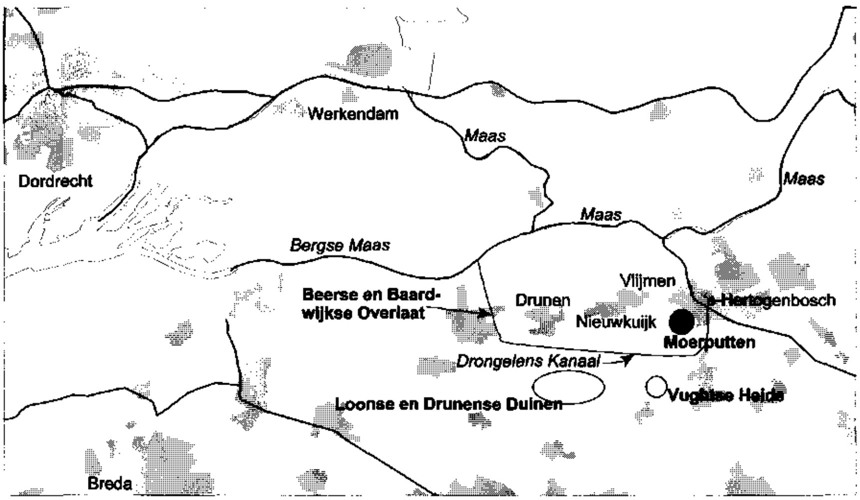


Figure 1.8: Map of locations, rivers and streams mentioned in the text.

In 1890 the railway between 's Hertogenbosch and Geertruidenberg was built. A railway bridge was constructed through the central part of the Moerputten, which was at the same time the narrowest part of the Beerze/Baardwijkse Overlaat, and around the bridge the peat was removed. As it was still necessary to keep open the possibility of high inundations in the retention area, the bridge is 7 m high and more than 50 m long. The railway was mainly used for transportation of materials and products of the leather and shoe-industry and coal until 1972, for people's transport only until 1950. After a period of incidental use the railway was closed in 1976 (Papenburg 1997). While the rails have been removed, the bridge is still present. It has the status of national industrial monument and is as such protected.

Figure 1.9: Farm on a hill between Vlijmen and 's Hertogenbosch while the region is flooded (*from: Van der Aalst 1989*).

Shortly after 1900 the 'Drongelens Kanaal' from Drongelen to 's Hertogenbosch was built for drainage of the agricultural areas. The Baardwijkse Overloop was closed. It meant an end to the high winter inundation levels in the Moerputten. The mean waterlevel in the winter decreased to about 2.25 m +NAP, but the area was still flooded regularly. The Bossche Sloot at the northwest and west side of the Moerputten was used to remove the water surplus to the stream Dieze.

Figure 1.10: Nature reserve Moerputten before 1960, looking from the railway bridge in Southeastern direction over the decoy to Drongelens kanaal (*from: Heemkundekring Onsenoort 1959*).

Due to its position in the landscape, there must have been a high upward seapage of ground water in the low lying area south of the Maas, pushing up base-rich and sodium-rich water from deeper soil layers. Therefore the groundwater level was generally quite high with about 2.00 m +NAP during summertime and more than that in the winter. The water quality was influenced by precipitation and mixing with river- and stream water, mainly during the winter. In these times until the railway bridge was built, the center of the Moerputten consisted of bog vegetation, where local peat diggers came to win turf. The basis for the present day star-like pattern of ditches and drains must have been created then. The outside edges and the area around were most likely wet meadows and pastures of the farmers living in the villages on the higher sandy ridges and on dwelling mounds. A photograph taken before 1960 gives a good impression of the landscape (figure 1.10) (Heemkundekring Onsenoort 1959). The butterfly fauna from 1900 to about 1960 gives evidence for an extended landscape with a mosaic of wet and dry places. As many as 51 species could be found then. Wet meadows were colonised by *Maculinea nausithous, M. teleius, Euphydryas aurinia, Clossiana selene* and *Coenonympha tullia*. In the heath *M. alcon* used to live, while on the drier meadows one could observe *Heodes tityrus, Pyrgus malvae* and *Mesoacidalia aglaya.* At forest edges *Carterocephalus palaemon, Nymphalis polychloros* and *Nymphalis antiopa* had their breeding areas (Wynhoff 1992). Until 1964 changes did not greatly affect the appearance of the Moerputten. It was still an open area with peat and wet meadows surrounded by many more wet meadows. Only the heathlands of Vughtse Heide on the sandy soils several kilometers to the south were separated by the Drongelens Kanaal. The area was obviously large and

Figure 1.11: The nature reserve Moerputten in its surroundings (*from: Grote Topografische Atlas van Nederland*).

diverse enough to support metapopulations of the butterfly species. During inundations, larval instars were not harmed or rescue sites were present to ensure recolonisation of empty places.

From 1964 on, the landscape was changed drastically by a land improvement project (a "ruilverkaveling") (Heemkundekring Onsenoort 1959). The Moerputten was hydrologically isolated and the groundwater level of the surrounding land was decreased by 1 m. The brewery of Heineken was permitted to pump 8 million m^3 water per year from the deep soil layers at a distance of 2.5 km east of Moerputten. The village of Vlijmen pumps up another 2.5 million m^3 per year at a distance of 3 km west of the nature reserve (Streefkerk 1990). It meant that the upward seepage almost stopped. The groundwater level decreased to 1.60 m +NAP in the summer and 2.00 m +NAP in the winter. Hence the floodings became restricted to nature reserve and "improved" the land (figure 1.11). For the populations of plant and animal species in the nature reserve it implied a spatial isolation from other populations and one by one the sensitive and sedentary species disappeared (Database Dutch Butterfly Conservation). Nowadays not more than 25 butterfly species are found, a reduction of 50% in 50 years (Wynhoff 1992). extremely wet winters only. Farmers settled in the low-lying area around the

As a consequence of the changes in the hydrology the vegetation of the Moerputten nature reserve is not low and open any more. Extended beds of *Phragmites*, *Typha* and tall *Carex* species surround the central lake. Around these, moist forests of *Alnus* and various *Salix* species are present nowadays where wet meadows used to occur when the area was still in agricultural use. The nature reserve started to get overgrown by forest in the 1960s (Van Engelen, pers. comm.) At the outer borders of the nature reserve, partially within the forest, different types of grasslands are found, of which the hay meadows with a high abundance of *S. officinalis* are most important as habitat of *Maculinea*. They have a vegetation that is classified as belonging to the alliance *Junco-Molinion*, locally with characteristics of *Arrhenaterion* on dry sites, or *Filipendulion* on moist sites (Ten Oever & Brongers 1994). Host ants of both *Maculinea* species are present.

Aims and outline of this thesis

The aim of this thesis is to study the reintroduction in 1990 of *Maculinea teleius* and *Maculinea nausithous* into the nature reserve Moerputten in The Netherlands. Population establishment and dispersal under natural conditions are monitored and explained. In the explanations, species-specific genetic composition and behaviour are incorporated.

The specific research questions to be answered are:

(a) What are the processes involved in the reintroduction and establishment of both *Maculinea* populations?

(b) How does the genetic composition of reintroduced populations change under natural conditions, and how are these changes influenced by the behaviour of the species concerned?

These questions will be examined for the two case-study species, *Maculinea nausithous* and *Maculinea teleius*. An overview of the study and the logical connections between the parts of it is given in figure 1.12.

To start with, a general overview of the recent distribution and the factors threatening populations (of all *Maculinea* species) is presented in chapter 2. This assessment illustrates the urgent need for conservation actions to prevent or at least minimize further losses. It underlines that not only is more knowledge about the ecology of the reintroduced species needed but also more work has to be done on the effects of conservation actions.

The Dutch populations were monitored in detail after the reintroduction. Population size, population dynamics, distribution and dispersal were determined by means of yearly transect counts and mark-release-recapture studies. These basic results are given in chapter 3.

The success of reintroductions is influenced by species-specific behaviour, especially the dispersal and colonisation behaviour. *Maculinea nausithous* as the more mobile species, shows dispersal behaviour and will colonise habitat patches with nests of its host ant *Myrmica rubra*. This butterfly species is more capable of avoiding less suitable habitat patches and can thus more successfully disperse and colonise empty habitat patches. For further planning at the landscape level and predictions about dispersal it is important to have more knowledge about whether ant-mediated oviposition can take place. If so, seemingly suitable locations with many *Sanguisorba* plants but no *Myrmica* ants could be avoided for ovipostion, but the females would rather deposit their eggs on host plants with host ants nearby. This was tested in a open-air insectory experiment with similar plots but different ant species on each plot, as described in chapter 4.

At the reintroduction site, the Moerputten nature reserve, *Maculinea teleius* and *M. nausithous* show a spatially separated distribution. Usually, the butterfly species co-occur on the same patches within their habitat. Factors determining habitat selection at the reintroduction site were investigated and at the same time, the hypotheses resulting from the experimental study were tested. Within the population of *Sanguisorba*, the vegetation was described in 1 x 1 m plots by means of vegetation relevés, vegetation structure measurements and measurements of several microclimatic variables. The occupancy of *Myrmica* ants was determined, too. First, oviposition was measured by counting the eggs on flowerheads. Since this method only gave good results for *Maculinea nausithous*, we also observed females of *Maculinea teleius* during oviposition. These field data and the distribution of the *Maculinea* populations throughout the years since reintroduction were analysed to find specific parameters explaining the habitat selection of both species. The results are given in chapter 5.

The distribution of both *Maculinea* species in and around the Moerputten nature reserve is limited. At present (2001), *Maculinea teleius* is still restricted to only one meadow, while *Maculinea nausithous* has established three local populations, but does not expand any further. The reasons for the limited dispersal may be, that the habitat quality of the empty plots is not sufficient for persistent colonisation, or that the distance between empty and colonised plots is too large to bridge. The results from studying habitat selection allowed for a detailed analysis of colonisation and abandonment patterns in the distribution of both butterfly species. The results are presented in chapter 6.

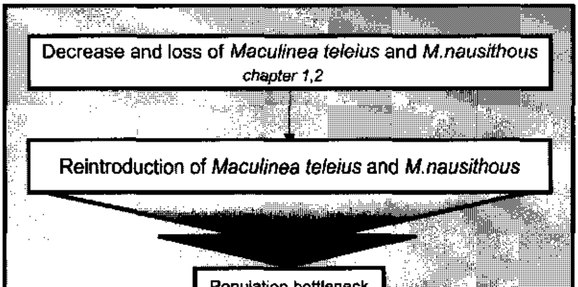


Figure 1.12: Relational diagram on the parts of this study.

The population studies revealed that after the reintroduction, *Maculinea nausithous* did not settle at the reintroduction habitat patch but on another. This occurred because of ecological factors. This behaviour resulted in a second potential bottleneck after the reintroduction. On the other hand, *Maculinea teleius* settled at the release-site and immediately increased in number and density. This species possibly did not go through any bottleneck in addition to the founder population. If this is correct, *Maculinea teleius* will be expected to show no or only little change in variation of quantitative or genetic characters. This was analysed by electrophoresis of allozymes. In contrast, *M. nausithous* will be more likely to show greater changes compared to the source population. The results of the allozyme electrophoresis are given in chapter 7.

The final synthesis (chapter 8) discusses the connections between the different parts of the study. Some additional data are added and conclusions are drawn.

The recent distribution of the European Maculinea species

Summary

The genus *Maculinea* is confined to the Palaearctic region. Four species occur from Central Europe to China and/or Japan, while *M. rebeli* is probably restricted to Europe. Although within many Western European countries the distribution of the species is well known, there are still many questions. The exact status of some of the species is still unclear and the amount of decline in this century is unknown. In Asia especially within the nations of the former USSR, the distributions of species are even less well known. However, as a basis for good research and effective nature conservation, knowledge on these subjects is needed.

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Introduction

All *Maculinea* species are restricted to the Palaearctic region and their centre of distribution is located in Central to Eastern Europe and West Asia. Five species are mentioned for Europe: *Maculinea arion* L. and *M. teleius* Bergsträsser from France and Spain to China; *Maculinea nausithous* Bergsträsser from Spain to Mongolia, and *M. alcon* Denis & Schiff. and *M. rebeli* Hirschke. The distribution and status of the latter two are somewhat difficult to determine: in former times the two were regarded to be subspecies of *M. alcon* s.l. and were rarely separated in distribution data. Consequently, in old literature it is often difficult, or even impossible to decide which species is treated. A sixth species *Maculinea arionides* M. occurs only in China and Japan and its status is unclear.

For all *Maculinea* species, the eastern boundaries of the distribution are poorly known, and there is little information on their distribution, status and ecology in many nations of the former USSR and neighbouring states. Some information can be found in Lee (1982), Bálint (1991a), Fukuda *et al.* (1992), Lukhtanov & Lukhtanov (1994), Корwунов & Горбунов (1995), but much has still to be found in the field and in many museum collections. The taxonomy of the *Maculinea* species is still unclear, especially with respect to the eastern part of their distribution. Further information dealing with the taxonomy can be found in Bálint (1985, 1986, 1991b and 1993) and in Sibatani *et al.* (1994), who also examines the status of several rare species in East Asia.

The main aim of this study is to give a realistic impression of the distribution of the five European *Maculinea*-species. A further aim is to identify the reasons for their decline.

Method

Maps were drawn on the standard 50x50 km-UTM-grid scale (Van Helsdingen *et al.* 1997) and based on published literature, national databases and data given by specialists. Only post-1980-data were included and data that were exact enough to be presented in the UTM-grids. Hence, "shaded areas" and fauna catalogues are not used in the maps, but mentioned in the text. An overview of the recent distribution by country is presented in table 2.1.
Detailed information on *Maculinea arion*, *M. teleius*, and *M. nausithous* can

be found in new data sheets produced by the European Invertebrate Survey and are used as background information for the Habitats Directives of the European Community (Thomas 1997, Wynhoff 1997a, 1997b). More details at regional and local level can be found in these data sheets.

Table 2.1: Occurence of *Maculinea* species in the European countries (After Van Swaay & Warren 1999). n, native; e, extinct; r, reintroduced, n*, data became available after paper was published.

	M. arion	*M. teleius*	*M. nausithous*	*M. alcon*	*M. rebeli*
Poland	n	n	n	n	n
Czechia	n	n	n	n	n
Slovacia	n	n	n	n	n
Germany	n	n	n	n	n
France	n	n	n	n	n
Switzerland	n	n	n	n	n
Austria	n	n	n	n	n
Yugoslavia	n	n	n	n	n
Slovenia	n	n	n	n	n
Hungary	n	n	n	n	n
Russia	n	n	n	n	n
Romania	n	n	n	n	.
Ukraine	n	n	n	n	.
The Netherlands	e	r	r	n	.
Turkey	n	.	n	n	.
Spain	n	.	n	n	n
Bulgaria	n	.	n	.	n
Croatia	n	n	n	n*	n*.
Italy	n	n	.	n	n
Lithuania	n	n	.	n	.
Belgium	n	e	.	n	.
Latvia	n	n	.	.	.
Estonia	n	.	.	n	.
Denmark	n	.	.	n	.
Sweden	n	.	.	n	.
Albania	n	.	.	n	.
Greece	n	.	.	n	.
Finland	n	.	.	.	.
Luxemburg	n	.	.	.	.
United Kingdom	r	.	.	.	.
Norway	.	.	.	.	.
Ireland	.	.	.	.	.
Sicily	.	.	.	.	.
Portugal	.	.	.	n*.	.

Results and Discussion

Distribution

All *Maculinea* species occur in Central Europe in a band reaching from the East of France through Germany, Switzerland, Austria, Czechia, Slovacia, the Balkan countries and further to the East. Except for *M. rebeli*, all species can be found in the region streching over the former Russian states until Mongolia, China and Japan. However, the Northern and Southern boundaries differ per species (Tolman & Lewington 1997).

Maculinea arion Linnaeus 1758 (Figure 2.1)

All over Europe, populations of *Maculinea arion* have experienced severe declines during the 20th century, especially in the northern part of the species' range. *M. arion* became extinct in the Netherlands in 1964 (Tax 1989) and in the UK in 1979 (Thomas 1994). It was also extinct in Belgium until summer 1996 when it recolonised a location in the South (Goffart 1997). Probably only 2 populations survive in Denmark (Stoltze 1996, Nielsen, pers. comm.). In Finland many local extinctions have occurred, with a few populations surviving on glacial outwash ridges near the south coast (Marttila *et al.* 1992). In Sweden, strong populations recently occurred on Gotland and Öland, while it is largely extinct on the mainland. Many populations have become extinct throughout Germany and northern France, although in southern Germany more populations exist than presented on the map. *M. arion* has also largely disappeared from some mountain ranges, for example the Jura. It is very localised, but common where it occurs in the Pyrenees, and remains widespread, but at low densities between c. 1500m - 2000m in the French, Italian and Austrian Alps and in the Cevennes (Thomas 1997). In Poland, the populations can be found in the Southern part of the country (Buszko 1997). In Estonia it is rare with at maximum 20 populations (Viidalepp 1995). Ivinskis (1993) calls it common for Lithuania, where it is restricted to dry pine forests in the eastern provinces.

Maculinea teleius Bergsträsser 1779 (Figure 2.2)

The distribution map for *M. teleius* overestimates the status in comparison to other species, as one dot on the map often represents just one population. Moreover, many populations are not only small but also isolated for many generations.

In France *M. teleius* has only a limited number of populations, mainly in

the northeastern regions (Lhonoré, 1992). The subspecies *burdigalensis* is probably extinct. Germany has isolated sites in the central and southern regions (Ebert & Rennwald 1991). However, for example in the Westerwald only 1% of the populations is large, while 85% are small or very small (<10 ind. per day) (Fischer & Kunz 1994). In Bavaria, larger populations and even meta-populations of *M. teleius* can be found in a number of regions (Bayerisches Landesamt für Umweltschutz 1995). In Switzerland and Austria the area occupied is restricted (Gonseth, pers. comm.). The last Italian populations, located in the southern foothills of the Alps, are also declining and threatened by the loss of suitable habitat (Balletto 1992).

In Hungary and Slowenia, *M. teleius* only occurs in the northern regions (Bálint 1991b, Jakšic 1988). In Poland, *M. teleius* is rarer and declining faster than *M. nausithous*. The only populations can be found in the South (Buszko 1997). At least some of them are very large. Finally, there are also populations in Lithuania (Ivinskis 1993), Czechia (Kudrna 1994), Romania (Popescu-Gorj 1987, Ruşti 1987), Bulgaria (Ganev 1985) & Moldova (Popov, pers. comm.). Much further east there are sites of this butterfly species in Western Siberia (Lukhtanov & Lukhtanov 1994, Woyciechowski, pers. comm.).

Maculinea nausithous Bergsträsser 1779 (Figure 2.3)

In Spain, *M. nausithous* only occurs in the province of Soria, North of Leon and near Madrid at an altitude 1100 to 1400m (Fernandez-Rubio 1991, Munguira & Martín 1993). In France the species occurs chiefly in the Northeast, Alsace and Bourgogne (Lhonore, 1992), but often only in a limited number of isolated populations. In Germany it is more widely distributed in the central part, Bavaria and the southern regions of Eastern Germany. In Baden-Württemberg there are still quite a lot of populations (Ebert & Rennwald 1991), and it also occurs in Westerwald and Spessart, the Palatinate, in the region around Stuttgart and Bavaria. Isolated sites can be found in the Eifel and the Lower Rhine Region. In the former East-Germany it occurs only in the southern regions and near Berlin (Weidlich & Kretschmer 1995). The Polish populations are mainly concentrated in the South. In some regions the species can be quite common, but it is still declining (Buszko 1997). In Czechia, it is restricted to the northern regions (Kudrna 1994). Records from Switzerland are concentrated in four regions: Neuchâtel, Berner Oberland, Lake Zürich and parts of the Rhine Valley (Gonseth 1987, SBN 1987). In Austria, where *M. nausithous* is rarer than *M. teleius*, local populations were recorded in the North and in the southeastern part of

Steiermark (Reichl 1992). The Hungarian populations can be found not far away in the northern and western regions of this country (Bálint 1991b, 1993). Several records are known from Moldova (Popov, pers. comm.) and Bulgaria (Ganev 1985). For Slovacia only old records are available (Hruby 1964). Furthermore, *M. nausithous* has been found in Northeast Turkey and in Western Siberia.

Maculinea alcon Denis & Schiffermüller 1775 (Figure 2.4)

The distribution of *M. alcon* shows a scattered pattern of several disjunct areas. In Denmark near the western coast of the mainland (Stoltze 1996) and at the utmost South of Sweden (Hammarstedt 1992), which are the most northerly populations in Europe. In The Netherlands there are two regions with populations of *M. alcon* with most occuring in the northern region (Tax 1989). This area is quite close to the populations in the moorland of Northern Germany (Rohlfs, pers. comm.). The Dutch populations on the sandy soils in the southern provinces are comparably close to the Flemish ones. In Flanders, the butterfly can be found in the region north of an imaginary line from Antwerp to Maastricht (Maes & Van Dyck 1996).

In France, *Maculinea alcon* also occurs in different regions. The largest populations are known from the Sarthe. In the Gironde region, populations are less dense, while they are smallest in Brittany and Contentin (Lhonoré, 1992). In Germany it is restricted to several regions where it can be locally quite common. The distribution in Central and South Germany is probably at least partly known, but is not yet published. In the former Eastern Germany, *M. alcon* is extinct (Reinhardt & Thust 1993). The distribution of *M. alcon* in Spain is restricted to isolated populations in Cantabrica and the western Sistema Iberico (Fernández-Rubio 1991) at altitudes up to 1300m, spatially isolated from *M. rebeli*.

In Switzerland most populations can be found east of the Alps from 400 to 900 m (Gonseth 1987). The largest spot on the map is formed by Northeast Austria, Slovenia and Northern Kroatia. More populations occur in North-Hungary, South-Poland, Moldova and Romania (Bálint 1985, Buszko 1997, Popov, pers. comm.). In Northeast-Turkey, *M. alcon monticola* was recorded (Hesselbarth *et al.* 1995).

Maculinea rebeli Hirschke 1904 (Figure 2.5)

The rarest European *Maculinea*-species is *M. rebeli*. In general, the distribution of this species extends from the Central Massif and the southeastern Alps in France and the eastern Pyrenees in Spain through Central European mountainous areas in Switzerland, the Dolomites and the Appenines to the east. All locations are at an altitude of 1300-2000 m (Higgins & Riley 1980).

In France, the majority of populations are located in the east of the country (SBN 1987). The Spanish populations are restricted to the central and western Pyrenees and the western Sistema Iberico at altitudes from 900 to 2000m (Fernández-Rubio 1991). The distribution is completely separated from *M. alcon*. In Swizerland *M. rebeli* only occurs in mountainous areas in the Jura and the Alps at altitudes of 800 to 2300 m (Gonseth 1987).

In Germany, isolated populations can be found in Eastern Westphalia (Meyer 1992) on the Swabian Alb, and in Bavaria (Ebert & Rennwald 1991, Dolek *et al.* 1998). In other mountainous areas in Germany, more populations probably occur. In Eastern Germany, *M. rebeli* can only been found in Thüringen (Reinhardt & Thust 1993). In Czechia, the species is restricted to the far south (Kudrna 1994). The only Polish population occurs in the Pieniny mountains (Buszko 1997). *M. rebeli* has not been found in Turkey nor in Siberia.

Status

The IUCN world status of *M. arion, M. alcon* and *M. rebeli* is 'vulnerable', while *M. teleius* and *M. nausithous* are considered to be 'endangered' (IUCN 1990, old categories). *M. arion* is listed in appendix IV, but not appendix II, of the EC Habitats Directive (Thomas 1997). *Maculinea teleius* and *M. nausithous* are listed in both appendeces (Wynhoff 1997 a, b), while *M. alcon* and *M. rebeli* are not included. Also, only three of the European *Maculinea* species are mentioned in the Bern Convention: *M. arion, M. teleius* and *M. nausithous*.

In almost all countries that have developed Red Lists, one or more of the *Maculinea* species have a well defined and protected status, although there are many differences between countries and states and/or provinces (Blab *et al.* 1984, Buszko 1997, Ebert & Rennwald 1991, Gepp 1983, Gonseth 1987, Maes & Van Dyck 1996, Munguira & Martín 1993, Wynhoff & Van Swaay 1995). The relatively best protected species is *M. teleius*, while *M. rebeli* is least protected. This is probably due to its still unclear taxonomical status. In most countries where (some of) the species are protected by law, nature conservation still faced the problem that habitats are not protected.

Reasons for decline

In most European countries, *Maculinea* species are declining. For example in The Netherlands four *Maculinea*-species occurred at the beginning of the century, but in the seventies all but one became extinct (Tax 1989). The reasons for the decline are often similar to other butterfly species but the highly specialised, myrmecophilous *Maculinea's* have been especially badly affected. Most populations have disappeared as a result of habitat destruction and habitat deterioration. Many habitats have also been destroyed by the development of suburbs, factories, infrastructure and recreational objects (Tax 1989, Settele 1990, Thomas 1991, Elmes & Thomas 1992, Munguira & Martín 1993, Thomas 1997). The habitat changes as a result of changes in agricultural techniques, such as increase in mowing frequency, drainage of patches or even complete regions, intensification of grazing or, alternatively, abandonment.

However, many populations depend on some form of disturbance by man to maintain their habitat through traditional methods of agriculture (Thomas 1997). This is shown for *M. arion* in the UK: about 50% of extinctions were caused by serious deteriorations of the site by intensive agriculture. The other half resulted from the abandonment of sites by farmers, leading to an increase in vegetation height and cover and a subsequent rapid loss of *Myrmica sabuleti* (Thomas 1994). In some countries, the decline of rabbits through myxomatosis made things even worse. Many populations of *M. nausithous* and *M. teleius* are affected in a similar way, either by intensification of agricultural use or by abandonment of sites (Ebert & Rennwald 1991, Maurin & Keith 1994). After a decrease in host ant density due to changes in the vegetation structure and in the microclimate at the colony sites, butterfly numbers decrease and finally the populations become extinct. At first, abandonment of meadows causes an increase in population density of *M. nausithous*, but if continued for a longer period, the vegetation will become too dense for the host ant *Myrmica rubra*.

Under certain climatological conditions and if the soil is not too dry or too wet, *M. alcon* and *M. rebeli* populations benefit from an extensive grazing regime by a limited number of sheep or cattle. Without this regime, vegetation cover gets too dense. The hostplant populations of *Gentiana cruciata* or *G. pneumonanthe* are no longer able to rejuvenate and the host ant species disappear (Kockelke *et al.* 1994). However, heavy grazing especially when the early larval instar caterpillars are still on the hostplant, is a serious threat to the populations (Scheper 1994).

In a more indirect way, air and water pollution also affect the vegetation composition and structure and this also causes deterioration in sensitive *Maculinea*-habitats (Munguira & Martín 1993). Habitat changes can sometimes be very subtle, but nevertheless destroy suitable butterfly patches completely. At present, biotope destruction is a major threat to *Maculinea* populations in Mediterranean Europe and in all eastern European Countries (Munguira & Martín 1993, Thomas 1997, M. Woyciechowsky pers. comm.).

Many populations of sedentary species are threatened by the fragmentation of their habitats in combination with the isolation of small habitat patches. Such isolated populations are very susceptible to chance extinction and as there is no exchange possible with other habitat patches, recolonisation will not occur. *Maculinea's* are generally not considered to be able to cross a distance of more than a few kilometers (Settele *et al.* 1996). Nowadays, many populations have already been isolated for decades and some of these have gone extinct without experiencing any change in their habitat. Climate change may also have a negative effect on stenoecious, sedentary species such as the *Maculinea's*.

In the Western part of its distribution, wet heathland represents the major habitat type of *Maculinea alcon*. During this century large areas of heath were drained and improved for agricultural use. The remnants suffer from eutrophication by air pollution. The vegetation changed in many cases from an open *Erica*-vegetation to a closed one, dominated by the Purple moorgrass (*Molinia caerulea*). There are strong indications that the butterfly avoids the larger Purple moorgrass areas. In this way fragmentation of populations takes place and former meta-populations get split up. In The Netherlands, there are at present only three metapopulations which are capable of surviving on the long term. The other small and isolated populations appear to be the first to become extinct. Even over a rather short time interval, between 1980 and 1995, extinction of several populations was observed (Scheper 1994, Wynhoff *et al.* 1996).

In several cases, this was most likely the result of a lowering of the ground water table and the absence of suitable management. The lowering of the water table has restricted many populations of *Gentiana pneumonanthe* (and *M. alcon*) to the lowest (and relatively wettest) areas of the nature reserves. This not only caused a fragmentation of populations within otherwise rather continuous heathlands, but also increased the risk of relatively long inundations of entire gentian-populations during winters with high rainfall. Long periods of inundation postpone the flowering of gentians, with the effect that the number of buds for oviposition will be reduced

(Wynhoff *et al.* 1996). This case study in The Netherlands is just an example; similar habitat deteriorations have occured all over Europe.

Collecting butterflies is often said to be a major reason for decline. As long as populations are large and subpopulations are connected, it is almost impossible to eliminate them just by collecting. However, collecting a relatively large number of individuals could have detrimental effects after habitat patches (1) have decreased in size and quality, (2) have become isolated and when (3) the butterfly numbers have already seriously decreased.

Conclusions

One or more *Maculinea*-species can be found in the majority of countries in Europe. *M. rebeli* is possibly restricted to Europe, the other four occur also further east as far as Mongolia or even China and Japan. Unfortunately, from Finland to Italy and from Spain to Poland, populations of all treated species are declining or have even become extinct. Meta-populations are reduced to core-populations and core-populations vanish. These processes executed a severe influence at the edges of distribution and in regions that were improved for agriculture. Nowadays, large populations and well functioning meta-populations are restricted to regions with a relatively low agricultural pressure, as can be found in Eastern European countries and probably in the states of the former USSR. In the West European countries extinctions have occured much more often, mainly due to intensification in agriculture and landuse. The remnants get more and more isolated and suffer from a high chance of stochastic extinction.

The maps (fig. 2.1-2.5) of the distribution of the *Maculinea*-species give a good impression of the species' range. However, gaps still need to be filled, for example mapping schemes in the countries in the Balkan, the former USSR, France and Italy would add a lot to the recent knowledge on the distribution of butterfly species. In some countries, data are already collected but have not yet been published.

In species conservation projects, detailed maps of the local distribution should be added. As such additional maps give the distribution on a more precise scale, they give better information on the size, the composition and the isolation of the populations.

Within Europe, almost all sites of *Maculinea*-species have been created by and depend on some kind of human activity in the form of extensive (often traditional) agriculture to keep the vegetation structure open and the ant nest

density sufficiently high. Next to habitat destruction, habitat changes as a result of improvements in agricultural techniques form a major threat to sites. This concerns not only intensification of agricultural use, but also abandonment. Therefore the protection of *Maculinea* populations almost always includes management of sites and thus protection of old cultural techniques.

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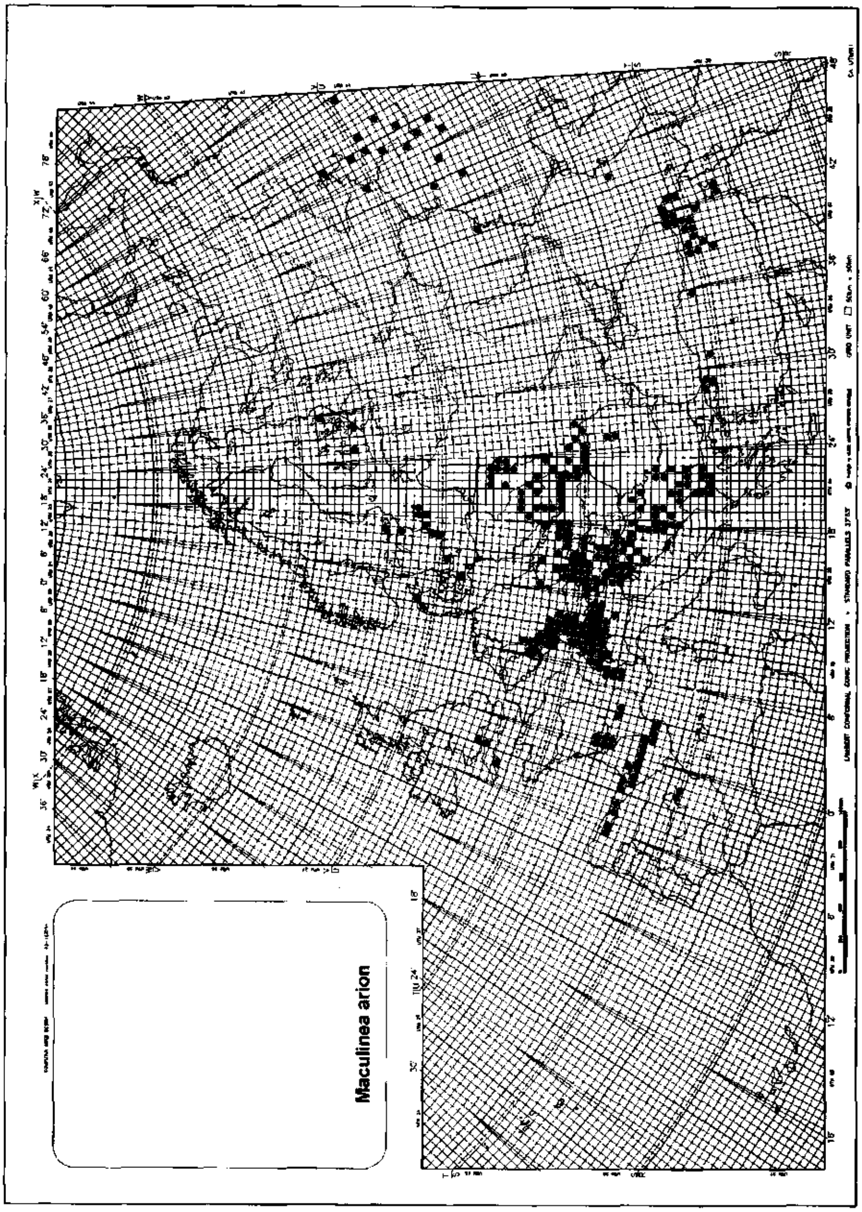

Figure 2.1: Distribution of *Maculinea arion* in Europe.

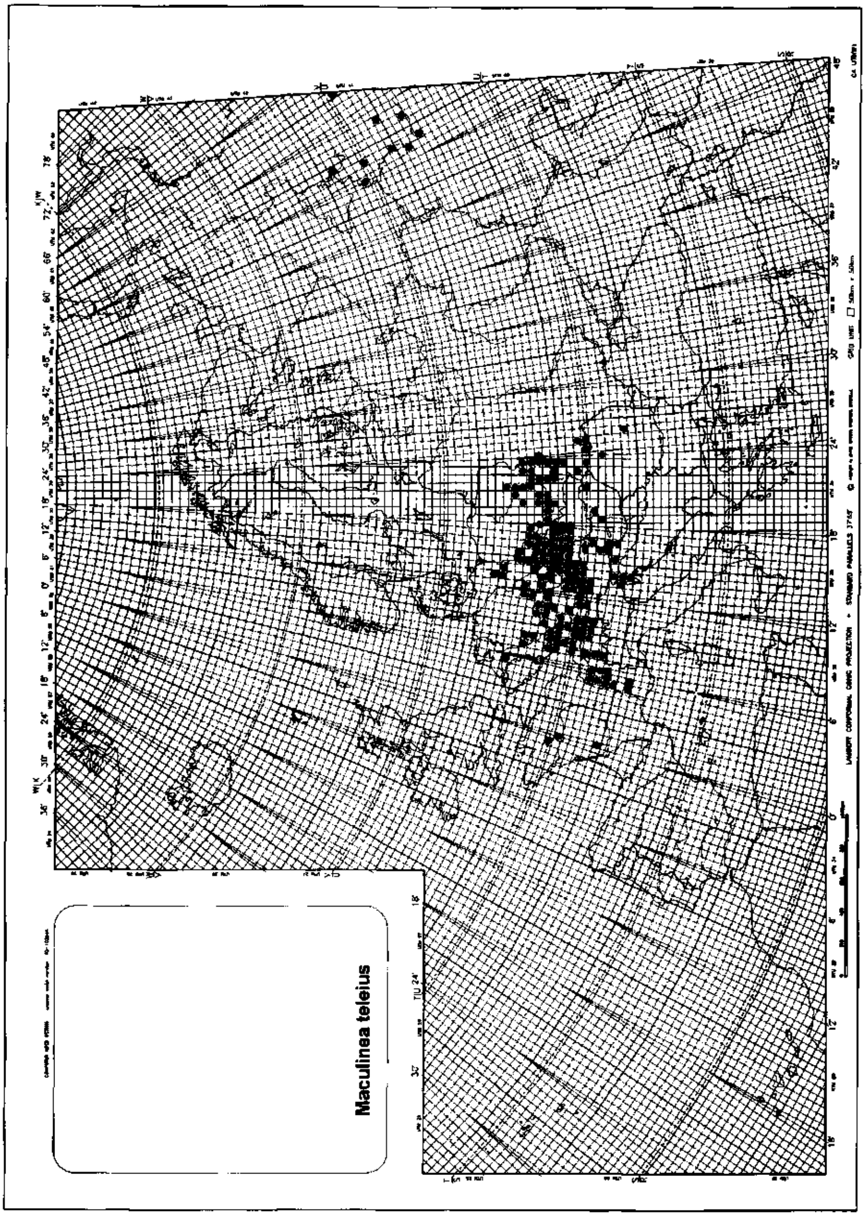

Figure 2.2: Distribution of *Maculinea teleius* in Europe.

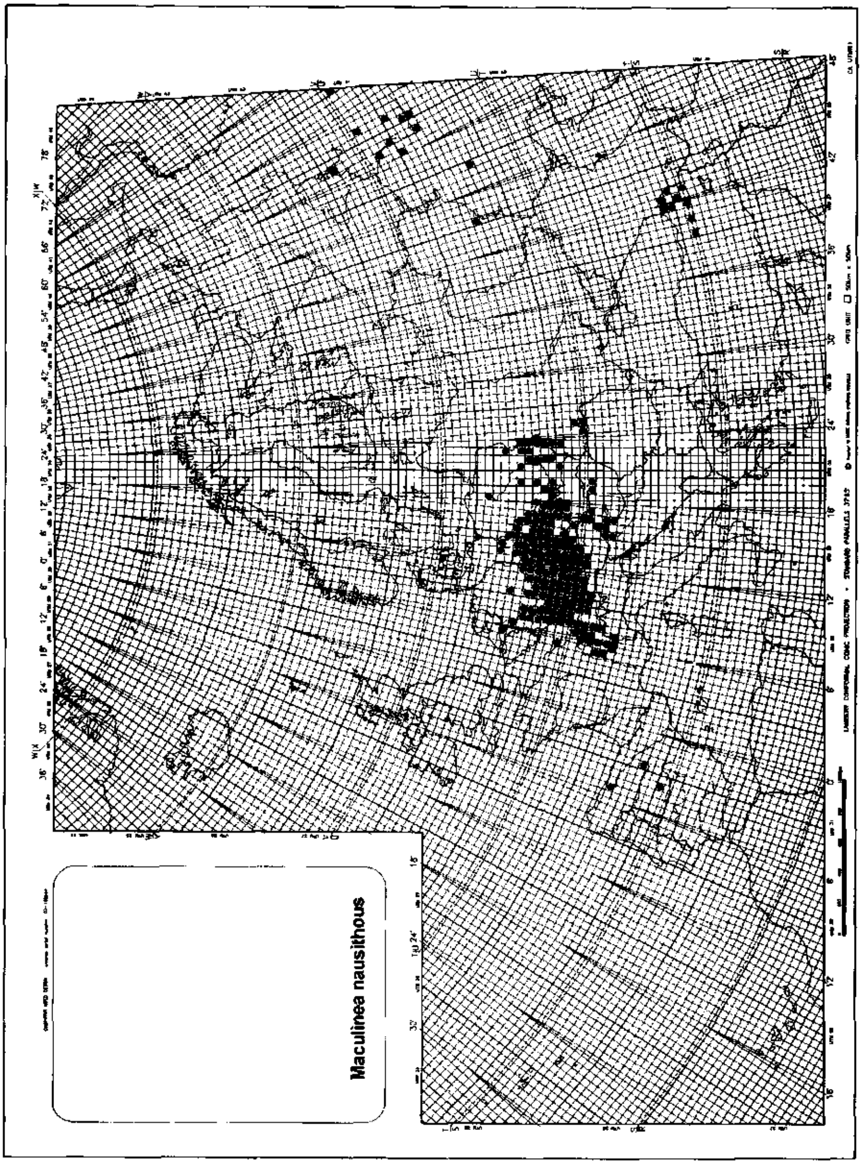

Figure 2.3: Distribution of *Maculinea nausithous* in Europe.

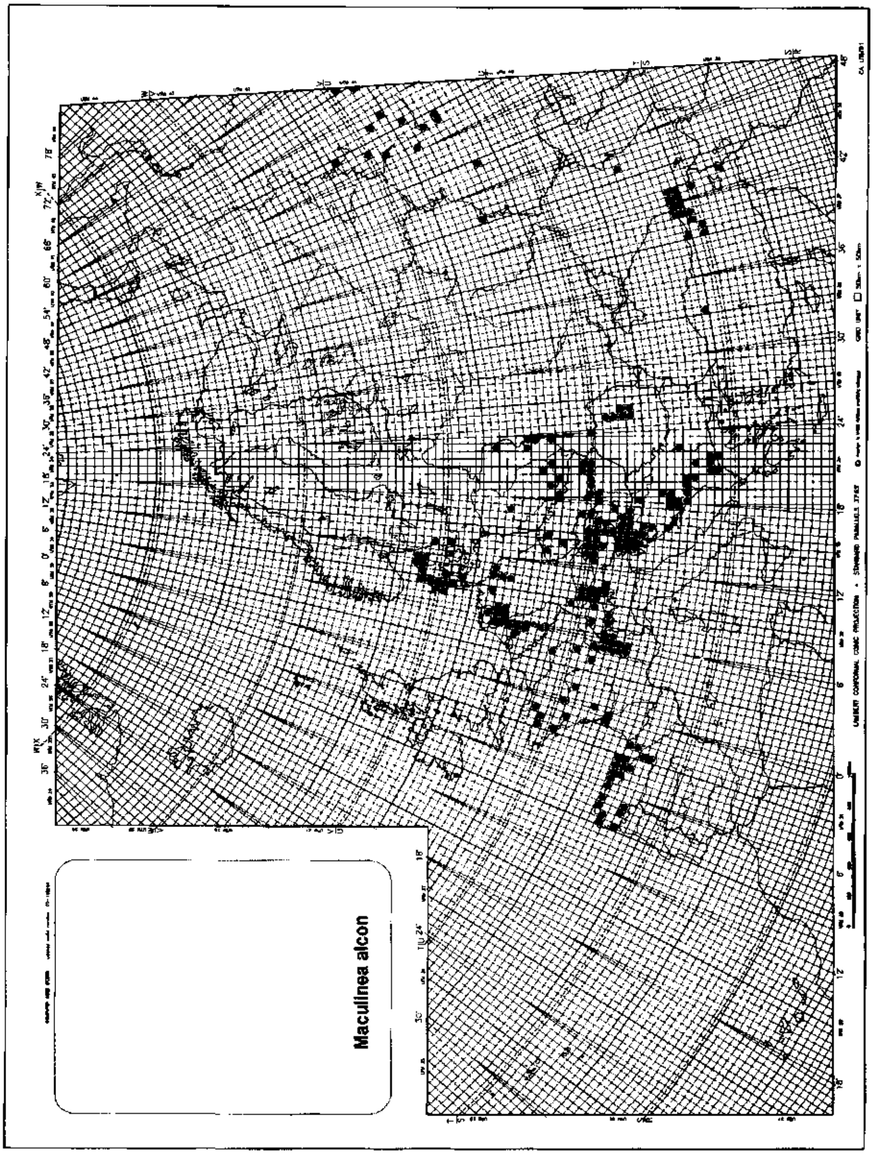

Figure 2.4: Distribution of *Maculinea alcon* in Europe.

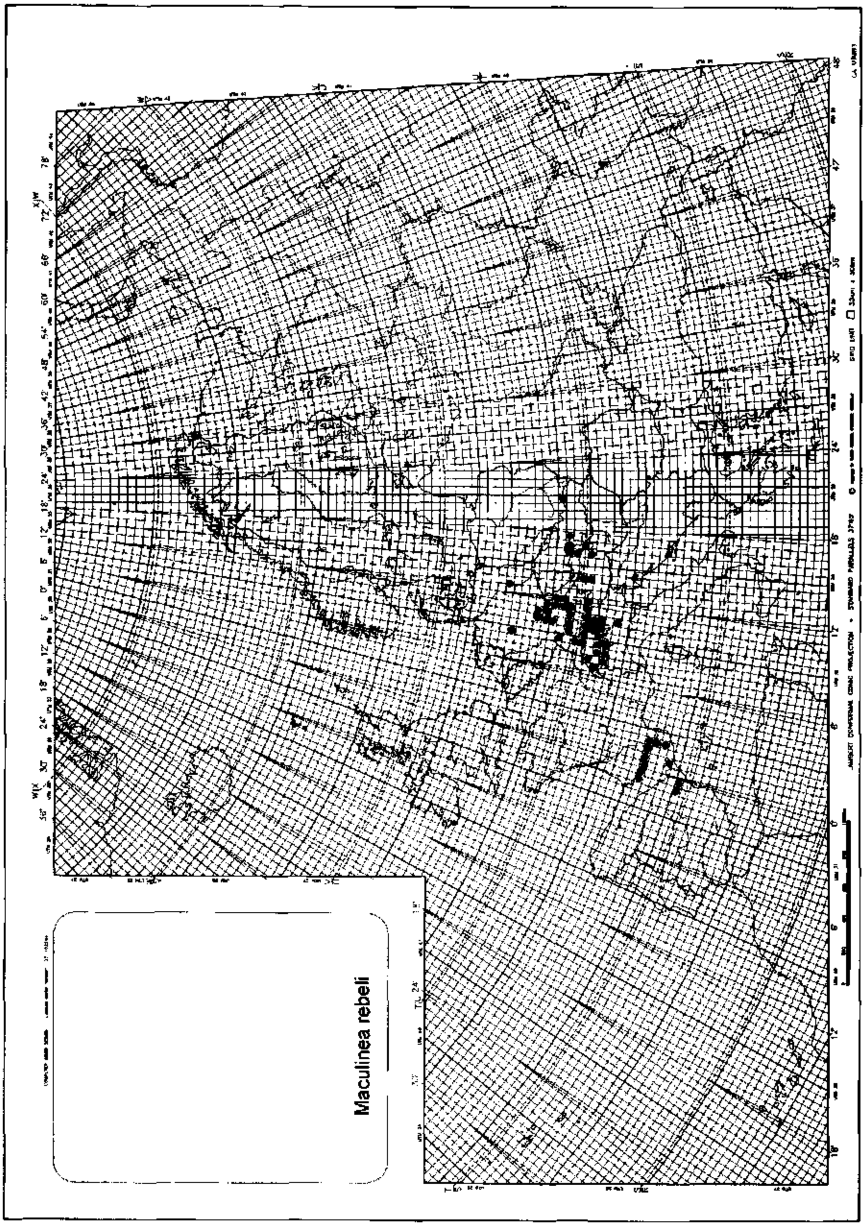

Figure 2.5: Distribution of *Maculinea rebeli* in Europe.

Population development of the Large Blue Butterfly species Maculinea teleius and M. nausithous after reintroduction

Summary

Details of a reintroduction of *Maculinea teleius* (Scarce Large Blue butterfly) and *Maculinea nausithous* (Dusky Large Blue Butterfly) into a nature reserve in The Netherlands are given. The introduced population of *M. teleius* expanded during the first three years. In contrast, the newly established population of *M. nausithous* declined in the year after the reintroduction but expanded later. After a considerable increase in numbers, this species can nowadays be seen in two subpopulations: one in and one outside the nature reserve. Although the results are quite positive with respect to the numbers of butterflies, some alarming features have also been found. Both species, but especially *M. teleius,* have a very low mean minimal life time, which makes them more susceptible to stochastic catastrophes. While the area populated by *M. nausithous* is still expanding, *M. teleius* can only be found on the meadow where it was released and has not dispersed at all. The possible consequences of reintroductions are discussed.

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extinction due to the capture of butterflies for the reintroduction was not a risk. Furthermore, the flowering period of *Sanguisorba officinalis* in South Poland is parallel to the flowering period in The Netherlands.

Habitat description of the reintroduction site

The only potential site for reintroduction of the *Maculinea* butterflies in The Netherlands was the nature reserve "Moerputten", located in a central position of the country (Wynhoff 1992). Other nature reserves within the former distribution area of *M. teleius* and *M. nausithous* were not acceptable for the reintroduction, either because the number and density of *S. officinalis* was too low, or the specific *Myrmica* host ant species could not be found.

The nature reserve "Moerputten" (115 hectares) is situated at the lowest place in its surroundings. The soil is loamy sand, covered by a peat layer which is locally overlain by sandy sediments deposited by inundations in former times. The present appearance of the nature reserve was to a large extent created by peat digging. The central lake is surrounded by tall beds of *Phragmites*, *Typha* and tall *Carex* species. Around these, moist forests of *Alnus* and various *Salix* species are present nowadays where wet meadows used to occur when the area was still in agricultural use. On the outer borders of the nature reserve, partially within the forest, different types of grasslands are found, of which the hay meadows with a high abundance of *S. officinalis* are the most important as habitat of *Maculinea*. They have a vegetation that is classified as belonging to the alliance *Junco-Molinion*, locally with characteristics of *Arrhenaterion* on dry sites, or *Filipendulion* on moist sites. Host ants of both *Maculinea* species are present.

Procedure of the Reintroduction

Butterflies for reintroduction were caught with a net and then placed three together in a small paper box. These boxes were kept cool in a car-refrigerator. The butterflies were transported by car to The Netherlands. On the warm evening of July 30th, 1990, a numbers of 33 males and 53 females of *M. teleius* and 22 males and 48 females of *M. nausithous* were released on the meadows of the Moerputten.

Evaluation of the reintroduction

Maculinea population data

The population size of both butterfly species was estimated by capturing, marking and recapturing adults during the whole flight-period in the Moerputten reserve and its surrounding. On two meadows a grid of 10 x 10 m squares was permanently placed, with a code for each square. Each adult caught was marked individually by a dot code on the wings using a Staedler Lumocolor Permanent pen, and immediately released at the point of capture. For each capture, a record was made of sex, mark-number and grid-square code of capture. Outside the grid, the site of capturing was drawn onto a map with a precision of about 10 m.

Butterflies were captured at regular intervals of two to three days. On each capture day, a path of approximately the same length over the meadows in the nature reserve was walked and inspected for adults. Walking speed was always kept the same and the track was never walked more than once a day, except for the area with the embankment population of *M. nausithous*. This area is situated along a small stream with rough vegetation, willows and elders where butterflies are difficult to access. Therefore the same path was walked four times (without capturing butterflies more than one time). By keeping the walking speed constant, the total capture time was relatively short at the beginning and end of the flight-period, but longer in the middle when more butterflies are on the wing. Butterflies are assumed to have an equal probability of capture. If the total time devoted to capture was kept constant over days, the probability of any particular individual being captured would be inversely density-dependent: in the optimum of the flight-period a lower percentage of the population would have been caught, which would have had consequences for the estimation of the population size. Directly after the peak of the flight-period other nature reserves, meadows, road verges and canal embankments in the surroundings of the "Moerputten" were also inspected for adults to investigate dispersal. If the weather was bad the intervals between the investigations were occasionally longer. The reintroduced populations were studied from 1990 to 1996. In 1993, only *M. nausithous* was captured and marked, in 1994 no mark-recapture study was done at all.

The flight-period of the reintroduced population was compared to historical records of flight-periods of the extinct populations in The Netherlands and to those of the source population. Data for these comparisons are comprized of dates of flight recordings of individuals of the extant populations but consist of dates of collections mentioned on cabinet specimens. All data were lumped into periods of five days each.

Estimation of population size

For the estimation of population size, two methods were used: the Minimal Number Alive (MNA)-method (Seufert 1993) and the method of Jolly (Begon 1979). This allows for the estimation of a low and a high limit of population size.

The Minimal Number Alive-method only works with marked individuals, but provides no estimation of the size of the unmarked part of the population. Next to the daily population size, the mean minimal life span per captured individual can be estimated. For 1990, the two days of age the butterflies had at the moment of release, were added.

The method of Jolly estimates the approximate population size over the marked and unmarked individuals (Jolly 1965). For the estimation the program JOLLY of the package CR15 was used.

Total population size was calculated plotting the daily population size estimated from the Jolly- or the MNA-analysis. The area under the curve was multiplied by the mean daily loss rate.

Myrmica ant nests

The density of ant nests was recorded by means of inspecting plots with a minimum size of 2 x 2 m at different localities in the nature reserve and the road verges. As a consequence of the mowing management of most grasslands, the subterranean ant nests are difficult to find. They were detected by disturbing the soil with a knife in a grid of 10 by 10 cm. Worker ants would leave their nest when it was disturbed. From each nest, some individuals were taken to the laboratory for species identification.

Sanguisorba officinalis

A spatial inventory was made of the distribution of all *Sanguisorba officinalis* plants in the nature reserve "Moerputten" as well as in the surrounding road verges. For the purposes of this study, a plant was defined as being one or more stems emerging from the same place in the soil.

Results

Population development of M. teleius and M. nausithous

After the reintroduction in 1990 the populations of each species developed in a different way. In 1991 and 1992 the number of *M. teleius* increased, followed by a decline to the numbers given for 1995 (figure 3.1a, 3.2a). In the years when no adults have been marked, the numbers of observed butterflies and the counts on the monitoring transect show that the populations size had been approximately the same as in 1995 (Wynhoff 1996). The second decline from 1995 to 1996 is caused by accidental mowing shortly after the peak flight-period of the only site where the butterflies occur and oviposition takes place.

In the course of the years directly after the release, the flight-period of this species gradually shifted forwards by a few weeks. A statistical difference in the timing of the flight-period between the Moerputten and the Kostrze-population can be shown (Mann-Whitney U-test: Z=-12,11; p<0,0001). The flight-period of the indigenous populations in The Netherlands endured until the end of August, with the peak in the last week of July. The peak population in Poland is in the middle of July with adults flying until late august with some still being observed in September (Figurny, pers. comm.). The flight-periods of the historical Dutch population differs from that of the Kostre-population (Mann-Whitney U-test: Z=-19,12; p<0,0001). The Moerputten population peaks in the second week of July, and with the beginning of August, the flight-period is already over. The flight-period of the new population is different from the historical one (Mann-Whitney U-test: Z=-22,53; p<0,0001). The development of the total population size per year is given in figure 2a. The total population size in 1993, 1994 and 1995 was about 300 individuals per year. This is most likely the maximum population size that can survive on the single occupied meadow.

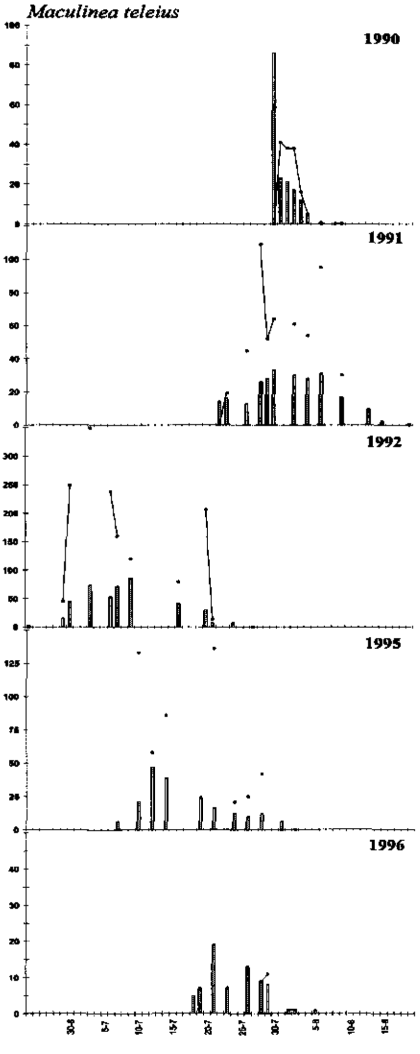

Figure 3.1a: Development in population size and flight-period of *Maculinea teleius* in the nature reserve Moerputten (The Netherlands) where the species had been reintroduced in 1990.
Bars: minimal daily population size estimated by Minimal Number Alive
Line: daily population size estimated by Jolly

M. nausithous experienced a second bottleneck after the first one induced by the reintroduction. After a sharp decrease in 1991, the butterflies settled at another site than the reintroduction spot and then considerably increased in numbers (Figure 1b, 2b). Also in this species a change in the flight-period compared to the source population can be shown (Mann-Whitney U-test: Z=-8,91; p<0,0001). There are also statistical differences between the historical and the new Dutch population (Mann-Whitney U-test: Z=-12,17; p<0.001) and betweeen the historical and the Kostrze-population (Mann-Whitney U-test: Z=-13,36; p<0,0001). The shift in the flight-period throughout the years is much less compared to *M. teleius* (figure 3.1a, 3.2a). The total population size per year since the reintroduction is given in figure 3.2b.

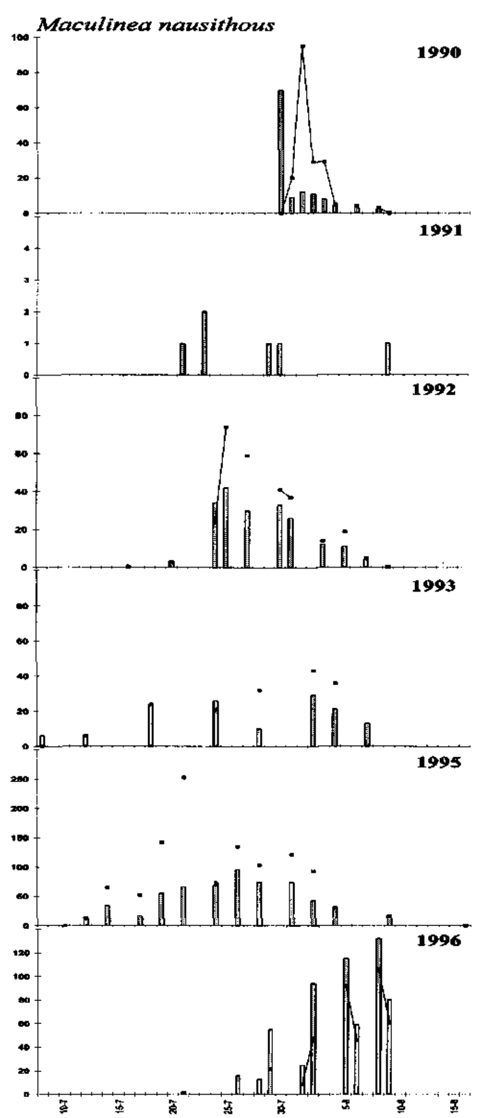

Figure 3.1b: Development in population size and flight-period of *Maculinea nausithous* in the nature reserve Moerputten (The Netherlands) where the species had been reintroduced in 1990.
Bars: minimal daily population size estimated by Minimal Number Alive
Line: daily population size estimated by Jolly

Life span

Although for the newly established populations of both species the general tendency with respect to the population size is positive (i.e. increase until equilibrium), the mean minimal life time per captured individual has decreased strongly (figure 3.2a and 3.2b). Even if the data from 1990 are excluded as two days under cool, dark and predator-free conditions almost certainly

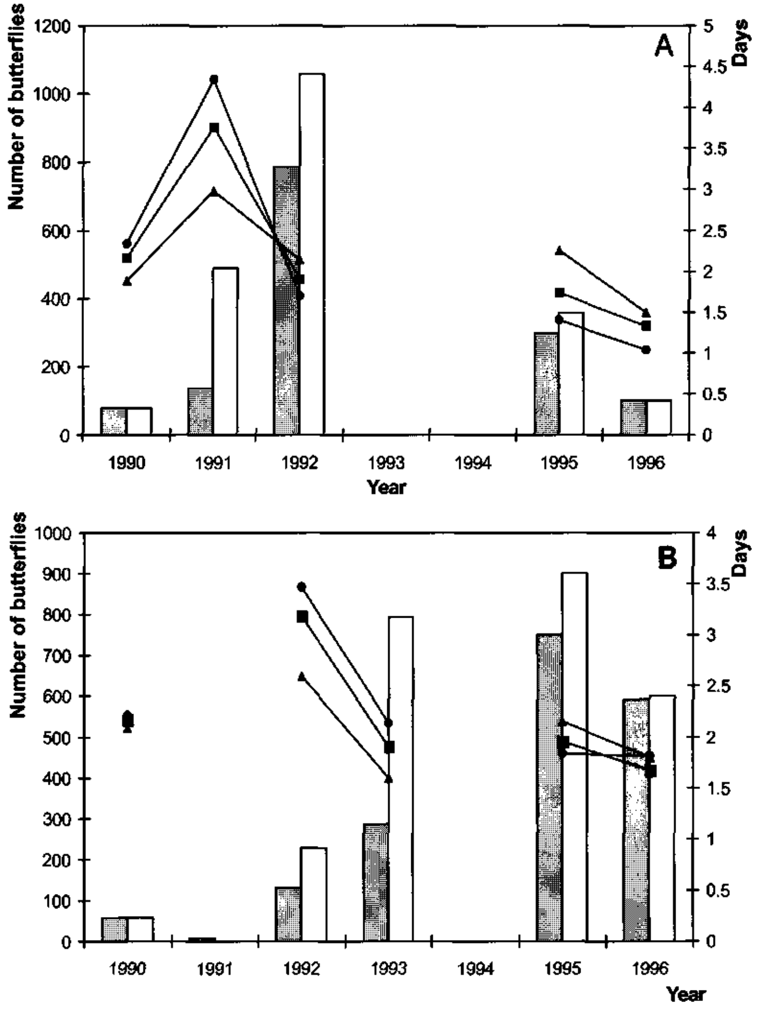

Figure 3.2: Annual population size and mean minimal life span of (A) *Maculinea teleius* and (B) *Maculinea nausithous* in the nature reserve Moerputten (the Netherlands) where both species had been reintroduced in 1990. In 1993 only *Maculinea nausithous* was captured and marked, in 1994 no mark-recapture study was done.

Bars: annual population size;

white estimated with the Minimal Number Alive-data;

grey estimated with the Jolly data.

Line: mean minimal life span estimated by MNA;

square, male and female adults;

circle, female adults;

triangle, male adults.

increase longevity, the same conclusion must be drawn. In the case of the female *M. teleius*, the mean minimal life time in 1996 was just one day. From all marked females, only one could be recaptured after two days.

Distribution of Maculinea teleius and Maculinea nausithous

Five years after reintroduction *M. teleius* is still restricted to the single meadow in the nature reserve, where the adults have been released (figure 3.3). The nest density of the host ant *Myrmica scabrinodis* was found to be between 0.4 and 1.3 nests/m². This was higher than in any other part of the Moerputten reserve (0-0.6 nests/m²). The density of the hostplant *S. officinalis* is 15 plants per m² with on average 30 flowerheads per plant. In 1991 and 1992, some adults were observed on the meadows at the western border of the reserve; but these meadows were mown in 1992 in the second week of the flight-period, one week later than the proposed date just before the start of the flight-period. Since then, less than one percent of the population has been observed outside their original release meadow each year. Also, no butterflies were recorded on road verges and canal borders with similar vegetation types and structure.

M. nausithous was released on a meadow with taller vegetation and high numbers of branched *S. officinalis* with many flowerheads per plant. However, they deserted this site to establish a population at an even rougher site on the northern side of the old railway embankment which runs through the nature reserve. However, the hostplant density is low. Not more than 30 plants with about 50 flowerheads per plant are available for oviposition. Since 1993, a second subpopulation exists on the road verges south of the Moerputten. This subpopulation is still increasing and is, since 1996, larger than the first population. Of all recaptured adults, 6 to 8 % per year was marked in the other subpopulation. Several individuals even migrated between the two sites more than once.

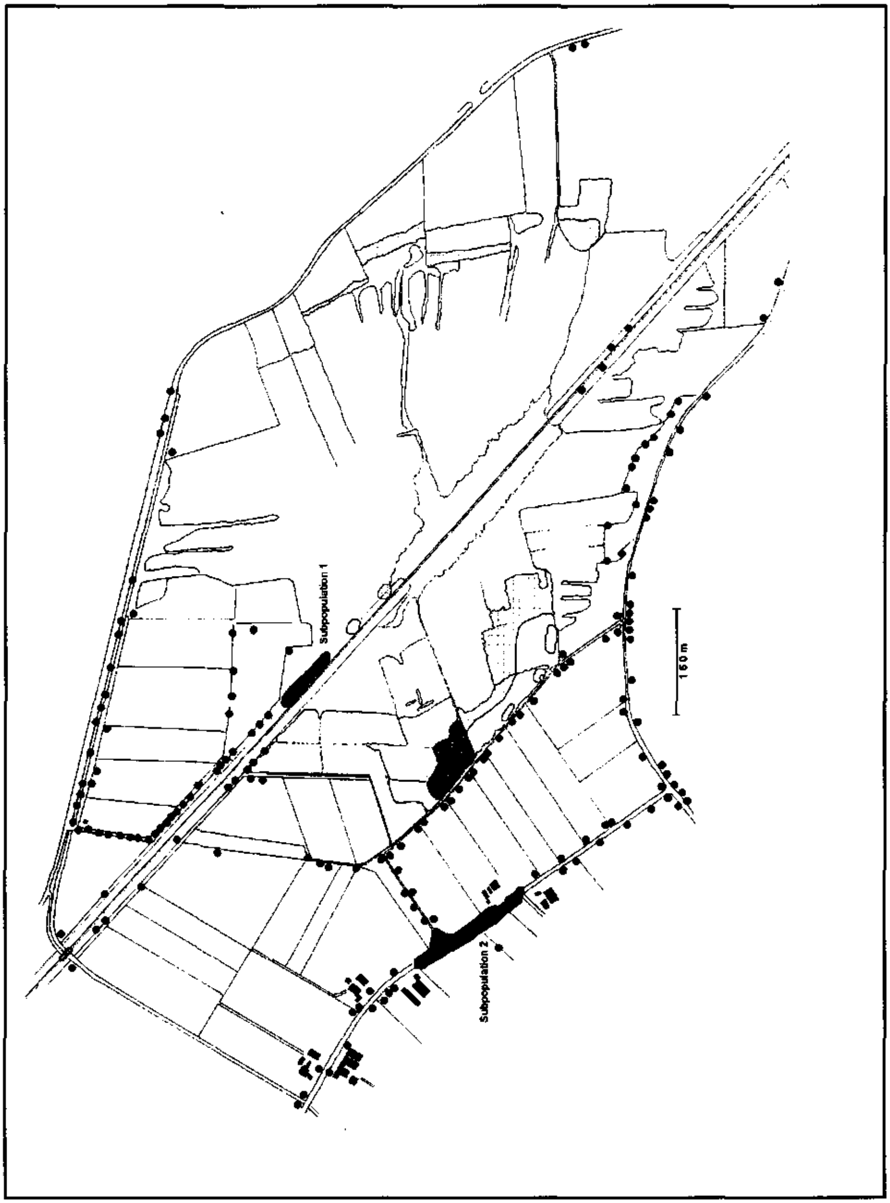

Figure 3.3: Distribution of (a) *Maculinea teleius* and (b) *Maculinea nausithous* in the nature reserve "Moerputten" (The Netherlands) where both species have been reintroduced in 1990.

black: *Maculinea nausithous*
dark grey: *Maculinea teleius*
light grey: *Sanguisorba officinalis*
dot: group of *Sanguisorba* plants

Discussion

Population development

Populations of *Maculinea* butterflies are generally considered to be closed populations. From case studies in many parts of Europe it is known that small populations can persist on small and isolated habitats for many years. If population size and flight-period between the newly established population in the Moerputten and natural populations are compared and if we take the criteria defined in the Dutch Red List of Butterflies (Wynhoff & van Swaay 1995) into account, we can conclude that the reintroduction was successful. A population of about 300 individuals of *M. teleius* per year can even be considered large, as especially in this species very, very small populations have been observed for many years on restricted sites (Fischer & Kunz 1994, Ebert & Rennwald 1991). However, such populations are susceptible to extinction due to stochastic events. The last remaining indigenous *Maculinea arion* population in Britain was of about the same size, thriving on a site with high habitat quality. Nonetheless, extinction occurred after a sequence of four years when mortalities were exceptionally high due to, amongst others, bad weather conditions (Thomas 1995). Hence, the number of years which a population was able to persist after reintroduction, is not very reliable to evaluate reintroduction success.

The population development of *M. teleius* in the Moerputten is closely similar to that of *M. arion* after reintroduction in Britain (Thomas 1995, Thomas, pers.comm.). Both populations increased for several years, as can be expected on good sites as long as there is unlimited growth. Then numbers dropped, in Britain through extreme drought and in The Netherlands through a wet and cold summer. Another factor causing the decline was most likely overexploitation of the host ant population. There are indications that the behaviour of *M. teleius*-caterpillars in ant nests is much like that of *arion*-caterpillars. Both butterfly species are able to kill off ant colonies as a consequence of scramble competition (Thomas *et al.* 1993). When host ants exist in small colonies with high densities in the field, it can be expected that the caterpillars feed on the brood of more ant nests subsequently, thereby creating major damages to the host ant population and lack of food for themselves. Eventually, even a new extinction might follow. In a stable system, the number, density and social structure of host ant nests will limit the size of the butterfly population, provided there is no lack of host plants. In the HCET-model of the *Maculinea rebeli* population of Panticosa (Spain) (Hochberg *et al.*, 1992, 1994) butterfly numbers increased rapidly for three generations after introduction of a female and then decreased to equilibrium numbers, very much like *M. teleius* appeared to do after reintroduction.

It is not possible to draw such conclusions from the development of the populations of *M. nausithous* after the reintroduction, as the (meta)population is still expanding. Limitations on population growth are caused by the small number of host plants in the embankment population and irregular management on the road verges-population. Up to 20 eggs per flowerhead, which is generally quite large, have been found (Fiedler 1990). This might lead to intraspecific competition in the early larval instars. Another consequence is that too many caterpillars are adopted by the same host ant nest, thus destroying the nest by feeding on the ant brood and leading to their own starvation.

The shift in the timing of the flight-periods shows that the phenology of the populations of both species have changed, probably as a direct response to the new (climatical) conditions of the reintroduction site. The difference between the species' growth development of the populations and the changes observed over the study period, suggest that the local adaptations might be due to selection on the introduced population. If this is indeed the case, then I recommend that in any future translocation a source population with as much genetical variation as possible should be used.

Life span

The life span of an individual is an important fitness character (Ross 1992, Stearns 1992). It has a great influence on its chance of successful reproduction. This is even more important in butterfly species that live under limiting climatical conditions and do not migrate (Thomas *et al.* 1998). Reduced longevity can be an effect of increased inbreeding (Orr 1994). In animal species that can be reared in the laboratory, data on longevity are easy to obtain. However, both *Maculinea* species cannot be reared under artificial conditions due to their specialised ant attendent life style, and therefore the only possibility to estimate longevity is to compare related field parameters. Mean minimal lifetime was used, because the two days of age the butterflies were when they were released in 1990, could be added. Since reintroduction, the mean minimal life time of both species has declined. This decline is also obvious, when the data from 1990 are excluded, because two days of transport under cool and dark conditions could have less impact on the life time than two days in the field. Reduced life time was not expected, as the effective population size between values of 106 (in 1990) and 672 individuals (in 1992) for *M. teleius* and 96 (in 1990) and 658 individuals (in 1995) for *M. nausithous*, calculated from the sex ratio of the captured adults, was high enough to reduce the risk of a loss of heterozygosity (lower than

5%: Hunter 1996), with the exception of *M. nausithous* in 1991. In this year the effective population size was limited to 8 individuals, and the estimated risk of loss of heterozygosity was 6.25%, whereas in the other years it was below 1%. However, mean minimal life time is lower in *M. teleius* where it approaches 1 day for the females. In field studies in Germany, a mean minimal life time of 1.5 days for *M. teleius* and 2.1 days for *M. nausithous* was found (Binzenhöfer, pers. comm. in Settele *et al.* 1996). These values are thought to be very low, because the recapture percentage was low and due to the size of the metapopulations, the intervals between captures were quite long. In the Moerputten, conditions for mark-recapture techniques were much more ideal. Probably, the life time of the adults is really reduced, which means that the populations are very sensitive to bad weather periods and therefore prone to stochastic events. In wet or cold summers, the females can survive for several days without depositing eggs. On the first available warm and sunny day, or sunny interval between periods of rain, they are able to lay many eggs within a short period af time, whereas under optimal weather conditions, the egg load per day is much lower.

Distribution of Maculinea teleius and Maculinea nausithous

Although many more *Sanguisorba* host plants and *Myrmica scabrinodis* ant nests can be found outside the single occupied meadow, *M. teleius* is completely restricted in its distribution to the release site. While marking the adults it happened frequently that marked specimens left the meadow and flew away over the edges of the willow forest or over the field at the southern border of the nature reserve. It might be expected that these butterflies were lost for the population but enable dispersal. However, in most cases when they were recaptured, it was on the same meadow again, but not outside the nature reserve, not even on the most suitable locations with many host plants and *Myrmica scabrinodis* nests. Only from 1990 to 1992, a small number of adults was found in the meadow west of the occupied one. After it was accidentally mown during the flight-period in 1992, this never occurred again. Through this event of mowing, a severe selection pressure against mobility might have been exerted, leaving only the very sessile adults for further successful reproduction. Although all road verges which look suitable for colonisation or at least migration of *M. teleius* have been inspected for butterflies each year, this species has never been seen outside the nature reserve on the road verges and canal borders, nor in neighbouring nature reserves. Binzenhöfer (pers. comm. in Settele *et al.* 1996) found in a metapopulation in Germany a maximum distance of 2.5 km between two observations of a marked butterfly and she even calculated that *M. teleius* was more mobile than *M. nausithous* living in the same landscape. Hence, it may be that these characters have been lost by the reintroduction.

The most striking aspects of the recent distribution of *M. nausithous* since the reintroductions are (1) the fact that they found the most suitable site in the nature reserve by themselves although this site was considered to be unreachable due to a band of willow and poplar vegetation, and (2) that they did not colonise apparently suitable sites between the embankment and the road verges. During a recent inventory of the ant fauna in 1996 no nests of *Myrmica rubra* were found, neither in most meadows of the Moerputten reserve, nor on sites between the present subpopulations. Furthermore, when adults were observed outside the two existing subpopulations, resting in the vegetation, we were almost always able to find nests of its host ant. Additionally, on sites close to the subpopulations, where no butterflies were observed at all since the reintroduction, no ant nests could be detected. These observations suggest that *M. nausithous* might be able to detect and locate host ant nests. The mobility and the interchange between the subpopulations are almost the same as found by Binzenhöfer (pers. comm. in Settele *et al.* 1996). The longest distance overcome by an adult was more than 5 km (Binzenhöfer pers. comm. in Settele *et al.* 1996) and included crossing landscape barriers such as agricultural land and forests. These data, together with the fact that the population around the Moerputten is still expanding over about 200 m of road verge per year, gives hope for a positive development in the future.

Conclusions for further reintroductions

- Before starting a reintroduction of butterflies, a considerable effort should be made to establish whether the characteristics of the release area can guarantee a long term survival of the new populations. It is also important to pay attention to possibilities of dispersal and establishment of a meta-population.
- The evaluation of the reintroduction success should not only be restricted to counting or estimating the number of butterflies by means of monitoring transects, but also some viability characteristics, such as life span, should be determined regularly.
- Genetical aspects should be integrated into all phases of the reintroduction procedure. It is important to select a large and well functioning source population, to translocate a high number of individuals and to facilitate a good colonisation of the reintroduction site by immediate increase of the (reproductive part of the) population.

- Species-specific management is very important to facilitate the colonisation. In the case of *Maculinea* butterflies, occupied meadows and road verges should not be mown from three weeks before the start of the flight-period until three weeks after the end of the flight-period. Furthermore, management should also take the habitat quality of the host ant species into account. However, once a large population has settled and does not have to cope with negative influences any more, the management of nature reserves should concentrate on the diversity of the complete system including all fauna and flora elements.

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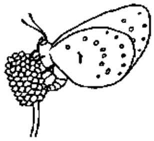


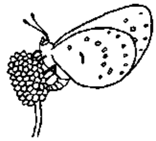
Oviposition by Maculinea nausithous and Maculinea teleius butterflies in relation to their host plant and specific host ants

Summary

Many lycaenid butterfly species are able to share habitats with ants by having evolved a myrmecophilous life style: the caterpillars provide sugary secretions for the ants and are in return protected from enemies. For obligately myrmecophilous butterflies whose caterpillars feed on ant larvae ant-related oviposition is expected but was rarely found. This study provides evidence for this phenomenon for the butterfly species *Maculinea nausithous* and *Maculinea teleius*. These butterflies are dependent on two sequential sources of larval food, namely the host plant *Sanguisorba officinalis* and for each butterfly species a different, specific, *Myrmica* host ant. In most occupied habitats the host plants are abundant while the host ant is relatively rare, so that most host plants are in fact unsuitable for larval survival due to a lack of ants in their close vicinity. It would give females a comparative advantage if they could detect host ants so as to deposit eggs on truly suitable *Sanguisorba* plants only.

Oviposition in response to the density of both these sources of food was studied by comparing adjacent patches differing in host plant and host ant nest density. The impact of host ant presence was tested in an open air insectory experiment where females of both species were free to choose between host plants on plots with the host ant species *Myrmica rubra* or *Myrmica scabrinodis* or without ants, while vegetation characteristics were similar between plots. For both *Maculinea* species, in the beginning of the flight period the presence of the respective host ant species has an influence on the oviposition of the butterflies. In the late flight period a heavier weighting of vegetation structure and flowerhead phenology characters was found.

Wynhoff I, MGA van der Heijden, JG van der Made, S Plat, HHT Prins, M van Steenis & M Woyciechowski. Submitted.



Introduction

The evolution of parasitic relationships is widespread within many types of insects and other invertebrates (Begon *et al.* 1996, Price 1980). Ants represent the major part of animal biomass in tropical climates and they are also important predators of small animal species in the temperate countries (Hölldobler & Wilson 1990). Therefore they are especially prone to being parasitised. Lycaenid butterflies have solved the problem of sharing habitats with ants by means of myrmecophily (Fiedler 1997). In the palaearctic, this concerns mostly unspecialised mutualistic relationships in which butterfly larval secretions provide additional food for the ants while the larvae benefit from protection from parasitoids and predators including the ants themselves. However, also specialised parasitism does occur. Unlike most western palaearctic lycaenid species, *Maculinea* butterflies are obligate parasites of ants. After a short period of feeding on the hostplant the larva proceeds as predator or competitor of ant brood in the host ants' nest (Thomas 1984a, Thomas *et al.* 1989).

Myrmecophily can have an effect on many life history traits, for instance caterpillars of facultative lycaenid species grow better when taken care of by ants (Fiedler & Saam 1994), but also mate location, fecundity, dispersal and oviposition are affected by myrmecophily (Fiedler 1997). Surviving in an ant nest means living as a larva between the risk of being discovered and predated upon or taking benefit from the dangerous host (Thomas *et al.* 1998). This brinkmanship is especially finely toned for the highly specialised obligate parasite entering the brood areas of their host. In order to exploit the ant nest as efficiently as possible, specific adaptations are needed to mimic the ant larvae in their behaviour and pheromones (for example Akino *et al.* 1999). It means that the better the adaptation to a specific host ant species has developed, the better nests of this specific ant species can be invaded but also the lower the chance is to survive in other ant species' nests (Begon *et al.* 1996). Because of this and because host selection carries such a strong fitness component, a strong selection on oviposition site choice is expected. The spatial pattern of oviposition should then overlap with the spatial distribution of host ant nests at a certain site. Caterpillars only have a chance of survival when the egg has been deposited within the limited home range of a *Myrmica* colony. Thus, a female would increase the number of surviving offspring if she oviposits in response to the presence of ant nests rather than in a spatially random pattern.

Ant-related oviposition is known for some other lycaenids such as *Plebejus argus*, *Ogyris amaryllis* or *Jalmenus evagoras*. For some butterfly species the pattern of distribution of oviposition is directly related to the presence of the ants (Atsatt 1981, Pierce & Elgar 1985, Smiley *et al.* 1988, Jordano *et al.* 1992). Not only visual cues but also volatile and non-volatile compounds can be involved (Baur *et al.* 1998, Honda *et al.* 1997, Henning 1983, Jutzeler 1989). However, although *Maculinea* butterflies are highly specialised ant parasites, it has been claimed that the females oviposit indiscriminately on any suitable foodplant regardless whether *Myrmica* ants are present (Thomas 1984b, Thomas *et al.* 1989).

In most habitats of *Maculinea nausithous* and *Maculinea teleius*, the hostplant *Sanguisorba officinalis* is abundant, while the colonies of the relevant host ant species are relatively scarce on local scale (Thomas 1984). The peculiar life history strategy in combination with the patchy distribution of *Maculinea nausithous* and *Maculinea teleius* populations on widespread seemingly suitable meadow habitats pleads against the random oviposition hypothesis.

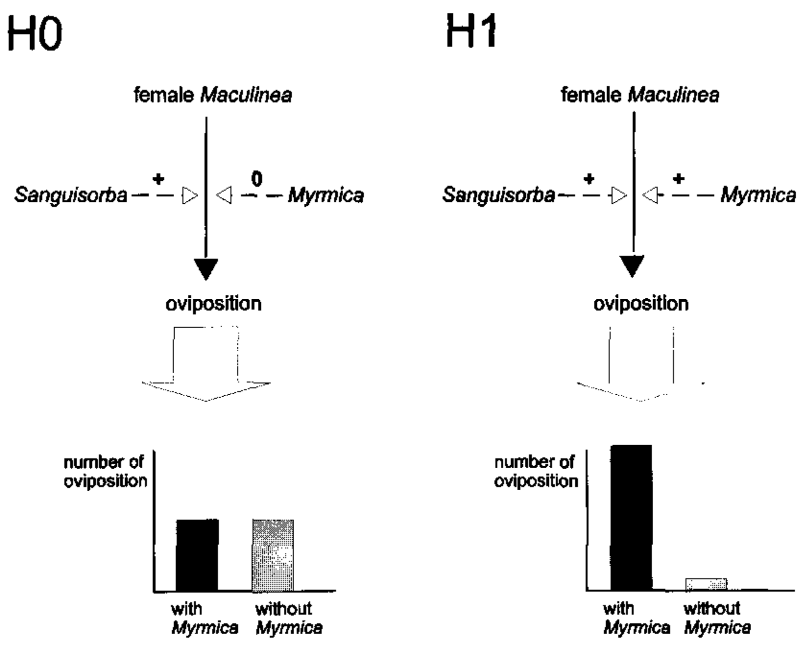
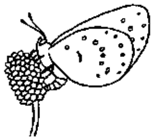


Figure 4.1: Hypothesis of the ovipositon experiment, in which was tested whether *Maculinea teleius* and *M. nausithous* prefer to deposit their eggs in plots with their host ant species rather than in plots with other *Myrmica* species or without ants.



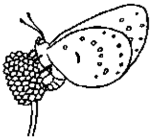
This study describes the impact of the presence of *Myrmica* host and non-host ant species on oviposition patterns of *Maculinea teleius* and *Maculinea nausithous*. If females deposit their eggs indiscriminately of the presence of *Myrmica* ants, the distribution of observed ovipositions should not be related to absence or presence of ants. If this is true a correlation with other parameters, such as for instance vegetation structure, may be expected. However, if females are able to detect ants and respond to their presence, then this should be revealed in the distribution patterns of ovipositions (figure 4.1). These alternative hypotheses were tested in the field and by means of an insectory experiment.

Methods

Ecology of Maculinea nausithous and *M. teleius*

Maculinea nausithous and *Maculinea teleius* oviposit on flowerheads of *Sanguisorba officinalis* (Thomas 1984a, Elmes & Thomas 1987). The early instar caterpillars feed on developing seeds. After two to three weeks, fourth instar caterpillars leave their host plant to be found by *Myrmica* worker ants and taken to their underground nests (figure 1.4). The caterpillars are obligate parasites of the ant nests, feeding mainly on ant larvae. Ultimately 99% of a butterfly's bodymass is acquired inside the ant nests, as was shown for *Maculinea rebeli* (Elmes *et al.* 1991a, b). The main and probably only possible host ant species for *Maculinea nausithous* is *Myrmica rubra*, whereas *Maculinea teleius* mainly lives in nests of *Myrmica scabrinodis* (Thomas *et al.* 1989). In most regions *Myrmica rubra* is of minor importance to this latter butterfly species. The number of caterpillars per *Myrmica* nest that develops into pupae also differs, primarily depending on the *Maculinea* species concerned, but also on nest size, nest composition and the *Myrmica* ant species involved (Elmes & Thomas 1987; Thomas & Wardlaw 1992; Elmes *et al.* 1991b; Thomas & Elmes 1998). In the large nests of *Myrmica rubra*, quite a high number of *Maculinea nausithous* caterpillars can successfully hibernate. In contrast, *Myrmica scabrinodis* has much smaller nests in which generally only one caterpillar of *Maculinea teleius* can survive (Weidemann 1995, Bink 1992, Thomas & Elmes 1998, own observations). The abundance of host ant nests is much more important than the abundance of host plants. However, a minimum number of host plants must grow within the foraging ranges of the colonies of the host ants so that the caterpillar can be found by the ants (Clarke *et al.* 1997).

The females of *Maculinea teleius* prefer very young flowerheads without



open flowers and a high percentage of flowers still enclosed by green sepals. In case of *Maculinea nausithous*, flowerheads selected for oviposition are generally older and longer. The oldest flowers on top are often already blooming and there is only a small proportion still covered by green sepals (Thomas 1984a; Figurny & Woyciechowski 1998). Furthermore, *Maculinea teleius* deposits only one egg at a time, while *Maculinea nausithous* often lays several eggs sequentially on the same flowerhead. The egg-load per flowerhead can be up to 20 and more (Fiedler 1990).

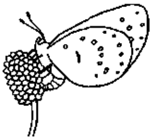
Field study and experiment

In the field, oviposition patterns were described for different patches within the same locality. We compared these patterns with vegetation parameters such as the density of *Sanguisorba officinalis*, the density of both *Maculinea* species and the nest densities of their respective host ants *Myrmica scabrinodis* and *Myrmica rubra*. The results of the field survey were tested in an experiment in an open-air insectory. The experiment consisted of six plots with identical vegetation with *Sanguisorba*. On four plots, *Myrmica rubra* or *Myrmica scabrinodis* colonies were released. *Maculinea* females were given free choice to deposit their eggs. The impact of the presence of host ant species on the oviposition behaviour of the females was studied for two periods: ovipositions during the first two days and during the whole experimental period by captures of caterpillars on the host plants. This way, the oviposition behaviour in the beginning can be distinguished from later ovipositions when the females could be confronted with a shortage of unoccupied flowerheads.

I Fieldwork

Study area

The area consists of a heterogenous mosaic of abandoned and extensively managed nutrient poor, botanically rich, wet meadows in the vicinity of the village Kostrza near Krakow (Poland) (50°15'N, 19°51'E, altitude 200m above sea level). Plant populations of these meadows are dominated by combinations of *Molinia caerulea*, *Festuca ovina*, *Trisetum flavescens*, *Sanguisorba officinalis*, and *Potentilla erecta*. The investigated area is part of the valley of the river Wisla with many populations of *Maculinea teleius* and *Maculinea nausithous*. *Sanguisorba officinalis*, *Myrmica scabrinodis* and *Myrmica rubra* are common, but densities vary between meadows (Skalski 1995, Woyciechowski 1991, Figurny & Woyciechowski 1998).



Data collection

The density of ant nests (Elmes & Wardlaw 1982) and of *Sanguisorba officinalis* depends on vegetation structure and plant species composition. With this in mind 21 homogeneous patches clearly different in these respects were chosen in May 1992. All patches were located within an area of 1.5 km^2 and ranged in size from 210 m^2 to 3600 m^2.

For each patch the following was determined:

1: number of *Maculinea teleius* and *M. nausithous* butterflies by point-transect-counts twice a week.

2: female density and number of ovipositions of *Maculinea teleius* and *Maculinea nausithous*.

3: density and size of *Myrmica scabrinodis* and *Myrmica rubra* nests.

4: vegetation relevés according to the Braun-Blanquet methodology.

5: vegetation structure measurements, including density of *Sanguisorba officinalis* flowerheads.

These data were collected in differently sized plots within the same homogeneous area of a patch. The number of ovipositions was determined for the whole patch. The density of both *Maculinea* species and of *Sanguisorba officinalis* and the vegetation composition and structure measurements were obtained in a 30 m^2 plot within each patch. Nest

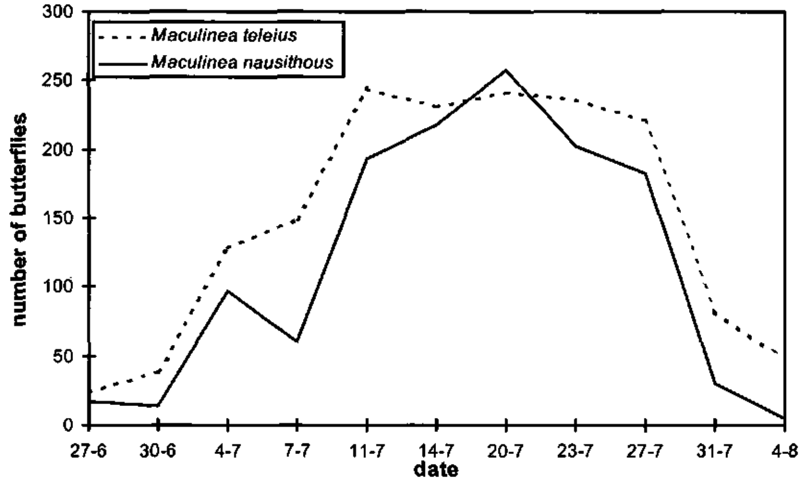


Figure 4.2: Point transect counts of *Maculinea nausithous* and *Maculinea teleius* (males and females) in 21 plots in the valley of Kostrza (Poland).

densities of both ant species were measured in a subplot of 16 m^2 within the 30 m^2 plot. The number of butterfly females entering a 30 m^2 plot over a period of 10 minutes was counted so as to estimate densities of both the *Maculinea* species. Eleven butterfly counts were done in each plot, twice a week from 30 June till 4 August 1992. The average of these eleven counts was used as mean density. In this period both *Maculinea* species are abundant (figure 4.2). The investigations in the patches were done during the main flight periods of both species with an equal impact of both on the results. The number of ovipositions was derived from 11 behaviour protocols which describe female behaviour of each *Maculinea* species per patch. Inside each patch the time needed to locate a female for a next behaviour protocol was equal for every patch (5 minutes waiting time).

The density of ant nests was determined once, in June 1992, by completely searching the 16 m^2 subplot. Ant nests were detected by pulling on plants and slightly disturbing the soil with a knife at a spatial scale of 2 cm^2. Per nest some of the worker ants that emerged after these disturbances were taken for identification in the laboratory. The density of *Sanguisorba officinalis* was determined by counting the number of plants and the number of flowerheads in bud and in flower. Both *Maculinea* species oviposit almost exclusively on buds (Thomas 1984a, Figurny & Woyciechowski 1998, pers. obs.). Six counts were done, weekly from 30 June till 4 August 1992. The average of these six counts was used as mean density for each plot. All field data were converted to densities.

Data analysis

The vegetation composition was analysed using a principal component analysis (Ter Braak & Smilauer 1998, Jongman *et al.* 1987), in which samples and species are ordinated along canonical axes according to similarities in occurrence. Plant species which were recorded in just one plot, were excluded from analysis. As a measure for vegetation composition, the sample scores of the plots for the four most important axes were taken for further analyses. Furthermore, the mean Ellenberg values for humidity, productivity and acidity were calculated for each sample. Ellenberg values are plant species specific indicator values for the relevé's habitat characteristics just mentioned (Kent & Coker 1992, Ellenberg 1982).

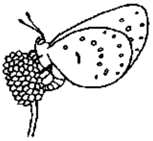
The total set of data was analysed by stepwise multiple regression, with the oviposition density of the butterfly species as dependent and all other variables as independent values. At the onset of the analysis it was unclear whether the *Maculinea* butterflies were able to detect their host ants' nests. A possibility was that there was no ant detection at all. It was also possible that ants from the genus *Myrmica* can be distinguished from other ant genera, or even that the specific host *Myrmica* species with the highest chance of survival for the offspring can be detected. Therefore, the total *Myrmica* ant nest density was included in the analysis, as well as the density of ant colonies per species, although these two factors are not independent. Apart from this, only independent factors were accepted for analysis.

II Insectory experiment

The experiment was conducted in 1997 in an open-air gauze insectory sized 4 x 12 m². In this insectory 6 plots were created according to the layout given in figure 4.3. Before starting the experiment all ant nests in the entire insectory were removed. At the outside edges wooden shelves with fluon were placed to prevent new colonisation by *Lasius* or *Myrmica* species from outside. At the beginning of June, vegetation sods (40 x 60 cm) with meadow vegetation including *Sanguisorba* plants were translocated from the nature reserve Moerputten in The Netherlands (51°41'N, 5°15'E, altitude 2 m above sea level, for further description see Wynhoff 1998) to the insectory. Each plot consisted of on average 31 *Sanguisorba* plants (range 23-35). At that time of the year *Sanguisorba* has not started flowering and is still quite robust. Later transplantation would lead to high mortality of plants. All plants and flowerheads were marked and coded with small paper labels. Around the plots, the vegetation consisted of different grass species. These grasses were mown regularly.

At the end of July, nests of *Myrmica scabrinodis* and *Myrmica rubra* were dug out in the nature reserve Moerputten and brought to the insectory. The ant nests were placed in the middle of the plots (figure 4.3) and the ants were given the possibility to settle themselves. To control possible ant migration, pit fall traps were placed at the edges of the plots. In order to prevent this migration, the ant colonies were fed abundantly each day with wingless *Drosophila* (larvae and adults), fruit and sugar cubes. The plots were separated from each other by 30 cm wide water-filled ditches to prevent ants from leaving their home plot.

Both *Maculinea* species prefer flowerheads of different phenological stage. As *Maculinea teleius*, contrary to *Maculinea nausithous*, might choose to avoid occupied flowerheads for oviposition, this species was released first in the insectory. In the early morning of August 4th, 16 young mated females and two males of *Maculinea teleius* were released into the insectory. They were distributed equally over the plots and were then left undisturbed. For oviposition the females could freely choose between 1452 flowerheads over six different plots. After a short time, the first females started to deposit eggs.



During two days 192 ovipositions were continously recorded (from 9.00 h to 19.00 h) and the flowerheads used were recorded too. Observation time per plot was kept equal. On August 8th, the last surviving individuals of *Maculinea teleius* were removed from the insectory. Then 16 young mated females and 3 males of *Maculinea nausithous* were released and during two days 99 ovipositions were recorded. The *Sanguisorba* plants and their flowerheads were measured. We measured the length and height of the flowerheads and estimated the percentage of green, red and flowering flowers per flowerhead. In the middle of August, 995 flowerheads were encapsuled in small gauze bags to prevent the caterpillars from leaving the flowerhead they had lived on. All flowerheads larger than 6 mm, and a subset of the smaller ones, were encapsuled. These flowerheads were examined daily for caterpillars. The caterpillars were removed and collected for other purposes, after which the flowerhead was encapsuled again. We recorded the butterfly species found and the code number of the occupied flowerhead. Five gauzed flowerheads were broken during handling.

Directly before the butterflies were released, on August 3rd one *Myrmica rubra* nest from plot 6 split up into two colonies and one of the sister colonies managed to enter plot 1, which was meant to be a plot with *Myrmica scabrinodis* alone. The *Myrmica rubra* colony was removed immediately, but nonetheless it may have stayed on the wrong plot for maximally 1.5 days. When analysing the data, it became obvious, that this short colonisation could not be neglected.

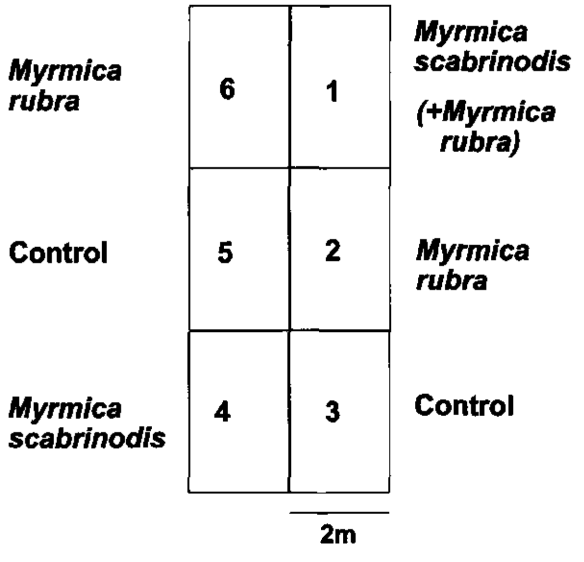
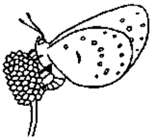


Figure 4.3: Layout of the open air insectory.

Data analysis

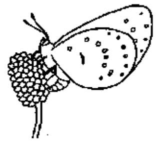
The collected data were analysed for two purposes. We investigated whether there are certain preferred or rejected flowerheads for oviposition based on the phenological state and shape of the flowerhead



regardless of presence or absence of ants. We assume that for an ovipositing female, the functional unit to which she responds, is the flowerhead with its characteristics. Therefore, a Mann-Whitney U-test was performed on a data base consisting of all flowerheads (n=1452) to test for differences between flowerheads with and without eggs. The results show whether there are important plant characteristics, which have to be treated as covariables in the further analysis of the ant treatment.

As one colony of *Myrmica rubra* had split up and invaded a plot of *Myrmica scabrinodis* four treatments were created: two control plots, one plot with *Myrmica scabrinodis*, two plots with *Myrmica rubra* and one with both *Myrmica* species. Therefore the analysis was conducted with the data base consisting of all flowerheads. The ant species present in the plot was taken as a character of a certain flowerhead in the experimental treatment. All data were tested for homogeneity of variances using Bartlett's test and for normality by the Kolmogorov-Smirnov test for goodness of fit. The assumptions for analysis of variance were repeatedly violated. Therefore the dependence of the ovipositions of both butterfly species on the treatment of the plot was tested by fitting a linear logistic regression, using all the flowerheads. In a multiple forward logistic regression procedure, the effects of vegetation characteristics and phenological state of the flowerhead on the oviposition were added. For the analysis of the caterpillar captures, the same logistic regression procedures as mentioned above were repeated with the data set of 990 gauzed flowerheads. Differences between the treatments were tested for significance by the method of Tukey's honestly significant difference (after running a GLM) (Sokal & Rohlf 1995, Norusis 1990).

It could be possible that one of the plots in the insectory would be preferred for oviposition because the vegetation or flowerhead phenology characteristics were favourable irrespective of the ant species present. Therefore we tested the relative distribution of the flowerhead characters over the six plots by means of a Kruskal-Wallis test before concentrating on the analysis of the oviposition behaviour. All statistical analysis was performed by using the SPSS version 8 package (Norusis 1990, Norusis 1993).



Results

I Field observations

In the Principal Component Analysis of the plant species composition of the plots in Poland, four axes with eigenvalues of 0.23 (axis 1), 0.15 (axis 2), 0.12 (axis 3) and 0.08 (axis 4) explained 58% of the variation in vegetation species composition. Including the sample scores for the four major axes from the Principal Component Analysis into the multiple regression it was found that there was no direct effect of plant species composition on the distribution of the *Maculinea* species, the *Myrmica* ants or the density of ovipositions. The ordination axes correlated positively with the Ellenberg values for productivity (axis 1: r=0.663, p=0.001), acidity (axis 2: r=0.748, p<0.001) and humidity (axis 3: r=0.509, p=0.019). The fourth axis was negatively correlated with nest size of the *Myrmica rubra* nests (r=-0.502, p=0.021), but only weakly so.

The results of the multiple regression showed for *Maculinea nausithous* that oviposition was, within the measured set of parameters, only explained by the density of the host ant species *Myrmica rubra* ($y = 0.20 + 2.06\,x$ with y = oviposition density of *Maculinea nausithous,* x = nest density of *Myrmica rubra*, standardised regression coefficient $\beta = 0.62$; $p = 0.003$; $r^2 = 0.38$)

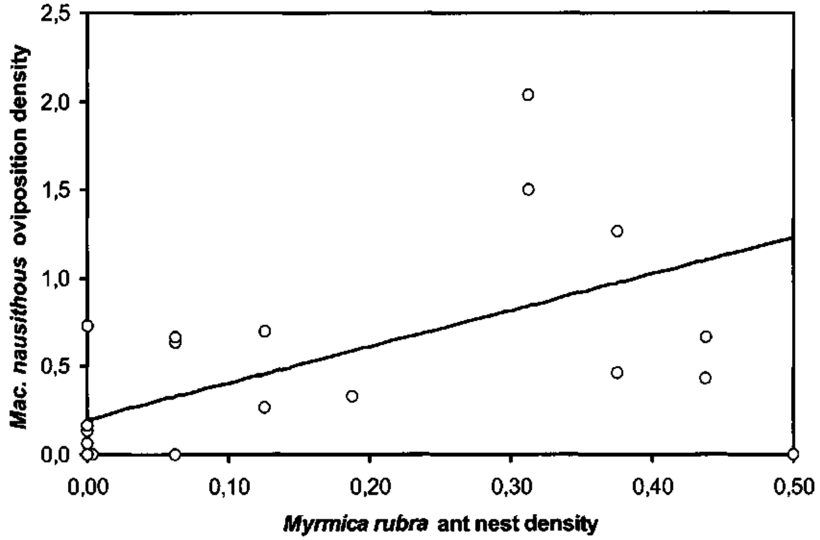
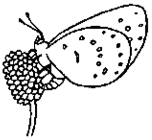


Figure 4.4: The oviposition density of *Maculinea nausithous* in relation to the density of its specific host ant *Myrmica rubra* in 21 plots in the Valley of Kostrza (Poland).

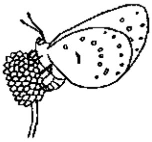


(figure 4.4). Nest density of this ant species and of females of *Maculinea nausithous* observed in the plot were positively correlated (r=0.447, p=0.042), but this effect was not significant in the regression. The density of *Sanguisorba* plants had no significant effect on oviposition of *Maculinea nausithous*. Nest densities of both host ant species were negatively correlated (r=-0.487, p=0.025).

Variation in the oviposition frequency of *Maculinea teleius* was explained by the density of the flowerheads in bud and plant height ($y = 1.61 + 0.002\ x_1 - 0.02\ x_2$ with y = oviposition density of *Maculinea teleius*, x_1 = *Sanguisorba* flowerheads in bud, x_2 = *Sanguisorba* plant height, standardised regression coefficients $\beta(x_1) = 0.53$; $\beta(x_2) = -0.52$; $p = 0.001$; $r^2 = 0.56$). There was no effect of female density. Patches with many *Maculinea teleius* females and patches with frequent ovipositions were not the same. Contrary to *Maculinea nausithous*, neither the density of ants of the genus *Myrmica* nor the density of the specific host ant *Myrmica scabrinodis* had a significant effect. The

Table 4.1: Comparison of flowerheads selected and rejected for oviposition by *Maculinea nausithous* and *Maculinea teleius*. All flowerheads of the six plots were lumped and the presence of ants in the plots was not included into the analyses.

Maculinea nausithous				without			
with caterpillars	caterpillars			Mann-Whitney U-test			
N	Mean	Std. Dev.		N	Mean	Std. Dev.	p
- flowering (%)	78	23	29	991	7	23	<0,0001
- red flowers (%)	78	45	29	991	10	19	<0,0001
- green flowers (%)	78	33	34	990	82	32	<0,0001
- height (cm)	78	97	13	1371	95	20	0,590
- length (mm)	78	13	4	1371	7	4	<0,0001
Maculinea teleius				without			
with caterpillars	caterpillars			Mann-Whitney U-test			
N	Mean	Std. Dev.		N	Mean	Std. Dev.	p
- flowering (%)	388	11	26	813	6	22	<0,0001
- red flowers (%)	388	21	26	813	7	18	<0,0001
- green flowers (%)	388	68	37	812	84	32	<0,0001
- height (cm)	388	96	17	1193	95	20	0,619
- length (mm)	388	10	4	1193	6	4	<0,0001



number of *Sanguisorba* flowerheads was, as expected, highly correlated both to the number of flowerheads in bud ($r=0.917$, $p<0.001$) and to the number of flowerheads in bloom ($r=0.928$, $p<0.001$). There was also a correlation of the density of *Sanguisorba* flowerheads with the density of females ($r=0.588$, $p=0.005$). The oviposition densities of both butterfly species were independent from each other ($r=0.355$, $p=0.138$).

Based on the results of the field work, we deduce that *Maculinea nausithous* might be able to detect the nests of its host ant, while *Maculinea teleius* apparently can not or does not use this information. Consequently, when the females of both species are given the opportunity to deposit eggs on vegetations similar in composition and structure but different in host ant species occupancy, eggs and caterpillars of *Maculinea nausithous* should be found on plots with their host ants, while those of *Maculinea teleius* should be distributed with equal frequency in all plots.

II Insectory experiment

In total, 118 caterpillars of *Maculinea nausithous* (7.4 per released female) and 605 caterpillars of *Maculinea teleius* (37.8 per released female) were found. Significant differences in phenological state of the flowerhead were found between flowerheads selected for oviposition by each of the *Maculinea* species and the ones rejected (table 4.1). For both *Maculinea* species, all characters having to do with the phenological state of the flowerhead, such as the percentage of green, red and flowering flowers were important in selecting flowerheads for oviposition. Furthermore larger flowerheads were preferred over smaller ones. Because of these differences between accepted and rejected flowerheads, the proportional frequency of flowerhead characters was checked for equal distribution over the plots. The plots were equal, with the exception of the relative frequency distribution of the green coloration of the flowerheads ($\chi^2=12.185$, $df=5$, $p<0.05$). Over the plots ($n=6$), there was no correlation between the number of ovipositions or caterpillars of both butterfly species and the number of flowerheads.

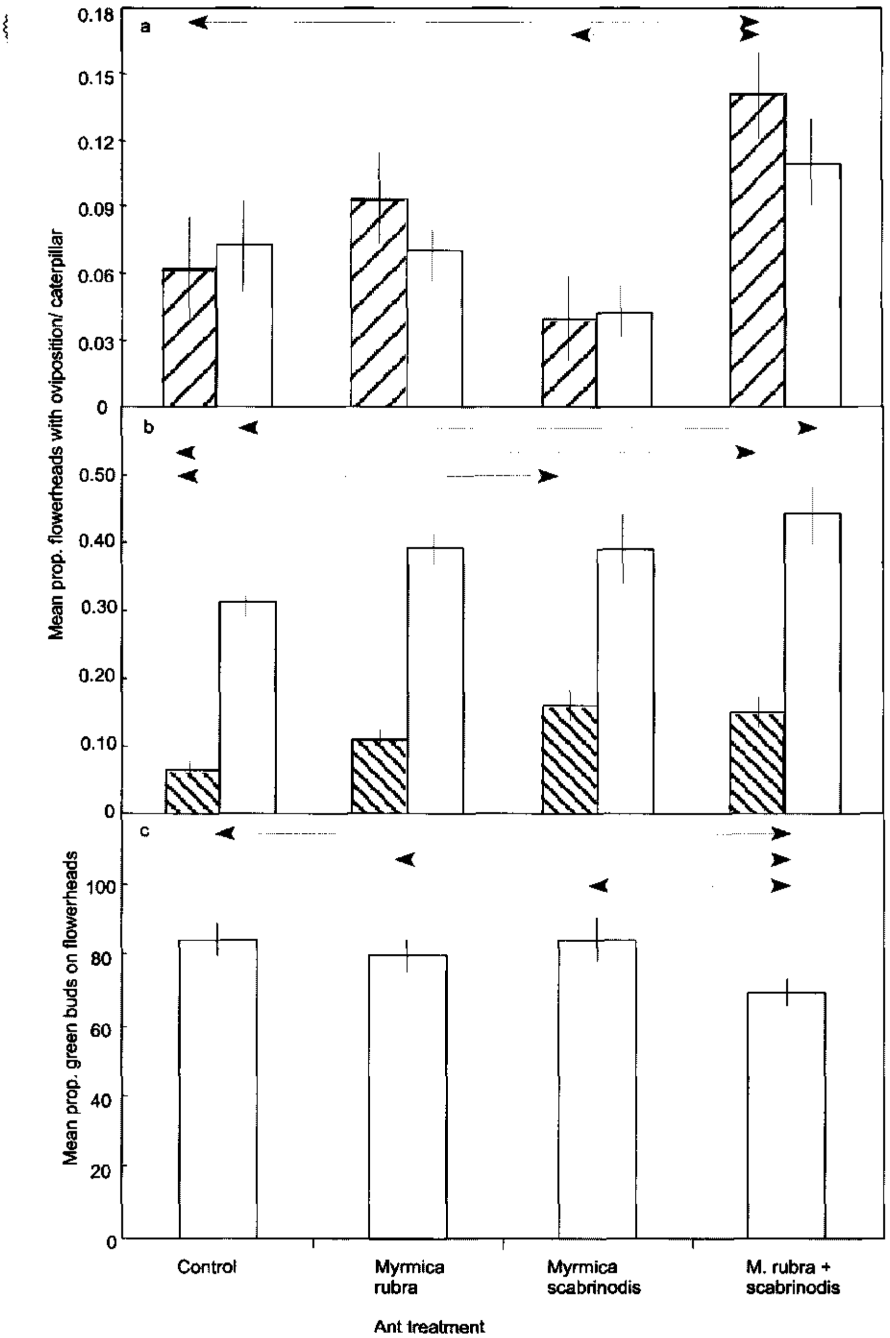


Figure 4.5: Mean proportion of observed ovipositions and captured caterpillars with standard error of (a) *Maculinea nausithous* and (b) *Maculinea teleius* in relation to different ant treatments.

cross hatched bars: ovipositions

open bars: caterpillars

(c) Mean proportion of green coloration per flowerhead in relation to different ant treatments.

The sample sizes are as follows: control: n=357, *Myrmica rubra*: n=596, *Myrmica scabrinodis*: n=131, *M.rubra + scabrinodis*: n=368.

The arrows represent significantly different means (within each type) (Tukey-test).

Influence of host ants on oviposition choice

In figure 4.5 the mean proportion of ovipositions by both butterfly species and the collected caterpillars under the four treatments is presented. For *Maculinea teleius* most ovipositions were observed on the one plot with the main host ant species *Myrmica scabrinodis*, but the other ant treatments were often accepted as well. Ovipositions of *Maculinea nausithous* took mainly place on the plot with both *Myrmica* species. The plots with *Myrmica rubra* and the control plots were intermediate and the flowerheads in the one with *Myrmica scabrinodis* were rarely chosen. Caterpillars were found in a similar distribution over the plots for both butterfly species, but with relatively more *M. teleius* caterpillars on the flowerheads of the plot with both ant species. Logistic regressions showed significant relations between the ant treatment and the oviposition of both butterfly species. Caterpillar captures of *Maculinea teleius* were also significantly explained by ant treatment while the distribution of caterpillars of *Maculinea nausithous* were independent from it (table 4.2). Contrary to our finding in the field study in Poland, in the experiment the oviposition of both butterfly species were correlated ($r=0.083$, $p=0.002$), as well as caterpillar captures ($r=0.160$, $p<0.001$).

For the interpretation of the results we have to keep in mind, that the number and the phenological state of the flowerheads were not equal between the plots or the treatments. Although the number of flowerheads per plot was rather different ($\chi^2=205$, df=5, $p<0.05$), it had no significant effect on oviposition or caterpillar density of either butterfly species. The percentage of green buds per flowerhead (fig. 5c) did have a significant influence. For *Maculinea nausithous* both oviposition and caterpillar captures were significantly affected by the proportion of green buds on the flowerheads. A low proportion of green coloration of a flowerhead resulted in relatively more ovipositions and more caterpillars were captured.

After incorporating flowerhead height and percentage of green buds on the flowerhead into the logistic regression procedure, the only significant effect on oviposition by *Maculinea teleius* was still given by ant treatment. The effect of the ants on caterpillar captures was now overruled by the height of the flowerhead in the vegetation and, in a negative sense, its proportion of green buds. In the case of *Maculinea nausithous*, the variation in ovipositions as well as caterpillar captures was significantly explained by the amount of green coloration of the flowerheads. The lower the percentage of green buds on the flowerhead, the higher the chances that it would be accepted for oviposition. When the plot with both *Myrmica*-species was excluded from analysis, the same results were obtained.

: Results of logistic regression analysis of presence/absence of ovipositions and caterpillars of *Maculinea nausithous* or *Maculinea teleiu*
ssion coefficients B and the coefficient for the intercept are given. The probability of observing an oviposition or finding a caterpillar of on
lied *Maculinea* species (M) is given by: $\ln[M/(1-M)]$ = constant $\Sigma\ \beta$ x variable.
cance of regression coefficients is designated as follows: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.
eatment compared with the others is in 1: control, 2: *Myrmica rubra*, 3: *Myrmica scabrinodis*.
.: Logistic regression method enter, simple contrast between treatments.
: Multiple forward logistic regression, simple contrast between treatments. Variables not in the equation are given in italics.

					partial regression coefficients			
			treatment					
	n	χ^2	df	p	1	2	3	y-intercept
ous ovipositions	1452	17.8	3	0.0005	0.44	-0.50	0.88***	-2.52***
oviposition	1452	16.2	3	0.001	0.63*	1.02**	0.91**	-2.04***
ous caterpillars	990	5.8	3	0.123	-0.05	-0.58	0.44	-2.59***
caterpillars	990	9.4	3	0.024	0.38*	0.37	0.58**	-0.49***

					partial regression coefficients					
						treatment				
	n	χ^2	df	p	green	height	1	2	3	y-intercept
ous oviposition	1452	187	1	<0.0001	-0.03***	0.01*	*0.64*	*0.99*	*0.21*	-1.20*
oviposition	1452	9	3	0.0253	*2.70*	*0.18*	0.56*	0.91**	0.66*	-1.74***
ous caterpillars	990	121	1	<0.0001	-0.03***	*1.99*	*0.92*	*0.75*	*0.76*	-0.49**
caterpillars	990	63	2	<0.0001	-0.01***	0.008*	*0.06*	*1.91*	*0.97*	-0.04

Table 4.2: Results of logistic regression analysis of presence/absence of ovipositions and caterpillars of *Maculinea nausithous* or *Maculinea teleius*. The regression coefficients B and the coefficient for the intercept are given. The probability of observing an oviposition or finding a caterpillar of one of the studied *Maculinea* species (M) is given by: ln[M/(1-M)] = constant Σ β x variable.

Significance of regression coefficients is designated as follows: * p<0.05, **p<0.01, ***p<0.001.

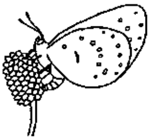
The treatment compared with the others is in 1: control, 2: *Myrmica rubra*, 3: *Myrmica scabrinodis*.

Part A: Logistic regression method enter, simple contrast between treatments.

Part B: Multiple forward logistic regression, simple contrast between treatments. Variables not in the equation are given in italics.

A					partial regression coefficients			
			treatment					
Variable	n	χ^2	df	p	1	2	3	y-intercept
M. nausithous ovipositions	1452	17.8	3	0.0005	0.44	-0.50	0.88***	-2.52***
M. teleius oviposition	1452	16.2	3	0.001	0.63*	1.02**	0.91**	-2.04***
M. nausithous caterpillars	990	5.8	3	0.123	-0.05	-0.58	0.44	-2.59***
M. teleius caterpillars	990	9.4	3	0.024	0.38*	0.37	0.58**	-0.49***

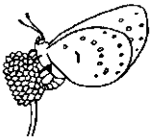
B					partial regression coefficients					
						treatment				
Variable	n	χ^2	df	p	green	height	1	2	3	y-intercept
M. nausithous oviposition	1452	187	1	<0.0001	-0.03***	0.01*	*0.64*	*0.99*	*0.21*	-1.20*
M. teleius oviposition	1452	9	3	0.0253	*2.70*	*0.18*	0.56*	0.91**	0.66*	-1.74***
M. nausithous caterpillars	990	121	1	<0.0001	-0.03***	*1.99*	*0.92*	*0.75*	*0.76*	-0.49**
M. teleius caterpillars	990	63	2	<0.0001	-0.01***	0.008*	*0.06*	*1.91*	*0.97*	-0.04



Discussion

Parasites are dependent on their hosts. Because of this strong dependence on their host, many parasites have developed systems to detect the host, thus favouring maximal survival of their offspring. For the butterflies *Maculinea nausithous* and *Maculinea teleius*, the host ants on which they parasitise, represent a main component of the habitat. In the studied part of the Wisla-valley in Poland, the respective host ant species are competitors. In general, in a vegetation with a constant plant species composition, only one *Myrmica* species occurs or dominates the ant community with only one *Maculinea* species parasitising upon it (Elmes *et al.* 1998). At present, under the dynamic conditions of management styles co-existence of several *Myrmica*-species and *Maculinea*-species is possible. Induced by changes in management, the *Myrmica* populations as well as the *Maculinea* populations have to change their local spatial distribution over the years to track changes in their habitat. Females have to distribute about 100 eggs each (estimated after Bink 1992) in an area with several millions of *Sanguisorba* flowerheads, of which only a small proportion grows within the foraging range of the host ants. The majority of these flowerheads are, thus, sinks for the caterpillars. Hence only a limited, frequently changing part of the population of hostplants will offer an opportunity for survival of the offspring.

The use of *Sanguisorba* flowerheads for *Maculinea* butterflies also changes with their phenology in time (Thomas 1984, Figurny & Woyciechowski 1998). A flowerhead is during its development at first a suitable egg deposition site for *Maculinea teleius* and then for *Maculinea nausithous.* Shortly after, it will be used as a main nectar source for both butterfly species, other lycaenid species and syrphids. Due to these changes in the use of the host plant, the local distribution patterns of the adults or even the females does not necessarily coincide with the oviposition patterns of the species, as was found by us for *Maculinea teleius* in the Kostrza-meadows. Within the flight period of the females, certain frequently changing locations in the habitat were in use as breeding areas and others as feeding areas in this area. In the case of *Maculinea nausithous* both female density and oviposition density were related to the density of the host ant *Myrmica rubra.* Because the flowerheads are older when *Maculinea nausithous* deposits its eggs, as compared to *Maculinea teleius*, areas of feeding and oviposition overlap more strongly. However, the significant parameters explained 38% of the variance of ovipositions; 62% was not explained and



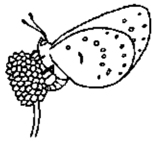
other factors are to be identified.

Behavioural observations show that visual cues are important in the first phase of searching a flowerhead for oviposition. Once a *Maculinea* female has found a suitable flowerhead, she relies on other cues. For other butterfly species it has been shown that chemical cues can be very important to decide whether an egg would be laid (Baur *et al.* 1998, Honda *et al.* 1997, Baylis & Pierce 1991). The importance of visual cues is also known for the presence of ants (Jordano and others, pers. comm.). However, *Maculinea teleius* and *Maculinea nausithous* do not search visually for ants. The oviposition behaviour does not include a walk along the stem of the host plant downwards to search for ant nests or ants. Most of the rather small ant nests are subterranean and not close to *Sanguisorba* plants. So, if the presence of ants plays a role for depositing eggs, it is likely that chemical cues are involved. For the *Maculinea* females it could be the scent of the volatile pheromones or the odour of their *Myrmica* host ant nests (Cammaerts *et al.* 1978, 1981; Hölldobler & Wilson 1990).

The field study and the experiment give strong suggestions that there is an impact of host ant presence on the oviposition by females of both *Maculinea nausithous* and *Maculinea teleius*. However, while in the field study *Maculinea nausithous* responded to the presence of ant nests, in the experiment *Maculinea teleius* did. Under field conditions there is a high number of flowerheads available in the first period of egg-laying, even when the female has very high requirements as to which flowerheads will finally be accepted. Later, when the best sites are occupied, she is forced to also deposit on less suitable but still acceptable flowerheads. The behaviour of *Maculinea teleius* in the insectory can be explained in such a way: When analysing the early ovipositions only, a convincing effect of the ant treatment was found. When analysing caterpillar captures, vegetation structure and flowerhead phenological state were the important cues. This tallies with the results of the field study in Poland. When all oviposition observations during the whole flight period are lumped, no significant effect of the ants was found. The interpretation appears to be that after having assured high chances of survival for the first of their offspring, the females prefer to deposit eggs on less suitable sites rather then stop laying eggs. Interestingly, the same phenomena have been shown for the oviposition preference of *Maculinea alcon*, which exploits *Myrmica* nests according to the cockoo-strategy (Van Dyck *et al.* 2000).

In case of *Maculinea nausithous*, in the field a clear response to the presence of ant nests was found, but this was not found in the experiment. The majority of flowerheads in the experiment turned out to be in fact too young for oviposition. This means that the females searched for the oldest available flowerheads and apparently rated this criterium higher than the presence of ants. This interpretation is supported by the positive correlation between ovipositions of both butterfly species in the experiment and by the low number of eggs deposited. Indeed, the females of *Maculinea nausithous* chose to deposit their eggs on flowerheads which already had been chosen by females of *Maculinea teleius* before, because these were obviously the oldest ones. Under natural conditions with a superabundance of hostplants, as in the valley of Kostrza, oviposition densities of both butterfly species will not be correlated with each other. After analysis, we therefore have to conclude that in the experiment for *Maculinea nausithous* it was not the presence of ants but it was the flowerhead phenology that was decisive for the females. Therefore the experiment should be repeated with *Sanguisorba* plants with flowerheads in an older phenological state.

On the contrary the results of the field study are convincing for *Maculinea nausithous*: it shows the highest degree of host ant specificity among all members of this genus. This *Maculinea* species is almost all over Europe confined to *Myrmica rubra* (Thomas *et al.* 1989 but see Munguira & Martín 1994). The advantage of deposition of eggs close to colonies of *Myrmica rubra* is related to the spatial scale of the distribution of the nests. This ant species tends to have large polygynous nests with a clumped distribution in the landscape. As the foraging range of the ants is restricted to a few meters around the nest site, it means that the majority of the much more evenly distributed *Sanguisorba* plants are in fact unsuitable to deposit eggs on. However, one ant nest can be successfully exploited by a number of caterpillars (Weidemann 1995, Ebert & Rennwald 1991, Thomas & Elmes 1998). Furthermore, *Maculinea nausithous* is the only *Maculinea* species which can for long periods survive in very small dynamic habitats like road verges and canal borders (Ebert & Rennwald 1991). Under such conditions, it seems natural that this rather mobile parasitic butterfly (Binzenhöfer in Settele *et al.* 1996, own unpublished data) can detect its host ant species, and by this succesfully colonise the really suitable locations, rather than loose many offspring by random oviposition on sinks of *Sanguisorba officinalis* plants without *Myrmica rubra*.



Acknowledgements

We thank Edytha Figurny for introducing us to the field site in Poland. The Dutch State Forestry Service kindly allowed us to translocate vegetation plots and ant nests from the nature reserve Moerputten. Ignas Heitkönig, André Schaffers and Han Olff gave statistical advice. Michiel Wallis de Vries corrected the English and Marjolein Spitteler made the drawings. Many thanks also to Chris van Swaay, Paul Loth and Jan van Groenendael for inspiring discussions and comments on an earlier draft of this manuscript.

Looking for the ants: habitat selection of two Maculinea butterflies and their Myrmica host ants

Summary

Obligate myrmecophilic butterfly species, such as *Maculinea teleius* and *M. nausithous* that live as a caterpillar in the nests of the ant species *Myrmica scabrinodis* and *M. rubra*, respectively, have narrowly defined habitat requirements. One would then expect that individuals are able to select for sites that meet their requirements. Both butterfly species occur in habitats where their initial larval resource, the host plant *Sanguisorba officinalis*, is abundant while the ant nests are in comparison less abundant. In the case of *Maculinea* butterflies, the presence of host ants close to the host plants determines the suitability of the host plant as being a true resource or a sink. In the proximity of a host plant which represents a resource, the caterpillar has a chance of being found by its host ant species, while in the proximity of a sink this *Myrmica* species is not present and therefore the caterpillar will die. The impact of presence or absence of host ant nests on the oviposition of females and on the distribution of the population was studied on a selection of more than 600 plots in and around a nature reserve where both species were reintroduced. We found that females of both species prefer to deposit eggs on host plants in the close vicinity of ant nests. Furthermore, adults select for plots with host ants as well. Plots with good vegetation characteristics but lack of ants were only occupied in years with high butterfly densities. We are able to reject the random oviposition hypothesis and argue that the recovery of small populations, such as after reintroductions or crashes in numbers of individuals, is supported by the ant-mediated oviposition.

Wynhoff I, F van Langevelde, H Olff, M Grutters, PM Brakefield & HHT Prins. Submitted.

Introduction

Habitat selection is a phenomenon shown by animals that are able to move among habitats and can choose where to live. It is a result of a combination of landscape features and behaviour of the animals. A complex form of habitat selection is the choice of specific oviposition sites in insects (Thomas 1983a, 1983b, 1991, Bourn & Thomas 1993, Ravenscroft 1994, Bergmann 1999, Gutiérrez *et al.* 1999). Selection will favour individuals that use habitats in which most progeny can reach the adult stage successfully. Through this selection ecologically specialised life-history traits are generated that can make use of this advantage (Krebs 2001, Resetarits Jr 1996). The more specialised the life history of a certain species is, the more narrowly defined the requirements for successful development of larval instars are. Butterflies which are specialised to parasitic larval instars are, therefore, expected to have narrowly defined requirements with respect to oviposition sites, because the choice of the oviposition site has a large influence on the survival probability of the caterpillars. Given these narrowly defined requirements, one would expect that individuals are able to select for sites that meet their requirements.

The caterpillars of obligate myrmecophile butterflies parasitise on ant species, a rare phenomenon within the butterflies of the Western palearctic (Fiedler *et al.* 1996). In Europe, only butterflies of the genera *Maculinea* and *Cigaritis* are able to exploit ant nests in such a way, thereby turning at least a portion of their predators into hosts. *Maculinea nausithous* and *Maculinea teleius* require two larval resources: host plants and host ants. Both species oviposit only on flowerheads of *Sanguisorba officinalis* (Thomas 1984a, Elmes & Thomas 1987). The early instar caterpillars feed on developing seeds. After two to three weeks, fourth instar caterpillars leave their host plant to be found by *Myrmica* worker ants and then taken to their underground nests where they feed on ant larvae and hibernate. While the caterpillars are adopted by any species of *Myrmica*, their survival until the imaginal stage depends largely on the species of host ant. The main and probably only host ant species suitable for *Maculinea nausithous* is *Myrmica rubra*, whereas *Maculinea teleius* mainly lives in nests of *Myrmica scabrinodis* (Elmes & Thomas 1987, Thomas *et al.* 1989, Thomas & Wardlaw 1990, 1992, Elmes *et al.* 1991b, Thomas & Elmes 1998). In the case of *Maculinea* butterflies, the presence of host ants close to the host plants determines the suitability of the host plant as a true source or a sink. By definition, at local level, in a source the birth rate exceeds the death rate, while in a sink the death rate exceeds the birth rate (Thomas & Kunin 1999). In the proximity of a host plant which is a source, the caterpillar has a chance of being found by its host ant species, whilst in the proximity of a sink this *Myrmica* species is not present and therefore the caterpillar will die. This is especially important since *Sanguisorba officinalis* is often very abundant and widely distributed compared to the host ants' nests. Consequently, many *Sanguisorba* plants are sinks for the caterpillars. If the females oviposit randomly with respect to *Myrmica* ants as proposed by Thomas *et al.* (1989), Hochberg *et al.* (1994) and Clarke *et al.* (1997), many females must oviposit on sinks. However, if they select for plots with host ants, then many seemingly suitable patches that are sinks, will be avoided. Selecting suitable oviposition sites and thereby increasing the survival probability of the offspring could be especially important in small populations, such as in recovery from stochastic catastrophes or establishment after reintroductions. For *Maculinea alcon*, there is some evidence that the presence of ants might be important for oviposition (Scheper *et al.* 1995, Van Dyck *et al.* 2000). This has also been shown for *Maculinea nausithous* and *Maculinea teleius* in an insectory experiment (Wynhoff *et al.* 2001a), but evidence from the field is lacking.

We investigate the impact of the presence of *Myrmica* ants on the oviposition behaviour and on the presence of *Maculinea nausithous* and *Maculinea teleius* in the nature reserve Moerputten (116 ha) and its surroundings in The Netherlands. In this area both butterfly species were reintroduced in 1990 after their extinction in 1976 (Wynhoff 1998, chapter 3). The 10 years of establishment provided us with data to study the selection of habitat by the two butterfly species. Our first hypothesis is that females of both butterfly species do not oviposit randomly with respect to the presence of host ants nests. We also use the presence of adult butterflies as an indicator of their selectivity since adult *Maculinea* butterflies live on average a few days (1.5 to 3 days within Europe) and the majority of their activity is dedicated to their reproduction. Therefore, the second hypothesis is that males and females are more often found on locations with nests of the host ant than without.

For the analysis, we divided the potential habitat into four categories. The first category includes the best habitat type: *Sanguisorba* grows in an attractive vegetation and the host ant species is present. We hypothesize that this habitat category is preferred for oviposition and is occupied by *Maculinea* butterflies in almost all years. In the second category, the vegetation characteristics are comparable to those in the first category, but host ants are absent. The plots in this category can be described as sinks. No oviposition is expected. Habitat with host ants but under unfavourable vegetation conditions to the butterflies represents the third category. If ant-mediated oviposition takes place, then these plots should be used for oviposition and colonised before those of the second category when butterfly density is high. The plots with inferior vegetation characteristics and lack of *Myrmica* ants fall into the fourth category. We expect that these plots are not used for oviposition and only occupied in years with very high butterfly densities.

Methods

I Data Collection

The field work was carried out in the nature reserve Moerputten (Wynhoff 1998b). The nature reserve is situated in the centre of the Netherlands in the province of Northern Brabant (51°41'N, 5°15'E, altitude 2 m above sea level, for further description see Wynhoff 1998b). It consists of a lake, surrounded by tall swamp vegetation and willow and elder forests. Around these, a ring of moist meadows at the higher locations is the potential habitat of the butterflies. The butterflies were released on the meadows at the southern side of the nature reserve. *Maculinea teleius* colonised the release site and expanded to a few meadows further west. However, after only three years this species was restricted to the release site, where it still occurs today. *Maculinea nausithous* seemed to have left the nature reserve after release, but was found a year later on the railway embankment crossing the reserve. This population increased rapidly and gave rise to the establishment of a new local population at a distance of about 600 m on the verges of a minor road. While the embankment population was rather constant for several years and then decreased in numbers, the new local population increased rapidly (Wynhoff 1998b). Another population was found two years later at a distance of 5 kilometers. This third population, again on road verges, is still very small. It is not included in this study.

Since the reintroduction in 1990, both *Maculinea teleius* and *Maculinea nausithous* were censused thoroughly. Butterflies were counted by means of transect counts and mark-release-recapture-studies (Wynhoff 1998b, Wynhoff *et al.* 2001b). Furthermore, all butterfly observations during field visits were recorded on detailed field maps. The nature reserve and the colonized road verges were visited at least once a week, but where possible every second to third day, during the whole flight period of both species. After the peak of the flight period, all road verges and ditch sides in the surrounding of the Moerputten were searched for *Maculinea* butterflies, too. We extracted the presence or absence of each *Maculinea* species per year in 1 x 1 m plots from the butterfly distribution data (total of 587 plots).

Maculinea butterflies only co-occur with their hostplant *Sanguisorba officinalis*. Therefore, we restricted our detailed research to those parts of the nature reserve and the road verges, where this conspicuous plant species was found. In addition to the host plant, *Myrmica* host ants are also required. The composition of the local ant fauna and the density of host ant nests depends on the vegetation structure and on the local microclimate (Elmes *et al.* 1998). With this in mind, 251 plots sized 1 x 1 m were chosen on all meadows at the southern side of the nature reserve and the road verges and ditch sides. We took 1 x 1 m plots, because this size represents the scale at which a female decides whether to deposit an egg or not. For each plot, the following was determined:

1: plant species composition according to the Braun-Blanquet methodology.
2: vegetation structure measurements such as maximum height and density of the vegetation cover and *Sanguisorba officinalis* plants and the *Sanguisorba officinalis* flowerheads and their phenology.
3: soil temperature 20 cm below soil surface and mean number of sunny hours per day.

All plots were marked in the field to enable tracing them back by the end of September. On 336 additional plots, only the vegetation structure during the flight period of the butterflies was determined.

Per plot, we established presence or absence of ants and determined the composition of the ant fauna by attracting them with sugar cubes.The sugar cube represents a model for the caterpillar to be found by worker ants. In the middle of the plots at the foot of a *Sanguisorba* plant, a sugar cube was placed on a concave glass plate covered with black plastic. We did this in the early morning hours before 8 o'clock, before the ants start their first activity period. After at least one hour, the bait was checked for worker ants visiting the sugar. From each species, several ants were collected for identification in the laboratory. Empty baits were left in the field and checked later again. In the evening, all baits were removed. We assume that the plots with undetected sugar cubes represent ant-free environments at the scale of our plot.

Data on oviposition behaviour were obtained by searching for deposited eggs in flowerheads of *Sanguisorba officinalis*. Direct observation of oviposition events is difficult, because it generally happens very quickly. Furthermore the eggs are deposited between the flowers on the inflorescence of the host plant and are therefore not visible from the outside. Therefore, we collected flowerheads of *Sanguisorba officinalis* in the last week of September in 1997 and 1998. At that time, the caterpillars have left their hostplant to be taken to nests of *Myrmica* ants where they hibernate. The flowerheads were dried at room temperature and dissected later to collect the eggs. Both species can be separated by a different surface pattern of the sculpturing on the egg. Per plot, 10-30 flowerheads were collected. For *Maculinea nausithous*, these gave sufficient data for a reliable index of female oviposition behaviour. However, in case of *Maculinea teleius* the number of flowerheads collected was still too small in comparison to the high density of flowerheads on the occupied meadow. Therefore, in 1999 a restricted number of females were followed and observed during oviposition. When an egg was deposited, we attracted ants in the way described above and made the same set of measurements. A random sample of *Sanguisorba* plants with flowerheads in a similar phenological state but not accepted for oviposition was also studied as a control. As it is already known that only certain phenological states are accepted for oviposition (Thomas 1984, Figurny & Woyciechowski 1998), we collected our control-sample only within flowerheads of this specific character and concentrated on examining the impact of host ants on oviposition. The observations were made on day 14, 18, 20, 21, 24 and 25 after the beginning of the flight period.

II Data analysis

Oviposition selection was analysed by fitting a logistic regression (Forward, Stepwise, Likelihood Ratio (Huisman *et al.* 1993)). The chance of finding at least one egg of *Maculinea nausithous* on one of the flowerheads in a plot was related to the presence of the host ant species, the number of *Sanguisorba* plants and their number of flowerheads and vegetation structure characteristics found in this plot. The oviposition observations of *Maculinea teleius* were analysed similarly.

The vegetation composition was analysed using a Detrended

Correspondence Analysis (Ter Braak & Smilauer 1998, Jongman *et al.* 1987), in which samples and species are ordinated along canonical axes according to similarities in occurrence. Plant species recorded in just one plot were excluded from analysis. As a measure for vegetation composition, the sample scores of the plots for the four most important axes were taken for further analyses. For the interpretation of the DCA axis, we related them to the ^{e}log-transformed abundances of plant species by means of stepwise linear regression. Furthermore, we calculated the mean Ellenberg values for humidity, productivity and acidity for each sample. Ellenberg values are plant species specific indicator values for the relevé's habitat characteristics just mentioned (Kent & Coker 1992, Ellenberg 1982).

After the reintroduction, there were years with high and low butterfly densities. However, the number of plots of the first habitat category available for the adults is limited. When there are many adults, it is likely that they also occupy plots of the second, third and even fourth category. Therefore, we also used the total number of occupied plots per year as an explanatory variable. Due to the constant management of the nature reserve and the road verges over the years, we consider the vegetation composition, vegetation structure, microclimate and ant fauna remain similar over all years. *Sanguisorba officinalis* being a perennial plant with a root lifespan of five to seven years, is considered to be constant in numbers of plants while the number of flowerheads per plant shows variation between the years. Therefore we omitted the number of flowerheads from the statistical analysis since it was measured in only one year.

For *Maculinea teleius,* all years since the reintroduction were included in the analysis because this species immediately spread in the nature reserve and increased in numbers. *Maculinea nausithous* passed through a bottleneck in numbers in 1991, therefore we began the analysis in 1992.

The total set of data consists of butterfly and host ant presence/absence data, the yearly population size of the butterflies, the number of occupied plots, the four DCA axes scores, vegetation structure and microclimate measurements. The latter three variables and the ant data vary between plots but are constant over the years. The butterfly population size and the number of occupied plots are constant over the plots but vary between years. The butterfly presence and absence data vary between both plots and years.

The presence or absence of a butterfly species was related to the data mentioned above, using logistic regression (Forward Stepwise, Likelihood Ratio (Huisman *et al.* 1993)). The resulting regression models are not intended to reveal the best possible. We used them to explain oviposition and distribution based on the ecological requirements of the species. Therefore, we first tested the influence of *Myrmica* host ant presence and the number of occupied plots on the response variables. Then, we added the other variables to find the best fitting model.

Results

In and around the nature reserve Moerputten, the studied plots are not equal with respect to host ant occurence. From 643 plots counted for the number of flowerheads, no *Myrmica* ants were attracted by the sugar baits on 391 (=61%). This means that from 31,065 flowerheads counted only 9706 flowerheads (=31%) were within reach of host ants. Thus, 21,359 flowerheads (=69%) were probably out of the normal foraging range of the worker ants. We found *Myrmica scabrinodis.* on 189 plots (=29%) with 6,534 flowerheads (21%). *Myrmica rubra* occurred on only 95 plots (=15%) with 4,863 flowerheads (16%). Indeed, the majority of flowerheads of the hostplant *Sanguisorba officinalis* are sinks. Only a small fraction of them can represent a potential resource for the *Maculinea* caterpillars.

Oviposition preference

We found 2,249 eggs of *Maculinea nausithous* on 4,404 flowerheads of *Sanguisorba officinalis.* The flowerheads were collected from 402 plots. Eggs were found in only 165 of these plots. On average 3.2 (SD=3.1, n=365) eggs were deposited on a single flowerhead, with the highest egg load of 20 found twice. We never found eggs of one of the *Maculinea* species in the breeding area of the other *Maculinea* species.

The best regression we found for the probability of finding eggs of *Maculinea nausithous* (p_{en}) on a certain plot was:

$$\text{Logit}(p_{en}) = -13.271 + 2.265\,T_1 + 2.398\,T_2 + 0.878\,T_3 - 0.070\,T_4 + 0.449\,T_5$$

where T_1 = presence of *Myrmica rubra* ($p<0.0001$), T_2 = vegetation height (cm) (^{e}log-transformed) ($p<0.0001$), T_3 = total number of flowerheads of *Sanguisorba officinalis* (ln-transformed) ($p<0.0001$), T_4 = number of

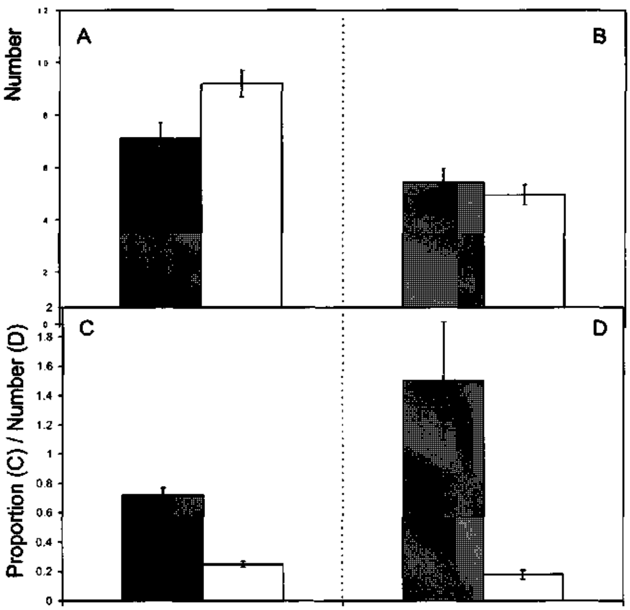


Figure 5.1: Mean number of *Sanguisorba officinalis* hostplants (A) and flowerheads (B), proportion of plots with eggs (C) and mean egg load per flowerhead (D) of the butterfly *Maculinea nausithous* in relation to the presence of the butterflies' host ant species *Myrmica rubra*.
Grey bars: plots with *M. rubra*, white bars: plots without *M. rubra*. The number of flowerheads is equal between categories, all others are significantly different at the level of p<0.0001 (n=311).

Sanguisorba officinalis plants ($p<0.05$) and T_5 = percentage of open soil (^{e}log-transformed) ($p<0.005$). The model explains 82% of the cases correctly (N=286, -2 Log Likelihood =200, χ^2=179, df=5, $p<0.0001$). The majority of eggs was found on *Sanguisorba* plants on plots where the host ant *Myrmica rubra* was also found. When the factor of ant-presence is removed from the model, the model was altered significantly with a change of 31.6 in the -2 Log Likelihood. *Myrmica rubra* appears to be quite rare in the study area, since it was found in only 25% of the plots used for this part of the analysis, together with 74% of the plots with eggs (figure 5.1). When based on the random oviposition hypothesis, 69% of the eggs were expected to be found on flowerheads in plots without *Myrmica rubra*, while only 26% of the eggs were deposited on locations without ants and can thus be considered as sinks. The number of eggs per plot is significantly different between plots with or without *Myrmica rubra*. On plots with the host ant, an average of 19 (CI(95%) = 9 to 29) eggs in the plot with 1.61 (CI(95%) = 0.8 to 2.5) per flowerhead were deposited. Empty plots only had on average 3 (CI(95%) = 1.9 to 3.8) eggs with 0.18 (CI(95%) = 0.11 to 0.24) per flowerhead. The number of eggs per plot (N = 323, Mann-Whitney U = 4755, $p<0.0001$) and the egg load per

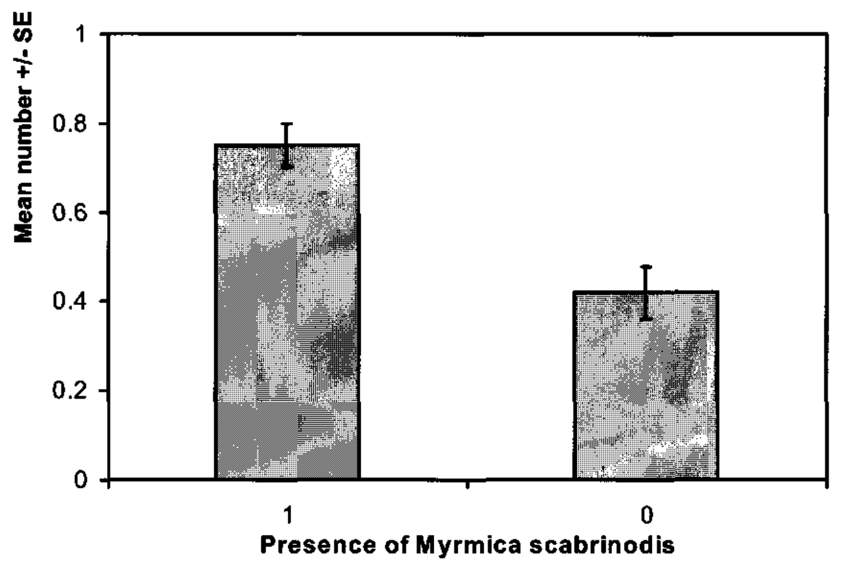


Figure 5.2: Mean proportion of ovipositions of the butterfly *Maculinea teleius* in relation to the presence of its host ant *Myrmica scabrinodis*. The difference between categories is significant (n=152, t=-4.310, p<0.0001).

flowerhead (N = 318, Mann-Whitney U = 4503, $p<0.0001$) are significantly different between plots with and without host ants (figure 5.1).

Ovipositions of *Maculinea teleius* were more frequently observed on phenologically suitable flowerheads in plots with ant nests than on flowerheads in the same phenologically suitable state but growing in plots without ants. The probability that an oviposition will take place at a certain suitable flowerhead, is related to the presence of ants in the following way:

$$\text{Logit}\ (p_{ot}) = -0.304 + 1.402\ T_6$$

where T_6 = presence of *Myrmica scabrinodis* ($p<0.0001$). This model (-2 Log Likelihood = 190, $\chi^2=17.1$, df=1, $p<0.0001$) explains 67% of the cases correctly. Apparently within the meadow where *Maculinea teleius* occurs, all flowerheads in a specific phenological state grow under similar conditions and in similar vegetation structure, since no other variables could be found to distinguish accepted flowerheads from a random sample other than the presence of *Myrmica scabrinodis* ants (figure 5.2).
As the presence of their specific host ant is thus shown to be important for

oviposition in both species, it is included in the subsequent analysis of the distribution of the butterfly species.

Habitat quality description of Maculinea teleius and Maculinea nausithous

In the Detrended Correspondence Analysis of the plant species composition of the plots, four axes with eigenvalues of 0.62 (axis 1), 0.28 (axis 2), 0.23 (axis 3) and 0.21 (axis 4) were found. DCA-axis 1 correlates with the Ellenberg productivity ($r=0.887$, $p<0.001$, $n=234$), Ellenberg acidity ($r=0.723$, $p<0.001$, $n=229$) and Ellenberg humidity ($r=-0.626$, $p<0.001$, $n=236$) indicator values. Next to these, correlations with some vegetation structure characteristics (vegetation height: $r=0.595$, *Sanguisorba* height: $r=0.524$, flowerheads per plot:$r=0.262$, all $p<0.0001$ and number of *Sanguisorba* plants:$r=-0.131$, $p<0.049$) and some microclimate variables (soil temperature: $r=0.253$, sunshine hours: $r=-0.587$, both $p<0.0001$) were also detected. The vegetation relevé's are ordinated from the most nutrient poor

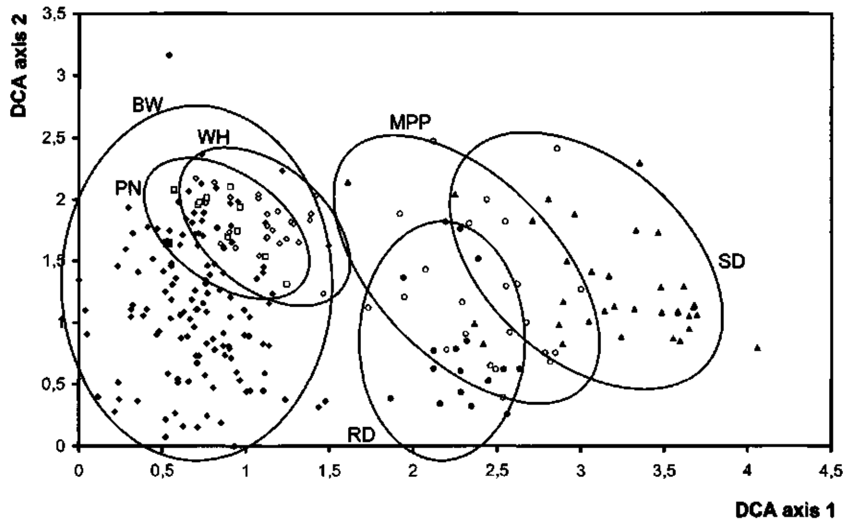


Figure 5.3: Vegetation relevés from the Moerputten nature reserve and surrounding road verges ordinated along two canonical axes. The meadows are designated BW, WH and PN, the road verges are RD and MPP, the railroad embankment is SD. *Maculinea teleius* inhabits only meadow BW. The two populations of *Maculinea nausithous* are situated on RD and SD, but not on MPP, which is situated in the middle of the occupied locations.

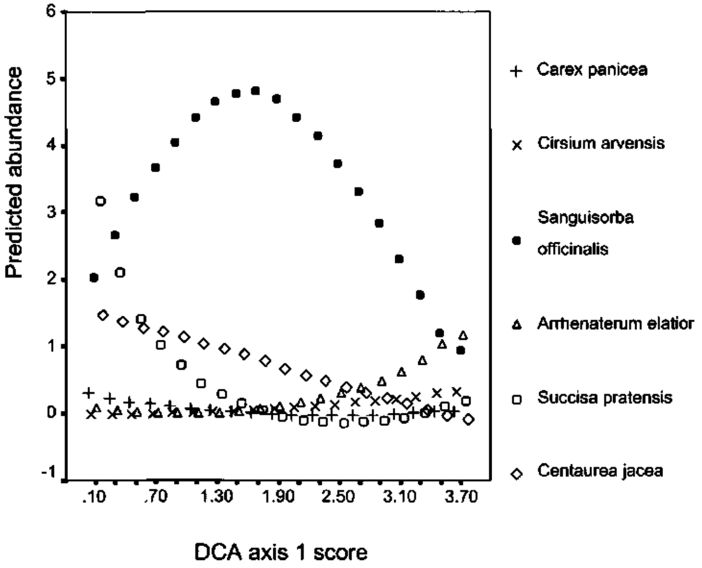


Figure 5.4: Predicted abundance of some indicative plant species along DCA axis 1.

meadows (BW, WH) at the left side in figure 5.3 via richer meadows and road verges (RD, MPP) to rugged vegetations at the railroad embankment (SD) at the right side in figure 5.3. The DCA axis1-scores can be interpreted in terms of abundances of the plant species (r^2=0.922, p<0.0001, F=100.3, df=25) in the following way:

DCA1 = 1.49 + Σ β_n ln(abundance) for 25 indicative plant species

The plant species *Succisa pratensis, Arrhenaterum elatior, Glechoma hederacea, Centaurea jacea, Galium mollugo, Holcus lanatus, Cirsium arvense and Phleum pratense* explained most of the variation. In figure 5.4, the predicted abundance of a number of these plant species and *Sanguisorba officinalis* is shown. At the left side plant species with high abundances are typical for nutrient poor situations, while at the right side species typical for more mesophilic conditions can be found. *Sanguisorba officinalis* had its highest abundance in the middle of the first ordination axis.

The probability (p_t) that an adult butterfly was present in a specific plot in at least one of the study years was related to the presence of its *Myrmica* host species, vegetation height, vegetation composition expressed as DCA-axis1 scores, and the number of occupied plots in a year (table 5.1). For both butterfly species, similar variables gave significant effects on the presence and absence of the adults. In the case of *Maculinea nausithous*, the mean Ellenberg value for humidity gave a slightly better explanation than height of the vegetation. Both are correlated (n=1710, r=-0.289, p<0.01). Therefore, we decided to work with height of the vegetation, since this, we think, can be more directly linked to the behaviour of the butterflies.

The spatial overlap at the scale of 1x1 m^2 from 1992 to 2000 between *Maculinea nausithous* and *Maculinea teleius* is only 0.66%, while the overlap between *Myrmica rubra* and *Myrmica scabrinodis* was estimated as 3.6%. Hence except for the presence of the ants, there are other factors important in the habitat requirements of the butterfly species. The most important variable determining habitat selection appears to be the DCA axis1. *Maculinea teleius* is restricted to the lower ranges (figure 5.5), while *Maculinea nausithous* shows a preference for higher values of that axis (figure 5.6). The host ant species show a similar pattern along the same axis, even though less strictly separated (figure 5.7) as compared to the butterfly species.

The second most important variable in habitat selection is the presence of the host ant species, followed by the height of the vegetation. The height of the vegetation is not significantly different between plots with or without *Myrmica rubra* (t-test, p=0.636), while *Myrmica scabrinodis* is more frequently found in short vegetation (t = 10.5, p<0.001). In the Moerputten nature reserve, a typical habitat of *Maculinea teleius* (meadow BW) consists of an open, short vegetation up to a height of about 50 cm on a comparably poor soil with a high density of the host ant *Myrmica scabrinodis*. The host plants need to be available, but high densities are not necessarily needed. Plots with only few flowerheads are likely to be occupied, while they are not found on plots with many flowerheads since these sites probably have higher and more rugged vegetation. In years with high population density, the butterflies can also been found on such marginal sites. In the same nature reserve, *Maculinea nausithous* prefers different conditions. The vegetation cover is taller and less open compared to sites with *Maculinea teleius*. With respect to

Table 5.1: Results of logistic regression analysis of presence/absence of *Maculinea teleius* or *Maculinea nausithous* in the nature reserve Moerputten and its surrounding. The regression coefficients β and the coefficient for the intercept are given. The probability of observing a butterfly of one of the studied *Maculinea* species (M) is given by: $\ln[M/(1-M)] = \text{constant} + \Sigma\, \beta \times \text{variable}$.

	Maculinea teleius	*Maculinea nausithous*
number of cases	1900	1710
-2 Log Likelihood	1637	552
$\chi 2$	637	342
df	5	4
% predicted correct	76	94

variable	β	S.E.	Wald	Sig.	β	S.E.	Wald	Sig.
constant	-3.750	0.395	90.092	0.000	-7.505	0.522	206.964	0.000
plotsize	0.019	0.002	76.678	0.000	0.018	0.005	14.692	0.000
DCA								
axis 1	4.232	0.687	37.984	0.000	1.766	0.156	128.032	0.000
(axis 1)^2	-3.082	0.422	53.420	0.000	.	.	.	.
Myrmica								
rubra	.	.	.	.	1.147	0.230	24.919	0.000
scabrinodis	0.439	0.131	11.313	0.001	.	.	.	.
vegetation height (cm)	0.027	0.011	5.788	0.016	-0.009	0.004	4.189	0.041

vegetation height there are plots with a vegetation cover higher than 1 m and plots where the vegetation is lower than about 50 cm. The first category comprises plots on the railroad embankment (SD) and alongside streams next to road verges (RD), while the latter category contains the road verges themselves (RD). *Myrmica rubra* has been captured in both types of vegetation, but not on the very open nutrient poor sites inhabited by *Maculinea teleius*.

For both butterfly species, plots of all four categories are available in the nature reserve and the surrounding road verges. Category 1-plots are almost always occupied, while category 4 plots are empty. The plots of category 2 and 3 show dynamic patterns of occupation and extinction. For *Maculinea teleius* more potential habitat is empty compared to *Maculinea nausithous*.

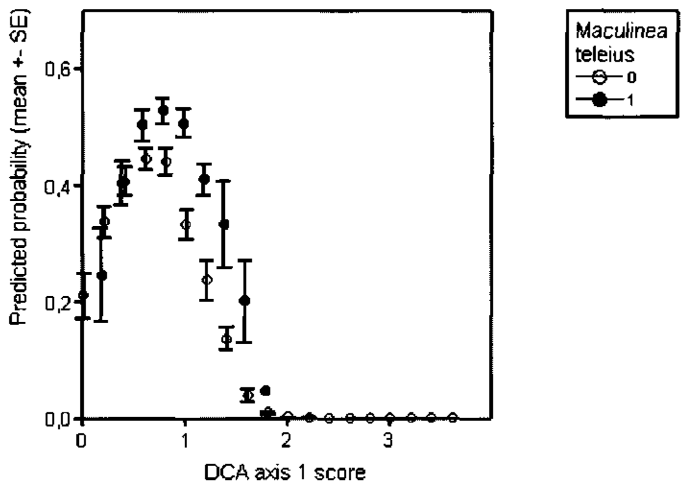


Figure 5.5: Probability of finding adult *Maculinea teleius* in relation to vegetation composition expressed as DCA-axis score 1. Occupied plots are indicated with filled symbols, empty plots with open symbols.

Discussion

Random or ant-mediated oviposition?

The results show that *Maculinea nausithous* and *Maculinea teleius* can cope with the fact that the hostplants are very abundant and the host ant nests are rare, which means that the majority of the host plants are sinks for the caterpillars. Among all *Sanguisorba* flowerheads in an acceptable phenological stage, the females select those for oviposition that grow close to a nest of their host ant species. At the reintroduction site, from the apparently suitable host plants, only a small fraction of less than 30% grows close to *Myrmica* ant nests. Thus, if random oviposition occured, a similar fraction of the eggs would be deposited on suitable sites, while the rest would be deposited on *Sanguisorba officinalis* plants that are sinks. Our results show that the females of both species oviposit more frequently in the proximity of their host ant nests and that adults of both species can be found more frequently close to host ant nests as well.

The results agree with observations of ovipositing females that frequently reject many flowerheads of several different hostplants before they accept one. Clearly, the selection is not limited to finding flowerheads in the right phenological stage (Thomas 1984, Figurny & Woyciechowski 1989). Our study supports the results of an experimental study on ant-mediated oviposition in both species (Wynhoff *et al.* 2001a, chapter 4). Based on these findings we reject the random oviposition hypothesis.

Even though selecting for host plants in the close vicinity of host ant nests, both *Maculinea* species deposit a proportion of their eggs on sinks. This can be caused by the fact that all flowerheads in the vicinity of ant nest are already occupied. Clearly, individuals are not able to find optimal oviposition sites with empty flowerheads close to ant nests when these are at relatively longer distances from their present position. We hypothesize that constraints in dispersal set conditions for the selection of habitat. In *Maculinea nausithous,* the fraction of eggs on flowerheads far away from ant nests was less than in *Maculinea teleius.* The reason might be that the egg load of *Maculinea nausithous* is much higher and the females are not deterred by already deposited eggs (Thomas 1984). The nests of *Myrmica rubra* are polygynous and much larger compared to those of *Myrmica*

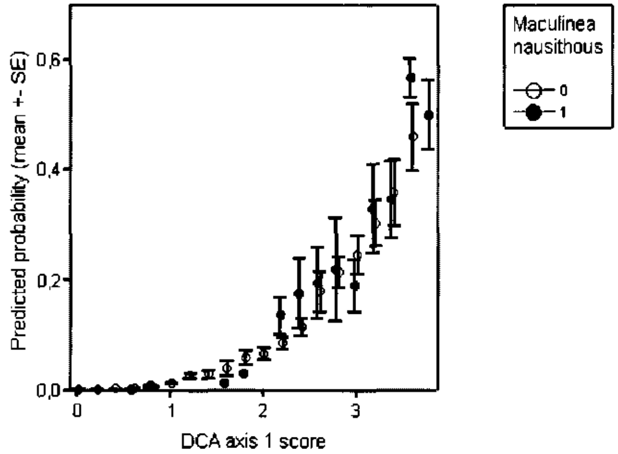


Figure 5.6: Probability of finding adult *Maculinea nausithous* in relation to vegetation composition expressed as DCA-axis score 1. Occupied plots are indicated with filled symbols, empty plots with open symbols.

scabrinodis and therefore the number of pupae surviving in one nest is higher. Moreover, as far as only a small number of caterpillars feeds on the same flowerhead, competition for food is less because the body weight of the caterpillar when reaching the L4 instar is lower in *M. nausithous* (Thomas 1984, Thomas & Elmes 1998, own unpublished data). Hence, the cost of increased food competition due to laying additional eggs on the same hostplant is counterbalanced by higher survival chances in the host ant nest.

Narrower restrictions to the oviposition site characteristics as compared to general habitat requirements are known for many butterfly species, but these restrictions are often described as limitations with regard to microclimate and/or vegetation structure (Thomas 1983a, Thomas 1983b, Thomas 1985, Warren 1987, Sparks *et al.* 1994, Bergmann 1999, Gutierrez *et al.* 1999). Ant-related oviposition, however, is known for myrmecophilous lycaenids in tropical regions such as *Jalmenus evagoras* and others. For some butterfly species, the pattern of distribution of oviposition is directly related to the presence of the ants (Atsatt 1981; Pierce & Elgar 1985; Smiley *et al.* 1988; Baylis & Pierce 1991, Jordano *et al.* 1992). We also demonstrated such direct impact of host ant presence on the oviposition of *Maculinea teleius* and *M. nausithous.*

Habitat selection in relation to the presence of Myrmica ants

The probability of finding adult *Maculinea* butterflies is higher on sites with certain vegetation characteristics and host ant nests than on similar sites where the host ants are absent. This is not only due to "staying home" behaviour, but at least with respect to the females a result of the active searching for host ant nests. The males spend most of their life time searching for females and are therefore also expected to be found close to host ants´ nests. An extraordinarily restricted distribution pattern despite the widespread distribution of the host plant is known for many other butterfly species as well (Thomas 1991, 1995, Gutierrez *et al.* 1999). However, in these studies habitat sites are unoccupied because the vegetation structure around host plants is unsuitable. For both *Maculinea nausithous* and *M. teleius*, plots are available which have suitable habitat with respect to vegetation characteristics but where the ants are missing (category 2 plots). The majority of these plots are not occupied by butterflies as well, even though the adults are potentially able to reach the plots (Settele 1998). In the Moerputten nature reserve, several marked individuals of *Maculinea nausithous* have been captured in both local populations at the railroad embankment and the road verges. Half of the studied meadows are situated between these two sites and must have been crossed by the dispersing adults. Even though these meadows have high densities of *Sanguisorba officinalis* and, at least locally, seem to be suitable for both *Myrmica rubra* and its parasite, they were not colonised neither were ovipositions observed. We argue that this is mainly due to the lack of host ants nests (category 2 plots). The results also indirectly support earlier findings that *Maculinea nausithous* has the highest degree of host ant specificity with almost entirely *Myrmica rubra* being parasitised. All other *Maculinea* species can survive in the nests of several often closely related *Myrmica* species at the same site and within different regions (Thomas *et al.* 1989, Elmes *et al.* 1994 but see Munguira & Martín 1994). *Maculinea nausithous* however, seems to avoid sites with suitable vegetation but with *Myrmica scabrinodis* instead of its true host ant species *Myrmica rubra*.

The mortality of a caterpillar before entering the *Myrmica* nest has been estimated as up to 40% (Thomas *et al.* 1998, own unpublished data: *Maculinea teleius* 50% and for *Maculinea nausithous* 47%). Of all the caterpillars which enter a *Myrmica* nest, about 80 to 90% will die before reaching pupation (Thomas *et al.* 1998). Only after hibernation is the mortality

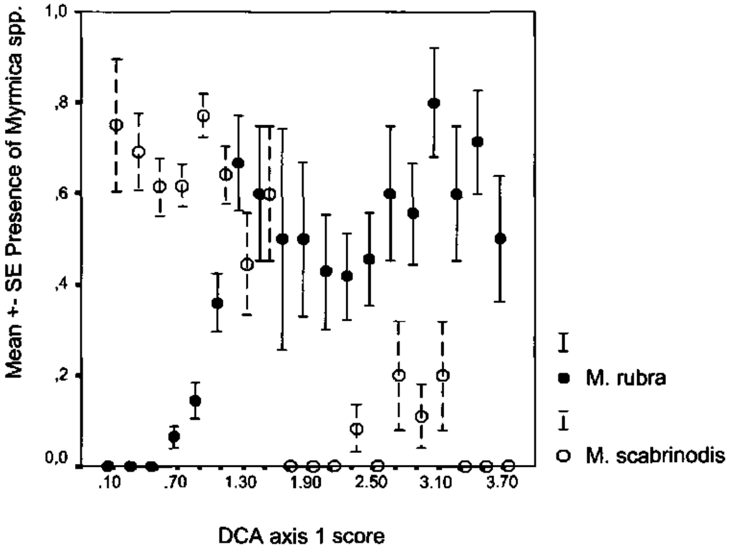


Figure 5.7: Proportion of plots with the host ants species *Myrmica rubra* (filled circle) and *Myrmica scabrinodis* (open circle) in relation to vegetation composition expressed as DCA-axis score 1.

quite low with most of the surviving caterpillars being able to pupate if there is enough food available (Thomas *et al.* 1998, Elfferich 1998, unpublished data of Figurny and IW). The reintroduction of both butterfly species was started with the release of about 50 females that were at least two days old. If we assume an equal egg production of 50 eggs per female and calculate the size of the population in the year after reintroduction as optimistic as possible (70% mortality through sinks, 40% mortality until adoption, 80% mortality in ant nest), then it is almost impossible to only double the number of translocated individuals. Surprisingly, in the initial years after reintroduction, butterfly numbers increased four-fold from one year to the next. This reinforces our finding that oviposition in these butterfly species is ant-mediated.

Consequences for co-occurence

Maculinea nausithous and *M. teleius* can occur on the same location with high densities and apparently thriving populations (Elmes & Thomas 1987, Ebert & Rennwald 1991, Figurny & Woyciechowski 1998, Settele 1998, Wynhoff 1998a). At such locations, the *Myrmica* ant species also co-occur. Consequently, when a female of a *Maculinea* species selects a flowerhead on a plant close to her host ants' nest, other *Myrmica* species could also have their nests within a short distance. The caterpillar behaves quite passively after leaving its hostplant. It will be adopted by the first worker ant that finds it, irrespective of the species. When it is taken by the wrong ant species, it will have a higher probability of later death in the ant nest. When spatial co-occurence of both butterfly and host ant species increases (the distribution of *Myrmica rubra* and *M. scabrinodis* show more overlap) then a larger proportion of caterpillars of both butterfly species is taken to the wrong ant nest. We hypothesize that when the spatial co-occurence of host ant nests increases, the probability of a caterpillar to be adopted by its specific host ant decreases and therefore its mortality increases. This effect is positively correlated to the densities of butterfly and host ant nests. Furthermore, it is expected to have more impact on *Maculinea teleius* as its host ant *Myrmica scabrinodis* has small nests in which generally only one caterpillar is able to survive.

The advantage of deposition of eggs close to colonies of the host *Myrmica* species is also related to the spatial scale of the distribution of the host plants. *Sanguisorba officinalis* is usually very abundant at sites with *Maculinea teleius* and to a lesser extent at sites with *Maculinea nausithous.* In contrast, *Maculinea alcon* and *Maculinea rebeli* require locally distributed host plant species that are often rare: *Gentiana pneumonanthe*, *G. asclepiadea* and *G. cruciata*. In their habitats, the host plant is less abundant than their host ant species (Ebert & Rennwald 1991, Hochberg *et al.* 1992, 1994, Van Dyck *et al.* 2000). In such circumstances, a relatively low proportion of host plants represent sinks due to lacking of host ants. Yet, there are indications for host ant mediated oviposition in *Maculinea alcon* (Scheper *et al.* 1995, Van Dyck *et al.* 2000). In their spatial model, Clarke *et al.* (1997) and Hochberg *et al.* (1994) assume random oviposition for *Maculinea rebeli.* For this species, the conclusions drawn would not be different when ant-mediated oviposition is assumed due to the in comparison limited number of host plants in the system. However, when having to cope with the situation that most host plants are sinks, it becomes important to select between the ones growing in the limited home ranges of the host ant nests and the ones growing outside, because this has direct consequences for the survival of the offspring. Furthermore, *Maculinea nausithous* is the only *Maculinea* species which can for long periods survive on very small and dynamic habitats such as road verges and canal borders (Ebert & Rennwald 1991). Under such conditions, it seems natural that this relatively mobile parasitic butterfly (Binzenhöfer in Settele *et al.* 1996, own unpublished data) can detect its host ant species, and successfully colonise suitable locations, rather than loose many offspring by random oviposition on sinks of *Sanguisorba officinalis* plants without *Myrmica rubra.*

Consequences for success of reintroduction

After their release, the butterflies were able to colonise the best locations first and establish on the sites where ecological requirements are best realised. After reintroduction, butterfly numbers have increased more than expected from one year to the next. *Maculinea nausithous* even managed to leave the reintroduction site and settle on a location with *Myrmica rubra*, that was thought to be out of reach of the butterflies, because it is separated from the release site by high poplars, willows and tall herb vegetation. For the success of the reintroduction, it is important to know whether the limited number of eggs of the founder females will be deposited according to the random oviposition hypothesis or according to the ant-mediated oviposition hypothesis. In the latter case, undesirable losses in the critical first founding years will be avoided and the chance of successful establishment will increase. For the same reasons, small populations of *Maculinea nausithous* and *M. teleius* and dispersing individuals profit from being able to select between sources and sinks. This is important for highly endangered animal species with a very specialised life cycle as these butterflies.

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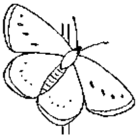


Landscape constraints in the dispersal of two parasitic butterfly species

Summary

In this paper, we investigate the population responses of two butterfly species, *Maculinea teleius* and *M. nausithous*, within a restricted set of patches in which the habitat quality is spatially heterogeneous. This differs from metapopulation studies where the dynamics of local populations and the exchange of individuals are studied in spatially disjoint habitat patches that are assumed to contain uniformly suitable habitat. The presence of both butterfly species largely depends on the presence of two larval resources: the host plant *Sanguisorba officinalis* and host ant nests. Although the adult individuals of both butterfly species can select sites that contain these resources, dispersal leading to colonisation of suitable sites at a larger spatial scale seems to be constrained. The question is whether the spread of these two butterfly species is limited by the quality or the spatial arrangement of their habitat. We addressed this question by investigating distribution shifts in the two butterfly species since their reintroduction and establishment in the nature reserve Moerputten and surroundings in The Netherlands. We examined for each species whether the colonisation of unoccupied locations and the abandonment of occupied locations are related to their degree of connectivity. According to the random sample hypothesis, no effects of the degree of connectivity are expected. Effects of the degree of connectivity on colonisation and abandonment suggest that these two processes are not random with respect to the degree of connectivity of the plots. We show that dispersal constraints at the individual level may affect habitat selection and have an effect on the colonisation and abandonment rates in the populations of the two *Maculinea* butterfly species and thus on their distribution. We discuss the consequences for conservation of the two species after reintroduction.

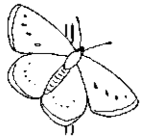
Van Langevelde F, I Wynhoff, H Olff, CAM van Swaay, PM Brakefield & HHT Prins. Submitted.

Introduction

Effects of the spatial arrangement of habitat on population dynamics have often been investigated in the context of metapopulations, where there are local populations that interact through dispersing individuals. One characteristic of metapopulations is the dynamic equilibrium between colonisation of patches of habitat and extinction of local populations (Verboom *et al.* 1991, Hanski 1999). Metapopulation studies are mainly based on local populations in spatially disjoint habitat patches that are assumed to contain uniformly suitable habitat. In several ways, however, these assumptions violate real world situations. For many species, local populations are difficult to distinguish and densities vary over space related to local habitat quality (Harrison 1994a, Lewis *et al.* 1997, Thomas & Kunin 1999). Within and between sites, habitat quality is often spatially heterogeneous which may affect habitat selection and movement patterns (Morris 1995, Doak 2000, Gutiérrez *et al.* 1999, Haddad 2000). We investigate the population responses of two butterfly species, *Maculinea teleius* and *M. nausithous*, within a restricted set of patches in which the habitat quality is spatially heterogeneous. In contrast to metapopulation studies with local populations in habitat patches, colonisation is here defined as the process that empty plots within a habitat patch become occupied.

The two butterflies are strictly sedentary, with *Maculinea nausithous* moving, on average, larger distances than *M. teleius* (Settele 1998). The presence of both butterfly species largely depends on the presence of two larval resources. First, both *Maculinea teleius* and *M. nausithous* deposit their eggs only on the host plant *Sanguisorba officinalis* (Thomas 1984, Elmes & Thomas 1987, Wynhoff *et al.* 2001a, chapter 5). The early instar caterpillars feed on developing seeds of the host plant. Second, both species are obligate myrmecophiles since their later instars are hosts in ant nests where they feed on ant larvae. After two to three weeks, fourth instar caterpillars leave their host plants to be found by *Myrmica* worker ants and are taken to their underground nests. *Maculinea teleius* mainly parasitises on the ant *Myrmica scabrinodis* while *Maculinea nausithous* can be found in nests of *Myrmica rubra* (Thomas *et al.* 1989). Wynhoff *et al.* (2001a, chapter 5) analysed the habitat selection of these butterflies, measured by the oviposition behaviour and the occurrence of adults, and showed that females of both butterflies are able to quite reliably select those host plants for oviposition which are in the proximity of nests of their specific ant host.

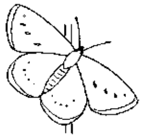
In 1990, the two butterfly species were reintroduced in the Moerputten nature reserve in the Netherlands following their national extinction in 1976



(Wynhoff 1998, chapter 3). Their reintroduction can be rated as successful since both butterfly species have established themselves at sites where they were released. However, although suitable habitat seems to be available, the distribution of the two butterflies is restricted: *Maculinea teleius* occurs only on the meadows where it was reintroduced, while *M. nausithous* has established populations on the railway embankment and the surrounding road verges (Wynhoff 1998, chapter 3). Within a habitat patch, females are able to preferentially lay their eggs in plots where both the food plant and the specific host ant occur (Wynhoff *et al.* 2001a, chapter 5). However, the species does not seem to have a high rate of colonisation of more distant plots where both resources also occur. The question is then whether such a spread of these two butterfly species is limited by the quality or the spatial arrangement of their habitat. We addressed this question by investigating distribution shifts in the two butterfly species since their reintroduction and establishment. By collecting data at the level of 1 × 1 m plots in all patches with host plants in and around the nature reserve, we studied the effects of within and between habitat heterogeneity on butterfly behaviour and distribution.

Metapopulation theory predicts that the degree of habitat connectivity is an important landscape feature that influences colonisation of unoccupied locations (Fahrig & Merriam 1985, Hanski 1994, With & Christ 1995, Gutiérrez *et al.* 1999, Van Langevelde 2000). In this context, connectivity is the degree to which the landscape facilitates or impedes movement among resource patches (Taylor *et al.* 1993). For the butterfly species, connectivity will be expressed as the nearest neighbour distance between plots. In conditions of low connectivity, dispersal may be constrained resulting in a low probability of colonisation of unoccupied locations. According to the random sample hypothesis (Connor & McCoy 1979, Haila *et al.* 1993, Andrén 1996, 1999), however, the simplest explanation for a low colonisation probability of unoccupied locations is their low habitat quality. Then, the colonisation pattern of locations with a low degree of connectivity is a random sample from equally large locations in contiguous habitat of the same quality. In particular, the random sample hypothesis predicts no effect of the degree of connectivity on distribution and colonisation. For the two butterfly species, we test whether the colonisation of unoccupied locations is related to their degree of connectivity.

Besides the quality of the habitat, the risk that a location becomes unoccupied is assumed to depend on the migration from other occupied locations, *i.e.*, the rescue effect (Brown & Kodric-Brown 1977). The rescue effect refers to increasing the size of local populations and hence decreasing



their risk of extinction with increasing rate of immigration. It implies a positive relationship between the fraction of occupied locations and the average size of local populations or densities. Thus, a negative relationship can be expected between the migration rate of a location and the probability that this location becomes unoccupied (Hanski 1999, Stacey *et al.* 1997). When the spatial arrangement of the habitat has no effect on the population responses, this rescue effect does not depend on the degree of connectivity of the locations. For the two butterfly species, we test whether the abandonment of occupied locations is related to their degree of connectivity.

Material and Methods

Study area

The study area covers the nature reserve of Moerputten (115 ha) and its surroundings, situated in the centre of the Netherlands in the province of Noord-Brabant (51°41'N, 5°5'E, altitude 2 m above sea level, figure 6.1, Wynhoff 1998, see chapter 1 for further information). The area consists of a lake, surrounded by tall swamp vegetation and willow and elder forests, and several moist meadows at the higher parts that contain the habitat of the butterflies. In 1990, the founding butterflies were released on the meadows at the southern border of the reserve (figure 6.1).

Maculinea teleius colonised the release sites and expanded to a few meadows further west. After three years, however, this species was restricted only to the release site, where it still occurs today. *Maculinea nausithous* apparently did not establish itself in the nature reserve after release, but was found a year later on the railway embankment crossing the reserve, SD in figure 6.1. The latter population rapidly increased and gave rise to the colonisation of habitat at a distance of about 500 m on the verges of the minor road RD (figure 6.1). While the railway embankment population was stable for several years and then decreased in numbers, the RD-population increased rapidly. Another local population was found 2 years later at a distance of 5 km. This third population, occupying road verges, is still very small. This unexpected large-scale colonisation is not included in this study since we focus on the local colonisations within the nature reserve and direct surroundings.

Butterfly data

Both *Maculinea teleius* and *M. nausithous* have been continuously monitored since the reintroduction. Butterflies were counted by means of transect counts and mark- recapture studies (Wynhoff 1998, Wynhoff *et al.* 2001a, 2001b, chapters 3, 5 and 7). Furthermore, observations of butterflies during field visits were recorded. The area of Moerputten and surroundings were visited at least once a week, and preferably every second or third day during the whole flight period of both species.

In the study area, 587 plots sized 1 × 1 m were randomly located on meadows, verges and patches with *Sanguisorba officinalis.* On the release meadow, BW, we used a 10 × 10 m grid square system, whereas the plots were located randomly in the other patches. The size of the plots represents the scale at which a female of each species decides whether to deposit an egg or not (Wynhoff *et al.* 2001a, chapter 5). In each plot, the presence or absence of an adult butterfly was recorded during the 10 years following reintroduction. A colonisation was recorded when a plot was unoccupied in a certain year and occupied in the next year. The opposite, abandonment of a plot, was recorded when a plot was occupied in a certain year and unoccupied in the next year. For *Maculinea teleius*, all years of observations were used in this study. For the colonisation analysis, the year of reintroduction was excluded since the butterflies could not spontaneously colonise the first-year occupied plots. In addition, *Maculinea nausithous* passed through a bottleneck of small numbers in 1991 and, therefore, the analysis begun with the census in 1992.

The degree of connectivity of a plot was measured as the Euclidean distance to the nearest-neighbour plot that was occupied (from centre to centre). To determine the relationship between the probability of both colonisation and abandonment of a plot in year t and its degree of connectivity, we used the distance to its nearest neighbour that was occupied in year $t - 1$.

Vegetation data

Since *Maculinea* butterflies only occur at sites with their host plant, we restricted the analyses to those plots with *Sanguisorba officinalis* (figure 6.1). For a subset of 251 plots, data on vegetation composition were collected through vegetation relevés according to the Braun-Blanquet method. In these plots, the maximum height of the vegetation was measured (in cm).

The vegetation composition was analysed using a Detrended Correspondence Analysis (DCA, Jongman *et al.* 1995), in which samples and species are ordinated along canonical axes according to similarities in occurrence. As a measurement for vegetation composition, the sample scores of the plots for the first 4 axes were taken for further analysis.

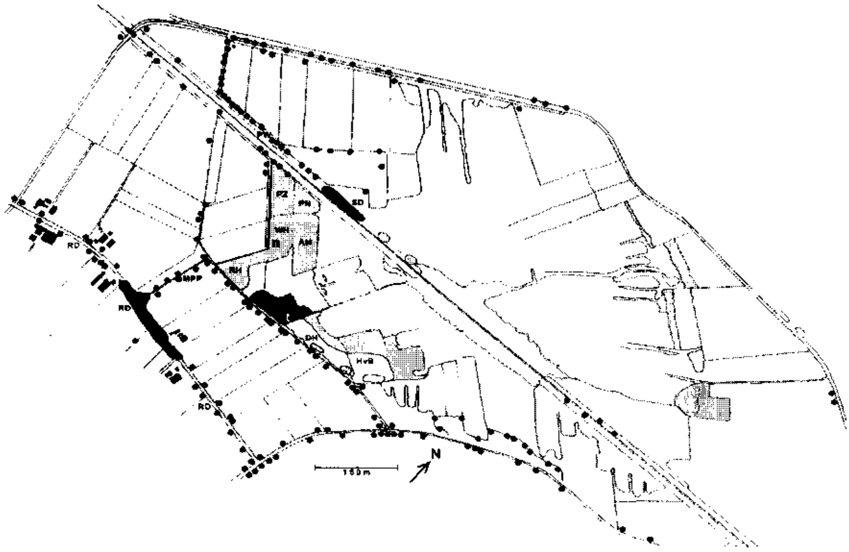


Figure 6.1: Study area of Moerputten and its surroundings in the centre of the Netherlands. Indicated with 'n' and 't' are the meadows were the two butterfly species, *Maculinea nausithous* and *M. teleius* respectively, were released in 1990. The host plant *Sanguisorba officinalis* occurs in the grey areas. Dots denote single plants or groups of plants. BW, WH and PN are the meadows, RD and MPP are the road verges and SD is the railroad embankment.

Furthermore, we calculated the mean Ellenberg values for moisture, productivity and acidity for each plot (Ellenberg 1992). For details of the analysis see Wynhoff et al. (2001a, chapter 5).

Ant data

The composition of the local ant fauna and the density of host ant nests depend on the vegetation structure and local microclimate (Elmes *et al.* 1998, Wynhoff *et al.* 2001a). Per plot, the presence of ant species was examined by attracting them with sugar cubes. The sugar cube represents the caterpillar waiting to be found by worker ants. In the centre of the plot at the foot of a *Sanguisorba officinalis* plant, a sugar cube was placed on a concave plate covered with black plastic. This was done in the early morning hours before the ants start their daily activity period (before 8 a.m.). After at least 1 hour, the baits were checked for worker ants visiting the sugar and the ant species were identified. In the evening, all baits were removed. Since

Myrmica ants have a restricted foraging range of 2 m around their nest site (Elmes *et al.* 1998), we assume that we only found workers from nests in the close proximity of the *Sanguisorba* plant during the short recording period. Moreover, we assume that the plots where sugar cubes remained undetected represented ant-free plots. Since the number of worker ants on the bait represents neither the density of ant nests nor the size of the nests in the plot, we only used the presence or absence of an ant species.

Statistical analysis

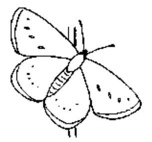
Regarding the abundant host plant *Sanguisorba officinalis* and the low nest density of the host ants, the quality of the habitat of both species is largely determined by the presence of the ant nests. In their habitat selection study, Wynhoff *et al.* (2001a, chapter 5) found that the probability that an adult butterfly was present in a plot, is related to the presence of their *Myrmica* host ant, the vegetation height and vegetation composition as represented by the first DCA-axis (Table 6.1). Based on these results, we describe the quality of the habitat for each of the butterfly species as follows:

$$Q_{tel} = \beta_0 + \beta_1 M_s + \beta_2\,DCA + \beta_3\,DCA^2 + \beta_4 VH \qquad \text{for } \textit{M. teleius} \text{ (eq. 1a)}$$

$$Q_{naus} = \gamma_0 + \gamma_1\,M_r + \gamma_2\,DCA + \gamma_3 VH \qquad \text{for } \textit{M. nausithous} \text{ (eq. 1b)}$$

where β_i and γ_i are the regression coefficients (Table 6.1), M_s and M_r the host ant presence of respectively *Myrmica scabrinodis* and *M. rubra*, DCA the first DCA-axis and VH the vegetation height. The first DCA-axis correlates with the Ellenberg productivity ($n = 234$, $r = 0.887$, $p < 0.001$), acidity ($n = 229$, $r = 0.723$, $p < 0.001$) and moisture ($n = 236$, $r = -0.626$, $p < 0.001$) (Wynhoff *et al.* 2001a, chapter 5). The vegetation relevés are ordinated from the most nutrient poor meadows (BW, WH), via richer meadows and road verges (RD, MPP) to the tall herb vegetation at the railroad embankments (SD) (Figure 6.2).

The colonisation of unoccupied plots and the abandonment of occupied plots by the two butterfly species were analysed by fitting a logistic regression. Since the probability that a plot of certain quality is colonised or abandoned in year *t* depends, among others, on the population size in that year, we used the total number of occupied plots as explanatory variable as well. In the regression analyses of both colonisation and abandonment, first the habitat quality and the total number of occupied plots was added. Then, the distance to the nearest-neighbouring occupied plot was added. Due to



the constant management of the nature reserve and the surrounding habitat, we consider the vegetation composition and structure and the presence of ant nests constant over all years in the analyses.

Results

In the study area, 274 colonisations of a total of 1920 unoccupied plots were observed for *Maculinea teleius*, while 256 of a total of 590 occupied plots were abandoned the next year. *Maculinea nausithous* colonised 50 of 1849 unoccupied plots. From 144 occupied plots, 51 were abandoned the

Table 6.1: Results of the logistic regression analyses of the presence/absence of *Maculinea teleius* and *M. nausithous* in the study area (Wynhoff *et al.* 2001a). The regression coefficients β_i and γ_i are given and used as in eq. 1a and 1b. DCA represents the first DCA-axis of the vegetation relevés, M_S and M_R is the presence/absence of a host ant nest (*Myrmica scabrinodis* and *M. rubra* respectively) and VH is the vegetation height in cm.
The probability of observing a butterfly of either one or the other *Maculinea* species (M) is given by: ln[M/(1-M)] = constant + Σ regression coefficient × variable.

	Maculinea teleius	*M. nausithous*
number of cases	1900	1710
% predicted correct	76	94

Maculinea teleius				
variable	β	*S.E.*	*Wald*	*Sig.*
Constant	-3.750	0.395	90.092	0.000
Population size	0.019	0.002	76.678	0.000
DCA	4.232	0.687	37.984	0.000
DCA^2	-3.082	0.422	53.420	0.000
M_S	0.439	0.131	11.313	0.001
VH	0.027	0.011	5.788	0.016
Maculinea nausithous				
variable	γ	*S.E.*	*Wald*	*Sig.*
Constant	-7.505	0.522	206.964	0.000
Population size	0.018	0.005	14.692	0.000
DCA	1.766	0.156	128.032	0.000
M_R	1.147	0.230	24.919	0.000
VH	-0.009	0.004	4.189	0.041

following year. The total population size widely fluctuated between sequential years: for *Maculinea teleius*, the average number of occupied plots is 75.3 with a standard deviation of 29.9 (n = 9), while *M. nausithous* has an average of 41.3 occupied plots with a standard deviation of 21.2 (n = 7).

The probability that an unoccupied plot is colonised is significantly related to the recorded habitat quality of the plot, the overall population size and its degree of connectivity (Table 6.2). For each butterfly species, a similar set of variables yielded significant effects on the colonisation probability. Increasing habitat quality leads to an increase in colonisation probability. However, plots with habitat of high quality can remain unoccupied when they have a low degree of connectivity since we found a decrease in colonisation probability with increasing distance to the nearest-neighbouring occupied plot. Plots of low habitat quality and high degree of connectivity may be colonised more frequently than expected based on the random sample hypothesis. Figure 6.3 gives the predictions for the colonisation probability of unoccupied plots by each butterfly species. This figure shows the distances that individuals of each species covered to colonise unoccupied plots: *Maculinea teleius*, on average, showed shorter distances

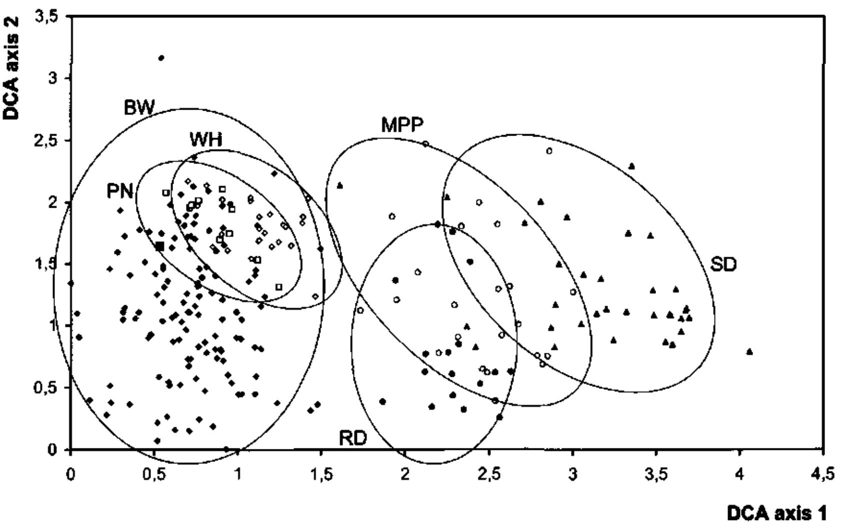


Figure 6.2: Vegetation relevés from the study area along the first two canonical axes. The meadows are BW, WH and PN, the road verges are RD and MPP, the railroad embankment is SD (see figure 6.1).

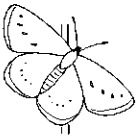


Table 6.2: Results of the logistic regression analyses of the colonisation of unoccupied plots by *Maculinea teleius* and *M. nausithous* in the study area. Q_{tel} and Q_{naus} represent the habitat quality for *Maculinea teleius* and *M. nausithous*, respectively (see eq. 1a and 1b). The distance refers to the distance to the nearest neighbour plot that was occupied in the previous year.

	Maculinea teleius	*M. nausithous*
number of cases	1920	1849
% predicted correct	87	97

Maculinea teleius *variable*	*regr. coef.*	*S.E.*	*Wald*	*Sig.*
Constant	-1.249	0.502	6.192	0.013
Habitat quality Q_{tel}	0.693	0.138	25.101	0.000
Population size	0.025	0.003	74.818	0.000
Ln(Distance)	-1.092	0.126	75.181	0.000

Maculinea nausithous *variable*	*regr. coef.*	*S.E.*	*Wald*	*Sig.*
Constant	-6.995	1.124	38.730	0.000
Habitat quality Q_{naus}	0.604	0.082	54.515	0.000
Population size	0.015	0.007	5.543	0.019
Ln(Distance)	-0.422	0.143	8.736	0.003

than *M. nausithous*. Colonisation distances are highly skewed, especially for *Maculinea teleius*. This species has a clumped distribution in the study area, resulting in many examples of larger distances to apparently suitable, but unoccupied plots (Table 6.4). The highest mean and maximum colonisation distances were measured in years following population increase, *e.g.*, in 1991 after reintroduction. Although a high population size increases the probability of colonisation, we could not find evidence that dispersal distances are density-dependent: individuals do not cover larger distances to colonise unoccupied plots when the population size is high. We tested this adding the interaction between the degree of connectivity and the overall population size to the regression model for *Maculinea teleius*, but it was not significant.

Maculinea nausithous has colonised plots at larger distances compared to *M. teleius* and has established two local populations. Therefore, the distances to unoccupied plots are shorter (Table 6.4). Again, no significant density-dependent colonisation distances were found.

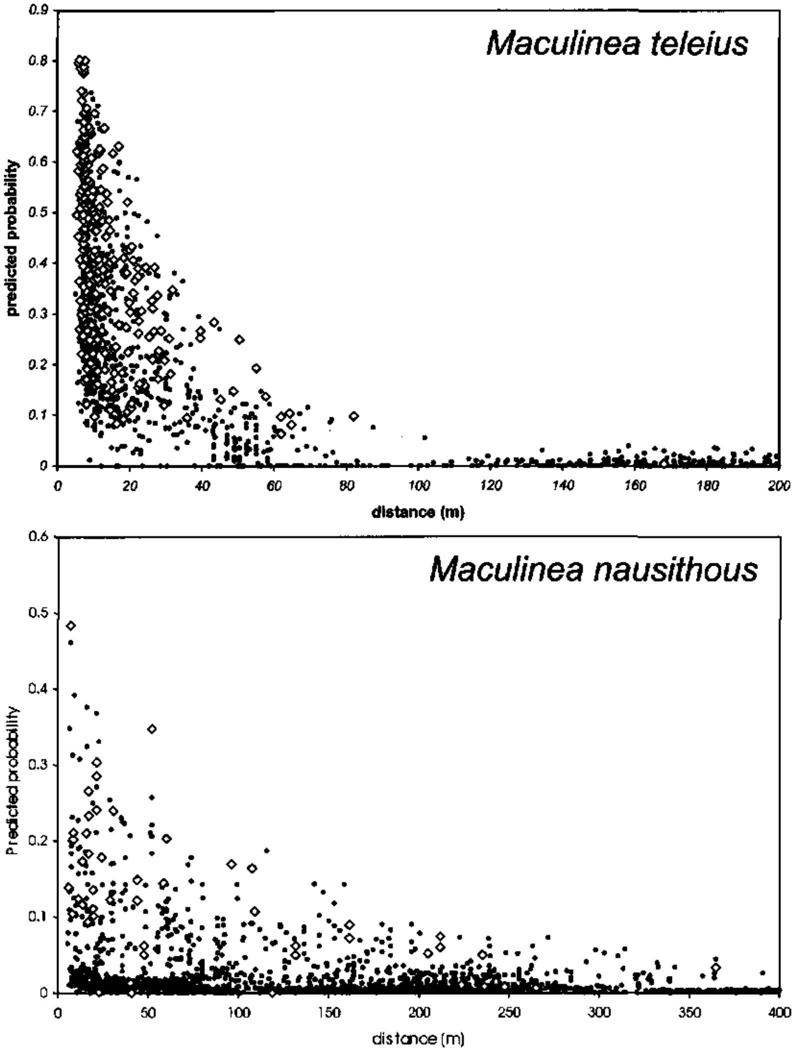
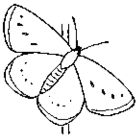


Figure 6.3: Predictions of the colonisation probability plotted against the observed distances of each unoccupied plot to its nearest-neighbouring plot that was occupied the previous year. Open squares denote colonised plots, dots refer to plots that remained empty.



As can be expected, the probability that an occupied plot is abandoned increases for both butterfly species when the quality of the habitat is lower. The probability of abandonment in *Maculinea nausithous* could only be related to habitat quality (Table 6.3). For *Maculinea teleius*, habitat quality of the plot, the overall population size and its degree of connectivity affect the abandonment probability. When the distance to the nearest neighbouring occupied plot increases, the probability that a plot is abandoned by *Maculinea teleius* increases, too (Table 6.3).

Discussion

The results show that both the quality and the spatial arrangement of their habitat limit the spread of the two *Maculinea* butterfly species. We can distinguish two scale levels at which these factors operate (Haila *et al.* 1993, Morris 1995): at the level of the adult individual, the spatial habitat arrangement may affect the selection of oviposition sites, whereas at the level of populations the exchange of individuals among local populations is affected. We found that although individuals can select their habitat, low quality plots are sometimes occupied and high quality plots can remain unoccupied especially when their degree of connectivity is low. This constrained dispersal has an effect on the rates of colonisation and abandonment, and thus on the local distribution of the species. Constraints in dispersal set the conditions for the selection of habitat. This phenomenon belongs to a central issue in ecology, namely the problem of scale: patterns at one scale may be manifested at another (Wiens *et al.* 1993, Levin & Pacala 1997).

The two butterfly species are highly specialised since they require host plants and host ant nests as larval resources. This specialisation appears to cause their restricted spatial distribution in the study area. Low quality of the habitat, in terms of the lack of one of the two larval resources, may then limit the spread of the butterflies when they occur in small local populations, such as after a reintroduction or a catastrophic event. Since the host plant *Sanguisorba officinalis* is abundant whilst there is a low nest density of the host ants, the quality of the habitat of both butterfly species is largely determined by the host ant. Nevertheless, both *Maculinea teleius* and *M. nausithous* are able to select for *Sanguisorba officinalis* in the proximity of host ant nests to deposit their eggs (Wynhoff *et al.* 2001a, chapter 5). For *Maculinea teleius*, however, Wynhoff *et al.* (2001c, chapter 4) found a

Table 6.3: Results of the logistic regression analyses of the abandonment of occupied plots by *Maculinea teleius* and *M. nausithous* in the study area. Q_{tel} and Q_{naus} represent the habitat quality for *Maculinea teleius* and *M. nausithous*, respectively (see eq. 1a and 1b). The distance refers to the distance to the nearest neighbour plot that was occupied in the previous year.

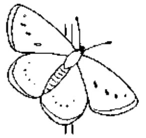
	Maculinea teleius	*M. nausithous*
number of cases	590	144
% predicted correct	68	72

Maculinea teleius				
variable	regr. coef.	S.E.	Wald	Sig.
Constant	-1.411	0.848	2.770	0.096
Habitat quality Q_{tel}	-0.619	0.210	8.679	0.003
Population size	-0.026	0.004	48.658	0.000
Ln(Distance)	1.976	0.337	34.483	0.000
Maculinea nausithous				
variable	regr. coef.	S.E.	Wald	Sig.
Constant	5.788	1.512	14.661	0.000
Habitat quality Q_{naus}	-0.648	0.152	18.247	0.000

positive relationship between ant nest presence and oviposition at the beginning of the flight period, but this relationship disappeared at the end of the flight period. Moreover, Wynhoff *et al.* (2001a, chapter 5) found that *Maculinea nausithous* deposited 26% of the eggs during the whole flight period at plots without ant nests. Although there are differences in oviposition behaviour between the two butterfly species (Thomas 1984, Figurny & Woyciechowski 1998), these findings strongly suggest that there are limitations on the selection of oviposition sites when the majority of suitable flowerheads in the proximity of butterflies have already been oviposited on. Based on our results, the observed limitations in habitat selection can be accounted for by constrained dispersal.

Connectivity and colonisation

For both *Maculinea nausithous* and *M. teleius*, we tested whether plots further away from occupied plots have a lower probability of being occupied. The random sample hypothesis provided the null hypothesis: there is no effect of the degree of connectivity on the probability that an unoccupied plot

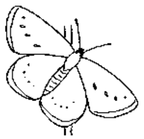


is colonised. The results show that, on the one hand, plots of low habitat quality and high degree of connectivity may be colonised more frequently than expected based on the random sample hypothesis. On the other hand, plots with high habitat quality but low degree of connectivity are less frequently colonised than expected. Thus colonisation is not random with respect to the degree of connectivity of the plots.

The random sample hypothesis has been previously examined for metapopulations of other species, *e.g.*, by Verboom *et al.* (1991) and Andrén (1996, 1999). These studies, however, focused on the question of whether the distribution of a species in a heterogeneous landscape is solely due to habitat loss or is also due to habitat fragmentation. Here, we discuss another dichotomy, namely whether the distribution of a species in a heterogeneous landscape is due to differences in habitat quality or also due to habitat fragmentation. Moreover in many studies of spatial population ecology, local populations can be distinguished and a constant habitat quality of the patches is assumed. In our study, the set of habitat patches, the meadows, is actually a spatially heterogeneous mosaic of plots with differences in vegetation structure and ant nest presence.

Table 6.4: Some characteristics of the degree of connectivity of plots that remain unoccupied and are colonised by *Maculinea teleius* and *M. nausithous* in and around the study area. The degree of connectivity is expressed in the distance (in m) to the nearest neighbour plot that was occupied in the previous year. The skewness is a measure of the asymmetry of a distribution: a large positive skewness (more than twice its standard error SE_{SK}) indicates a long right tail. This means that the majority have dispersed short distances (see figure 6.3).

	n plots	Median distance	Minimum distance	Maximum distance	Skew-ness	SE_{SK}
M. nausithous						
All	1849	143	5	655	0.620	0.06
Empty	1799	147	5	655	0.061	0.06
Colonised	50	42	6	442	1.560	0.34
M. teleius						
All	1920	81	5	922	1.190	0.06
Empty	1646	171	5	922	1.011	0.06
Colonised	274	9.8	5	263	7.900	0.30



The colonisation events of *Maculinea teleius* mainly took place within the meadow where the butterflies have occurred since their release in 1990. The very small colonisation steps (Figure 6.3, Table 6.4) indicate an expansion – retraction process. This is most likely caused by stochastic demography due to weather conditions. The probability that an individual will leave this meadow is low. This agrees with the displacement of individuals in a mark-recapture study. Even though the butterflies are quite active, they tend to stay at particular sites within the meadows, resulting in a mean displacement of less than 1 m per day (in a 10 × 10 m grid). The maximum displacement found was 260 m in 10 days for males and 639 m in 7 days for females (unpublished data from 1991). Similar data have been found in other populations (Settele 1998), with a single displacement of more than 5 km. Dispersal to unoccupied, suitable habitat plots at longer distances seems to be constrained and this is likely to limit the distribution of *Maculinea teleius*. The few long distance movements may be very important for the persistence of the population as they enable exchange between local populations (cf. Quinn & Hastings 1988).

Maculinea nausithous has been found to colonise unoccupied plots less frequently, but it covers, on average, longer distances. The latter is shown by the longer colonisation distances (Figure 6.3, Table 6.4) and by mark-recapture studies. Mean daily displacement was 6 m for females and 8 m for males (unpublished data from 1991) and displacements exceeding 1 km have only rarely been found, but more frequent than in *M. teleius* (Settele 1998). However, with a maximum colonisation distance of 442 m, we conclude that the spatial arrangement of the habitat limits the distribution of *Maculinea nausithous*.

Connectivity and abandonment

The rescue effect reduces the risk of extinction or abandonment by migration from other occupied plots. The results in *Maculinea teleius* suggest the existence of such a rescue effect: the probability of abandonment of an occupied plot decreases with both overall population size and the degree of connectivity of the plot. With increasing overall population size (more occupied plots), more individuals tend to move to a particular plot which leads to an increase in its local population density and hence a decrease in the risk of abandonment of this plot.

Females partly select flowerheads without ant nests in the proximity, especially at the end of the flight period (Van Dyck *et al.* 2000, Wynhoff *et al.* 2001c, chapter 4). They select for empty flowerheads to oviposit, but they are restrained to cover large distances to search for these flowerheads. In those plots without ant nests, no caterpillars pupate and abandonment will be recorded unless the searching females again oviposit their eggs on the flowerheads. Partly, this may appear as a rescue effect although in fact source-sink relationships appear. This may be more obvious in *Maculinea teleius* then in *M. nausithous* since the latter can oviposit several eggs on the same flowerhead.

Hanski *et al.* (1995) show the existence of the rescue effect in a metapopulation of the butterfly *Melitaea cinxia*. They could distinguish this effect for different population sizes, finding a reduction in local extinction in small populations and not in large populations. The rescue effect was also demonstrated in patchy populations of *Aphantopus hyperantus* (Sutcliffe *et al.* 1997).

Implications for conservation

Although individuals of *Maculinea teleius* and *M. nausithous* are able to select plots with the two larval resources, dispersal is constrained due to the spatial arrangement of the habitat. This limits the spread of small populations, in our case following reintroduction of the species. Although the population dynamics of both butterfly species are affected, we expect that *Maculinea teleius* is less widely distributed than *M. nausithous* since they cover smaller distances (Figure 6.3). This agrees with the observed distribution pattern over the years. Moreover, we predict that the population of *Maculinea teleius* could be more stable than *M. nausithous* since we found, on average, a lower extinction risk due to the rescue effect in *M. teleius*. This also agrees with the observations to date since the variation in overall population size is higher in *Maculinea nausithous* than in *M. teleius*.

Conservation of *Maculinea teleius* should concentrate on improving local habitat quality, as the persistence of this species is dependent on the situation of the core population. The constrained dispersal in this butterfly species resembles almost flightless moth *Itame andersoni* (Doak 2000) and the flightless moth *Orgyia vetusta* (Harrison 1994b). Given the low dispersal abilities, expansion of the resident population can only be expected on high quality patches in the close proximity. Plots with high quality habitat at longer distance are more likely to be colonised if high quality stepping stones are available in between (Haddad 2000). Conservation of *Maculinea nausithous* by creating a spatial network of suitable habitat plots seems to be more effective since this species is able to cover longer distances. Local populations of this species are generally smaller and depend more on exchange of individuals.

Studies in spatial population ecology focus on effects of the degree of connectivity while habitat quality is subject in habitat selection studies. An explicit link between the two is often lacking (Lima & Zollner 1996). In our study, we show that dispersal constraints at the individual level may affect habitat selection and have an effect on the colonisation and abandonment rates in the populations of the two *Maculinea* butterfly species and thus on their distribution. Understanding the scaling of the processes is relevant for conservation since it contributes to insight in the bottlenecks in the distribution of species. Therefore, such links between processes at individual and population level should be an urgent topic in conservation biology.

Acknowledgements

We are very grateful to Paul Kreijger who counted the butterflies according to the Dutch Butterfly Monitoring Scheme and gave us valuable records of dispersing *Maculinea* butterflies and potential habitats. Maria Asunción Hidalgo Lopez collected the ant samples at the meadow BW. Marcel Grutters, Andrea Grill, Maarten van Steenis, Gerrit Klomp and Menno van Zuijen helped with the vegetation measurements and the other ant samples. Victor Mensing helped us a lot with the data input. The Dutch State Forestry Service kindly gave us permission to conduct this study in the nature reserve Moerputten. The contribution of FL was financially supported by the Netherlands Organisation for Scientific Research (NWO) (Stimulation Program Biodiversity "Development of strategies for conservation and restoration of biodiversity in agricultural areas").

Genetic variation is retained after reintroduction of the butterflies Maculinea nausithous and Maculinea teleius

Summary

Reintroduction is an increasingly important tool in nature conservation after populations of rare animals have disappeared from nature reserves. The demography of the founder populations is often well studied, but there are only a few case studies of the genetic consequences of the potential bottleneck. The rare butterfly species, *Maculinea nausithous* and *M. teleius*, both obligate ant parasites, were reintroduced to a Dutch nature reserve in 1990 using butterflies collected in Poland. The population increase was monitored and five years after reintroduction a genetic analysis was performed. The reintroduced populations of both butterfly species did not suffer an initial bottleneck in terms of a very low number of founder individuals. Population numbers increased rapidly and loss of genetic variation was only mild. Low levels of electrophoretic variation of the source populations did not appear to have a negative impact on the establishment from the founder populations.

There were shifts in the flight periods after release and the mean minimal life time of the adults decreased. In both *Maculinea* species significant differentiation at the Aco-K locus between source and founder population was found. These observations suggest that both species experienced selection due to the changed ecological conditions in The Netherlands.

Wynhoff I, H de Vos, MP van Zuijen, HHT Prins & PM Brakefield 2001. Submitted.

Introduction

Many populations of threatened animals have disappeared due to the destruction of their habitat or due to major habitat changes resulting in significantly lower survival probabilities for the species concerned (Hunter 1996, Settele *et al.* 1996, Soulé 1986). If habitats are not completely destroyed, conservation biologists may wish to reconstruct them and reintroduce lost species (Fischer & Lindenmayer 2000). There have been many such fauna restoration projects, for example with numerous butterfly species (Oates & Warren 1990), the lynx (Breitenmoser & Haller 1993) and the beaver (Nolet & Baveco 1996). Although reintroduction may give the appearance of a readily available tool in nature conservation, success is not guaranteed. In fact, most reintroduction projects in butterflies have been failures (Oates & Warren 1990). Sometimes reintroductions failed because habitat quality was not good enough for population persistence. In other cases no reason could be found. Usually only the demography of the founder population has been monitored and not the genetic consequences of the founding event, even though they can have a strong impact on success. For the founders a reintroduction can be compared to a bottleneck: due to the severely reduced population size there is likely to be a decrease of genetic variation and loss of alleles compared to the original population (Hartl & Clark 1989, Maynard Smith 1998). This has been demonstrated in bottleneck-experiments with butterflies and other invertebrates under laboratory conditions (McCommas & Bryant 1990, Saccheri *et al.* 1999). Reintroduced populations are frequently fully isolated and remain small, so that their genetic variation is completely dependent on the effective founder individuals. Founder populations are comparable to the remnants of population fragmentation, their behaviour follows the same rules (Gilpin 1991, Olivieri & Gouyon 1997). Loss of genetic variation can lead to reduced fitness, especially under changing environmental conditions (Hunter 1996). The impact of isolation as a consequence of meta-population degradation on population genetic structure has been studied in many butterfly species (Goulson 1993, Haag *et al.* 1993, Britten *et al.* 1995, Johannesen *et al.* 1996, Johannesen *et al.* 1997, Meglécz *et al.* 1997). In contrast, we know much less about the effects of bottlenecks under natural conditions (but see Brookes *et al.* 1997, Barascud *et al.* 1999).

In 1990 the Europe-wide endangered butterfly species *Maculinea teleius* and *M. nausithous* were reintroduced in the nature reserve "Moerputten" near the city of Den Bosch in the province of Northern Brabant (The Netherlands) (Wynhoff 1998a). The last indigenous populations in The

Netherlands were lost in the late 1970s (Tax 1989). The founder individuals of the reintroduced populations of both species were collected from large meta-populations in a stream valley in Poland. After the reintroduction the populations of both species developed in a different way. The density of *Maculinea teleius* immediately increased at the reintroduction site. The flight-period of this species shifted towards a few weeks earlier over the first three years. *Maculinea nausithous* on the other hand, appeared to experience a second bottleneck. It didn't establish at the release site but rather elsewhere in the nature reserve at a distance of up to 200 m from the release site. It has formed a small metapopulation consisting of two subpopulations. Furthermore, there has been only a slight change in the flight-period compared to the source population (Wynhoff 1998a). Both species have experienced a reduction in mean minimal life time relative to those typical of the source population. Here we report on the use of gel electrophoresis to compare source populations and introduced populations for genetic variation after five years of separation. We investigated whether the reintroduction has involved a true bottleneck event in the founder populations, leading to significant loss of genetic variation. We compare the results from genetic analysis with the known dynamics of adult populations after reintroduction.

Materials and methods

Ecology of Maculinea teleius *and* M. nausithous

In contrast to other butterfly species in Europe, the larvae of *Maculinea* species are obligate parasites of ants. *Maculinea nausithous* and *Maculinea teleius* oviposit on flowerheads of *Sanguisorba officinalis* (Thomas 1984, Thomas *et al.* 1989, Elmes & Thomas 1987, Wynhoff 1998). The early instar caterpillars feed briefly on developing seeds. After two to three weeks, fourth instar caterpillars leave their host plant to be found by *Myrmica* worker ants and taken to their underground nests. The caterpillars feed mainly on ant larvae. The main host ant species for *Maculinea nausithous* is *Myrmica rubra*, whereas *Maculinea teleius* mainly lives in nests of *Myrmica scabrinodis* (Thomas *et al.* 1989, Wynhoff *et al.* 2001, chapter 5). The details of the life history of these species result in highly specialised habitat requirements (Wynhoff *et al.* 2001, chapter 5, Clarke *et al.* 1997; Figurny & Woyciechowski 1998; Thomas & Elmes 1998, Thomas & Wardlaw 1992).

The study populations

The source populations for the reintroduction of *Maculinea teleius* and *M. nausithous* are large meta-populations in the valley of the river Wisla in the vicinity of the village Kostrza near Krakow (Poland) (50°15'N, 19°51'E, altitude 200m above sea level, figure 7.1). Further information of these populations is given by Skalski (1995), Woyciechowski (1991) and Figurny & Woyciechowski (1998). The size of the total meta-population of each of these species is approximately between 5,000 and 10,000 individuals per year. Although these populations have been declining for many years, we can assume that they have not undergone any substantial loss of genetic variation through any recent bottleneck.

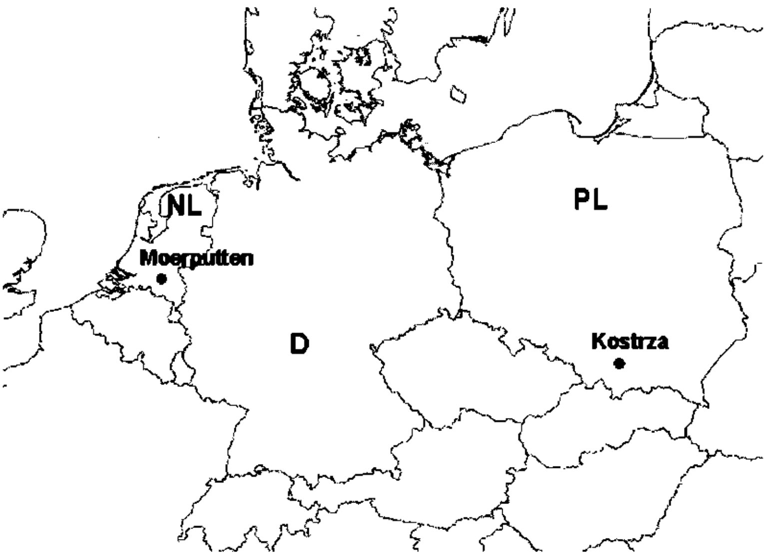


Figure 7.1: Map indicating the location of the source (Kostrza) and the founder (Moerputten) populations of the reintroduction of *Maculinea teleius* and *M. nausithous*. Abbreviations for countries: PL=Poland, D=Germany, NL=The Netherlands.

In 1990 a random sample of *Maculinea teleius* and *Maculinea nausithous* was taken for reintroduction into the nature reserve Moerputten in The Netherlands (51°41'N, 5°15'E, altitude 2 m above sea level, for further description see Wynhoff 1998a). On 30 July 1990, 33 males and 53 (mated) females of *M. teleius* and 22 males and 48 (mated) females of *M. nausithous* were released. Females probably only mate once as only freshly eclosed females are ever found in copula. The reintroduced populations were studied in each year. Annual population sizes were estimated by means of mark-release-recapture techniques or by walking monitoring transects (Wynhoff 1998a). For *M. nausithous* the reintroduction bottleneck has endured for two generations, but since 1991 the new population has increased and extended its flight area. The population of *M. teleius* immediately increased, but the butterflies remained confined to the single meadow where the introduction was made. In 1995 a second bottleneck occured due to accidental mowing of almost the complete habitat in the beginning of August. The majority of the eggs and the young larval instars on the flowerheads were killed or removed. The population size decreased to less than 60 adults in the subsequent summer but is recovering very well since then (Wynhoff, unpublished data).

Procedure of electrophoresis

Cellulose acetate electrophoresis was used to survey genetic polymorphism (system developed by Helena Laboratories (Hebert & Beaton 1993)). All insects were homogenised, each in a volume of 0.04 ml buffer solution of 0.01 M Tris, 0.001 M NaEDTA, 0.001 M $MgCl_2$, 0.01 M Maleic acid and 4mg NADP/100 ml at pH 7.4. Preparation and electrophoresis were performed in a cool chamber. Details of electrophoresis are given in table 7.1a and 7.1b. For staining an agar overlay was used. Staining recipes are given in Richardson (1986).

Twenty-two different enzymes were tested, but most were not scorable or were monomorph. Six loci (α-glycerophosphatase dehydrogenase (GPDH), Aconitase (Acon), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), glucose phospho-isomerase (PGI) and phosphoglucomutase (PGM)) showed a scorable banding pattern and were used for genetic analysis (see table 7.3). During staining, the gels were photographed. Counting of alleles took place on the gels as well as on the photographs.

Table 7.1a: Enzyme loci analysed in this study with Enzyme Commission numbers, buffer system and voltage, time and starting place of electrophoresis.

Locus	E.C. nr.	voltage (V)	time (min)	starting place	running buffer
PGM	2.7.5.1	200	45	cathodal	TG
PGI	5.3.1.9	200	40	central	CP
MDH	1.1.1.37	200	60	central	P
ACON	4.2.1.3	200	30	central	TG
IDH	1.1.1.42	200	60	central	TM
GPDH	1.1.1.8	150	75	cathodal	P

Table 7.1b : Composition of running buffers.

running buffer	molarity (M)	pH	composition of running buffer
TG	0.025	8.5	3.03 g/l Trismabase, 14.41 g/l glycine
CP	0.01	6.4	1.78 g/l $Na_2HPO_4.2H_2O$, 0.48 g/l citric acid
P	0.02	7.0	2.06 g/l $Na_2HPO_4.2H_2O$, 1.16 g/l $NaH_2PO_4.2H_2O$
TM	0.05	7.8	6.06 g/l Trismabase, 2.32 g/l malic acid

Calculation of genetical variables

Allele frequencies were calculated by means of Biosys-1 (Swoffort & Selander 1989). Standard errors were calculated with the SPSS package (Norusis 1993). The observed heterozygosity is derived from Biosys-1, the expected heterozygosity was calculated by GDA (Lewis & Zaykin 1999). We tested the difference between them with a X^2-test for goodness of fit (Biosys-1). The standard error for both values was calculated according to Weir (1996). For the calculation of the Hardy-Weinberg equilibrium we used Genepop (Raymond & Rousset 1995). Linkage disequilibrium was also tested by the Markov-chain method of Genepop. Significance was tested using sequential Bonferroni tests (Sokal & Rohlf 1995). For a pairwise test of genetic differentiation we used the Markov-chain method with 1000 dememorisations and 50 batches in Genepop (Raymond & Rousset 1995). For p-values the Fishers' exact test was used.

F-statistics were calculated using the program GDA (Lewis & Zaykin 1999). For the mean value per locus over both populations we used the method described by Weir & Cockerham (1984). The inbreeding coefficient F_{IS} was calculated in detail for the loci of both populations within the species. For the estimation of the inbreeding coefficient, only those which were polymorphic in both populations within the species were included (for *Maculinea teleius*: IDH-2, PGM, PGI and ACO-K; for *M. nausithous*: ACO-K, PGM, ACO-A, PGI and IDH-1). The difference between the F_{IS} values was tested with the Wilcoxon signed ranks test for paired samples (Sokal & Rohlf 1995). All F-statistics values are accompanied by their respective 95% confidence intervals estimated by bootstrapping with 1000 replications.

Sample collection for electrophoresis

The Moerputten populations in The Netherlands were sampled in both 1995 and 1996 because of their limited size. It was, however, not possible to collect many individuals of *Maculinea teleius* in 1996, so the sample size is smaller. The Polish Kostrza populations were sampled in 1996 (table 7.2). Butterflies in Poland were captured and put into liquid nitrogen for transport. They were then stored in a freezer at -80°C until analysis. The butterflies collected in The Netherlands were transported alive and immediately stored in the freezer.

Table 7.2: Numbers of sampled butterflies of *Maculinea nausithous* and *Maculinea teleius* in the respective years of sampling.

Site	year	*Maculinea nausithous*	*Maculinea teleius*
Kostrza (P)	1996	41	50
Moerputten (NL)	1995	39	21
Moerputten (NL)	1996	19	0

Procedure and evaluation of reintroduction

Details of the reintroduction are given in Wynhoff (1998a). For the estimation of the population size two different methods were used. Firstly, in several, but not all, years butterflies were marked and recaptured. These data were used to estimate the daily population size as well as the yearly population size by means of two methods: the Minimal Number Alive-method and the method of Jolly (Begon 1979, Amler *et al.* 1999). In this way, a low and a high estimate of population size can be given. Transect counts within the Dutch Butterfly Network were performed in all years (Van Swaay & Veling 1996). *Maculinea teleius* has been counted yearly from 1990 on. By combining transect counts and mark-recapture results, an estimation of the yearly population size is possible. Thus, data from the years when both methods were used, formed the basis for a linear regression, which was then used to extrapolate the yearly population size for years with only transect counts. Since *Maculinea nausithous* established itself on a site other than the release site, we selected additional new transects in 1995.

Estimation of effective population/census size

The effective population size is generally defined as the size of an ideal population that results in a given variance in allele frequencies or amount of inbreeding. Usually it is smaller than the estimated population size. In our case it is not possible to estimate the effective population size by means of the results of the electrophoresis, because the sample size is too small due to the rareness of the butterflies. Furthermore the species appeared to be too monomorph to acquire reliable estimates (Schwartz *et al.* 1998). Therefore we calculated whether a bottleneck effect was to be expected based on the census data and the sex ratio (Hartl & Clark 1989, Avise 1994, Maynard Smith 1998). We can use these population estimates to give upper bounds to the expected rate of loss of genetic variation. For the first year, 1990, it is assumed that all translocated females had already mated before they were captured. Both species are protandrous. After eclosion males almost immediately start searching for females (Pfeiffer *et al.* 2000). They even mate with females who have not yet unfolded their wings. Furthermore, only the males mate several times, while the females usually mate just once. Due to the high population density at the source site, it is very likely that all females mated with different males.

The mean effective population size (based on census data) over a period of years is estimated by calculating the harmonic mean over all effective census sizes of all years between 1990 and 1995 (Avise 1994). We assume random mating and an equal chance for all individuals for progeny in the next generation. This assumption is violated in the first and last days of the flight period when the sex-ratio is biased to one of the genders, but it is likely to be true for most of the time. A low bound of the effective census Ne_{min} size is based on the Minimal Number Alive data, a maximal effective census size Ne_{max} on the Jolly estimates. *Maculinea teleius* was marked and recaptured in 1990, 1991, 1992 and 1995. For the intermittent years when only transect counts took place, the population size was estimated by interpolation based on linear regression (Sokal & Rohlf 1995). For the sex ratio, the mean value of the years before and after was calculated. For *Maculinea nausithous*, the same procedure was followed. However, as the transect counts started later and on other places then where mark-recapture took place, the error in our estimate is larger. Mark-release-recapture studies of *Maculinea nausithous* took place in 1990, 1992, 1993 and 1995.

From the effective census size, the upper bounds of the chance of loosing heterozygosity through genetic drift can be calculated by means of the following formula:

$$H_{ret} = (1-(1/2Ne))^t$$

with : H_{ret} = retained heterozygosity after bottleneck
Ne = effective census size
t = number of generations

Results

Genetic variation of Maculinea teleius *and* M. nausithous *populations*

The genetic variation is very similar between species and populations. The most common alleles are the same in each population. Allele frequencies are also similar. There is only limited evidence for loss of alleles. For *M. teleius* only the locus IDH-2 in the Kostrza-populations segregated for three alleles, PGM, PGI, ACO-A and ACO-K for two alleles, while IDH-1, MDH-1, MDH-3 and GPDH were monomorphic (appendix 7.1). In the Moerputten-population the rare alleles of IDH-2 and ACO-A appear to have been lost. While 11 different genotypes were detected in the Kostrza-population, only 6 genotypes were found in the Moerputten-population. However, the sample size was only about half as large. So, from Moerputten, some of the apparent loss may be an artefact.

In the case of *M. nausithous* only the loci MDH-1 and IDH-2 are monomorphic over both populations. PGI separated with three alleles, all the other enzymes with two. One allele of MDH-2 and of GPDH are not found in the Moerputten population. In MDH-3 a rare allele was found in the Moerputten sample but not in the Kostrza sample (appendix 7.1). Twenty genotypes were detected in each population.

A reduction in the number of alleles can be expected as the result of the reintroduction bottleneck. One locus for *M. teleius* and two for *M. nausithous* have become fixed for the common allele five years after reintroduction. The mean heterozygosity is low in all four analysed populations (appendix 7.2). There were no significant differences between source and reintroduced population except for one locus. The heterozygosity-values for *M. nausithous* were slightly higher than for *M. teleius*. Overall with the possible exception of a limited loss of rare alleles in each species there is no loss of genetic variation.

Only one locus in each species was not in Hardy-Weinberg-proportion for a single sample. The IDH-2 locus is not in equilibrium in the Kostrza population of *M. teleius*, while the MDH-3 locus deviates in the Moerputten sample of *M. nausithous*. No linkage disequilibrium was found in any of the population samples of both species.

Population development after reintroduction

From the regression analysis of transect counts and mark-recapture studies of *M. teleius* a reliable approximation of the total population size per year could be yielded ($N_{min}=a*N_{trans}$, $a=16.3$, $SE_a=0.74$, $p<0,0001$, $r^2=0.99$; $N_{max}=b*N_{trans}$, $b=24.4$, $SE_b=1.3$, $p<0.0001$, $r^2=0.99$) (figure 7.2). The minimal and maximal population sizes of *M. teleius* and *M.nausithous* are presented in figure 7.3. The population structure of *M. teleius* was quite similar in each year from 1991 to 1995, when the sampling for the genetic analysis took place. The population increased from the reintroduction event up until 1992. Then, possibly due to overexploitation of the ant nests, the butterfly population decreased to about 300 individuals. Over all years on average 62.7(± 7.7)% of the captured butterflies were females. The mean effective population size derived from the census size is as high as 94.5 (± 2.5)% of the total census size.

The population of *M. nausithous* suffered a second potential bottleneck in 1991 when only 8 individuals were encountered. Since then the population increased approximately linearly. The sex ratio was close to equality (59.7(± 7.8)% females). The mean effective census size is again quite high: 95.9 (± 4.5)% of the population size. However, due to the second bottleneck, the mean effective population size is only 38 to 40 individuals. A detailed overview of the population development of *Maculinea teleius* and *M. nausithous* is given in Wynhoff (1998a).

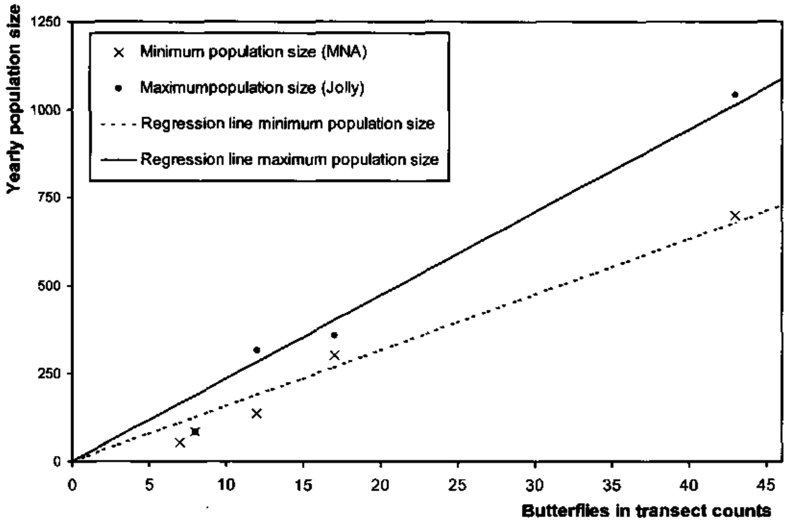


Figure 7.2: Regression line of transect counts and mark-recapture studies of *Maculinea teleius.*

Table 7.3: Predicted effect of bottleneck passed by the reintroduced populations of *Maculinea teleius* and *M. nausithous* in 1990. Ne: effective census size; H_{ret}: heterozygosity retained after bottleneck.

	Maculinea teleius	*Maculinea nausithous*
Nr. of introduced females	53	48
Nr. of introduced males	33	22
Ne (2x females)	106	96
H_{ret}	0.995	0.995

Predicted effect of reintroduction bottleneck

Based on the number of translocated individuals, the expected maximum proportion of heterozygosity retained after the artificially introduced bottleneck can be estimated following the above mentioned formula (table 7.3). We also estimated the expected loss for the case that all females would have mated with the same male, which is very unlikely. Furthermore, a limited number of females may have been able to oviposit. However, we observed many ovipositions from different females immediately after release.

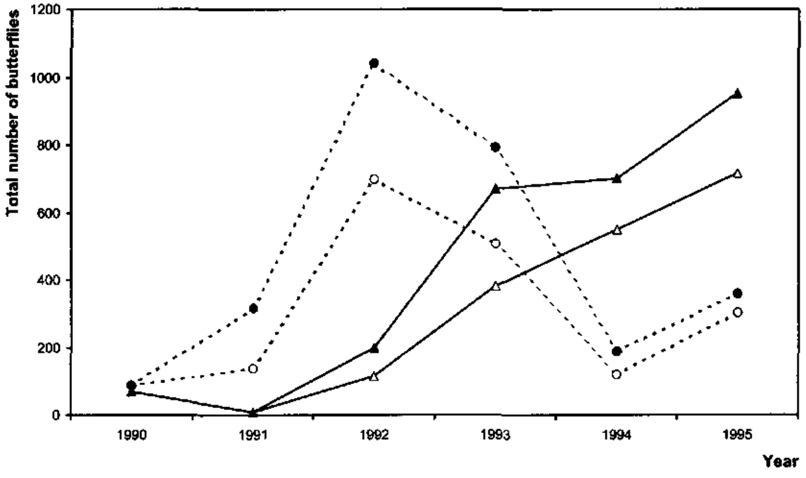


Figure 7.3: Population development (in total population numbers per year) of *Maculinea teleius* (circles) and *Maculinea nausithous* (triangles) after reintroduction in 1990.

open symbol: minimum population size
closed symbol: maximum population size

Thus, the number of translocated individuals is likely to have been high enough to prevent any significant loss of genetic variation due to the reintroduction bottleneck. However, we wished to check this using the direct estimates of genetic polymorphism.

Genetic differentiation of Kostrza and Moerputten-populations: the possible bottleneck effect

Although being separated for only five generations the populations of Kostrza and Moerputten have already become differentiated at the ACO-K locus (Fisher's Exact p=0.0062 for *M. nausithous* and p=0.0003 for *M. teleius*). At this locus the F_{ST}- value as a measure of population subdivision is much higher compared the value found for the other loci (appendix 7.3). Over all loci the populations do not show significant differentiation, Nei's genetic distance between the populations is for *M. teleius* estimated at 0.003 and for *M. nausithous* at 0.004. Thus the data suggest that some direct selection has occurred on polymorphism at the ACO-K locus or that there is strong linkage disequilibrium and hitch-hiking.

The F_{IS} value represents deviation from local panmixis and as such can be interpreted as an inbreeding coefficient. If a population has passed a bottleneck the F_{IS} value is expected to increase. For both species the mean F_{IS} values calculated for the polymorphic loci present in source and founder population, are not significantly different from zero in the Polish source populations. In the Moerputten populations, they were negative and differed significantly from zero (figure 7.4). However, no significant difference to the source population's F_{IS} values was found (Wilcoxon paired samples: p=0.068 for *M. teleius* and p=0.225 for *M. nausithous*). The observed heterozygosity was higher in the Moerputten population compared to the Kostrza population, though not significantly. If the F_{IS} values for both species are pooled (to estimate an overall inbreeding effect of the reintroduction), there is an overall decrease in inbreeding coefficient (Wilcoxon paired samples p=0.038). Except for *M. teleius* at the Moerputten site (r=-0.999, p=0.001,not correlated. A decrease in F_{IS} in combination with an increase in heterozygosity can be achieved by outbreeding or heterozygous advantage.

Discussion

Effects of reintroduction bottleneck

When a small group of animals or plants is isolated for whatever reason from their ancestral population, a divergence of the separated gene pools is

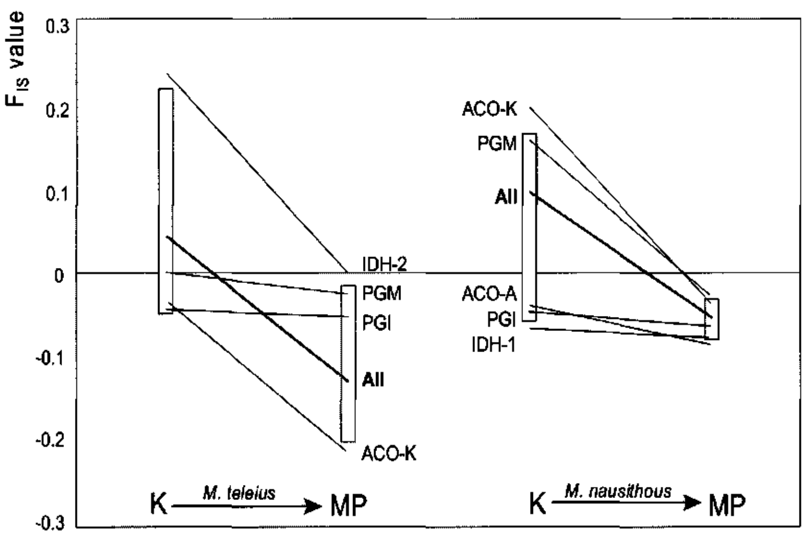


Figure 7.4: Changes in inbreeding coefficient F_{IS} of source population and new population of *Maculinea teleius* (left side) and *M. nausithous* (right side) after five years of separation since reintroduction in 1990. Bars represent 95% confidence intervals estimated by bootstrapping (1000 replications).
K = Kostrza (source population), MP = Moerputten (reintroduced population)

expected, with the smaller or more isolated group tending to loose variation. Saccheri *et al.* (1998) were able to show that this process is also detectable in the field for the butterfly species *Melitaea cinxia*. After a severe reduction in effective population size, genetic drift is an important evolutionary force and rare alleles tend to be lost through chance. However, a bottleneck effect is only detectable when the bottleneck has reduced the size of the breeding population usually severely and especially when this has persisted over n=4), F_{IS} and heterozygosity were several generations (Saccheri *et al.* 1996, Van Oosterhout *et al.* 2000, Van Hooft 2001). For *Maculinea nausithous* and *M. teleius* the separation of gene pools since reintroduction has endured for only five generations at the moment of sampling. Large genome-wide genetic changes are unlikely after such a limited time span in regions where ecological conditions are supposed to be similar and when the effective number of translocated individuals was quite high. The analysed allozymes indicate that the losses have indeed been mild and with respect to this, the reintroduction did not represent a severe bottleneck to the populations. Loss of heterozygosity, as was found for the butterfly *Proclossiana eunomia* (Barascud *et al.* 1999), has probably been prevented by the high number of founders and the successfull, rather rapid establishment. If nearly all of the founding females laid viable eggs any expected loss of genetic variation would be minimal and be impossible to detect using limited electrophoretic data. However, if only a few individuals were successful in leaving offspring and/or subsequent generations were of very small effective size, the genetic effects would be likely to be detectable. Furthermore, in one experimental study, post-bottlenecked populations showed an increase in heterozygosity through heterozygous advantage, even though alleles had been lost by suffering a strong bottleneck (Leberg 1992). Both reintroduced populations of *Maculinea*, however, have lost several rare alleles. Even in *M. nausithous*, which is thought to have experienced smaller population sizes during establishment, of the six alleles detected at a frequency of <10% in Poland, four were detected in the Moerputten population after 1991 (and an additional rare allele which was undetected in the Polish sample). These data also indicate that in this species the effective population in 1991, the year after release, was higher than the 8 butterflies observed in the field.

Implications of low genetic variation

Both studied *Maculinea* species show a low but not exceptionally low level of genetic variation as compared to that found for polymorphic enzyme loci of other butterfly species (Barascud *et al.* 1999; Napolitano & Descimon 1994; Goulson 1993; Brookes *et al.* 1997; Gadeberg & Boomsma 1998; Van Dongen *et al.* 1998; Clarke & O'Dwyer 2000; Figurny-Puchalska *et al.* 2000). In reality, genetic variation might even be lower because there is a bias in the allozymes chosen for the study: most monomorphic enzyme systems have been omitted.

Having sufficient genetic variation is often thought to be a prerequisite to successfully translocating a part of a population to another site and retaining potential for adaptive evolution to changing conditions (Hunter 1996). Thus, even though they are seriously threatened (Van Swaay & Warren 1999; Wynhoff 1998b), *Maculinea* species might intrinsically not be very suitable for reintroduction. Figurny-Puchalska *et al.* (2000) also found low levels of genetic variation in the Kostrza-populations, but other Polish and Russian populations were more polymorphic. It is possible that the source population could have been subject to a relatively recent bottleneck event, as has been indicated for other butterfly species too (Meglécz *et al.* 1999). However, the success of the reintroduction at Moerputten shows that even though both species show a low level of polymorphism using the limited methodology of allozym electrophoresis, it was not a constraint to their successful establishment. In the Wisla valley both *Maculinea* species are abundant but *M. teleius* occurs in higher numbers than *M. nausithous*. Hence based on census sizes for *M. teleius* more variation would be expected but less was found. Perhaps, due to its specialised ecology, this species is more sensitive to catastrophes. *M. nausithous* seems to be more robust: even though it has experienced smaller numbers, genetic losses have been less compared to *M. teleius*. In Poland, this robustness was expressed in the lack of genetic differentiation with increasing spatial scale (Figurny-Puchalska *et al.* 2000).

On the other hand a low level of heterozygosity cannot always be considered a problem for the viability of populations or for the fitness of individuals, as there are many rare and common species lacking a high level of heterozygosity while other rare or endangered species have been shown to have normal or high levels of heterozygosity (Avise 1994). So in general, a prediction of survival of bottlenecked populations merely based on their genetical variation is not possible. Dunham *et al.* (1999) suggest that it is likely that species with limited within population variability, or non-equilibrium population structure may be well adapted to persist in the face of extreme isolation or small population size. This may be the case for species that inhabit naturally isolated, but relatively stable habitats. Obligate ant parasite butterfly species, which are adapted to specific habitat conditions and cannot survive in a changing environment, such as *M. teleius* living in *Myrmica scabrinodis* nests on wet meadows may have such adaptations as well. The example of the reintroduction of the beaver to Sweden shows that a species with low genetic variation can survive and even spread sucessfully over many years and generations (Ellegren *et al.* 1993). This case of the *Maculinea*-butterflies also demonstrates through the increase in population size, the balanced sex ratio and the low chance of loosing heterozygosity in the founder population, that the Kostrza populations as source population for a reintroduction project was a good choice, at least in the short term. It is probably most important to enable a good start for a reintroduction project by releasing many founder individuals and by finding a release site which fullfills all ecological demands of a species.

Possible selection on the founder populations

Although the variation in these *Maculinea* species is rather low, at least for a very limited group of enzyme loci, there is no reason to suppose that this will impede adaptive evolution in response to the different ecological conditions in The Netherlands. Indeed we observed evidence for such a process both for one of the enzyme loci (ACO-K) and for phenology. Changes in the allozyme frequencies were not dramatic and only occurred at one locus. At the ACO-K locus the populations of *Maculinea nausithous* and *M. teleius* show a significant differentiation between the ancestral Kostrza-population and the newly founded Moerputten population. The population studies at the reintroduction site also revealed that there was a gradual forward shift in the flight period from of *M. teleius* and to a lesser extent in *M. nausithous*, with the flight period in the Netherlands peaking earlier than in Poland and than the historical Dutch populations (Wynhoff 1998a). The observation of a gradual shift suggests that this is unlikely to have resulted purely from a response in phenotypic plasticity. Furthermore, the mean minimal life time per captured individual has decreased strongly in the Moerputten population of both species (Wynhoff 1998a). These changes point in the direction of different ecological and environmental conditions and the potential for selection. Conditions at the new site were probably quite different from those in the Wisla-valley in Poland favouring adaptation to the new environment. Selection has also been shown to influence enzyme polymorphism in other butterfly species (McKechnie *et al.* 1975, Watt 1977, Watt *et al.* 1986, Goulson 1993).

Genetic differentiation between populations

Within Europe, *Maculinea nausithous* is more common than *M. teleius* and the population size is also often much higher. Furthermore, *M. nausithous* can survive for long periods with small populations of less than 100 individuals on marginal habitats such as road verges and canal borders (Settele 1998; Wynhoff 1998b) while *M. teleius* is restricted to larger and less dynamic sites. Populations of *M. nausithous* are considered to be more connected with higher rates of interchange. Hence, in the source population the F_{ST} value, representing the variance in allele frequencies between subpopulations and therefore being a measure of population subdivision, should be lower in *M. nausithous* as compared to *M. teleius*. This is indeed confirmed by our analysis.

After five years of separation, no significant differentiation expressed in F_{ST} value between source and founder population in either species could be detected. If the differentiating Aco-K is excluded from the F-statistics, values are closer to zero, but the conclusion still holds. On a higher regional level significant population structure in both species by a relatively high F_{ST} value has been suggested (Figurny-Puchalska *et al.* 2000), but there was no isolation by distance.

Effects of inbreeding

Small populations are especially prone to loss of genetic variation by inbreeding, which can be shown by a significant positive F_{IS} value. The probability of matings between relatives increases in smaller randomly mating populations. For both *Maculinea* species, the significant negative F_{IS}

in the comparably smaller, newly founded Moerputten population indicates that inbreeding may be avoided. This is unexpected and at the same time could perhaps be important as the founder populations are completely isolated. Apart from chance, negative F_{IS} values can be explained in two ways:
(i) outbreeding: relatives avoid mating with each other,
(ii) heterozygote advantage: in the new environment the heterozygote has a higher fitness compared to the homozygote. If outbreeding or heterozygous advantage were an explanation for the decrease in F_{IS} value, it should be accompanied by an increase in heterozygosity. In both species this was not found. Furthermore, inbreeding affects all loci similarly, hence F_{IS} values should be similar at all loci in a given population. We did not observe such a pattern in any of the populations. The decrease in F_{IS} values is consistent in both species, with the steepest decrease in the loci with the highest value. When we accept selection as responsible for the changes in the Moerputten population compared to the Kostrza population, then the Aco-K allele has to be excluded from the F-statistics. The trend then remains similar for *M. nauithous.* In the case of *M. teleius* in the Moerputten population, the detected differences are only very weak. Thus the F_{IS} values may hint at some active or inactive avoidance of inbreeding in the Moerputten population, although the mechanism is unknown.

Conclusions

We conclude that the reintroduced populations of *Maculinea nausithous* and *M. teleius* in the Moerputten did not pass through a bottleneck in terms of a low number of founder individuals. The low levels of genetic variation of the source populations did not have a negative impact on the settling and subsequent increase of the founder populations. The number of translocated founder individuals and the quality of the release site have a higher impact on success than the genetic constitution of the founders. The differentiation between source and founder population since reintroduction, in the allozymes as well as in flight period and life time, are probably due to selection. In reintroduction projects with this type of species, it is important to create as soon as possible a metapopulation at the release site. In this way stochastic extinction of subpopulations can be compensated for because in the metapopulation, gene exchange between subpopulations is possible.

Acknowledgement

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Appendix 7.1: Allele frequencies of *Maculinea teleius* and *M. nausithous* at Kostrza and Moerputten. *(N)* = sample size, n_A = total number of alleles, n_L= mean number of alleles per locus, H = mean heterozygosity per locus (direct count), P_{DC} = mean percentage of polymorphic loci (no criterion), P_{95} = mean percentage of polymorphic loci (95% criterion).

		Maculinea teleius		*Maculinea nausithous*	
Locus	Allele	Moerputten	Kostrza	Moerputten	Kostrza
		frequency±SE	frequency±SE	frequency±SE	frequency±SE
PGM	*(N)*	21	50	58	41
	A	0	0	0.276±0.042	0.220±0.046
	B	0.952±0.033	0.990±0.010	0.724±0.042	0.780±0.046
	C	0.048±0.033	0.010±0.010	0	0
PGI	*(N)*	21	48	58	41
	A	0.071±0.040	0.052±0.022	0.043±0.019	0.049±0.024
	B	0.929±0.040	0.948±0.026	0.914±0.026	0.927±0.029
	C	0	0	0.043±0.019	0.024±0.017
ACO-K	*(N)*	21	50	58	41
	A	0.190±0.061	0.040±0.020	0.957±0.019	0.793±0.045
	B	0.810±0.061	0.960±0.020	0.043±0.019	0.207±0.045
ACO-A	*(N)*	21	50	58	41
	A	0	0.090±0.029	0.086±0.026	0.049±0.024
	B	1.000	0.910±0.029	0.914±0.026	0.951±0.024
IDH-2	*(N)*	21	50	58	41
	A	0.024±0.024	0.030±0.017	0	0
	B	0.976±0.024	0.960±0.017	1.000	1.000
	C	0	0.010±0.010	0	0
IDH-1	*(N)*	21	50	58	40
	A	1.000	1.000	0.922±0.025	0.927±0.033
	B	0	0	0.078±0.025	0.073±0.029
MDH-3	*(N)*	21	50	58	41
	A	1.000	1.000	0.017±0.012	0
	B	0	0	0.983±0.012	1.000
MDH-2	*(N)*	21	50	58	41
	A	0	0	0	0
	B	1.000	1.000	1.000	0.988±0.012
	C	0	0	0	0.012±0.012
GPDH	*(N)*	21	50	57	40
	A	1.000	1.000	1.000	0.988±0.017
	B	0	0	0	0.013±0.012
MDH-1	*(N)*	21	50	58	41
	A	1.000	1.000	1.000	1.000
	n_A	14	16	17	18
	n_L	1.40±0.16	1.60±0.22	1.70±0.21	1.80±0.20
	H	0.067±0.038	0.044±0.019	0.100±0.042	0.100±0.035
	P_{DC}	40	50	60	70
	P_{95}	20	20	40	40

Appendix 7.2: Observed (H_{obs}) and expected heterozygosity (H_{exp}) per locus for *Maculinea teleius* and *M. nausithous*. (Total number of alleles: 10).* = no test available.

Site	locus	H_{obs} mean±SD	H_{exp} mean±SD	χ^2	df	p
Maculinea teleius						
Moerputten						
	PGI	0.143±0.076	0.136±0.066	0.081	1	0.776
	PGM	0.095±0.064	0.093± 0.059	0.026	1	0.873
	IDH-2	0.048±0.047	0.048± 0.045	0.000	1	1.000
	ACO-K	0.381±0.106	0.316± 0.067	0.998	1	0.318
	mean	*0.067±0.038*	*0.059±0.030*			
Kostrza						
	PGI	0.104±0.044	0.100±0.040	0.115	1	0.735
	PGM	0.020±0.020	0.020±0.020	0.000	1	1.000
	IDH-2	**0.060±0.034**	**0.078±0.041**	*		
	ACO-A	0.180±0.054	0.165±0.045	0.431	1	0.521
	ACO-K	0.080±0.038	0.078±0.035	0.064	1	0.800
	mean	*0.088±0.018*	*0.044±0.017*			
Maculinea nausithous						
Moerputten						
	PGI	0.172±0.050	0.163±0.044	0.461	1	0.927
	PGM	0.414±0.065	0.403±0.037	0.043	1	0.836
	MDH-3	**0.000±0.000**	**0. 034±0.033**	*		
	IDH-1	0.155±0.048	0.144±0.040	0.362	1	0.547
	ACO-A	0.172±0.050	0.159±0.041	0.461	1	0.497
	ACO-K	0.086±0.037	0.083±0.034	0.093	1	0.760
	mean	*0.100±0.023*	*0.099±0.016*			
Kostrza						
	PGI	0.146±0.048	0.140±0.050	0.211	3	0.976
	PGM	0.293±0.071	0.347±0.056	1.050	1	0.305
	MDH-2	0.024±0.024	0.024±0.023	0.000	1	1.000
	IDH-1	0.146±0.056	0.141±0.048	0.211	1	0.646
	ACO-A	0.098±0.046	0.094±0.042	0.080	1	0.777
	ACO-K	0.268±0.069	0.333±0.057	1.619	1	0.203
	GPDH	0.025±0.025	0.025±0.024	0.000	1	1.000
	mean	*0.100±0.019*	*0.110±0.017*			

Appendix 7.3: Estimates of Wright's F-statistics. The mean values per locus are given. The confidence intervals of the mean value over all loci are obtained by bootstrapping. The F_{IS} values are presented in figure 7.4.

Locus	F_{IT}	F_{ST}
Maculinea teleius		
PGM	-0.0043	0.0174
IDH-2	0.1742	-0.0175
PGI	-0.0621	-0.0131
ACO-A	-0.0293	0.0511
ACO-K	-0.0085	0.1225
mean	*-0.0002*	*0.0489*
Bootstrapping over loci (C.I. 95%)		
upper	0.0822	0.0990
lower	-0.0414	-0.0114
Maculinea nausithous		
MDH-2	0.0022	0.0044
PGI	-0.0642	-0.0081
MDH-3	1.0000	-0.0062
PGM	0.0406	-0.0025
ACO-A	-0.0705	0.0010
ACO-K	0.2353	0.1172
GPDH	0.0023	0.0046
IDH-1	-0.0922	-0.0096
mean	*0.0519*	*0.0202*
Bootstrapping over loci (C.I. 95%)		
upper	0.1845	0.0751
lower	-0.0494	-0.0067

Synthesis

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On the warm evening of July 30th, 1990, the first official butterfly reintroduction in The Netherlands took place. Jan van der Made, Chris van Swaay, Ivo Raemakers and I released *Maculinea teleius* and *Maculinea nausithous* butterflies on the meadows of the nature reserve Moerputten. Now, ten generations later, after warm and wet summers have passed, the time has come to make an evaluation. Was the reintroduction of these butterflies a success or a failure, is reintroduction a useful and applicable technique in nature conservation? Are *Maculinea* butterflies still under threat of extinction? What have we learned about their ecology while monitoring and studying the newly established populations? In this chapter I will put all the small pieces of the *Maculinea* story together so as to obtain a more complete picture.

The success of the reintroduction

After the reintroduction, the newly founded populations of *Maculinea teleius* and *M. nausithous* behaved differently. The introduced population of *M. teleius* expanded during the first three years. Then, adult numbers decreased and stabilised on the occupied meadow BW (figure 6.1, figure 7.3). This process happened twice, since in 1995 the meadow was accidentally mown in the flight period. It resulted in the reduction of adults in 1996 to the level of the founder population at the reintroduction. No other meadow was occupied, since there was almost no long distance dispersal after 1993. Occasional dispersal was observed but was not followed up by the establishment of a new subpopulation. Apparently, in the existing conditions at meadow BW, the yearly population size has found its limit with about 300 to 400 individuals (Wynhoff 1998 b, chapter 3). An increase in population size is most likely only possible through dispersal and establishment at unoccupied patches.

In contrast, the newly established population of *M. nausithous* declined in numbers in the year after the reintroduction but expanded later. After a considerable increase in numbers, this species colonised the road verges at the southern side of the nature reserve. Later, a third subpopulation was established at a distance of 5 kilometers to the west (Wynhoff 1998 b, chapter 3). In The Netherlands, a reintroduction of butterfly species is considered successful when the introduced population can persist for at least five years without further intervention (Wynhoff & Van Swaay 1995). In a new edition of the Red List, both *Maculinea teleius* and *M. nausithous* would not be listed as extinct species any more.

Unfortunately, adult numbers of *Maculinea nausithous* are now decreasing, a decline which began a few years ago. At the embankment, this is due to vegetation succession through which the occupied patch deteriorates while there is no suitable location within reach of the butterflies available to relocate the population. On the road verges, the decline is clearly due to human activities. Management by (local) authorities and local residents and reconstructions of the verges have destroyed large parts of the vegetation which reduced survival of the reintroduced species and the host ants. This development clearly shows, that the reintroduction of rare butterfly species into agricultural landscapes is risky. Local authorities and residents should be informed and their full support should be gained. After all, they shape the habitat in which the newcomers have to survive (Oostermeijer & Wynhoff 1996, Wynhoff & Jansen 2000).

In the Moerputten nature reserve, *Maculinea teleius* and *M. nausithous* occur spatially separated, while in many regions they sympatrically inhabit the same meadow, for example in the Kostrza source population (Ebert & Rennwald 1991, Wynhoff 1998b, Figurny & Woyciechowksi 1998). At the reintroduction site, a typical habitat of *Maculinea teleius* consists of *Molinietalia* vegetation with a strong *Junco-Molinion* aspect. It consists of a rather short cover up to a height of about 50 cm on a comparably poor soil with a high density of the host ant *Myrmica scabrinodis*. The nutrient poor vegetation communities belong to *Junco-Molinion* with high abundances of *Succisa pratensis* and *Juncus conglomeratus*. Locally at the meadow BW, where there are high abundances of *Cirsium dissectum* and *Carex panicea,* the vegetation can be classified as *Cirsio dissecti-Molinietum*. In The Netherlands, it is found on moist, slightly acidic soil, that is inundated during winter time.

Within these vegetation types, the host plant *Sanguisorba officinalis* can occur in high densities with on average 10 (SD=7) plants with a mean of 17 (SD=15) flowerheads per square meter on the meadow BW. On the other meadows at the southern side of Moerputten, the density of plants as well as flowerheads is higher. However, density and quality of host ants' nests are the main factors determining butterfly behaviour, butterfly density and survival of the larval instars (see chapter 4 to 6). There can be a considerable variation in host ant nest density within and between sites. The source population in Kostrza persisted on *Myrmica scabrinodis* nest densities between 0.1 and 1.2 nests per square meter. At the Dutch site, densities are approximately the same with 0.80 nests per square meter (Wynhoff 1992). At the continental scale, the most important host ant species is *Myrmica* *scabrinodis*, but also *Myrmica rubra* can be exploited as a secondary host (Chardon & de Boer 1994, Ebert & Rennwald 1991, Thomas *et al.* 1989, Figurny, pers. comm.). At the reintroduction site, however, *Maculinea teleius* appaers to be restricted to *Myrmica scabrinodis.* As the behaviour of *Maculinea teleius* caterpillars in the ant nests is similar to *Maculinea arion* (Thomas & Elmes 1998), a high density of relatively small nests of *Myrmica scabrinodis* probably yields the highest survival.

The vegetation on the meadows WH, AH, PN, PZ, DH and HvB consists of a mozaic of *Molinietalia* and *Arrhenateretalia* types with patches rich in herbs or rich in sedges and grasses. Low lying locations under influence of base-rich seepage show aspects of *Calthion palustris* with *Lychnis flos-cuculi, Rhinanthus angustifolius* and *Lotus uliginosus.* On higher and drier sites the typical hay-communities *Arrhenaterion elatioris* and *Alopecurion pratensis* occur closely together. The density of plants and flowerheads of *Sanguisorba officinalis* is higher than on the occupied meadow BW, however, the host ant *Myrmica scabrinodis* occurs only locally and with a lower ant nest density on these meadows. If the occurence of *Maculinea teleius* and *M. nausithous* were only dependent on the availability of the preferred flowerbuds in an acceptable phenological stage during the flight period, as proposed by Thomas & Elmes (2001), both butterfly species should be thriving there sympatrically.

In the same nature reserve, *Maculinea nausithous* prefers different conditions than *Maculinea teleius* does. On preferred sites, a species-poor *Arrhenaterion elatioris* vegetation, also with characteristics of *Artemisietea,* is found. The vegetation structure is taller and less open compared to sites with *Maculinea teleius.* On the roadverges MPP and RD, these latter types also occur, even though typical herb species are missing and locally vegetations resemble *Cynosurion* and *Plantaginetea majoris.* The communities from the railway embankment SD include *Arrhenateretalia* patches with typical members of the *Artemisietea* and *Galio-Urticetea,* such as *Urtica dioica* and *Cirsium arvense. Sanguisorba officinalis* occurs in all these vegetation types, albeit in various abundances (Ten Oever & Brongers 1994, Zuidhoff *et al.* 1996, Schaffers & Sýkora 2000). On the embankment, a mean density of 3.7 (SD=3.9) plants was found, with as much as 42.5 (SD=41.8) flowerheads per square meter. On the road verges, fewer plants but more flowerheads were available for the butterflies (plants: 8.6±6.5, flowerheads 77.8±53.7).
While for *Maculinea rebeli* a positive relationship between the population size of the host plant *Gentiana cruciata* and that of the butterfly was found (Kéry *et al.* 2001, Elmes *et al.* 1996, Hochberg *et al.* 1994), such a relationship

does not exist for *Maculinea teleius* and *M. nausithous* and their respective hostplant (chapter 5).

At the site naturally colonised by *Maculinea nausithous*, the highest nest density of its host ant *Myrmica rubra* was found, with up to 6 nests per square meter. On all locations colonised by this butterfly species, *Myrmica scabrinodis* was also found. However, sites with only the latter ant species were not colonised. At the Kostrza source population site, the mean density of *Myrmica rubra* is 0.4 nests per square meter. However, at that particular location, both ant species co-occur.

The successful reintroduction also shows that the communication between the "Polish" caterpillars and the "Dutch" ants is functioning. Lycaenid larvae which enter ant nests to predate on ant brood could never do so without having the appropriate communication means (Fiedler *et al.* 1996). The caterpillars of *Maculinea rebeli*, an advanced predator of *Myrmica schencki*, produce surface chemicals which enable them to be mistaken for their host ants' brood and be taken to the nest. The mimetic chemicals resemble those employed by *Myrmica* to recognize conspecifics and are very similar to those of the specific host ant species (Akino *et al.* 1999). For another advanced parasite, *Maculinea alcon*, differences in these pheromone-like chemicals were found between populations parasitising on different *Myrmica* host ant species in Denmark (Nash, pers. comm.). This phenomenon suggests adaption at local level. Obviously, in the primitive parasites *Maculinea teleius* and *Maculinea nausithous*, pheromones of parasite and host ant are similar within large parts of their range. The same can be concluded for Danish *Maculinea arion* butterflies which were released into an English site with *Myrmica sabuleti* (Thomas 1989, 1991, 1995). Furthermore, the lack of parasitoids in the reintroduced butterfly populations might also give an additonal reason for the rapid increase of adult numbers. However, in the large source populations, the impact of *Maculinea* specific parasitoids is small or restricted to certain years (Chardon & De Boer 1994), and we don't think that the parasitoid free space has contributed much towards the success of the reintroduction.

Oviposition behaviour and habitat selection

An egg of a *Maculinea* butterfly is not being deposited until the female has selected a suitable oviposition site. The intention to deposit an egg is already detectable in the way a female flies: she flutters slowly and low in the vegetation between the flowerheads that emerge from the dense vegetation cover. The flight direction changes frequently. Often, a flowerhead of *Sanguisorba officinalis* in an acceptable phenological state is approached but when close, the female suddenly changes direction and flutters to another one. Sometimes, a flowerhead is inspected more intensely by flying several loops around it, sometimes the females would alight and inspect it with her antennae or the abdominal tip. All this time a rejection is still possible and usually several flowerheads are rejected before a suitable one has been found to proceed with egg-laying. This behaviour is very similar in *Maculinea nausithous* and *M. teleius*. The acceptable phenological state of the flowerhead when finally depositing an egg, is different between the species (Thomas 1984a, Figurny & Woyciechowski 1998). It is important for the oviposition decisions of the females. However, next to this, the complicated life cycle of both butterfly species suggest that the presence of host ants is of equal importance.

In many occupied habitats the host plants are abundant while the host ant is relatively rare, so that most host plants are in fact unsuitable for oviposition due to a lack of ants in their close vicinity. Survival of the progeny is initially dependent on the availability of host ants' nests in the close proximity of the host plant. Therefore, it would give females a comparative selective advantage and fitness increase, if they could detect host ants so as to deposit eggs on truly suitable *Sanguisorba* plants only. For the source population at the Kostrza site, pooling all data for the whole flight period, we found that *Maculinea nausithous* females aggregate and oviposit in places where nests of their host ant, *Myrmica rubra* are present (figure 8.1). Neither the density of *Sanguisorba officinalis* plants, nor the number of flowerhead in bud or in flower appeared to help to explain the oviposition density. *Maculinea teleius* females oviposit independently of the density of the nests of the host ant *Myrmica scabrinodis*. The oviposition density was explained by the number of host plants and the number of flowerheads in bud. Most ovipositions were detected on patches with low vegetation. These differences lead to the hypothesis that *Maculinea nausithous* can detect its host ant, while *Maculinea teleius* reacts to vegetation characteristics (Wynhoff 2001, chapter 4).

The hypotheses mentioned above were further tested in an insectory experiment, where females of both species were free to choose between host plants on plots with *Myrmica rubra* or *Myrmica scabrinodis,* or without ants. We initially observed the ovipositing females. Two weeks later, all flowerheads were enclosed in small netting sacs to capture the emerging caterpillars. Oviposition and caterpillar captures show that *Maculinea teleius*

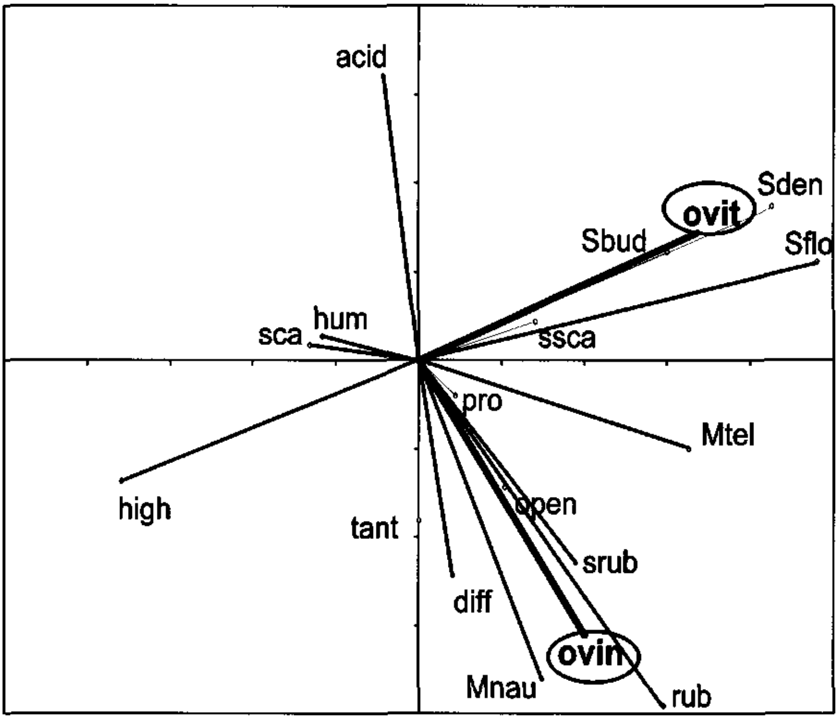

Figure 8.1: Result of PCA analysis of oviposition observations of *Maculinea nausithous* (=ovin) and *M. teleius* (=ovit). Explanatory variables are: mean number of *M. nausithous* (=Mnau) and *M. teleius* (=Mtel) butterflies, number of *Myrmica rubra* (=rub) and *Myrmica scabrinodis* (=sca) nests, total number of *Myrmica* nests (=tant), number of *Myrmica rubra* (=srub) and *Myrmica scabrinodis* (=ssca) workers, number of *Sanguisorba officinalis* plants (=Sden), *Sanguisorba* flowerheads in bud (=Sbud), *Sanguisorba* flowerheads in bloom (Sflo), height of *Sanguisorba* plants (=high), height difference between *Sanguisorba* and sward (=diff), Ellenberg value for humidity (=hum), acidity (=acid) and productivity (=pro) and the percentage of bare soil (=open).

selects hostplants on plots with *Myrmica*. *Maculinea nausithous* responds to vegetation structure and flowerhead phenology characteristics, because the deveplopment of the offered hostplants was very early. For both butterfly species the presence of their host ant species is important in selecting flowerheads for oviposition. This effect is clear for the first ovipositions while later phenological characteristics of the flowerheads may become more important. Thus, the results of the experiment provide further support for the presence of the host ant species having an impact on the oviposition behaviour of the females. However, they fail to give indisputable evidence.

Habitat selection of Maculinea teleius and Maculinea nausithous

Maculinea teleius and *M. nausithous* that parasitize as caterpillar in the nests of the ant species *Myrmica scabrinodis* and *M. rubra* respectively, have narrowly defined habitat requirements (see chapter 1). Therefore, one would expect that individuals are able to select for sites that meet their requirements. Both butterfly species occur in habitats where their initial larval resource, the host plant *Sanguisorba officinalis*, is abundant while the ant nests are in comparison less abundant. From this unequal occurrence in resources and because the home range of the *Myrmica* colonies is only limited, it follows that a part of the host plant population represents a sink when selected for oviposition. In the case of *Maculinea* butterflies, only host plants within the home range of a host ants nest represent a true resource, while host plants out of the home range are sinks. After all, in the proximity of a host plant being a resource, the caterpillar has a chance of being found by its host ant species, while in a sink this *Myrmica* species is not present and therefore the caterpillar will die. On a selection of more than 600 plots in and around Moerputten nature reserve, the impact of presence or absence of host ant nests, vegetation composition, vegetation structure and microclimate on the oviposition of females and on the distribution of the population was studied. It appears that females of both species prefer to deposit eggs on host plants in the close vicinity of ant nests. Furthermore, adults also select for plots with host ants. Plots with good vegetation characteristics but lack of ants were sometimes occupied, but only in years with high butterfly densities. Compared to the results of the oviposition experiment (chapter 4), these data are much more convincing. They show, that it is the ants that are largely determining habitat quality and that distribution patterns are a result of an active choice of the females rather than a sieve in the survival of the final instar caterpillars before they enter the host ant nests. Hence, the random oviposition hypothesis has to be rejected in favour of the ant-mediated oviposition hypothesis. *Maculinea* butterflies, at least *Maculinea teleius, M. nausithous* and *M. alcon*, are, like most parasites, able to find their host. A straight forward calculation with the limited knowledge available on the mortality of the larval instars shows that the success of the initial phase of the reintroduction is largely attributed to the ant-mediated oviposition. Adding an extra mortality of 70% due to random oviposition on sinks to the high mortality that the larval instars already experience, would have made a reintroduction almost impossible (chapter 5).

For *Maculinea teleius* we have also additional anecdotal evidence suggesting ant-mediated oviposition from field observations. Sometimes, though only rarely, females were observed when they left the meadow BW. We followed these females without apparently disturbing them. In several cases the female started the typical flight behaviour when looking for hostplants and were observed depositing an egg. After oviposition, we always searched for *Myrmica* ant nests in the direct surroundings of the chosen plant. We always found one within a distance of 2 m. Unfortunately, these ovipositions took place on meadows with traditional mowing in the beginning of August, when the caterpillars are still on the hostplant and will be removed with the hay.

The spring and the early summer of the year 2000 were very cold and wet. Therefore the *Sanguisorba officinalis* plants were not in bud when the flight period of *Maculinea teleius* started. The first females had mated but almost no flowerheads were available to deposit eggs. During two days we observed five ovipositions on the leaves and stems of *Sanguisorba officinalis* and *Vicia sepium*. With sugar baits next to the stem of the used host plant we attracted *Myrmica* ants within a short period of time. Apparently, even when accepting unsuitable hostplants, the females still favour locations with their host ants. However, this behaviour is unambiguously statistically testable only in the beginning of the flight period, when in years with normal weather conditions there is an overabundance of hostplants available. Later when many flowerheads are already occupied with one egg, others further away from ant nests will be accepted too (Van Dyck *et al.* 2000, Wynhoff *et al.* chapter 4). The females of *Maculinea teleius* rather accept a lower adoption chance than leaving the meadow and finding host plants with *Myrmica scabrinodis* nests nearby. Thus, when all data over the whole flight period are combined, for this species no correlation between oviposition events and presence of host ant nests will be found. This was indeed the case in the source population in Kostrza (chapter 4) and in the Moerputten population (Wynhoff, unpublished data from 1993).

However, our data also show, that vegetation characteristics are also an important aspect of habitat selection. Thus, those host ant nests, which are located in vegetation that are avoided or used to a lesser extent by the butterflies, can escape parasitism (Hochberg *et al.* 1994). Since infestation of a *Myrmica* colony through *Maculinea* predation increases its risk of extinction, the empty sites can be recolonized by nests in unfavourable vegetation.

In the Moerputten nature reserve, the microdistribution of *Myrmica rubra* is correlated with the microdistribution of *Maculinea nausithous* eggs, and, independently, the distribution of the adults of the same species. Again independently, the same has been found for *Maculinea teleius* and *Myrmica scabrinodis*, albeit only for the beginning of the flight period. So, the ant species is an exclusive resource for both butterfly species respectively. The fact that host ants can be detected, has beneficial consequences with respect to increasing the survival probability of the offspring (which *Maculinea teleius* only partly makes use of). It also opens the discussion to whether the butterflies are able to reduce their host ant nest density so much that they may initiate their own local extinction. Apparently, on the one hand the persistence of the butterfly population depends on local refuges for the host ant species. These can be found in habitat without *Sanguisorba officinalis* and on locations where the host plants develop buds in the acceptable phenological stage only after the flight period of the butterfly. On the other hand the amount of co-occurence of the *Myrmica* species influences local mortality. The more non-host *Myrmica* nests there are in the close proximity of the host plant, the more caterpillars will be adopted by them, thereby decreasing the extent of invasion into ant nests and the mortality. Through inefficient feeding behaviour and scramble competition, high mortalities occur in overcrowded ant nests (Thomas & Elmes 1998, Thomas & Wardlaw 1992). The consequences of the spatial distribution of host and non-host *Myrmica* colonies have been studied for *Maculinea rebeli*, an advanced predator using the rare host plant *Gentiana cruciata* (Hochberg *et al.* 1992, 1994, Elmes *et al.* 1996, Clarke *et al.* 1997). However, for *Maculinea teleius* and *M. nausithous*, the availability of host plants and host ants is different and the behaviour of the butterflies is different, too. Therefore, new predictions should be tested in a differently parameterised model.

Dispersal and the establishment of a possible metapopulation

The persistence of many populations is generally increased when they consist of a system of several local subpopulations which exchange individuals with each other. It generates advantages with respect to environmental and demographic stochasticity and decreases the chance of genetic impoverishment (Hanski & Gilpin 1997, Hanski 1999). The functioning of such a metapopulation is on the one hand dependent on landscape characteristics such as the number of connected patches, the distances between them and how easy these distances can be overcome. On the other hand, species specific characters determine whether there will be exchange between subpopulations and how intense this exchange will be. Within the same set of plots used to study the habitat selection of *Maculinea nausithous* and *M. teleius* after their reintroduction in the new environment, we investigated their colonisation and abandonment pattern. While in the classical metapopulation approach, dynamics of local populations and patterns of exchange between spatially disjoint patches of uniformly suitable habitat are studied, we decided to concentrate on a finer spatial level and study the same patterns on heterogeneous habitat within one patch and between patches as well.

Although the individuals of both butterfly species can select sites that contain both their resources, dispersal leading to colonisation of suitable sites at a larger spatial scale seems to be constrained. This could be caused by the fact that the habitat quality of the unoccupied plots is too low, or because the distance necessary to reach these plots is too long. Effects of the degree of connectivity on colonisation and abandonment suggest that these two processes are not random with respect to the degree of connectivity of the plots. For both butterfly species, both habitat quality and connectivity affect colonisation patterns. Plots with high habitat quality are more likely to be colonised than plots with low habitat quality, however, with a low degree of connectivity, their colonisation probability decreases. Dispersal constraints at individual level may affect habitat selection and have an effect on the colonisation and abandonment rates in the populations of the two *Maculinea* butterfly species and thus on their distribution. *Maculinea nausithous* colonizes unoccupied plots less frequently but covers langer distances. *Maculinea teleius*, however, shows only very small colonisation distances, which appear to show expansion - retraction processes within heterogeneous plots in one particular habitat patch. Larger displacements are found only very rarely in this species. These data fit well with our findings in mark-recapture studies (Wynhoff 1998 b, chapter 3, chapter 6) and with the published literature (Settele *et al.* 1996).

Given the constrained dispersal and the landscape in and around Moerputten nature reserve, the conservation of *Maculinea teleius* should concentrate on improving local habitat quality on the occupied meadow and the meadows in the close vicinity. A metapopulation will only be established, if high quality habitat patches and stepping stones at short distance from occupied sites will be created. Conservation of *Maculinea nausithous* by creating a spatial network of suitable patches seems to be likely to be more effective, since this species can cover longer dispersal distances. Most likely in former times, these processes of colonisation and abandonment of habitat patches took place quite regularly and the *Maculinea* populations were in dynamic equilibrium with their environment. In bad times, retraction to core populations took place, from where empty patches could be colonized after their recovery. However, nowadays Moerputten nature reserve represents only a small island of nature in an intensively used agricultural landscape. It is almost impossible that a metapopulation with a structure needed by *Maculinea* butterflies, could exist within the borders of a nature reserve. This ia only possible when the butterflies are given the possibility to spread into the agricultural landscape. Clearly, such a metapopulation would put new challenges to nature conservation.

How do they find the host ants?

Our results show that *Maculinea* females are able to find locations with their respective host ant species (chapter 4, chapter 5). We can only speculate about the mechanisms which they use to do so. Behavioural observations indicate that visual cues are important in the first phase of searching a flowerhead for oviposition. Once a *Maculinea* female has found a flowerhead in a suitable phenological state, she relies on other cues. For other butterfly species it has been shown that chemical cues can be very important to decide whether an egg would be laid (Baur *et al.* 1998, Honda *et al.* 1997). Baylis and Pierce (1991) have shown that females of the ant-tended lycaenid species *Jalmenus evagoras* prefer to lay egg batches on fertilized rather than unfertilized host plants. The importance of visual cues is also shown for the presence of ants (Jordano *et al.* 1992, Jordano, pers. comm.). However, *Maculinea teleius* and *Maculinea nausithous* do not search visually for ants. Most of the rather small ant nests are subterranean and not between the roots or directly next to *Sanguisorba officinalis* plants. The oviposition behaviour does not include a walk along the stem of the host plant downwards to search for them. So, if the presence of ants plays a role for depositing eggs, it is likely that chemical cues are involved. For the *Maculinea* females it could be the scent of the volatile pheromones or the odour of their *Myrmica* host ant nests.

Pheromones are important for the intra- and interspecific communication of ants. Although in most cases contact-chemoreception is very important, chemoreception over longer distances by volatiles has been shown to occur frequently (Hölldobler & Wilson 1990). For *Myrmica rubra* and *Myrmica scabrinodis* a number of volatile and non-volatile pheromones from the Dufour gland have been identified and their function in interspecific communication has been partly clarified (Cammaerts *et al.* 1978, 1981). While the very volatile compounds are not species-specific, the less volatile compounds used for marking of territories are. It would be very interesting to test the behaviour of gravid *Maculinea* females when being confronted with these odours. *Myrmica rubra* workers occasionally visit higher parts of trees and herbs when searching for aphids. During these visits they could leave trails of non-volatile pheromones on the flowerheads of *Sanguisorba officinalis*, which could be detected by the *Maculinea* females. From our field work, we have the impression that this happens so rarely that it cannot explain the observed phenomena. It is more likely that one of the volatile components of the pheromones or other chemicals related to nest odours are involved in the oviposition behaviour of *Maculinea* butterflies.

Genetics of reintroducing Maculinea butterflies

Genotype

After the reintroduction of *Maculinea teleius* and *Maculinea nausithous,* both the demography of the founder populations as well as the genetic consequences of the bottleneck were studied. Population numbers increased instantly and loss of genetic variation was only mild. The low levels of genetic variation of the source populations did not have a negative impact on the settling and increase of the founder populations. Apparently the number of translocated founder individuals and the quality of the release site have a higher impact on success than the genetic constitution of the founders. Hence, also with respect to genetic heterogeneity, no bottleneck effects could be detected (chapter 7).

In both *Maculinea* species, only at the Aco-K locus significant differentiation between source and founder population was found. Taking into account that the flight periods shifted forwards and the mean minimal life time of the adults has decreased, we come to the conclusion that the founder populations have experienced selection due to the changed ecological conditions at the founder site.

Both founder populations have a significant negative F_{IS} value (=inbreeding coefficient), which means that they may in some way be able to maintain higher levels of heterozygosity than would be expected in a random mating population. Further research is necessary to elucidate the processes responsible for this phenomenon. Still, with low levels of genetic variation, the chance of loosing variation through stochastic events is high. Therefore it is important to create metapopulations of reintroduced species at the release site to compensate for local stochastic extinction of subpopulations.

On the other hand a low level of heterozygosity in itself cannot always be considered a problem for the viability of populations or for the fitness of individuals, as there are many rare and common species lacking a high level of heterozygosity, such as the northern elephant seal (*Mirounga angustirostris*), the South African subspecies of the cheetah (*Acinonyx jubatus*) or the Florida tree snail (*Liguus fasciatus*), as an example of a non-vertebrate species. In many cases it has been shown that this was the result of historic bottlenecks, but it is not necessarily the cause for recent bottleneck events (Avise 1994). Other rare or endangered species have been shown to have normal or high levels of heterozygosity, such as the flightless parrot (*Strigops habroptilus*) and the Przewalski's horse (*Equus przewalski*). So in general, a prediction on survival of bottlenecked populations merely based on their genetical variation is not possible (see also Van Hooft 2001). Dunham *et al.* (1999) suggest that it is likely that species with limited within population variability, or non-equilibrium population structure may be well adapted to persist in the face of extreme isolation or small population size. This may be the case for species that inhabit naturally isolated, but relatively stable habitats. Obligate ant parasite butterfly species, which are adapted to specific habitat conditions, such as *M. teleius* living in *Myrmica scabrinodis* nests on wet meadows, may have such adaptations as well. The example of the reintroduction of the beaver to Sweden shows that a species with low genetic variation can survive and even spread sucessfully over many years and generations (Ellegren *et al.* 1993). This case of the *Maculinea*-butterflies also demonstrates through the increase in population size, a balanced sex-ratio and the low chance of loosing heterozygosity in the founder population, that the Kostrza populations were good source populations for a reintroduction project. Obviously it is important to enable a good start of a reintroduction project by finding a release site which fullfills all ecological demands of a species and by releasing many founder individuals.

However, it is obvious that both species, but especially *M. teleius,* have a very low mean minimal life time. This makes them more susceptible to stochastic catastrophes.

Phenotype

It is not only the bands from the allozymes, when they appear during the exciting moments after the staining agar has been poured out over the white cellulose acetate plates that provide genetic information. The spots on the wings being cut from the bodies before homogenizing them for the electrophoresis, will tell their own story, too. Size, shape and number of the spots can be measured and compared between populations to reveal relationships and to find out, whether a possible bottleneck through reintroduction has become visible in different wing phenotypes. Spot pattern variation has been shown to have a high heritability in several butterfly species (Brakefield 1989). Furthermore, indications for the biogeographic origin in historical times can be deduced (Taberlet 1998).

The Pleistocene period (2,400,000 to 13,000 years BP) consisted of cyclic alternations dominated by major ice ages and short warm interglacials (Taberlet 1998, Hewitt 1996, Roy *et al.* 1996). Northern Europe as far as 52 °N parallel, including most of Britain, was covered by the Scandinavian ice sheet. The major mountain ranges had large ice sheets, too, while between them the plains of Europe were tundra, steppe tundra and cold steppe on permanently frozen soil. Flora and fauna retreated from the cold to refuge areas in the south, for example Italy, the Balcans or Turkey, and recolonised Central and Northern Europe again in the warmer periods. While some species withdrew to one refuge area, others were split up into several refuges and evolved in different ways during the period of separation. Later, through landscape barriers, postglacial spread could take place along different dispersal routes. Through the long periods of separation conditions were created that allow the evolution of subspecies under influence of different selection through different environmental conditions. Furthermore, rapid long distance (leptocurtic) dispersal will involve population bottlenecks and therefore loss of alleles. It means that populations at the edge of the range will tend to be more homozygous compared to those in the centre or closer to the refuges. In conclusion, during the climatic oscillations of the Pleistocene, conditions were favourable for the evolution of divergence in species at higher spatial scale, while at lower spatial scale the loss of heterogeneity can be expected (Taberlet 1998, Hewitt 1996, Avise 1994). Phenotypic and genotypic differences between populations can be interpreted in such biogeographical context. A study on wing patterns enables us to include more individuals and more populations, since it can be done without killing butterflies. Moreover, we could include historic material from butterfly collections.

For this part of the study, we used wings of *Maculinea teleius* from the source population in Kostrza (Poland), the reintroduced population in the Moerputten nature reserve (The Netherlands) and a population in the Westerwald region (Germany). The latter is geographically closest to the Netherlands. From other small and protected populations of *Maculinea* butterflies, samples were collected by making slides of anaesthetised individuals. The butterflies were put to sleep one by one with carbon dioxide to make a picture. Within 15 minutes they recovered again. We took photos in the population of the Marais de Lavours (France) and additionally from all populations used for the allozyme study. Furthermore we used cabinet specimen and all *Maculinea teleius* butterflies of the National Collection of the Museum for Natural History Naturalis at Leiden. These include the historical Dutch populations in Helvoirt (which can be considered a part of the old Moerputten population), the riverine meadows of Roer and Swalm, several butterflies from the Vosges (F) and the extinct Belgian populations of Vilvoorde/Epeghem.


Figure 8.2: Wing pattern of *Maculinea teleius*, with measured spots associated by their number.

Digitised images of the wings, the cabinet specimen and the slides were produced. Then, we measured area, perimeter and distance between numbered spots on the left hindwing (figure 8.2). We recorded how many spots number 8 were present (0-2) and how many spots we could find on the left fore wing. We measured 336 males and 251 females, of which we used 256 males and194 females for a Principal Component Analysis (PCA). Alas, the measurements from the slides appeared to deviate too much from the others and were therefore excluded. Considerable phenotypic heterogeneity within and between populations was found with the only exception of those with a very small sample size (Chindrieux, Vosges).

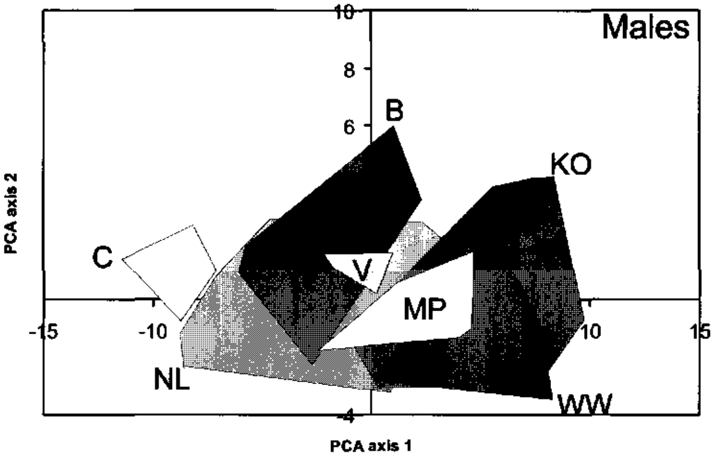

Figure 8.3: Wing pattern of male *Maculinea teleius*, expressed in PCA coordinates. Abbreviations denote the populations (B=Vilvoorde (B), C=Chindrieux (F), KO= Kostrza source population (PL), MP= reintroduced Moerputten population (NL), NL=historical Dutch populations, V=Vosges (F), WW=Westerwald (D)).

Populations differed from each other in spot size, wing size and spottiness. The largest butterflies with the most complete wing pattern were found in Chindrieux in France. The extinct populations in the Netherlands also consisted of large individuals with many spots. Conversely, butterflies from the source populations at Kostrza had comparably smaller wings. Spots were smaller, too, and spots nr. 1 and 9 and to a lesser extent 8 and 3 were missing. These differences were consistent in males and females. For both sexes, the first four PCA axes were significant in explaining the variation of the wing pattern measurements (figures 8.3 and 8.4).

Then, populations were grouped together into an Eastern and a Western group, with the populations from Kostrza, Moerputten and Westerwald together in the Eastern group and the old Dutch, Belgian and Vosges populations into the Western groep. Within one group, no significant differences in PCA axis 1 coordinates could be found (West(♀): F=1.74, n=86, p=0.165, East(♀): F=1.64, n=100, p=0.171, West(♂): F=2.77, n=132, p=0.03, East(♂): F=2.15, n=119, p=0.098) with exception of the males from the Western group. This was due to the population from the Vosges, which also had a very small sample size of only 4. In conclusion, most of the

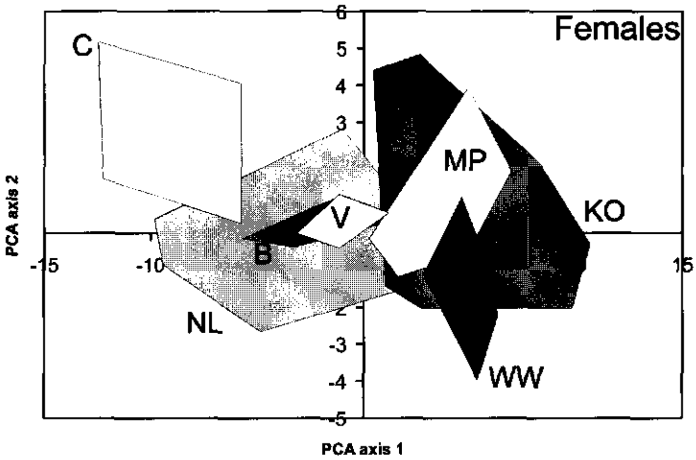

Figure 8.4: Wing pattern of female *Maculinea teleius*, expressed in PCA coordinates. Abbreviations denote the populations (B=Vilvoorde (B), C=Chindrieux (F), KO= Kostrza source population (PL), MP= reintroduced Moerputten population (NL), NL=historical Dutch populations, V=Vosges (F), WW=Westerwald (D)).

variation is due to the difference between the Western and the Eastern group. The reason for this phenomenon can be found in postulated differences in biogeographical origin since the ice ages.

It is likely that both groups originate from different ice age refugia (figure 8.4). *Maculinea teleius* occurs only north of the Pyrenees. Mountain populations in Zwitserland and Austria are also located relatively low, not exceeding 1600 m (SBN 1987). Clearly, mountain ranges are effective distribution barriers to this butterfly. The Western group could have survived cold periods west of the Alps in a refuge area, which was centered in the Rhone delta. Due to the lower sea level, the delta of the Rhone reached much further into the Mediterranean than it does now (Kurtén 1972). This refuge area (W) might also have had extensions to the regions of the tundra at the northern border of the Alps. According to Lukhtanov & Lukhtanov (1994), *Maculinea* species occur on riverine meadows in the taiga-zone. Such meadows could have existed along melting water streams from the Alpine glaciers. The summers in the late Pleistocene steppe tundra vegetation were warmer than in the recent time tundra vegetation, and open graslands including *Sanguisorba officinalis*, used to exist in such vegetation (Gamble 1999, Prins 1997, Kurtén 1972). Schmitt & Seitz (in press) found

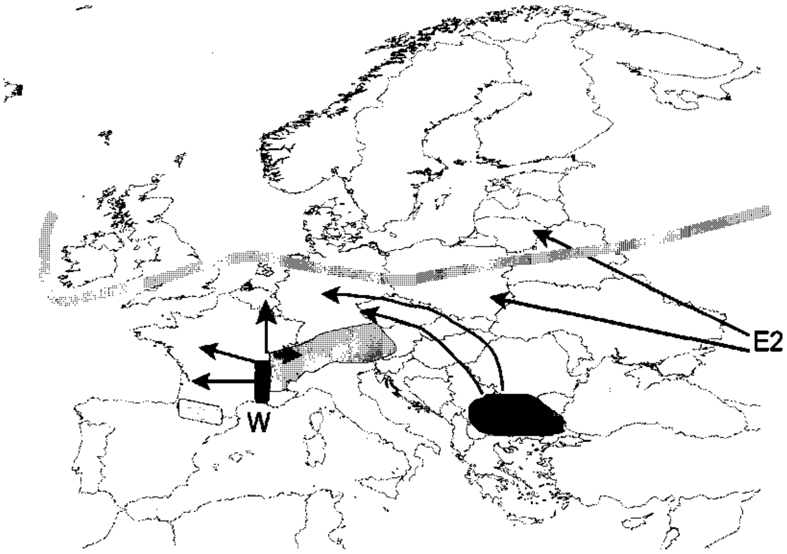

Figure 8.5: Putative location of refuge areas during last glaciation and later migration routes of *Maculinea teleius*. Broad line: southern border of Scandinavian ice sheet, dotted: glaciers, hatched: refuge areas: W = Western refuge, E1 = Eastern refuge 1, location of Eastern refuge 2 is unknown, arrows: colonisation routes.

strong indications for the existence of Central European refugia for the butterfly species *Erebia medusa*, and it is likely, that *Maculinea teleius* survived the last ice age in a similar region. With climate warming, populations spread to Central France, to the South, to the Langres and via the Vosges to Belgium and The Netherlands. It was also possible to invade into the northern valleys of the Alps after the large glaciers melted (figure 8.5).

The Eastern group, consisting of the population of Kostrza, the Moerputten daughter population and the Westerwald population, is possibly from a Central Asian refuge area. Their dispersal route would have been much longer (Hewitt 1996, Taberlet 1998), which would explain the losses in phenotypic characters compared to the Western group, as well as the loss of genotypic variation in the populations in the South of Poland compared to those in Russia (Figurny *et al.* 2000). However, our own data show higher levels of genotypic heterogeneity in the Kostrza population. While the Kostrza and Westerwald populations have differentiated significantly in allozyme

variation, as shown by a significant F_{ST}-value (F_{ST}=0.136, CI(95%): 0.233 to 0.039), the Westerwald population of *Maculinea teleius* shows more allozyme polymorphism rather than less. Significant population differentiation was found at three loci: MDH-2, PGM and Aco-K. Thus, a combination of phenotypic and genotypic data points in the direction of more refuge areas in the East (E1 and E2). A putative refuge (E1) could have been located in Rumania and Bulgaria (see also Kurtén 1972), as was also found for the grasshopper *Chorthippus parallelus* (Cooper *et al.* 1995), and the butterfly *Polyommatus coridon* (Schmitt & Seitz (in press b). From that region, Slovacia, Czechia, Poland and Central Germany up to the valley of the river Rhine could have been colonised by the *Maculinea* butterflies. Also, an invasion of alpine regions could have been possible. In the Alps, migration routes might have met each other and a hybrid zone could have been formed (see also Taberlet 1998 and Hewitt 1999). However, the number of studied populations is too low to give evidence for any of our hypotheses except for the separation into at least two lineages. It would be interesting to study populations in Rumania, Russia and Czechia to test whether the assumption of (at least) two different refuges in the East is supported. Since the Alps could have been colonised by the Western or the Eastern lineage or both, populations there also deserve further study. Finally, it seems that both lineages almost met each other at the river Rhine in Germany. A similar phenomenon has been found for the white stork *Ciconia ciconia*, where an apparently panmictic population exists of two lineages which meet in the North of The Netherlands and Germany (Bairlein 2001). If this is true, then the populations in the Palatinate should resemble those of France, while the populations in Bavaria would be more like those in Poland. As small and endangered populations would be involved in such a study, it is recommended to standardise the method of making slides from anaethetised butterflies, or use digital cameras.

The results of the wing pattern analysis of *Maculinea teleius* do not give evidence for a bottleneck due to the reintroduction. The reintroduced Moerputten population is not different from the Polish source population nor in phenotype or in genotype. It also does not show any tendency to shift in wing patterns towards that of the extinct Dutch butterflies. However, it shows, that the butterflies from the historic population from the reintroduction site resemble much more those from Belgium and France than those from the Westerwald region. The Westerwald populations are geographically closest to the reintroduction site and would normally have been the first choice to serve as source population. We decided to use the much bigger Kostrza population, and doing so, butterflies from a different biogeographical origin were translocated. The founder population, being adapted to climatic conditions in the south of Poland, must have experienced considerable selection pressure. It is likely that this was expressed in the shift of the flight period, the decrease in mean minimal life time (Wynhoff 1998, chapter 3) and the significant differentiation between source and founder population at the Aco-K locus (Wynhoff *et al.* 2001, chapter 7). However, ecologically significant traits have most likely not been affected in such a way that it could jeopardize the success of the reintroduction.

Nonetheless, if another extinct butterfly species or other animal would be reintroduced to The Netherlands, it is recommended to first investigate the genetics of (a series of possible) source populations and biogeographical origin of species to avoid excessive selection pressure. The *Maculinea* population might have adapted easier to the reintroduction site when a source population from France would have been selected. When the adults would have a longer life span, they would probably be less sensitive to environmental stochasticity.

Retaining genetic diversity in a metapopulation of Maculinea

The effective population size (Ne), which is an important character of a population with respect to retaining genetic diversity, is reduced with fluctuations in population size, but it is also affected by the "architecture" of the metapopulation (Gilpin 1991, Ray 2001). Subdivision into local interconnecting subpopulations, generally has a positive effect on persistence time, when the extinction of subpopulations is independent from each other. However, models predict that the more different the local populations are in size, the more the metapopulation resembles a single large population, which lowers persistence due to an increasing effect of environmental stochasticity. It has been shown that an uneven subdivision of a metapopulation reduces the effective population size more than expected (Ray 2001). This means that in metapopulations consisting of equal numbers of individuals, founder effects are expected to happen more frequently in systems of uneven units than in those of equally sized units. In such a situation two dispersing individuals are more likely to come from different populations. When a metapopulation consists of a very large and several very small populations, the founders are more likely to be both from the large population. If the large population goes extinct, only a part of their genetic diversity will survive in the small subpopulations since these have experienced a bottleneck in numbers and genetic drift (Ray 2001). Hence it is important not only to establish metapopulations but also to manipulate their structure in order to preserve as much as possible genetic variation.

In the case of *Maculinea* butterflies with their restricted dispersal, the distance between subpopulations deserves particular attention. From mark-recapture studies we know that for *Maculinea nausithous*, the exchange of individuals over 500 m was 8 to 10 % in years with high population densities (chapter 3, chapter 7). Such high migration rates increase the persistence of subpopulations and avoid genetic drift, albeit also reducing genetic differentiation (Ray 2001). For *Maculinea teleius*, distances between subpopulations most likely need to be shorter for the same rate of exchange. Consequently, to increase population persistence and genetic diversity, several large connected subpopulations should be established. Based on our experience, for *Maculinea nausithous* the distance between them should not be more than 1 km, while for *Maculinea teleius* they should be within 500 m of each other. Even though both butterfly species could be insensitive to genetic drift as a consequence of low population sizes, populations should be maintained as large as possible.

The creation of such a fine scale metapopulation within the highly fragmented and agriculturally used landscape, must be a serious problem for the reintroduced populations. Given the constraints in dispersal and the highly stochastic negative effects of management of road verges and canal borders, it is uncertain whether the populations will be able to establish new populations by themselves. The best way to ensure persistence of the resident populations by increasing the number of interconnected subpopulations without taking the risk of any further loss would then be the application of an extended reintroduction program.

Genetic diversity can best be encouraged in metapopulations among a network of habitat patches. However, such a network would soon surpass the borders of nature reserves and invade the landscape used by humans. As habitat patches outside nature reserves are, at least in The Netherlands, under controle of land managers and resident farmers, they have substantial influence on the persistence and genetic diversity of populations. In theory, they can maintain populations by preventing deterioration of habitat and decline in numbers. Furthermore they can reduce the frequency of human disturbance to local populations. Locally, on the short term, this may be as important for populations surviving on the road verges between the farms as the management of nature reserves (see also Gibbs 2001). For the Burchell zebra in South Africa, the first conservation program based on genetic information has been developed and implemented (Bowland *et al.* 2001). This program aimes at a genetically based translocation scheme to retain genetic variability and avoid inbreeding rather than landscape architecure and management. For *Maculinea* butterflies under the given conditions, it is recommended to also start a similar program, but with the integration of landscape management, since this is often the more effective way to tackle the problem in insects.

Conservation of Maculinea butterflies at local level

The protection of *Maculinea teleius* populations should be concentrated on a favourable management of the host ant population, because the population dynamics of the butterfly is depending on the density and spatial pattern of its host ant (chapter 4, 5 and 6). Females oviposit on *Sanguisorba* plants close to host ant nests, and the presence of *Myrmica scabrinodis* is an essential factor with regard to habitat selection. Due to the limited dispersal, the persistence of populations is at the moment probably more dependent on conditions at the patch level than on the metapopulation level. Thus, conservation measures should first concentrate on improvement of habitat quality. In addition, the population size per site should be as high as possible to buffer against stochastic extinction, since small insect populations are at a higher risk of extinction due to chance events than large populations (Thomas & Jones 1993) and small populations experience higher losses of genetic variation (Gilpin 1991).

The nutrient poor to mesophilic meadow vegetation (*Molinietalia* with strong *Junco-Molinion* aspect and species rich *Molinio-Arrhenateretea*) represents the vegetation composition of the *Maculinea teleius* habitat. This vegetation needs a regular mowing regime of one time per year. If the hydrological conditions with seepage of base-rich soil water and a high waterlevel (but dry during summer!) persist and as long as the mowing regime is kept the same, the meadow BW with this vegetation forms a relatively stable site. For the benefit of *Myrmica scabrinodis*, it is essential to always have parts of the meadow with an open vegetation. Mowing of habitats with *Maculinea teleius* should be done before the middle of June or after the middle of September. In the first case there is sufficient regrowth of *Sanguisorba officinalis* before the adults appear. In the second case, the caterpillars are in the ant nests before mowing and removing hay (Schurian 1984, Wynhoff 1992, Thomas 1995). In the nature reserve, both *Myrmica scabrinodis* and *Maculinea teleius* will then have a relatively safe future, as long as they are restricted to the meadow BW, even though the butterfly species is very sensitive to stochastic negative influence. In the nature reserve many more patches are available with high habitat quality and presence of *Myrmica scabrinodis* nests. Occasionally butterflies reach these patches and ovipositions have also been observed. However, mowing the vegetation in the beginning of August preempts successful colonisation. Since the establishment of a metapopulations is needed, mowing should be shifted forward to the beginning of June or later to September. In this way the suitable habitat of *Maculinea teleius* would increase substantially, which would increase their long term persistence. It is also recommended that comparably large areas of habitat should be managed to promote a similar habitat to ensure a high carrying capacity with respect to the *Myrmica* ants (Clarke *et al.* 1997). Nature conservation organisations should be given the support and the fundings to perform such a fine scale, but quite expensive management.

Due to the very low mobility of *Maculinea teleius*, further expansion outside the nature reserve is not to be expected in the present situation in the near future. Observations of long distance dispersal are very rare. For the establishment of a metapopulation a fine grained network of stepping stones with hostplants and host ants connecting habitat patches is needed, which is difficult to realize on road verges. If another network of populations of *Maculinea teleius* should be established at another location, the only possible way to do so would be by reintroduction.

In the Moerputten nature reserve, *Maculinea nausithous* is a species of relatively transient successional habitats of abandoned meadow vegetation. The host ant *Myrmica rubra* does not occur in the species rich *Molinietalia* meadows, but finds its highest densities when these vegetations are abandoned. The tall herb vegetation encompasses highest ant nest densities and is used for searching by egg-laying females. For the benefit of *Myrmica rubra* it is essential to always have parts with rough vegetation within a mosaic of different vegetation succession stages of abandonment. A 3 -5 year turnover of mowing is proposed to guarantee a high diversity of uncultivated grassland in one habitat. This can be achieved by rotational mowing. However, it is difficult to keep such a type of habitat suitable within the limitations of management plans of nature reserves. Due to the dynamics of this type of tall herb vegetation and the relative mobility of the adults, *Maculinea nausithous* would also profit substantially from a metapopulation system of habitat patches in the nature reserve and on the road verges. As this butterfly occurs in patches of a highly dynamic nature, its persistence is much more dependent on the persistence of the metapopulation as a whole, rather than on the persistence of a single occupied habitat patch.

In contrast to *Maculinea teleius*, *Maculinea nausithous* is able to persist on small habitat patches. Dispersing adults use corridors, and attention should be given to the creation and management of connecting elements in the landscape. The management of road verges and stream borders should be done in the same way as the management of meadows. In addition, the population size per site should be as high as possible to buffer against various kinds of (environmental, genetical or demographical) stochasticity.

Conservation of *Maculinea* butterflies at regional level

It might be clear that reintroducing two parasitic *Maculinea* butterfly species into The Netherlands appears to be risky and exceeds merely realisation of the ecological requirements of the species. Both butterflies and their host ants depend on high quality habitat kept by low intensity agricultural management. However, they are released into a nature reserve, which is only a small part in a landscape, dominated by and formed with the purpose of high intensity agriculture. When aiming at the long term persistence of the newly founded populations, the adults have to leave the nature reserve and find their way on verges among corn fields and pastures. The expansion to the road verges south of Moerputten has proven, that high quality patches are available. The adults are able to detect them and use these landscape elements for further dispersal. Thus, habitat quality of the road verges, ditch sides and embankments around the nature reserve of Moerputten are very important. They fulfil a role as disperal corridor but also as a temporary or semi-permanent habitat (Oostermeijer & Wynhoff 1996). But at the same time, since *Sanguisorba officinalis* and the *Myrmica* ants are sensitive to management, the butterfly populations became very vulnerable to public management. To prevent problems and to increase *Myrmica* ant nest density and facilitate further dispersal of the *Maculinea* populations, the Province of Northern Brabant designated a *Maculinea* Action Plan Area (Provincie Noord-Brabant 1997, figure 8.6). In this area the management of

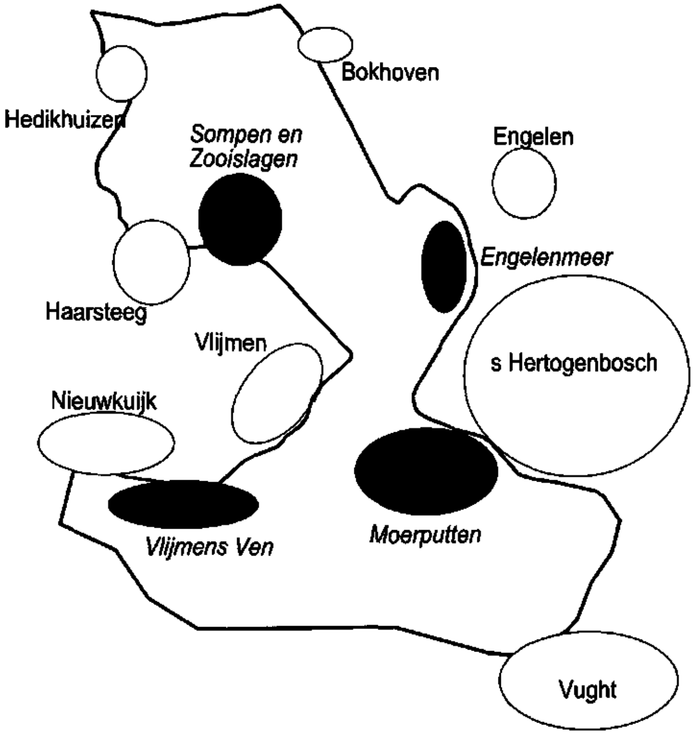

Figure 8.6: *Maculinea* Action Plan Area in the Province of Northern Brabant. Black circles: nature reserves, white circles: villages and cities, black line: Action Plan Area.

nature reserves and public property areas was adapted to the ecological requirements of the *Myrmica* ant species. Hence, the vegetation in the nature reserves and on grounds under public ownership, such as road verges, were managed in a way that encouraged initially a colonisation by the host ant species and later by the *Maculinea* butterflies. Four actions were formulated: (a) adaptations in the management of nature reserves, (b) extensions of nature reserves, (c) adaptations in the management of, for instance, road verges, canal borders, ditch sides, embankments, and (d) monitoring of actions and effects. The action mentioned under (c) is essential for regional conservation.

Adaptation in the management means mainly that areas are mown less often. It is of vital importance that occupied *Maculinea* habitat is not mown at all during the butterfly's flight period. Mowing only one side of road or ditch at a time reduces the disturbance of mowing to the whole insect fauna. Especially for the benefit of *Myrmica rubra,* strips of vegetation were left unmown for one or two years. Furthermore, rough vegetation with flowers was mown only late in the year.

Participants in the agreement of the *Maculinea* Action Plan are (a) the Municipalities of Heusden, 's Hertogenbosch and Vught, (b) State Forestry and "Natuurmonumenten", (c) Polder (Waterschap) de Maaskant, (d) Province of Northern Brabant, (e) Ministry of Agriculture, Nature Conservation and Fisheries, Department South, (f) Dutch Butterfly Conservation and (g) the local farmers.

In the designated Action Plan Area (figure 8.5), 177 plots with hostplants were searched for ants for a period of 15 minutes to obtain relative density measurements. The first inventory was done in 1995 (Oostermeijer & Wynhoff 1996, Wynhoff *et al.* 2000). In 1997, the management changed according to the *Maculinea* Action Plan and in 2000 the ants were counted again to check whether there were effects of the changes in mowing regime (Wynhoff & Janssen 2000). The first investigation of the ant fauna in the *Maculinea* Action Plan Area has shown, that *Lasius niger* is by far the most common ant species. A negative correlation between the nest densities of this species and *Myrmica rubra* (Spearman $r=-0.1772$, $p<0.05$, $n=177$) and *Myrmica scabrinodis* (Spearman $r=-0.2345$, $p<0.005$, $n=177$) shows that these species do not occur together but rather compete for habitat space (Oostermeijer & Wynhoff 1996, Wynhoff *et al.* 2000). Changes in the management for the benefit of the *Maculinea* butterflies should therefore increase abundance and nest densities of *Myrmica* species (and *Lasius flavus*) while *Lasius niger* should decrease.

Since the management of the road verges in the Action Plan Area has been changed, the ant fauna has indeed changed (figure 8.7). The mowing frequency has been reduced which resulted in less disturbance. In general, significantly less plots were occupied by ants (Pearson $\chi= 9.76$, $df=1$, $p<0.005$) and on many plots the number of ant nests had decreased as well. The pioneer species *Lasius niger* is now less abundant than before and nest densities are reduced, too. The number of plots with *Myrmica* species has not increased. Therefore it seems like they have not been able to take over

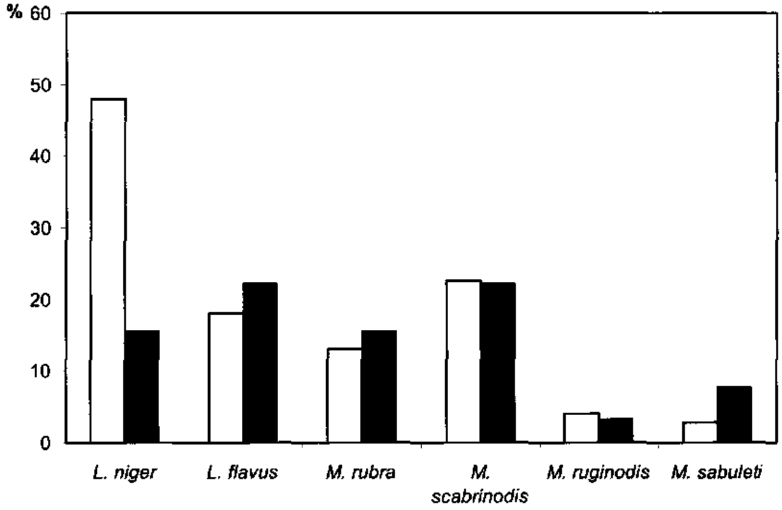

Figure 8.7: Changes in relative occupancy of plots with ants from 1996 (white bars) to 2000 (black bars) in the *Maculinea* Action Plan Area (n=180). Significant changes were found in *Lasius niger* (p<0.0001) and *Myrmica ruginodis* (0.01<p<0.05) (Wilkoxon Signed Rank test).

the empty space. However, the abundance of the ants has not changed equally in the whole Action Plan Area. *Lasius flavus* and *Myrmica rubra* show an overall slight increase of occupied plots. For *Myrmica rubra*, newly colonised plots and plots with an increase in ant nest density are distributed equally over the whole study area, while the plots with extinctions are concentrated at two locations: the road verges directly south of the Moerputten nature reserve (RD) and a part of the canal borders of Drongelens Kanaal. These two sites were treated differently from the others. The road verges of the Ruidigerdreef changed severely after the ditch sides were stripped of vegetation and reconstructed. The original vegetation consisted of mainly *Phragmites communis*, in which there were many nests of *Myrmica rubra* and some *Myrmica scabrinodis*. Since the ditch side reconstruction, *Myrmica* ants are missing, as well as *Maculinea* butterflies. The borders of Drongelens Kanaal used to be mown but are grazed by sheep nowadays. *Myrmica rubra* is very sensitive to trampling. The reduction in nest sites of this species might be explained by the change in management.

In general, the aims of the adaptations in the management of public property within the *Maculinea* Action Plan Area have been achieved. Except for two locations, a decrease of *Lasius niger* was found in combination with an increase in *Myrmica* species and *Lasius flavus*. Through the reductions in mowing frequency, more suitable sites for *Maculinea nausithous* and *Maculinea teleius* can be created. The more mobile *M. nausithous* can disperse better and found new subpopulations om the improved road verges. For *M. teleius* we expect positive effects only in the longer term (Settele 1998).

The great changes at Ruidigerdreef also show how vulnerable the implementation of an agreement such as the *Maculinea* Action Plan is. Even though improvements in habitat quality can be achieved by the management, local actions within the recent flight area of the *Maculinea* butterflies can still have tremendous effects on the affected subpopulations. At the moment the butterfly metapopulation system is still too small to compensate for that. Therefore it is still necessary to put further effort into the information and motivation campaigns to all participants of the agreement. In addition, the obligations signed for in the agreement should be corroborated by all participants.

In conclusion......

The five butterfly species of the genus *Maculinea* have received major attention from European lepidopterists because of their unusual life cycle as parasites of *Myrmica* ant nests and their declining status in most of their range (chapter 2). The uninterrupted decline of these species has been a cause to develop a European *Maculinea* action plan to protect them in their total European range (Munguira & Martín 1999). *Maculinea nausithous, Maculinea teleius and Maculinea arion* are species mentioned in the appendices II and IV of the Habitat Directives. This fact puts extra impact on the need to strictly protect populations and their habitats. Any action to protect a population of any *Maculinea* wherever in Europe is therefore important and should be supported (Wynhoff & Munguira 2001).

In the European *Maculinea* Action Plan, reintroductions are considered to be important in Western Europe, especially when conservation of *Maculinea teleius* is concerned. Species are not expected to readily adapt to large scale landscape changes due to fragmentation of habitat and climate warming, therefore reintroduction programs are increasingly important. Our experiences can help in the elucidation of problems of reintroducing highly specialised species and in the conduction of reintroduction projects in such a way that the chance of success can be increased. As a consequence of landscape architecture and the very limited dispersal of the *Maculinea* species (and other comparable species with low mobility), it seems that the establishment of a metapopulation is difficult, if not impossible. Since a metapopulation is required to realize persistence, founder populations should be reintroduced as metapopulations or metapopulations should be established by additional translocations.

The reintroductions of *Maculinea teleius* and *M. nausithous* were conducted according to the recommendations of the IUCN. Translocating a large number of founder individuals appeared to be more important for the success than the genetic variation of the source population. Both *Maculinea* species seem to be protected from losses through chance caused by low numbers of reproducing individuals, but they experienced selection under the new environmental conditions at the reintroduction site. However, since sufficient high quality habitat was available and adults were apparently able to select for such patches, populations could increase quite rapidly.

Constraints in dispersal and deterioration of habitat through human interference are a danger to a long term persistence. When populations expand beyond the borders of nature reserves, their persistence is strongly linked to landuse. The conclusion must be that reintroduction projects can be successful. But however careful their scientific foundations have been, they are prone to fail when not accompanied by extensions and motivating campaigns aimed at local people, local authorities, nature conservationists and reserve wardens (figure 8.8).

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Summary

The five butterfly species of the genus *Maculinea* have received major attention from European lepidopterists because of their unusual life cycle as parasites in *Myrmica* host ant nests. In most of their range, *Maculinea* butterflies have shown a remarkable decrease in numbers of populations and individuals per population. This decline is mainly due to agricultural improvement as well as abandonment, both resulting in marked changes in habitat. Furthermore many populations were lost due to habitat destruction for houses, infrastructure, afforestation and land drainage. This resulted in a severe decline of their distribution ranges and an increase of fragmentation and isolation of the surviving populations.

Maculinea teleius (scarce large blue butterfly) and *M. nausithous* (dusky large blue butterfly) occur on mesophile to nutrient poor moist meadows. For *Maculinea teleius*, habitats are mown once to twice a year, depending on their productivity, while *Maculinea nausithous* also thrives in taller herb vegetation that is mown every third to fifth year. In The Netherlands, both species used to occur mainly in the riverine landscapes of the provinces Limburg and Northern Brabant. They went extinct in the 1970s.

In 1990 both *Maculinea* species were reintroduced in the nature reserve Moerputten in Northern Brabant, following the recommendations of the IUCN. With a very high density of *Sanguisorba officinalis* and the host ant species *Myrmica rubra* and *Myrmica scabrinodis* present, this nature reserve was found to be the only feasible reintroduction site. As a source we used the metapopulations of both butterfly species near the village Kostrza in the valley of the river Wisla south of Krakow in Poland. These metapopulations consist of several thousands of adults per year and would not be endangered if a high number of individuals were taken away.

The reintroduced populations of *Maculinea teleius* and *Maculinea nausithous* behaved differently after release. The population of *M. teleius* expanded during the first three years. Then, after a decrease in numbers due to overexploitation of the host ant population, the population size levelled at 300 adults per year. This happened twice, since in 1995 the occupied meadow was accidentally mown in the flight period. *Maculinea teleius* appeared to show only restricted dispersal. Until the present day, the only occupied meadow is the one where the butterflies were releasd in

1990.

The newly established population of *M. nausithous* declined in the year after the reintroduction but expanded later. After several years with very high population size, the number of butterflies is again decreasing. The butterflies left the meadow where they were released and settled on the railway embankment crossing the nature reserve. This appeared to be the site with the highest density of *Myrmica rubra* nests, the specific host ant species for this butterfly. Two years later, the road verges at the street Ruidigerdreef at the southern side of the Moerputten nature reserve were colonized. This second subpopulation also spread and increased in numbers. These subpopulations were separated by about 500 m but exchanged 8 to 10% of adults during the flight period. Again two years later, a third subpopulation at a distance of more than 5 km was founded. This subpopulation is still very small.

Although the results are quite positive with respect to the numbers of butterflies, some features of potential concern have also been found. Both species, but especially *M. teleius,* have a very low mean minimal adult life span, which makes them more susceptible to stochastic catastrophies. Furthermore, it has been found that the flight periods of the reintroduced populations differ from that of the source populations and from that of the historic Dutch populations. *Maculinea teleius* is on the wing during the month of July, *Maculinea nausithous* from the middle of July to the middle of August. In the former Dutch populations and in Kostrza, a normal flight period of both species endures until the end August.

Maculinea nausithous and *Maculinea teleius* butterflies are dependent on two sequential sources of food, namely the host plant *Sanguisorba officinalis* and for each butterfly species a different, specific, *Myrmica* host ant. The eggs are deposited on *Sanguisorba officinalis* of certain phenological stages differing between the butterfly species. The L1 to L3 instar caterpillars feed on the hostplant for two to three weeks. Then, they drop to the ground to be collected by worker ants of *Myrmica* species. In the ant nests they feed on ant grubs, hibernate and pupate. *Maculinea nausithous* parasitizes on the nests of *Myrmica rubra*, while *Maculinea teleius* stays in the nests of *Myrmica scabrinodis. Myrmica* ants have a limited home range, normally not exceeding 2 m around the nests. Therefore, in most occupied habitats the host plants are abundant while the host ant is relatively rare, so that most host plants are in fact unsuitable for oviposition due to a lack of ants in their close vicinity. It would give females a comparative advantage if they could detect host ants so as to deposit eggs on truly suitable *Sanguisorba* plants only. However, *Maculinea* butterflies are thought to oviposit randomly with respect to the occurence of host ants (=random oviposition hypothesis). The phenology of a chosen flowerhead and vegetation characteristics are thought to be the most important variables a females reacts upon when depositing an egg. We tested oviposition in response to the density of both the sources of food (=ant-mediated oviposition hypothesis) by comparing adjacent patches differing in host plant and host ant nest density. This part of the reserach was performed at the Kostrza site. *Maculinea nausithous* females appeared to aggregate and oviposit in places where nests of their host ant, *Myrmica rubra* are present. *Maculinea teleius* females oviposit independently of the density of the nests of the host ant *Myrmica scabrinodis* but rather respond to vegetation and flowerhead characteristics. Based on these differences we formulated the hypothesis that *Maculinea nausithous* can detect its host ant, while *Maculinea teleius* reacts to vegetation characteristics.

The idea of ant detection was then further tested in an insectory experiment. Females of both species were free to choose between host plants on plots with *Myrmica rubra* or *Myrmica scabrinodis,* or without ants, while vegetation characteristics were similar between plots. First, we took notes of oviposition observations, later the flowerheads were encapsulated with small curtain bags to capture the caterpillars. The oviposition observations show us how females react when they can choose optimal sites in a overabundance of empty flowerheads. The caterpillar captures include the choices when the best flowerheads are already occupied. For both *Maculinea* species, in the beginning of the flight period the presence of the respective host ant species has an influence on the oviposition of the butterflies. For *Maculinea teleius*, caterpillar captures also show that females prefer hostplants on plots with *Myrmica. Maculinea nausithous* later responds to vegetation structure and flowerhead phenology characteristics, because the development of the offered hostplants was very early. Hence, first indications have been found that for both butterfly species the presence of their host ant species is important in selecting flowerheads for oviposition. This effect is clear for the first ovipositions while in the late flight period a heavier weighting of vegetation structure and flowerhead phenology characters was found.

Given the narrowly defined habitat requirements of *Maculinea teleius* and *Maculinea nausithous* that live as a caterpillar in the nests of the ant species *Myrmica scabrinodis* and *M. rubra* respectively, one would expect that individuals are able to select for sites that meet their requirements. In contrast to the situation in the insectory, in the field their first larval resource, the host plant *Sanguisorba officinalis*, is abundant while the ant nests are in comparison less abundant. Therefore, a part of the host plant population represents a sink when selected for oviposition due to the limited home range of the ant nests. In the case of *Maculinea* butterflies, the presence of host ants close to the host plants determines the suitability of the host plant as being a true resource or a sink. Only in the proximity of a host plant being a resource, has the caterpillar a chance of being found by its host ant species. In the proximity of a sink the required *Myrmica* species is not present and therefore the caterpillar will die. The impact of presence or absence of host ant nests on the oviposition of females and on the distribution of the population was studied on a selection of more than 600 plots in and around the Moerputten nature reserve. We tested the oviposition of *Maculinea nausithous* by counting empty egg shells on flowerheads that were collected in September. Since this method was not useful for *Maculinea teleius*, we observed females of this species during the first half of the flight period. It appeared that females of both species prefer to deposit eggs on host plants in the close vicinity of ant nests. Then, the habitat selection of the adults was tested at a higher spatial level by analysing the distribution since 1990 on plots. The vegetation composition, vegetation structure, ant fauna and microclimate of these plots was described and measured in detail. The analysis clearly shows, that adults select for plots with host ants as well. Only in years with high butterfly densities, plots with good vegetation characteristics but lack of ants where occupied, too. These data enable us to reject the random oviposition hypothesis. Furthermore, it is argued that the success of the reintroduction is largely attributed to the ant-mediated oviposition.

It is puzzling that, although the adult individuals of both butterfly species can select sites that contain both their larval resources, dispersal leading to colonisation of suitable sites at a larger spatial scale seems to be constrained. It appears that high quality plots are not easily colonized when they are far away from occupied plots. In contrast, low quality plots are easily colonized when they are close to occupied high quality plots. Dispersal constraints at the individual level affect habitat selection and have an effect on the colonisation and abandonment rates in the populations of the two *Maculinea* butterfly species and thus on their distribution. *Maculinea nausithous* colonizes over longer distances than *Maculinea teleius*, but both species are limited with respect to covering distances. For *Maculinea teleius* the pattern of colonisation and abandonment resembles een expansion-retraction pattern within the heterogenous plots on the occupied meadow BW. The limited dispersal implies consequences for the populations. The meadow BW should be kept at high quality for *Myrmica scabrinodis* and *Maculinea teleius*. This butterfly can only reach adjacent meadows while long distance dispersal is not to be expected. For *Maculinea nausithous* it is recommended to establish a metapopulation. It means that changes in the management in the nature reserve but also on verges and canal borders in the surrounding are needed.

Five years after the reintroduction, a genetic analysis of source and founder poulations was performed. The reintroduced populations of both butterfly species did not pass a bottleneck in terms of a low number of founder individuals. Population numbers increased very rapidly and loss of genetic variation was only mild. The source populations appeared to have only low levels of genetic variation (in allozyme electrophoresis) but this did not have a negative impact on the settling and increase of the founder populations. Apparently the number of translocated founder individuals and the quality of the release site have a higher impact on success than the genetic constitution of the founders.

In both *Maculinea* species only at the Aco-K locus significant differentiation between source and founder population was found. Taking into account that the flight periods shifted forwards and the mean minimal life time of the adults has decreased, we come to the conclusion that the founder populations have experienced selection due to the changed ecological conditions at the founder site. This conclusion is supported by an analysis of the wing patterns of the founder population, the reintroduced population, the historic Dutch population and additional ones in France, Belgium and Germany. The historic Dutch, the Belgian and the French population are alltogether phenotypically different from Polish, German and Moerputten population. We postulate that this is due to different Pleistocene refugia west and east of the Alps. Apparently, butterflies from a different biogeographical origin were translocated to a location outside their naturally colonized range. The founder population, being adapted to climatic conditions in the south of Poland, must have experienced considerable selection pressure.

Both founder populations have a significant negative F_{IS} value, which means that they are in some way able to maintain higher levels of heterozygosity than would be expected in a random mating population. Further research is necessary to elucidate the processes responsible for this phenomenon.

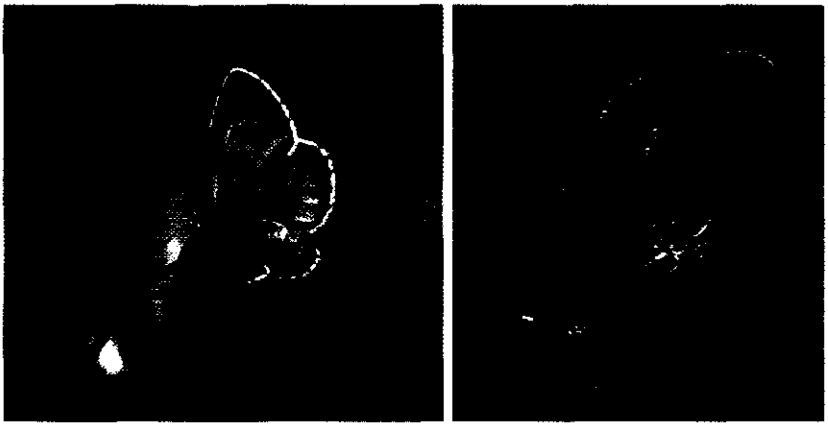
For a long term persistence, the reintroduced *Maculinea* populations need to establish metapopulations to protect them from environmental, demographic and genetic stochasticity. Even though adults appeared to be able to find their host ants, their dispersal and colonisation ability was very restricted. *Maculinea teleius* can establish a metapopulation within the borders of the nature reserve. Next to ensuring high quality habitat on the occupied meadow by constant management, adjacent meadows should be managed in the same way so as to enable colonisation by dispersing adults. Colonisations of other nature reserves is not to be expected without further reintroductions. *Maculinea nausithous* is dependent on the road verges in the surrounding of the nature reserve as habitats for subpopulations of a network. Thus, this species is very sensitive to the management by order of official authorities and by residents. A local *Maculinea* Action Plan has shown, that after changes in management, the ant fauna on the verges has improved. However, at the same time, *Maculinea* habitat has been destroyed by illegal private activities. Again, colonisation of sites at a longer distance is only possible by means of reintroduction projects.

In conclusion, constraints in dispersal and deterioration of habitat through human interference are a danger to a long term persistence of the *Maculinea* populastions. As most nature reserves are small and embedded in an agricultural landscape, butterflies have to leave nature reserves and spread between them. Their persistence is thenstrongly linked to landuse. Reintroduction projects, if performed carefully, can be a useful tool in nature conservation to compensate for large scale changes such as climate warming, and they can be successful. But however careful their scientific foundations have been, they are prone to fail when not accompanied by thourough, well used information and motivating campaigns aimed at local people, local authorities, nature conservationists and reserve wardens.

Samenvatting

In Europa komen vijf soorten blauwtjes van het genus *Maculinea* voor. Door hun bijzondere levenswijze als parasieten in de nesten van *Myrmica* mieren hebben zij de aandacht van veel vlinderdeskundigen op zich gevestigd. Helaas vertonen alle soorten binnen grote delen van hun verspreidingsgebied een sterke achteruitgang in aantallen populaties en ook in populatiegrootte. De achteruitgang wordt veroorzaakt door de modernisering in de landbouw, waardoor veel percelen intensiever worden gebruikt en andere worden verlaten. In beide gevallen verandert het leefgebied van de vlinders in sterke mate. Daarnaast zijn leefgebieden verloren gegaan door de aanleg van woonwijken en infrastuctuur, door de aanleg van productiebossen en door verdroging. Veel van de resterende leefgebieden liggen in een versnipperd landschap waardoor en isolatie van populaties plaatsvindt.

Het pimpernelblauwtje (*Maculinea teleius*) en het donker pimpernelblauwtje (*Maculinea nausithous*) komen voor op matig voedselrijke tot voedselarme vochtige hooilanden en verlaten hooilanden. Het leefgebied van het pimpernelblauwtje moet, naar gelang de productiviteit van de bodem, een tot twee keer per jaar gemaaid worden. Het donker pimpernelblauwtje komt daarnaast voor in ruigere vegetatie,



Het pimpernelblauwtje (links) en donker pimpernelblauwtje zijn in heel Europa bedreigd en vallen onder de habitat-richtlijn.
Maculinea teleius (left) and M. nausithous are threatened all over Europe and are both Habitat-directive species.

die slechts om de drie tot vijf jaar wordt gemaaid. In Nederland kwamen beide soorten voor in de rivier- en beekbegeleidende graslanden van Limburg en Noord-Brabant. Ze zijn in de zeventiger jaren verdwenen uit Nederland.

In 1990 werden beide *Maculinea* soorten geherintroduceerd in het natuurgebied Moerputten in Noord-Brabant (in het kader van het Beschermingsplan Dagvlinders van het Ministerie van Landbouw, Natuurbeheer en Visserij), volgens de voorschriften van het IUCN. De Moerputten bleek de enige voor herintroductie geëigende locatie, omdat de waardplant *Sanguisorba officinalis* (grote pimpernel) in hoge dichtheden aanwezig was en de waardmieren *Myrmica rubra* (rode steekmier) en *Myrmica scabrinodis* (ruwknoopmier) eveneens werden aangetroffen. Als bronpopulaties fungeerden een aantal met elkaar in verbinding staande populaties van beide vlindersoorten rondom het dorpje Kostrza in de vallei van de rivier de Wisla ten zuiden van Krakow in Polen. Deze zogenaamde metapopulaties bestaan uit duizenden vlinders per jaar en zouden geen schade ondervinden van het weghalen van een flink aantal vlinders voor de herintroductie.

Na de herintroductie ontwikkelden de nieuwe populaties van pimpernelblauwtje en donker pimpernelblauwtje zich nogal verschillend. De populatie van het pimpernelblauwtje breidde zich de eerste drie jaar behoorlijk uit. Daarna volgde een flinke teruggang, veroorzaakt door te hoge belasting van de mierennesten, waarna de populatie schommelde rond 300 vlinders per jaar. Dit proces herhaalde zich, nadat in 1995 het hooiland per ongeluk begin augustus werd gemaaid. Het pimpernelblauwtje bleek heel honkvast. Tot vandaag is alleen dat hooiland gekoloniseerd waarop de vlinders bij de herintroductie in 1990 zijn vrij gelaten.

Het donker pimpernelblauwtje liet na de herintroductie eerst een teruggang in aantal zien, voordat de nieuwe populatie begon te groeien. Na enkele jaren met bijzonder veel vlinders nemen de aantallen nu weer af. Op het hooiland waar ze vrij zijn gelaten komen ze niet meer voor. Er vestigde zich eerst een populatie op de spoordijk die dwars door het natuurgebied loopt. Later bleek dat dit de plek was waar de hoogste nestdichtheid van de specifieke waardmier *Myrmica rubra* voorkwam. Twee jaar later werden de wegbermen van de Ruidigerdreef ten zuiden van de Moerputten gekoloniseerd. Ook deze subpopulatie nam snel in omvang toe. Uit vangst-terugvangst onderzoek bleek dat 8-10% van de teruggevangen vlinders afkomstig was uit de andere subpopulatie. Weer twee jaar later werd een derde subpopulatie op een afstand van ruim 5 km gevormd. Deze subpopulatie is echter klein gebleven.

Wat de aantallen vlinders betreft, lijken de resultaten vrij positief, maar er zijn ook enkele punten van zorg. Beide soorten, maar vooral het pimpernelblauwtje bleken gemiddeld een zeer korte levensduur te hebben. Hierdoor worden zij gevoelig voor onvoorspelbare tegenslagen. Verder bleek dat de vliegtijd van de geherintroduceerde populaties verschilt van die van de Poolse bronpopulaties. Ook de vliegperiode van de oude Nederlandse populaties was later. Het pimpernelblauwtje vliegt nu vrijwel alleen in de maand juli, het donker pimpernelblauwtje vliegt van midden juli tot midden augustus. Vroeger in Nederland vlogen en in Kostrza vliegen de soorten tot eind augustus en soms nog later.

Het pimpernelblauwtje en het donker pimpernelblauwtje zijn afhankelijk van twee voedselbronnen, te weten de waardplant *Sanguisorba officinalis* en voor iedere vlindersoort een andere specifieke *Myrmica* waardmierensoort. De eitjes worden afgezet op *Sanguisorba officinalis*, waarbij de soorten echter verschillende ontwikkelingsstadia prefereren. De rupsen in het eerste tot derde stadium voeden zich met deze waardplant gedurende twee tot drie weken. Vervolgens laten ze zich op de grond vallen en worden door *Myrmica* werksters meegenomen naar het mierennest. In het mierennest voeden ze zich verder van mierenlarven. Ze overwinteren en verpoppen er. Het donker pimpernelblauwtje parasiteert in de nesten van *Myrmica rubra*, terwijl het pimpernelblauwtje verblijft in de nesten van *Myrmica scabrinodis*. De werksters hebben een klein foerageergebied en komen normaal niet verder dan twee meter van hun nest. In veel leefgebieden komt de waardplant algemeen en in hoge dichtheden voorkomt, terwijl de mierennesten in verhouding zeldzaam zijn. Hier zijn veel waardplanten eigenlijk ongeschikt om er eitjes op af te zetten, omdat er geen mierennesten dichtbij zijn. Vrouwtjes zouden daarom een voordeel hebben, als ze waardmier(nesten) zouden kunnen detecteren, zodat ze hun eitjes alleen afzetten op daadwerkelijk geschikte waardplanten. Er wordt echter van uitgegaan, dat *Maculinea* vrouwtjes hun eitjes met betrekking tot de mieren willekeurig af zetten (=*random oviposition hypothesis)*. De ontwikkeling van de bloemhoofdjes en de

De Bijenwei (BW) in de Moerputten: leefgebied van het pimpernelblauwtje. De vlinders vinden er alles om te kunnen overleven: veel waardplanten en nectarplanten en zeer veel nesten van de ruwknoopmier *Myrmica scabrinodis*.

The meadow BW (Bijenwei) in the Moerputten: home of Maculinea teleius. A high density of the hostplant, many nectar sources and many nests of the specific hostant Myrmica scabrinodis *can be met at this site.*

vegetatiekenmerken ter plaatse zouden de meest belangrijke eigenschappen zijn, waarop een eiafzettend vrouwtje reageert. Er is getoetst of de eiafzetting niet toch een repons op zowel bloemhoofd- als mierennestdichtheid zou kunnen zijn (=*ant-mediated oviposition*). Dit werd gedaan door bij het dorpje Kostrza vlakken te vergelijken, die in waardplant- en waardmiernestdichtheid verschilden. Het bleek dat de vrouwtjes van het donker pimpernelblauwtje zich voornamelijk daar ophouden en eitjes afzetten, waar veel nesten van hun waardmier *Myrmica rubra* aanwezig zijn. De vrouwtjes van het pimpernelblauwtje daarentegen zetten hun eitjes onafhankelijk van de dichtheid aan

De bloemrijke bermen van de Ruidigerdreef zijn tegenwoordig het belangrijkste leefgebied voor het donker pimpernelblauwtje. De nesten van de rode steekmier *Myrmica rubra* bevinden zich in het hoge gras.

The roadverges of the road Ruidigerdreef, south of the Moerputten, are at present the major habitat for Maculinea nausithous. The nests of its specific host ant Myrmica rubra *are restricted to the borders with high vegetation.*

De rupsen van de pimpernelblauwtjes worden door knoopmieren meegenomen naar hun nest. Iedere soort mierenblauwtje is in meer of mindere mate gespecialiseerd op een waardmierensoort.

A caterpillar of a Maculinea butterfly is taken to the nest of a Myrmica-ant. The survival chance is highest in it's specific host ants' nest.

waardmiernesten af. Zij reageren op vegetatie en bloemhoofdkenmerken. Op basis van deze verschillen is de hypothese geformuleerd, dat het donker pimpernelblauwtje wel zijn waardmier kan detecteren, terwijl het pimpernelblauwtje meer op vegetatie-eigenschappen reageert.

Deze hypothese is getoetst in een experimentele opstelling in een gazen vlinderkas. Vrouwtjes van beide soorten werden vrijgelaten en konden voor de ovipositie een keuze maken uit waardplanten in vakken met *Myrmica rubra*, *Myrmica scabrinodis* of zonder mieren. De eerste honderd eiafzettingen (=*oviposities*)

Het vrijlaten van de vlinders in de Moerputten.
The release of the butterflies in the Moerputten.

In het oorspronkelijk leefgebied bij Krakau in Polen komen de pimpernelblauwtjes voor in een grote metapopulatie.
Near Krakow in Poland the Maculinea's occur in a large metapopulation.

werden geobserveerd en genoteerd. Deze laten conclusies toe over de reacties van de vrouwtjes als zij geconfronteerd worden met een grote hoeveelheid potentiële ei-afzetplaatsen. Als de beste bloemhoofdjes bezet zijn, zullen ze ook voor de minder optimale hoofdjes kiezen. Nadat de vrouwtjes dood waren werden de bloemhoofdjes met kleine gazen zakjes omsloten, zodat alle rupsen gevangen konden worden. Hierbij worden ook de latere, minder optimale keuzes meegenomen.

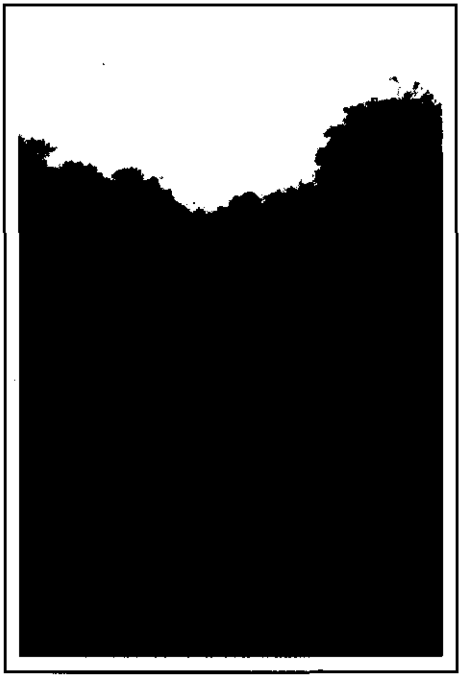
Uit het experiment blijkt dat de vrouwtjes van beide soorten vlinders meer eitjes afzetten op vlakken waar hun waardmier voorkomt. Bij het pimpernelblauwtje worden ook de meeste rupsen op vlakken met waardmieren gevangen. Bij het donker pimpernelblauwtje daarentegen bleken de rupsenvangsten verklaard te worden door de vegetatiestructuur en het bloeistadium van de bloemhoofdjes. Dit kwam doordat de aangeboden waardplanten in een te vroeg ontwikkelingsstadium waren: de vrouwtjes van het donker pimpernelblauwtje prefereren oudere bloemhoofdjes. Ook in dit experiment zijn dus aanwijzingen gevonden voor het belang van mieren op de eiafzettingen, maar een duidelijk verband werd alleen in het begin van de vliegperiode gevonden.

Het pimpernelblauwtje en het donker pimpernelblauwtje stellen hoge eisen aan hun leefomgeving. Het ligt in de verwachting dat de vlinders de plekken die aan hun ecologische eisen voldoen, kunnen selecteren. In tegenstelling tot de situatie in de vlinderkooi is in het veld de waardplant *Sanguisorba officinalis* in hoge dichtheid aanwezig, terwijl de waardmieren minder algemeen zijn. Dit houdt dus in dat een deel van de waardplanten ongeschikt is voor de ei-afzet (= een "sink" vertegenwoordigen), omdat de mieren een beperkt foerageergebied hebben en deze planten niet bereiken. De rupsen worden niet door mieren gevonden en gaan dus dood. Voor de vlinders zijn alleen die planten echte waardplanten ("resource") die binnen het foerageergebied van een mierennest staan. Alleen de rupsen op deze planten hebben een kans om door een werkster gevonden en geadopteerd te worden. Het effect van de aan- of afwezigheid van mierennesten op de eiafzetting van de vrouwtjes en het voorkomen van de populaties werd bestudeerd op meer dan 600 proefvlakken in de Moerputten en de omgeving ervan. Op deze plots werden vegetatiesamenstelling, vegetatiestructuur, mierenfauna en microklimaat in detail gemeten en beschreven. Oviposities van het donker pimpernelblauwtje zijn gemakkelijk aan te tonen door de eirestanten op de bloemhoofdjes van de waardplant te tellen, nadat deze eind september zijn geplukt. Deze methode voldeed niet voor het pimpernelblauwtje, zodat bij deze vlinder vrouwtjes gedurende de eerste helft van de vliegtijd werden gevolgd. Bij beide soorten prefereerden de vrouwtjes waardplanten in de buurt van *Myrmica*-nesten voor de ovipositie.

Vervolgens werd de habitatselectie op een hoger ruimtelijk schaalniveau getoetst door de verspreiding van de vlinders sinds 1991 per plot te analyseren. Ook op dit schaalniveau werd selectie voor plots met mieren aangetoond. Alleen in jaren, waarin de populatiedichtheid van de vlinders hoog was, werden ook plots met geschikte vegetatie maar zonder mieren bezet. Op basis van deze resultaten kunnen we ervan uitgaan, dat de eiafzetting van pimpernelblauwtje en donker pimpernelblauwtje afhankelijk van mierenaanwezigheid plaats vindt. Eitjes worden bij voorkeur dicht bij mierennesten afgezet, en het succes van de herintroductie is zeker ten dele te danken aan dit gedrag.

Het is opvallend dat ondanks de selectie van proefvlakken met beide voedselbronnen voor de rupsen, de kolonisatie van geschikte plekken op hogere ruimtelijke schaal beperkt blijft. Geschikte plekken op grote afstand van bezette plekken blijken minder gekoloniseerd te worden dan verwacht. Daarentegen worden plots met een slechte habitatkwaliteit op korte afstand van bezette plots gemakkelijk bezet. Beperkingen in de verbreidingscapaciteit op individueel niveau belemmeren de habitatselectie. Dit heeft een effect op de kolonisatie en het verlaten van proefvlakken van de gehele populatie, en dus ook in het aangetroffen patroon van voorkomen. Het donker pimpernelblauwtje koloniseert over grotere afstanden dan het pimpernelblauwtje, maar beide soorten zijn toch zeer honkvast. Bij het pimpernelblauwtje laten de kolonisaties en lokale extincties een patroon van uitbreiden en terugtrekken binnen de heterogene plots op de Bijenwei zien, het enige bezette hooiland. Voor de populatie als geheel zijn er consequenties verbonden aan deze beperkte verbreiding. De hoge habitatkwaliteit voor het pimpernelblauwtje en zijn waardmier *Myrmica scabrinodis* op de Bijenwei moet behouden blijven. Deze vlinder kan hooguit naburige hooilanden bereiken, terwijl dat over grotere afstanden niet te verwachten valt. Het donker pimpernelblauwtje daarentegen kan wel een metapopulatie opbouwen in de omgeving van de Moerputten. Dit houdt in dat niet alleen in het natuurgebied maar ook op wegbermen en slootranden in de omgeving bij inrichtings- en beheersmaatregelen met de habitateisen van deze vlinder, zijn waardplant en waardmier rekening gehouden moet worden.

Vijf jaar na de herintroductie werd een genetische analyse van de bronpopulatie in Polen en de geherintroduceerde populatie in de

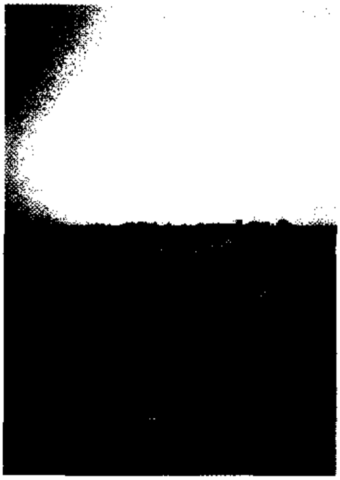


Het donker pimpernelblauwtje heeft een voorkeur voor een ruigere vegetatie dan het pimpernelblauwtje, zoals hier op de noordzijde van de spoordijk in de Moerputten.
M. nausithous prefers a tall herb vegetation, such as on the railway embankment in the Moeputten.

Moerputten uitgevoerd. Bij beide geherintroduceerde populaties zijn de aantallen vlinders na de vrijlating niet sterk teruggevallen. De populatiegroottes zijn voldoende snel toegenomen en er werd alleen een gering verlies aan genetische variatie vastgesteld. De bronpopulatie had slechts weinig genetische variatie (detecteerbaar door eiwit-electrophorese), maar er werd geen negatief effect hiervan op de vestiging en uitbreiding van de geherintroduceerde populaties vastgesteld. Blijkbaar waren de aantallen vrijgelaten vlinders en de kwaliteit van de herintroductie-locatie van groter belang voor het herintroductiesucces dan de genetische variatie van de bronpopulatie.

Het juiste beheer van de bermen bepaalt voor een belangrijk deel de overlevingskansen van het donker pimpernelblauwtje. Als er op het verkeerde moment gemaaid wordt zijn er geen waardplanten beschikbaar als de vlinders vliegen. Bij aangepast maaibeheer kan moderne landbouw samen gaan met kwetsbare vlindersoorten.

The right management of the roadverges is an important factor in the survival of Maculinea nausithous. *If sites are mown at the wrong moment no larval foodplants are available for oviposition. But with small adaptations in the management, modern agriculture and vulnerable butterfly species can occur side by side.*



De in onbruik geraakte spoorbrug over het meertje in de Moerputten.

The railway bridge over the centrallake in the Moerputten nature reserve is not in use anymore.

Bij beide *Maculinea* soorten werden alleen voor een bepaald eitwit (Aco-K) genetische verschillen tussen bron- en herintroductiepopulatie gevonden. In combinatie met de verplaatsing van de vliegperiode en de korte levensduur van de vlinders leidt dit tot de conclusie dat de

Rondom het meer komt nu veel wilgen- en elzenbroekbos voor, waar vroeger blauwgraslanden waren, vergelijk fig. 1.10, pag. 37.

At present moist forests occur on the former meadows around the lake, compare fig. 1.10, page 37.

Niet alle ogenschijnlijk geschikte hooilanden met veel grote pimpernel worden gekoloniseerd. Dat kan worden veroorzaakt door de afwezigheid van *Myrmica*-mieren, of door een te grote afstand tot een bezette plek.

Not all meadows with lots of Sanguisorba officinalis *are colonised. The host ant species might be absent or the distance to an occupiedplot is too large to bridge with normal dispersal behaviour.*

geherintroduceerde populaties op de nieuwe locatie selectie hebben ondervonden. Deze conclusie wordt ondersteunt door de analyse van de vleugelpatronen van bronpopulatie, Moerputten-populatie, de historische Nederlandse populaties en populaties in Frankrijk, België en Duitsland. De groep bestaande uit de oude Nederlandse, Belgische en Franse populaties verschilt in zijn uiterlijk van de groep bestaande uit de Poolse, Duitse en de Moerputten populaties. Gesteld kan worden dat dit het gevolg is van verschillende ijstijdrefugia ten westen en ten oosten van de Alpen. Bij de herintroductie zijn vlinders van een bepaalde andere biogeografische oorsprong verplaatst naar een plek buiten hun op natuurlijke wijze gekoloniseerde verspreidingsgebied. De verplaatste vlinders waren aangepast aan de klimatologische omstandigheden in het zuiden van Polen en hebben selectiedruk ondergaan op hun nieuwe vliegplaats.

Beide geherintroduceerde populaties hebben een negatieve inteeltcoëfficient F_{IS}. Dit wil zeggen dat zij op een of ander manier in staat zijn om een hogere mate van variatie te handhaven dan op basis van toevallige paringen verwacht kan worden. Het is niet duidelijk hoe dit phenomeen totstandkomt, en verder onderzoek is nodig om dit op te helderen.

Voor de levensvatbaarheid op lange termijn zouden de geherintroduceerde *Maculinea* populaties metapopulaties moeten kunnen ontwikkelen, zodat ze beter beschermd zijn tegen demografische, genetische en omgevingscatastrofes. Ook al zijn de vlinders in staat om plekken met hun waardmieren op te sporen, hun verbreiding en kolonisatievermogen zijn zeer beperkt. Het pimpernelblauwtje is alleen in staat om een metapopulatie binnen de grenzen van het natuurreservaat op te bouwen. Hiervoor moet ten eerste de hoge habitatkwaliteit op het bezette hooiland door constant beheer bewaard worden. Daarnaast moeten naburige hooilanden op een vergelijkbare wijze beheerd worden zodat zij gekoloniseerd kunnen worden. Uitbreiding naar andere natuurgebieden is alleen door middel van herintroducties mogelijk. Het donker pimpernelblauwtje is voor het opbouwen van een populatienetwerk afhankelijk van de wegbermen en slootranden in de omgeving van het natuurreservaat. Deze soort is dus zeer gevoelig voor het beheer zoals dat door lokale overheden en aanwoners wordt uitgevoerd. In het kader van het Convenant Pimpernelblauwtjes zijn beheersveranderingen uitgevoerd en is aangetoond, dat de mierenfauna op deze wijze verbeterd kan worden. Maar tegelijkertijd is ook duidelijk geworden, dat het leefgebied van de *Maculinea's* door illegale activiteiten gemakkelijk vernield kan worden. Ook het donker pimpernelblauwtje kan gebieden op grotere afstand niet zelfstandig bereiken en zou daar geherintroducerd moeten worden.

Samenvattend: Veel leefgebieden van de pimpernelblauwtjes zijn door toedoen van de mens verdwenen. Resterende terreinen zijn achteruitgegaan in kwaliteit. Hierdoor en door beperkingen in het verbreidingsvermogen van beide vlindersoorten worden populaties op lange termijn in hun levenvatbaarheid aangetast. Omdat de meeste natuurreservaten maar klein zijn en ingebed zijn in agrarisch landschap, moeten de vlinders de reservaten wel verlaten en zich ertussen vestigen en verbreiden. Hun overlevingskans is daar sterk gebonden aan het landgebruik. Als herintroductieprojekten voorzichtig en op wetenschappelijke basis worden uitgevoerd, kunnen zij belangrijk zijn voor de natuurbescherming. Negatieve effecten op grote schaal, zoals ten gevolge van klimaatsverandering, kunnen ermee ten dele worden gecompenseerd. Maar hoe zorgvuldig de wetenschappelijke uitvoering ook is, herintroductieprojekten hebben een lage kans van slagen als niet ook gedegen voorlichtingscampagnes naar de lokale bevolking, de lokale autoriteiten, natuurbeschermingsorganisaties en natuurbeheerders worden meegenomen.

Zusammenfassung

In Europa kommen fünf Bläulingsarten der Gattung *Maculinea* vor. Durch ihre besondere Ökologie als Parasiten in den Nestern von *Myrmica*-Ameisen erfreuen sie sich der besonderen Aufmerksamkeit vieler Lepidopterologen. Alle fünf Arten zeigen innerhalb ihres Verbreitungsgebietes einen starken Rückgang an Populationen wie auch vielfach bezüglich ihrer Populationsgrössen. Diese Rückgänge werden hauptsächlich durch landwirtschaftliche Nutzungsintensivierung einerseits sowie das Brachfallen von Extensiv-Grünland andererseits verursacht. In beiden Fällen ändern sich die Lebensräume der Falter grundlegend. Zudem werden Lebensräume durch Siedlungsbau, Infrastrukturmassnahmen, Aufforstungen und Entwässerung zerstört. Die verbleibenden Populationen werden durch Fragmentierung ihrer Lebensräume und Populationensisolation beeinträchtigt.

Der Helle Wiesenknopf-Ameisenbläuling (*Maculinea teleius*) und der Dunkle Wiesenknopf-Ameisenbläuling (*Maculinea nausithous*) besiedeln Feuchtwiesen, feuchte bis frische Mähwiesen und jüngere Brachen. Wiesen mit Vorkommen des Hellen Wiesenknopf-Ameisenbläulings müssen, je nach Produktivität, ein- bis zweimal jährlich gemäht werden. Der Dunkle Wiesenknopf-Ameisenbläuling toleriert daneben auch Brachen, die nur alle drie bis fünf Jahre gemäht werden. In den Niederlanden kamen beide Arten auf Wiesen entlang von Flüssen und Bächen in den Provinzen Limburg und Nord-Brabant vor. In den siebziger Jahren verschwanden die letzten Populationen.

Im Rahmen eines Wiedereinbürgerungs-projektes wurden im Jahre 1990 Falter beider Arten im Naturschutzgebiet Moerputten in Nord-Brabant freigelassen. Die Wiedereinbürgerung wurde nach den Regeln der IUCN (International Union for Conservation of Nature and Natural Resources) ausgeführt. Es stellte sich heraus, dass dieses Naturschutzgebiet der einzig mögliche Ort in den Niederlanden für ein derartiges Projekt war, da nur hier die Wirtspflanze *Sanguisorba officinalis* in hoher Dichte vorkommt und beide Wirtsameisen (*Myrmica rubra* und *Myrmica scabrinodis*) nachgewiesen werden konnten. Die freigelassenen Falter stammen aus grossen Metapopulationen im Tal des Flusses Wisla in der Nähe des Dorfes Kostrza südlich von Krakau in Polen. Die Metapopulationen bestehen aus mehreren Tausend Faltern pro Jahr und wurden durch das Wegfangen der Falter für das

Wiedereinbürgerungsprojekt nicht beeinträchtigt.

Nach der Wiedereinbürgerung zeigten beide Arten unterschiedliche Populationsentwicklungen. Der Helle Wiesenknopf-Ameisenbläuling nahm in den folgenden drei Jahren zu. Anschliessend nahm die Population stark ab, was möglicherweise durch Überbelastung der Ameisennester verursacht wurde, um sich danach auf eine Anzahl von rond 300 Faltern pro Jahr einzupendeln. Dieser Prozess wiederholte sich noch einmal, da 1995 durch einen unglücklichen Zufall das Habitat im August gemäht wurde. Die Art hat sich bislang räumlich nicht ausgebreitet. Bis heute kommt sie nur auf der einen Wiese vor, auf der die Falter ursprünglich freigelassen wurden.

Der Dunkle Wiesenknopf-Ameisenbläuling durchlebte im Jahr nach der Wiedereinbürgerung zunächst einen starken Rückgang, bevor die Anzahl der Falter in den folgenden Jahren stieg. Die Schmetterlinge haben die Wiese, auf der sie freigelassen wurden, verlassen und sich auf dem Bahndamm, der mitten durch das Naturschutzgebiet läuft, angesiedelt. Es stellte sich heraus, dass hier die höchste Dichte der spezifischen Wirtsameise *Myrmica rubra* vorkam. Nach zwei Jahren wurden die Böschungen der Strasse Ruidigerdreef an der Südseite des Naturchutzgebietes kolonisiert. Diese zweite Subpopulation breitete sich relativ schnell in Anzahl und Fläche aus. Beide Subpopulationen liegen mehr als 500 Meter von einander entfernt. Im Rahmen von Fang-Wiederfangstudien zeigte sich, dass zwischen 8 und 10% der wiedergefangenen Falter in der jeweils anderen Subpopulation markiert wurden. Wieder zwei Jahre später etablierte sich eine dritte Subpopulation in einem Abstand von mehr als 5 km, die jedoch immer noch sehr klein ist.

Trotz der im allgemeinen positiven Resultate zeigten sich einige besorgniserregende Fakten. Beide Arten, besonders der Helle Wiesenknopf-Ameisenbläuling, haben eine kurze Lebenserwartung, wodurch sie empfindlich gegenüber stochastische Katastrophen sind. Ausserdem hat sich die Flugzeit der wiedereingebürgerten Populationen, verglichen mit den Mutterpopulationen, zu einem früheren Zeitpunkt hin verschoben. Dies zeigte sich auch im Vergleich mit den Flugzeiten der historischen holländischen Populationen. Aktuell fliegt der Helle Wiesenknopf-Ameisenbläuling von Anfang bis Ende Juli, der Dunkle Wiesenknopf-Ameisenbläuling von Mitte Juli bis Mitte August. Bei den früheren holländischen Populationen und in Kostrza dauert die Flugzeit beider Arten bis Ende August und länger.

Die Larven der Wiesenknopf-Ameisenbläulinge nutzen zwei verschiedene Ressourcen, die Wirtspflanze *Sanguisorba officinalis* und eine, je nach Art unterschiedliche, *Myrmica*-Wirtsameisen. Die Eier werden einzeln oder zu mehreren auf die Blütenköpfchen des Grossen Wiesenknopfes abgelegt, wobei die Falterarten verschiedene phänologische Stadien der Blütenköpfe bevorzugen. Die L1- bis L3-Raupen ernähren sich von der Wirstpflanze, auf der sie zwei bis drei Wochen verbleiben. Dann lassen sie sich zu Boden fallen, wo sie von den Arbeiterinnen unterschiedlicher *Myrmica*-Arten adoptiert und in die Ameisennester mitgenommen werden. Im Ameisennest ernähren sich die Raupen parasitisch von Ameisenlarven. Hier findet die Überwinterung und im folgenden Frühjahr die Verpuppung statt. Der Dunkle Wiesenknopf-Ameisenbläuling parasitiert in den Nestern von *Myrmica rubra*, während der Helle Wiesenknopf-Ameisenbläuling hauptsächlich in den Nestern von *Myrmica scabrinodis* zu finden ist. Die Arbeiterinnen der Ameisenvölker bleiben im allgemeinen immer in der Nähe des Nestes und entfernen sich selten mehr als 2 Meter von diesem. Da in den meisten Bläulings-Lebensräumen der Grosse Wiesenknopf in hoher Dichte vorkommt, während die Ameisennester viel seltener sind, ist nur ein Teil der Wiesenknopfpflanzen als Wirtspflanzen geeignet, der im Fouragierbereich eines Ameisennestes liegt. Die Weibchen der Bläulinge hätten darum einen grossen Vorteil, wenn sie ihre Eier nur auf solchen Pflanzen ablegen würden, die aufgrund der Anwesenheit der spezifischen Wirtsameise ihre Funktion als Wirtspflanze erfüllen können. Allerdings wird bisher davon ausgegangen, dass die *Maculinea*-Weibchen ihre Eier in Bezug auf die Ameisen willkürlich ablegen (=*random oviposition hypothesis*). Der phänologische Zustand des gewählten Blütenköpfchens und die Eigenschaften der Vegetation in der direkten Umgebung seien für das Weibchen von ausschlaggebender Bedeutung. Wir haben getestet, ob das tatsächlich der Fall ist, oder ob das Weibchen neben den Eigenschaften des Blütenköpfchens und der Vegetation auch auf die Anwesenheit bzw. Dichte von Ameisennestern reagiert (=*ant-mediated oviposition*). Hierzu wurde die Eiablage-Dichte auf Probeflächen mit unterschiedlichen Wirtspflanzen- und Wirtsameisendichten in der Umgebung des Dorfes Kostrza verglichen. Es zeigte sich, dass die Weibchen des Dunklen Wiesenknopf-Ameisenbläulings sich bevorzugt dort aufhalten und Eier ablegen, wo die Nestdichte der Wirtsameise *Myrmica rubra* hoch ist. Bei dem Hellen Wiesenknopf-Ameisenbläuling konnte jedoch keine Verbinding zwischen Eiablageorten und Wirtsameisendichte gefunden werden. Hier waren die Eigenschaften der Vegetation und der Blütenköpfchen wichtiger. Aufgrund dieser Unterschiede stellen wir die Hypothese auf, dass der Dunkle Wiesenknopf-Ameisenbläuling die Anwesenheit seiner Wirtsameisen feststellen und darauf reagieren kann, während der Helle Wiesenknopf-Ameisenbläuling bei der Ablage der Eier auf Vegetationseigenschaften reagiert.

Diese Hypothese wurde in einem Insektarium experimentell getestet. Weibchen beider Falterarten konnten bei der Eiablage frei zwischen Wirtspflanzen mit *Myrmica rubra*, *Myrmica scabrinodis* oder ohne Ameisen in vergleichbarer Vegetation wählen. Erst wurden die Eiablagen bobachtet und notiert. Später wurden die Blütenköpfchen in kleine Säckchen eingeschlossen, um die Raupen zu fangen. Die Eiablage-Beobachtungen erlauben Rückschlüsse auf das Verhalten der Weibchen, wenn ihnen ein Übermass an unbelegten Wirtspflanzen zur Verfügung steht. Bei den Raupenfängen werden auch die Eiablage-Entscheidungen einbezogen, als bereits ein Teil der Wirtspflanzen belegt war. Bei beiden *Maculinea*-Arten spielte zu Anfang der Fluzeit die Anwesenheit der jeweiligen Wirtsameise eine grosse Rolle für die Eiablagen. Bei dem Hellen Wiesenknopf-Ameisenbläuling zeigten auch die Raupenfänge, dass die Weibchen vorzugsweise Wirtspflanzen in Probeflächen mit Ameisen belegten. Bei dem Dunklen Wiesenknopf-Ameisenbläuling liess sich auch ein Einfluss der Vegetationseigenschaften nachweisen, der darauf zurückzuführen war, dass die angebotenen Wirtspflanzen zu jung waren. Folglich gibt es erste Hinweise, dass für beide Arten die Anwesenheit der Wirtsameisen bei der Wahl eines Blütenköpfchens für die Eiablage eine Rolle spielt. Dieser Effekt ist zu Beginn der Flugzeit deutlich ausgeprägt, während später die Vegetationsstruktur und der phänologische Zustand des Blütenköpfchens gleichfalls von Bedeutung sind.

Wie bereits zuvor ausgeführt wurde, können die Ameisenbläulinge aufgrund ihrer spezifischen Ökologie nur ein Teil der ihnen potentiell zur Verfügung stehenden Wiesenknopfpflanzen sinnvoll nutzen. Pflanzen ausserhalb der Aktionsradien von Ameisennestern stellen Populationssenken dar, da die hier befindlichen Raupen keine Überlebenschance haben. Die Bedeutung der An- und Abwesenheit von Ameisennestern auf die Eiablage und die Anwesenheit von Faltern wurde im Freiland anhand von mehr als 600 Probeflächen im Naturschutzgebiet Moerputten und seiner Umgebung untersucht. Eiablagen des Dunklen

Wiesenknopf-Ameisenbläulings wurden anhand von Eischalenresten auf den Blütenköpfchen des Grossen Wiesenknopfes nachgewiesen. Die Blütenköpfchen wurden Ende September gesammelt. Da diese Methode für den Hellen Wiesenknopf-Ameisenbläuling nicht geeignet war, wurden die Weibchen dieser Art in der ersten Hälfte der Flugzeit direkt bei der Eiablage beobachtet. Die Weibchen beider Arten legten ihre Eier vorzugsweise auf Blütenköpfchen in der Nähe von Wirtsameisennestern ablegen. Die Habitatwahl der Falterpopulation wurde untersucht, indem die An- und Abwesenheit der Falter auf den Probeflächen seit 1991 analysiert wurde. Die Zusammensetzung der Vegetation, die Vegetationsstruktur, die Ameisenfauna und das Microklima wurden erfasst bzw. gemessen. Die Resultate zeigten, dass sich auch die adulten Falter bevorzugt auf Flächen mit Ameisen aufhielten. Nur in Jahren mit sehr hoher Falterdichte wurden auch Flächen mit geeigneten Vegetationseigenschaften, wo aber die Ameisen fehlen, besetzt. Diese Resultate bestätigen, dass die Falter ihre Wirtsameisen entdecken können und die Eiablage nicht zufällig stattfindet. Der Erfolg des Wiedereinbürgerungsprojektes ist unter anderem darauf zurückzuführen, dass Verluste durch zufällige Eiablage vermieden werden.

Es bleibt erstaunlich, dass die Falter beider Arten offenbar in der Lage sind, Flächen mit beiden larvalen Futterquellen aufzuspüren, aber dennoch auf Landschaftsebene Flächen hoher Habitatqualität unbesetzt bleiben. Eine Analyse der Abstände zwischen besetzten und unbesetzten Patches zeigte, dass Patches mit hoher Habitatqualität unbesetzt bleiben, wenn der Abstand zum nächsten besetzten Patch zu gross ist. Patch von niedriger Habitatqualität werden hingegen schnell kolonisiert, wenn sie nahe bei besetzten Patches hoher Habitatqualität liegen. Limitierte Dispersionskapazitäten führen daher zu Einschränkungen in der Habitatwahl der Population. Der Dunkle Wiesenknopf-Ameisenbläuling kann zwar grössere Strecken zurücklegen als der Helle Wiesenknopf-Ameisenbläuling, doch bei beiden Arten werden geeignete Lebensräume in grossem Abstand zu besiedelten Flächen nicht kolonisiert. Beim Hellen Wiesenknopf-Ameisenbläuling beschreibt das Kolonisations-und Extinktionsmuster einen Ausbreitungs- und Rückzugsprozess innerhalb der einzigen besetzten, heterogenen Mähwiese BW. Die eingeschränkte Dispersionsfähigkeit führt zu Konsequenzen für die Populationen. Für den Hellen Wiesenknopf-Ameisenbläuling und seine Wirtsameise *Myrmica scabrinodis* ist es dringend erforderlich, dass die hohe Habitatqualität auf der Mähwiese BW erhalten bleibt. Diese Art kann höchstens benachbarte

Mähwiesen erreichen, während lange Wanderstrecken nur selten zurückgelegt werden. Für den Dunklen Wiesenknopf-Ameisenbläuling sollten die Voraussetzungen geschaffen werden, eine Metapopulation aufbauen zu können. Hieraus folgt, dass das Biotopmanagement nicht nur innerhalb des Naturschutzgebiets, sondern auch ausserhalb an Strassenböschungen und Bachufern geändert werden sollte.

Fünf Jahre nach der Wiedereinbürgerung wurde ein genetischer Vergleich der Mutter- und Tochterpopulationen mit Hilfe von Allozym-Electrophorese durchgeführt. Bei den wiedereingebürgerten Populationen beider Arten konnte "Flaschenhals" infolge der geringen Anzahl an Gründerindividuen nachgewiesen werden. Die Individuenzahl hat in beiden Populationen schnell zugenommen, und es konnte nur ein geringfügiger Verlust an genetischer Variation entdeckt werden. Bereits die Mutterpopulationen wiesen eine geringe genetische Variabilität auf, was allerdings keinen negativen Einfluss auf den Wiedereinbürgerungserfolg hatte. Offensichtlich haben die Anzahl der Gründerindividuen und die Habitatqualität des neu zu kolonisierenden Gebietes einen grösseren Einfluss auf den Erfolg als die genetische Konstitution der Mutterpopulation.

Nach der Wiedereinbürgerung hat bei beiden Arten nur auf dem Locus Aco-K eine genetische Differenzierung gegenüber der Mutterpopulation stattgefunden. Da sich die Flugzeit verschoben und die Lebenserwartung der Falter abgenommen hat, kommen wir zu der Schlussfolgerung, dass die Gründerpopulation unter erheblichem Selektionsdruck durch die veränderten Umweltbedingungen gestanden haben muss. Diese Schlussfolgerung wird unterstützt durch die Analyse der Fleckenmuster auf den Flügelunterseiten. In diese Studie wurden ausser Mutter- und Tochterpopulation auch die historischen holländischen Populationen und Populationen aus Frankreich, Belgien und Deutschland einbezogen. Im Phänotyp unterschied sich die Gruppe aus den historischen holländischen, den belgischen und französischen Populationen von der Gruppe der polnischen, deutschen und der Moerputten-Populationen. Diese Unterschiede sind wahrscheinlich auf unterschiedliche eiszeitliche Refugien westlich und östlich der Alpen zurückzuführen. Offensichtlich wurden Tiere eines bestimmten biogeographischen Ursprungs in das Areal der Populationen eines anderen biogeographischen Ursprungs transportiert. Die Gründerpopulation, die an die klimatologischen Verhältnisse in Polen angepast war, wurde dadurch veränderten

Selektionsbedingungen ausgesetzt.

Bei beiden Gründerpopulationen wurde ein negativer Inzuchtkoeffizient festgestellt. Also sind die Populationen in der Lage, eine höhere Heterozygosität zu bewaren, als aufgrund von Zufallspaarungen erwartet werden kann. Es ist unklar, mit welchem Mechanismus dieses Phänomen erklärt werden kann, weitere Studien müssen dies zeigen.

Zur Erhöhung der längerfristigen Überlebenswahrscheinlichkeit empfiehlt es sich, den wiedereingebürgerten *Maculinea*-Populationen Möglichkeiten zur Entwicklung von Metapopulationen zu bieten, so dass sie mögliche negative Auswirkungen von demographischen und genetischen Zufallseffekten sowie der Umweltstochastizität kompensieren können. Die Falter sind zwar in der Lage, geeignete Lebensräume (inklusive Wirtsameisen) zu entdecken, doch ihre Dispersionsfähigkeit ist begrenzt. Der Helle Wiesenknopf-Ameisenbläuling kann innerhalb der Grenzen des Naturschutzgebietes eine Metapopulation aufbauen. Hierzu muss einerseits die hohe Habitatqualität auf der einzigen besetzten Mähwiese durch konstantes Biotopmanagement erhalten bleiben, andrerseits müssen benachbarte Mähwiesen in gleicher Weise bewirtschaftet werden, so dass auch diese erfolgreich kolonisiert werden können. Andere Naturschutzgebiete werden mit grosser Wahrscheinlichkeit nicht besetzt werden, es sei denn durch weitere Wiedereinbürgerungsprojekte. Der Dunkle Wiesenknopf-Ameisenbläuling besitzt die Dispersionskapazität, selbstständig eine Metapopulation mit Habitatverbund aufzubauen. Hierbei ist er abhängig von der Habitatqualität der Strassenböschungen und Bachränder in der Umgebung der Naturschutzgebiete, da diese als Trittsteinbiotope genutzt werden. Daher kann diese Art leicht durch das Management dieser Landschaftselemente geholfen werden, wie es durch öffentliche Organisationen und Anrainer ausgeführt wird. Ein lokal begrenztes *Maculinea*-Hilfsprogramm hat gezeigt, dass im Rahmen eines öffentlich ausgeführten Biotopmanagements die Ameisenfauna auf Böschungen nach den ökologischen Anforderungen der Bläulinge entsprechend verbessert werden kann. Leider wurde aber gleichzeitig auch deutlich, dass *Maculinea*-Habitat leicht durch illegale Aktivitäten zerstört werden kann. Auch für den Dunklen Wiesenknopf-Ameisenbläuling gilt, dass die Kolonisation neuer Lebensräume über grössere Abstände nur durch Wiedereinbürgerungsprojekte erfolgen kann.

Wir können zusammenfassen, dass durch die limitierte Dispersionskapazität und anthropogen verursachte Habitatverschlechterungen und -verluste die längerfristige Überlebenswahrscheinlichkeit der *Maculinea*-Populationen vermindert wird. Da die meisten Naturschutzgebiete klein sind und als isolierte Inseln in der agrarisch geprägten Landschaft liegen, müssen die Falter sie auf Dauer verlassen und sich dazwischen ansiedeln und verbreiten. Ihre Überlebenswahrscheinlichkeit ist dann in grossem Masse abhängig von der Landnutzung. Wenn Wiedereinbürgerungsprojekte umsichtig und auf wissenschaftlicher Basis ausgeführt werden, können sie eine wichtige Rolle als Instrument des Naturschutzes spielen. Negative Effekte grossflächiger Umweltveränderungen, wie z.B. Klimaerwärmung, könen auf diese Weise unter Umständen gemildert werden. Aber wie sorgfältig die wissenschaftliche Ausführung auch ist, Wiederbürgerungsprojekte sind erst dann langfristig erfolgreich, wenn Informationscampagnen für die lokale Bevölkerung, öffentliche Organisationen, Naturschutzorganisationen und Naturschützer in das Gesamtprojekt einbezogen werden.

Thank you, dank je wel, Danke, gracias!

Dit boekje draagt slechts mijn naam, maar de basis ervan kon alleen stevig zijn door de bijdragen van velen: mijn familie, vrienden, studenten en collega's. Er zijn honderden vlinders gevangen en eitjes geteld, duizenden bloemhoofdjes gemeten, honderden vegetatie-opnamen gemaakt en mieren gevangen, en er is nog zoveel meer gebeurd door en dankzij jullie! Thank you, dank je wel, Danke, gracias!

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Paul Kreijger en in de beginjaren Ans Raadsen hebben op de monitoring route in de Moerputten iedere week van April tot Oktober vlinders geteld. Paul heeft ook nog vele kilometers per fiets naar pimpernelblauwtjes gezocht. Zo zijn belangrijke gegevens verzameld.

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Last, but not least: Mari im fernen Kanada. Weit weg und doch so nah. Inzwischen sind es 30 Jahre Freundschaft, und das merkt frau, wenn es darauf ankommt. Danke!

Curriculum vitae

Irma Wynhoff werd geboren op 25 december 1960 in Hasselt (Duitsland). In 1980 behaalde zij haar Abitur aan het stedelijk lyceum Johanna-Sebus-Schule te Kleve. Ze begon in hetzelfde jaar met haar studie biologie aan de Katholieke Universiteit in Nijmegen, Nederland. Dat leidde ertoe dat ze zich definitief in dat land vestigde. In haar studie concentreerde ze zich volledig op de ecologie en studeerde af in drie vakken: Plantenecologie en vegetatiekunde, Dierecologie en natuurbeheer, en als derde vak Aquatische ecologie. Tijdens de studie, die tot 1988 duurde, werd het interesse in vlinders gewekt, en later heeft ze voornamelijk aan ecologische vraagstellingen omtrent de levenswijze van deze insectengroep gewerkt. Haar eerste project omvatte het "Beschermingsplan Dagvlinders" voor het Ministerie van Landbouw, Natuurbeheer en Visserij. Naast een grote hoeveelheid kleine projecten begon ze in 1989 met de voorbereiding van de herintroductie van twee op mieren parasiterende vlindersoorten, het pimpernelblauwtje en het donker pimpernelblauwtje. In 1990 werd, eveneens ondersteund door het Ministerie van Landbouw, Natuurbeheer en Visserij de herintroductie uitgevoerd. Irma werd gegrepen door de complexiteit van het vlinder-mier-systeem en stortte zich op de evaluatie van de herintroductie en de ontrafeling van enkele ecologische problemen van deze vlinders. Tot 1995 voerde zij haar werkzaamheden uit als parttime gastmedewerkster van de Vakgroep Terrestrische Oecologie en Natuurbeheer en de inmiddels opgerichte Vlinderstichting. In 1995 begon zij, naast haar werk op de Vlinderstichting, met een promotie-project bij Professor Herbert Prins van de Leerstoelgroep Natuurbeheer in de Tropen en Ecologie van Vertebraten, Wageningen, en Professor Paul Brakefield, Instituut voor Ecologische en Evolutionaire Wetenschappen, Leiden. In dit project werden de voorlopige conclusies in detail uitgewerkt en getoetst. Daarnaast werd haar de mogelijkheid geboden om naast ecologische ook genetische vraagstukken te bewerken. Het resultaat ervan is dit proefschrift. Tevens heeft zij twee boeken en een CD-rom over vlinders gepubliceerd.

Irma is getrouwd met Chris van Swaay. In 1991 en 1993 heeft haar leven nieuwe dimensies gekregen door de geboorte van haar dochter Eva en haar zoon Bosse. Zij gaat graag in zonnige streken camperen. In haar vrije tijd luistert ze naar en danst ze op Flamenco muziek. In deze muzieksoort heeft ze zich ontwikkelt tot een echte "aficionao".

Druk: Van Gils Grafisch Service Centrum, Wageningen.

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