The Ecology and Evolution of Melitaeine Butterflies

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The thesis is based on the following articles:

I   Wahlberg, N. & Zimmermann, M. 2000. Pattern of phylogenetic relationships among members of the tribe Melitaeini (Lepidoptera: Nymphalidae) inferred from mtDNA sequences. – Cladistics 16, in press.

II   Wahlberg, N. 2000. The phylogenetics and biochemistry of host plant specialization in melitaeine butterflies (Lepidoptera: Nymphalidae). – Submitted manuscript.

III   Wahlberg, N., Klemetti, T., Selonen, V. & Hanski, I. 2000. Metapopulation structure and movements in five species of checkerspot butterflies. – Manuscript.

IV   Wahlberg, N., Moilanen, A. & Hanski, I. 1996. Predicting the occurrence of endangered species in fragmented landscapes. – Science 273: 1536-1538.

V   Wahlberg, N., Klemetti, T. & Hanski, I. 2000. Dynamic populations in a dynamic landscape: the metapopulation structure of the marsh fritillary butterfly. – Manuscript.

These are referred to by their Roman numerals in the text.

Contributions

The following table shows the major contributions of authors to the original articles.

	I	II	III	IV	V
Original idea	NW, MZ	NW	IH, NW	IH	TK, IH, NW
Study design	NW	NW	NW	IH, NW	TK, NW
Methods and implementation	NW	NW	NW	IH, AM, NW	NW, TK
Empirical data gathering	NW, MZ	NW	NW, J-PB, TK, VS	NW, MP	TK
Manuscript preparation	NW	NW	NW	NW, IH	NW

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Contents

Introduction

The study of evolution is often focused on some particular characters in a set of related species. By comparing different but related species one can hope to partition the effects of adaptation and constraint on a character of interest. The shared ancestry of species may confound such comparisons, but by taking into account information on the genealogical relationships of the species, one can hope to make the data conform to the assumptions of statistical analyses (Wanntorp et al. 1990; Harvey and Pagel 1991; Harvey 1996).

The comparative approach has been used over a long period of time since Darwin (1859) first proposed the theory of evolution by natural selection. There have been two different traditions in the field of comparative analysis, called the descent and guild traditions (Harvey and Pagel 1991). Taxonomists group species according to common ancestry, while ecologists group species according to a common way of life (guilds). These two traditions are now being united to ask questions about the processes of evolution. For instance, are members of a guild of species similar in their ecology due to identity by descent, or due to parallel or convergent evolution?

The comparative approach is basically a study in adaptation. Many ecologists have noticed that different species have the same adaptations in similar environments. Different species may have the same adaptations mainly for two reasons; they may share a common ancestor (identity by descent) or natural selection may have worked on the different species independently in a similar way (parallel or convergent evolution) (Harvey and Pagel 1991). However, since the knowledge of the evolutionary history of species and species groups is at best sketchy (usually the fossil record is rather inadequate), what one observes is the current state of unknown evolutionary development. In the past few decades systematic methods have enabled taxonomists to build phylogenetic hypotheses which show the best approximation of this evolutionary development for a species group (Hennig 1965; Kitching et al. 1998).

Phylogenetical hypotheses can be used by comparative biologists to study common evolutionary patterns across species and to infer which characters may have evolved in particular species as adaptations to the surrounding environment (Harvey and Pagel 1991). Taking a historical perspective can also help us understand the ecology of single species living in a changing world. By comparing a group of related species, we can identify evolutionary constraints on ecological features we might be interested in, such as host plant choice in butterflies. My aim in this thesis is to make a contribution towards a better understanding of the evolutionary and ecological patterns observable in a group of butterflies belonging to the tribe Melitaeini.

Checkerspot butterflies (melitaeines) have played a major role in helping to understand the population biology of insects ever since Paul Ehrlich began his work on *Euphydryas editha* in the late 1950's (Ehrlich 1961; Ehrlich et al. 1975). Work on melitaeines has been extended into many areas of population biology, from population ecology and genetics to the evolution of host plant use and host-parasitoid interactions. Most recently one melitaeine species, *Melitaea cinxia*, has become the focal species of extensive studies on metapopulation dynamics (Hanski 1999).

The melitaeines are a distinct group of butterflies in the family Nymphalidae and comprise about 250 species (Higgins 1941, 1950, 1955, 1960, 1981). The species are distributed widely in Europe, Asia, North and South America, but are absent from Africa south of the Sahara and Australia. According to the most recent classification by Harvey (1991), melitaeines form the tribe Melitaeini in the subfamily Nymphalinae, which includes two other tribes, the Nymphalini and Kallimini. The Kallimini are postulated to be the sister group of the Melitaeini based on larval morphology (Harvey 1991) and DNA sequence data (Brower 2000b).

The melitaeines have been taxonomically revised extensively by L. G. Higgins over four decades (Higgins 1941, 1950, 1955, 1960, 1978, 1981). He divided the butterflies into

three main groups for which no morphological intermediate forms are known (Higgins 1981). One group comprises the species belonging to the genus *Euphydryas*, which differ from all other melitaeines by the structure of the genitalia and features of their life history. The second group is much less homogenous and includes melitaeine species belonging to the genera *Melitaea*, *Chlosyne* and 9 smaller genera. The third group consists of species belonging to the *Phyciodes* group, which Higgins (1981) split into 12 genera.

The relationships of species in this group of butterflies are just beginning to be discovered (**I**), opening up possibilities of detailed comparative studies. The ecologies of many species are well known, which helps to formulate relevant hypotheses that can be tested. In my thesis, I attempt to understand the ecology and evolution of melitaeine butterflies by investigating patterns found in a wide range of hierarchical levels. I start from the highest level, the level of the entire tribe, by investigating the evolutionary relationships of species and species groups (**I**). I then proceed to use the results of (**I**) to infer possible evolutionary patterns in an ecological trait, the use of host plants (**II**). Moving down to the level of species occuring in Finland, I study the similarities and dissimilarities in movement patterns of five species using standard ecological methods, but analyze these results with a new model (**III**). Related species tend to be similar ecologically, and I use this assumption to describe the metapopulation dynamics of an endangered species with information from a common related species (**IV**). Finally, I investigate the metapopulation dynamics of a single species in a dynamic landscape and arrive at a conclusion that one cannot rely entirely on current models to analyze the metapopulation dynamics of species that inhabit dynamic landscapes (**V**). By using comparative methods, we will eventually be able to understand the diversity of species in this tribe and perhaps be able to extend the results to other groups of insects.

Systematics and biogeography

Despite the intensive taxonomic work on the melitaeines, nobody has attempted to build a phylogeny for the entire group. In part this is due to the difficulties of finding informative morphological characters. Many characters are invariant (which is why the group is so distinct), while the characters that vary tend to be hypervariable or form continuous clines that make their coding very difficult (Higgins 1941; Scott 1994, 1998). These problems now have an apparently easy solution: DNA sequences (Simon et al. 1994; Caterino et al. 2000). With the advent of the polymerase chain reaction (PCR), DNA sequence data has become accessible to just about anybody, with the advantage that it does not require much experience to generate a large amount of data. This is in stark contrast to morphological data, which requires many years of experience for the researcher to be able to make statements of homology (Sperling 2000).

Constructing phylogenetic trees based on DNA sequence data is not as easy as generating it. The methodology of sequence analysis, especially for phylogenetic purposes, is in a state of flux at the moment (Steel and Penny 2000). There are currently three major schools of thought in sequence analysis: the cladistic, phenetic and probabilistic schools. Each of these promote a different way of building phylogenetic hypotheses for DNA sequence data. The cladistic school seeks to find a cladogram that explains the data in a way that minimizes the number of changes using the Principle of Parsimony (Farris 1970; Kitching et al. 1998) (known as the maximum parsimony or MP method). The phenetic school clusters those sequences that are most similar to each other, usually using some form of model to account for different forms of base transformations (Saitou and Nei 1987) (known as the neighbour-joining or NJ method). The probabilistic school attempts to model the evolution of sequences through time, assigning each inferred change a probability and trying to find the most probable tree according to the given model

Figure 1. A phylogenetic hypothesis for the tribe Melitaeini based on sequences of two genes in the mitochondrial genome. The different shadings on the branches show a biogeographical hypothesis for the Melitaeini, which is the most parsimonious solution to optimizing distribution onto the preferred phylogenetic hypothesis. The generic and subtribal classification represents our recommended classification.
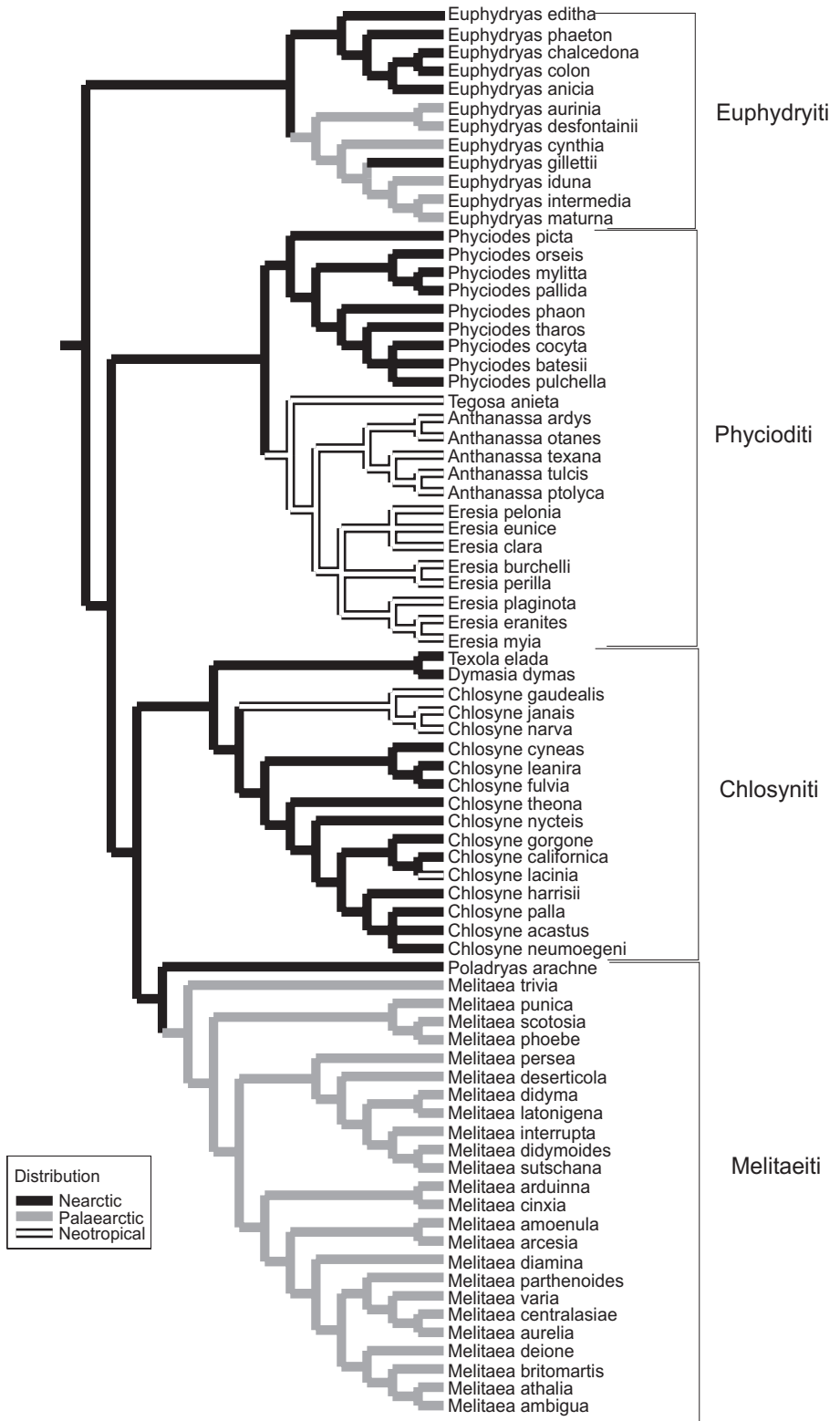
(Felsenstein 1973; Goldman 1990) (known as the maximum likelihood or ML method).

To the novice, the choice between these three ways of analyzing sequence data may be overwhelming, and indeed many recently published papers have used all three methods and then arbitrarily chosen the results that seem the best. Some researchers advocate the use of all three methods, claiming that if they give the same results, one can be more confident in the conclusions (e.g. Kim 1993). However, a critical overview of all three methods shows that there are theoretical as well as practical problems with some of the methods, making their use questionable.

The NJ method has been shown to be very sensitive to the order in which data are fed into the algorithm, and is designed to produce only one tree, regardless of the quality of the data (Saitou and Nei 1987; Farris et al. 1996). This is not a desirable property as there is no objective way to assess how well the given tree is supported by the data. The ML method has been shown to be robust and statistically consistent if the underlying model of evolution is known (Goldman 1990; Steel and Penny 2000). However, to know the underlying model of evolution is to know the phylogenetic tree, and thus this method is best used to study the evolution of nucleotide sequences after a phylogenetic hypothesis is available (e.g. Campbell et al. 2000). The MP method assumes that nature can be represented by a hierarchical classification that can be estimated from internested sets of synapomorphies (unique characters that describe a group of species) that are replicated in a given data set (Platnick 1979; Brower 2000a). The algorithms that have been developed to find such hierarchical classifications are designed to keep all the trees that explain the data equally parsimoniously. As the quality of the data set increases, one should converge on the true tree. It is this method that I have chosen to use to analyze our data set (**I**).

Once a phylogentic hypothesis is available, it can be used to investigate the systematics of the group in question. So far, molecular phylogenies have not been used much to test the classifications of insects that are based on morphological characters (Caterino et al. 2000), though this situation is changing

Table 1. The classification of the tribe Melitaeini based in part on (**I**) and in part on the morphological work of Higgins (1941, 1950, 1955, 1960, 1981) and Harvey (1991).

Family	Subfamily	Tribe	Subtribe	Genus	No. of species
Nymphalidae	Nymphalinae	Melitaeini	Euphydryiti	*Euphydryas*	14
			Phycioditi	*Phyciodes*	11
				Tegosa	14
				Anthanassa	27
				Eresia	ca 80
				Phystis	1
			Chlosyniti	*Chlosyne*	ca 30
				Texola	1
				Dymasia	1
				Microtia	1
			Melitaeiti	*Melitaea*	ca 50
				Poladryas	2
			Incertae sedis	*Gnathotriche*	5
				Atlantea	4
				Fulvia	2
				Antillea	2

(e.g. Mitchell et al. 2000). Our molecular phylogeny of the Melitaeini is based on 77 melitaeine species and 3 outgroup species (**I**). For each species we sequenced 1422 bp of the cytochrome oxidase I gene and ca. 536 bp of the 16S ribosomal RNA gene in the mitochondrial genome. Our work suggests that there are at least four groups of species that should be given equal rank (Fig. 1). These are the *Euphydryas* group (subtribe Euphydryiti), *Phyciodes* group (Phycioditi), *Melitaea* group (Melitaeiti) and *Chlosyne* group ("Chlosyniti").

The sequence divergences of the COI gene were equal between the four groups of species and we could not conclusively find the relationships of the four groups. Within the four groups, we found several genera to be paraphyletic, that is a given genus does not describe a natural group of related species, but includes species belonging to another genus within the group. Our phylogenetic hypothesis is robust enough to make statements on the classification of melitaeines and we recommend that 12 genera be synonymized (**I**, Zimmermann et al. 2000). Though our phylogeny is by no means complete, especially concerning the Neotropical species of Phycioditi, I suggest that the classification should follow that in Table 1.

The molecular phylogeny has interesting implications for the broad historical biogeography of the tribe (**I**). It would appear that the melitaeines originated in the Nearctic (Fig. 1), where the basal members of all four major groups are extant. The Neotropics have been colonized three times, once in the *Phyciodes* group and twice in the *Chlosyne* group. The colonization by the ancestor of the Neotropical *Phyciodes* clade has led to a species radiation, as the clade putatively contains about 120 species (only 14 species were included in the analysis of the molecular data in **I**), which is almost half of all species in the Melitaeini. The Palaearctic has been colonized twice, once by the *Euphydryas* group and once by the *Melitaea* group. There has been one recolonization of the Nearctic in the subgenus *Hypodryas* of the *Euphydryas* group (Zimmermann et al. 2000).

The relationship between melitaeines and their host plants

Phytophagous (plant-eating) insects are a very species rich group of organisms (Strong et al. 1984) and butterflies are no exception, with about 20000 species described (Ackery et al. 1999). Most phytophagous insect species are highly specialized on one or a few host plant species and even the generalist species are not able to eat everything. These two observations have intrigued researchers for several decades – why is phytophagy such a successful way of living and what are the advantages of specialization? The answers to these questions are just beginning to emerge, but there is still much controversy about the processes involved in the evolution of host plant use in insects (Schoonhoven et al. 1998).

In a seminal paper on insect-plant interactions, Ehrlich and Raven (1964) suggested that plants evolving novel secondary compounds (chemicals thought to be involved in plant defenses) are able to escape predation, thus setting the stage for species radiations. Any insect then evolving resistance to these secondary compounds is confronted with an abundance of potential host plants and another species radiation can take place. The hypothesis of Ehrlich and Raven (1964) suggests that there is coevolution between insects and their host plants, i. e. both groups affect each other in an evolutionary context. Through the process of coevolution (also termed an evolutionary arms race between insects and plants), insects have become highly specialized, with most species utilizing only one or a few plant species.

However, the reciprocality of the selective responses has been questioned, based on observations that while plants exert strong selective pressures on insects, insects rarely exert strong selective pressures on plants (Jermy 1976, 1984; Schoonhoven et al. 1998). This observation has led to the the hypothesis of sequential evolution, which posits that insects are merely following the evolution of plant secondary compound without directly affect-

Table 2. The occurrence of iridoids in plant families used by melitaeine butterflies as host plants according to Jensen (1991).

Melitaeine subtribe	Host plant family	Type of iridoids found	Number of melitaeine species known to use as hosts
Euphydryiti	Scrophulariaceae	iridoid glycosides	1
	Plantaginaceae	iridoid glycosides	7
	Caprifoliaceae	seco-iridoids	7
	Adoxaceae	seco-iridoids	1
	Dipsacaceae	seco-iridoids	2
	Oleaceae	seco-iridoids	2
	Valerianaceae	seco-iridoids	2
	Lamiaceae	iridoid glycosides	2
	Orobanchaceae	iridoid glycosides	5
	Gentianaceae	seco-iridoids	1
Chlosyniti	Scrophulariaceae	iridoid glycosides	1
	Orobanchaceae	iridoid glycosides	4
	Acanthaceae	no iridoids	5
	Asteraceae	no iridoids	8
	Amaranthaceae	no iridoids	1
Phycioditi	Verbenaceae	iridoid glycosides	1
	Acanthaceae	no iridoids	9
	Asteraceae	no iridoids	9
	Urticaceae	no iridoids	1
	Convolvulaceae	no iridoids	1
Melitaeiti	Scrophulariaceae	iridoid glycosides	2
	Plantaginaceae	iridoid glycosides	14
	Valerianaceae	seco-iridoids	1
	Lamiaceae	iridoid glycosides	1
	Asteraceae	no iridoids	4
	Orobanchaceae	iridoid glycosides	1
	Gentianaceae	seco-iridoids	1

ing it, i. e. speciation in phytophagous insects can be brought about by plants but speciation in plants is not caused by insects feeding on them.

An investigation of these hypotheses requires that patterns of host plant use are placed in an historical perspective. In (**II**) I have studied the evolutionary history of host plant use in melitaeine butterflies by using the phylogenetic hypothesis from (**I**). Larvae of melitaeine species are found on a rather restricted range of host plants (**II**). Most species (or populations) are oligophagous or even monophagous on plant species belonging to 16 families (Table 2). Fourteen families belong to the subclass Asteridae, and are found in two distinct clades (Olmstead et al. 1993;

2000b; Angiosperm Phylogeny Group 1998). The families Scrophulariaceae, Lamiaceae, Plantaginaceae, Oleaceae, Acanthaceae, Verbenaceae, Gentianaceae, Orobanchaceae and Convolvulaceae belong to one clade (the asterid I clade); and Asteraceae, Adoxaceae, Caprifoliaceae, Valerianaceae and Dipsacaceae belong to the other clade (asterid II clade). The remaining two families are entirely unrelated to the previous families, Urticaceae belongs to the subclass Rosidae and Amaranthaceae belongs to the subclass Caryophyllidae. Eleven of these families are united by the presence of secondary compounds known as iridoids (Jensen et al. 1975; Jensen 1991). Four families, Asteraceae, Convolvulaceae, Urticaceae and Amarantha-

ceae, do not contain iridoids.

The relationship between melitaeines and their host plants has been much studied since Singer (1971, 1983; White and Singer 1974) discovered that different populations of a single species *Euphydryas editha* preferred different host plants. Bowers (1981, 1983b) showed that the host plants of *Euphydryas* species in North America all contained iridoids. Bowers (1980, 1981) was intrigued by the fact that *Euphydryas* larvae and adults are aposematically coloured and found that they were unpalatable and even emetic to vertebrate predators. The reason behind the unpalatability of the butterflies is the ability of larvae to sequester iridoids (Bowers and Puttick 1986). Iridoids are known to be very bitter tasting compounds and have been used as insecticides against generalist insect herbivores (Hegnauer 1964; Seigler 1998).

Iridoids have been found to be important feeding stimulants for *Euphydryas chalcedona* larvae (Bowers 1983b). The larvae refused to feed on pure artificial diet, but when catalpol (an iridoid glycoside) was added to the artificial diet, the larve fed actively (Bowers 1983b). This finding led Bowers (1983b) to postulate that the ability to utilize iridoids by *Euphydryas* species has enabled them to colonize a variety of plant families containing iridoids. My study (**II**) suggests that this hypothesis is relevant to the entire tribe of Melitaeini.

The ability of melitaeine species to sequester iridoids has been studied in several species. Iridoid glycosides are a diverse group of chemical compounds (over a thousand different compounds have been recorded) and can be divided into two groups of compounds with similar structures. These are iridoid glycosides and seco-iridoids (Jensen 1991; Seigler 1998). So far, all iridoids that have been recorded to be sequesterable by melitaeine species are iridoid glycosides (Bowers and Puttick 1986; Stermitz et al. 1986, 1994; Franke et al. 1987; Belofsky et al. 1989; L'Empereur and Stermitz 1990b; Mead et al. 1993; Bowers and Williams 1995). In fact, there are two iridoid glycosides that are most often sequestered; catalpol and aucubin.

The ability to sequester iridoid glycosides has been recorded in 5 species of *Euphdryas* (Bowers and Puttick 1986; Stermitz et al. 1986, 1994; Franke et al. 1987; Gardner and Stermitz 1988; Belofsky et al. 1989; L'Empereur and Stermitz 1990a), 2 species of *Chlosyne* (Mead et al. 1993; Stermitz et al. 1994), *Poladryas minuta* (L'Empereur and Stermitz 1990b) and *Melitaea cinxia* (Lei and Camara 1999). Bowers and Williams (1995) found that though the larvae of *Euphydryas gillettii* are found mainly on plants containing seco-iridoids, they were unable to sequester these compounds. *Euphydryas gillettii* larvae were able to sequester iridoid glycosides from other plants that postdiapause larvae sometimes feed on.

When a historical perspective is adopted, it becomes apparent that iridoid glycosides have had a substantial impact on the evolution of host plant use in melitaeine butterflies (**II**). When the presence of iridoid glycosides in the host plants of extant melitaeine species is mapped onto the phylogeny of the butterflies, it can be seen that this trait is very conservative, i. e. there does not seem to be much switching back and forth between character states. In contrast, when the use of host plant families is mapped onto the phylogeny, the patterns are much more dynamic (see Fig. 3 in **II**). The evolutionary dynamics of host plant use are evident as the widening of host plant range in clades using plants containing iridoid containing plants and as host shifts to chemically dissimilar plants.

The patterns I have described in (**II**) are generated by the behavior of individuals over evolutionary time. In melitaeines it is the ovipositing female that is the most crucial stage of host plant choice, as newly hatched larvae are not able disperse over distances longer than a few cm (Moore 1989). All melitaeine species that have been studied have shown similar oviposition behavior to the well-studied *Euphydryas editha* (Singer 1994; Thomas and Singer 1998), e. g. *Euphydryas maturna* (Wahlberg 1998), *Melitaea cinxia* (Kuussaari et al. 2000) and *Melitaea diamina* (Wahlberg 1997). Detailed studies on the host plant preferences of females have shown that the fe-

males are choosing plant individuals rather than plant species to oviposit on (Ng 1988; Singer and Lee 2000). This means that an individual of one plant species may be preferred over an individual of another plant species, which in turn may be more acceptable than another individual of the first species. This implies that females are choosing to oviposit on a plant individual based on the biochemical profile of that plant individual.

Going up from the level of the individual butterfly to a population of butterflies, we find that most females prefer to oviposit on one species of host plant (Singer et al. 1994; Kuussaari et al. 2000), indicating that biochemical profiles are usually plant species specific, but not always (Singer and Lee 2000). An environmental perturbation may introduce a new plant species or change the phenology of an existing plant species and the biochemical profile of these species may be more acceptable to some ovipositing females (Singer et al. 1993; Thomas and Singer 1998). If the new host supports higher larval survival, the average female preference of the population may change rapidly (Singer et al. 1993). Note that environmental perturbations have been common especially for Nearctic and Palaearctic species over the past 5 My (glacial periods).

Moving up from the level of populations to an entire species (which is the smallest unit in this study), one finds that different populations have become specialized on different host plant species usually in the same plant family. This pattern is very clear in many Holarctic species and the apparently monophagous Neotropical species may just represent a dearth of information from this region. Within one species that has been studied (*Euphydryas editha*), the evolution of host plant use appears to have been very dynamic, with several host plant genera being lost and recolonized several times by different populations of the butterfly (Radtkey and Singer 1995).

As one moves up still further to the level of clades of species, my study (**II**) has shown that the apparent dynamism of host plant genera utilization within species is reflected in

the dynamism of host plant family utilization in related species. It is at this level that one should see signs of coevolution over longer periods of time. It is very clear that parallel phylogenesis has not occurred in melitaeines and their host plants, as there is dynamism of host plant utilization both at the species level and at the clade level. Mitter and Farrell (1991) stress that the ages of the insect and host plant clades should be similar, but most of the host plant families in this case are likely to be much older than the melitaeines, e.g. Acanthaceae 19 My, Asteraceae 31 My, and Caprifoliaceae 47 My (Eriksson and Bremer 1992). In (**I**) we speculate that the tribe Melitaeini originated at the beginning of the ice ages ca. 5 Mya, based on low sequence divergences and the biogeography of the tribe.

The question then is whether the melitaeines have coevolved with their host plants in a broader sense. Coevolution implies that the insects should affect the fitness of the host plants in a negative way. Most Holarctic melitaeine species feed on herbs of small size and in some cases the larvae are able to kill individuals of their host plants (Parmesan 2000). Only one study has explicitly studied the effect of melitaeine herbivory on the fitness of the host plants (Parmesan 2000). This study showed a surprising result that when plant density was low, herbivory had a significant negative effect on the fitness of the host plant, but when plant density was high, herbivory had no effect on host plant fitness. This indicates that competition between plants may be a stronger evolutionary force than herbivory by insects. Also, insects are usually distributed patchily in the landscape and do not affect the plant population as a whole in a certain area. Thus plants with similar genotypes to those that are eaten are able to escape predation. This situation is common in batch laying melitaeines, which are highly localized as larvae in a given habitat patch.

The most likely explanation for melitaeine host plant use is that the butterflies have colonized an already diverse assemblage of plants. Melitaeines have not coevolved with iridoid containing plants, but rather have been able to circumvent the plants' defences and

are now able to exploit the plants. Whether insects have been instrumental in the evolution of iridoids has not been answered by this study, but that melitaeines have not been instrumental is clear. Some ancestral populations have specialized on plants containing seco-iridoids in addition to those containing iridoid glycosides. One lineage has speciated to form the *Euphydryas* group and two lineages have not speciated (yet?). This again suggests that the ancestral populations have evolved a way to circumvent the negative effects seco-iridoids and thus the butterflies have merely followed the plants, rather than caused the evolution of a more potent plant defence.

There are three groups of melitaeine species that depart from the general pattern of using host plants containing iridoids, and all three groups use host plants in the Acanthaceae or Asteraceae (**II**), which do not contain iridoids at all (Jensen et al. 1975; Jensen 1991). The three groups of species are in the genera *Melitaea*, *Chlosyne* and *Phyciodes*. Whether the host plants in these two families confer some sort of protection to the larvae feeding on them is still an open question. Adult *Chlosyne harrisii* were found to be palatable to a bird predator (Bowers 1983a), while larvae of *C. lacinia* were found to be unpalatable to amphibian predators (Clark and Faeth 1997). Both species feed on plants belonging to Asteraceae.

In the case of the *Chlosyne* group, species ancestral to the *C. nycteis* clade used host plants in Orobanchaceae, which are hemiparasitic plant species. These plants are able to take up plant secondary compounds from their hosts (Stermitz et al. 1989). If the hosts of the ancestral Orobanchaceae that the *Chlosyne* species fed upon were plants belonging to Asteraceae, the butterfly would be exposed to the secondary compounds of Asteraceae. This may have then facilitated the colonization of Asteraceae. In the other two groups, the host plant affiliations of ancestral species is unclear, and thus my hypothesis remains to be tested in a more rigorous fashion.

It is clear that the host plants of melitaeines contain many compounds other than iridoids. How sensitive melitaeines are to these other secondary compounds has not been studied much. Stermitz et al. (1989) report that quinolizidine alkaloids that the hemiparasitic host plant of *Euphydryas editha* obtain from another plant, do not affect most larvae. Some larvae are however affected by these compounds. My study (**II**) has shown that the presence of iridoids in the host plants of melitaeines is a phylogenetically conservative character. Further research should concentrate on finding other chemicals that influence host plant use in these butterflies.

Population structure and dynamics

Population structure in melitaeines

A striking feature of melitaeine populations that was noted already at the beginning of this century is their patchy nature (Ford and Ford 1930; Ehrlich 1961). This patchiness is highlighted by the often very sedentary behaviour of individuals within a habitat patch (Ehrlich 1961, 1965; Warren 1987; Harrison 1989; Hanski et al. 1994). A consequence of this sedentary behaviour is that populations occupying different habitat patches often fluctuate in size independent of each other (e.g. Ehrlich et al. 1975; Ehrlich and Murphy 1981; Hanski et al. 1995a). Sometimes populations in certain patches may go extinct, but these extinctions can be balanced by occasional colonizations of empty patches (Harrison et al. 1988; Hanski et al. 1995a).

The population structure described above is known as a metapopulation, or a population of populations (Levins 1969). Metapopulations and their dynamics have become very popular subjects of study recently, especially in butterflies (Thomas and Hanski 1997). Melitaeines are ideal subjects for metapopulation studies mainly because suitable habitat patches are usually easily delimited from the surrounding habitat and the

presence or absence of a species is fairly easy to assess (Harrison et al. 1988; Hanski et al. 1995a; **IV, V**).

All studied melitaeine species exhibit a fragmented population structure. Such species are *Euphydryas editha* (Ehrlich et al. 1975; Thomas et al. 1996), *E. chalcedona* (Brown and Ehrlich 1980), *E. gillettii* (Debinski 1994), *E. anicia* (White 1980), *E. phaeton* (Brussard and Vawter 1975), *E. aurinia* (Warren 1994; Lewis and Hurford 1997; **V**), *E. maturna* (**III**), *Melitaea cinxia* (Hanski et al. 1994, 1995a, 1995b, 1996), *M. diamina* (**IV**), *M. didyma* (Vogel 1996; Vogel and Johannesen 1996) and *M. athalia* (Warren 1987; **III**). What is evident from the list above is that the population structures of any *Chlosyne* or *Phyciodes* species have not been studied in detail. Dethier and MacArthur (1964) report that *C. harrisii* inhabits a network of old fields in a forested region, suggesting that this species has a metapopulation. On the other hand, a mark-recapture study on *C. palla* suggests that this species occupies a much larger area and moves more than *Euphydryas* or *Melitaea* species (Schrier et al. 1976).

Are melitaeines then more susceptible to a metapopulation structure than other butterflies? Apparently not. The metapopulation structure is fairly common in butterflies (Hanski and Kuussaari 1995; Thomas and Hanski 1997). Hanski and Kuussaari (1995) estimated that 65% of the 94 species of butterflies living in Finland have a metapopulation structure. What characteristics in the melitaeines can be thought to affect their population structure? There are two major factors; the occurrence of discrete local populations and the magnitude of adult movements between local populations.

What makes melitaeine local populations discrete in space? Most studied species are found in habitats that are well separated from the surrounding habitat, such as open meadows in a forest matrix (e.g. Warren 1987; Hanski et al. 1996b; **IV, V**). Two important resources often occur together in such habitats, adult nectar sources and larval host plants. The size of the area occupied by a local population may sometimes depend on how the two resources are distributed relative to each other (Gilbert and Singer 1973).

Ehrlich (1961, 1965) found that an *E. editha* colony inhabiting seemingly uniform habitat was in fact highly aggregated. These aggregations formed three local populations that fluctuated in size independently of each other (Ehrlich et al. 1975). Even though there was no physical barrier between these populations, very few butterflies moved from one population to another. This observation led Ehrlich (1961) to propose some sort of "intrinsic barriers to dispersal". It was later discovered that the populations were situated in places where the larvae had access to alternative host plants after the senescence of the primary host plant, thus allowing larvae to develop up to diapause (Singer 1972). Singer (1972) proposed that individuals are selected for sedentary behaviour, because adult butterflies were unable to recognize the alternative host plant and thus suitable habitat.

Other populations of *E. editha* are found in habitats where larval host plants and adult nectar sources can occur several hundred meters apart from each other (Gilbert and Singer 1973). Adults in these populations are much more vagile, moving between both resources with ease. Consequently, the area covered by local populations at these sites is much larger than at the site described previously. Further studies showed that at least one colony of *E. editha* exists as a mainland-island metapopulation (Harrison et al. 1988). In this colony, one population exists on a habitat patch with an area of over 2,000 ha and it numbers maximally in the hundreds of thousands of individuals. This large local population is surrounded by smaller habitat patches of which those closer to the "mainland" population are more likely to be occupied. The risk of extinction of the mainland population is thought to be minimal, while the surrounding smaller populations are thought to go extinct frequently. Empty habitat patches are colonized mainly from the mainland population (Harrison et al. 1988).

Many melitaeines are known to exist in classical metapopulations, where all local

populations have a substantial risk of extinction (Hanski et al. 1995a; **IV, V**). One system has been particularly well studied at the metapopulation level. This is the *Melitaea cinxia* metapopulation in the Åland Islands in SW Finland (Hanski et al. 1994, 1995a, 1995b, 1996b; Hanski 1999). *Melitaea cinxia* inhabits a patch network of roughly 3,000 patches, of which 300 – 500 have been occupied at any one time (Hanski et al. 1995a; Hanski 1999). The entire network has been surveyed twice a year since 1993, yielding a time series that has brought many insights into how metapopulations work.

It has been shown beyond doubt that *M. cinxia* exists in a stochastic balance between extinction of local populations and colonization of empty patches (Hanski et al. 1995a; Hanski 1999). Over the years it has become apparent that the metapopulation on the Åland Islands actually consists of many metapopulations in semi-independent patch networks, given the acronym SIN by Hanski et al. (1996). The metapopulations in these SINs fluctuated independently of each other, but there appears to be some gene flow between them (I. Saccheri, pers. comm.). Semi-independent patch networks have been recorded in *M. diamina* as well (**IV**).

Other species have also been studied in the metapopulation perspective. *Melitaea didyma* occurs as a metapopulation at the northern edge of its range in Germany (Vogel 1996; Vogel and Johannesen 1996). The species apparently occupied all the suitable habitat available, as no extinctions or colonizations were observed. The same appears to be true for Finnish populations of *M. athalia* and *E. maturna* (**III**). In these species suitable habitats occur quite densely, so even though the butterflies are not more mobile than *M. cinxia* (**III**), they occupy almost all available habitat.

The dynamics of species inhabiting fragmented landscapes have been successfully analyzed using a stochastic model known as the incidence function model (Hanski 1994). The incidence function model assumes that the extinction risk of a local population is related to the patch area, and that colonization of empty habitat patches is related to the connectivity of the patches. Details of the incidence function model are given in (**IV, V**). In short, the incidence function model has 5 parameters that are estimated from observed patch occupancies. The parameters describe the annual risk of local population extinction, how fast the extinction risk increases with decreasing patch area, the effect of distance between patches on colonization of empty patches, the efficiency with which colonizations occur and the relationship between patch area and expected population size. To estimate parameter values for the incidence function model from the occupancy pattern of one year, one has to assume that the metapopulation is at a stochastic steady state. This is a potentially problematic assumption for endangered species, whose populations have most likely declined strongly over past years. We applied the incidence function model to two endangered species, *Melitaea diamina* (**IV**) and *Euphydryas aurinia* (**V**), in order to assess their conservation status in Finland.

In the case of *M. diamina*, we investigated how well the parameter values of three different species of butterfly were able to predict the observed occupancy pattern of the endangered species (**IV**). The best results were given by the parameter values for the most closely related species, *M. cinxia*. Our study draws attention to the historical component in the ecologies of different species. The most likely reason why our study was successful is that the two species *M. diamina* and *M. cinxia* share a relatively recent ancestor. This implies that there are constraints on the evolution of ecological parameters such as movement ability (**III**). Our study represents the first attempt in what promises to be an intriguing area of research, comparative metapopulation biology. A rigorous approach is needed to investigate whether some aspects of the ecology of species living in a fragmented landscape are constrained by phylogeny, and which features are free to evolve in a changing world.

In most studies of metapopulations, the habitat patch network is assumed to be static, i. e. the quality of patches does not change

with time and patches do not disappear. However, butterflies often inhabit successional habitats and their patch networks are therefore dynamic, with patches appearing and disappearing in the landscape. Our study of *E. aurinia* is a good example of such a system (**V**). This species lives in a patch network of meadows and small-scale clearcuts in the forest. The meadows can be considered to be static, though currently they are becoming overgrown due to changes in agricultural practices. Clearcuts are distinctly transient habitat patches, being suitable for at most 12 years. The same approach to analyzing the dynamics of *E. aurinia* as was used in (**IV**) does not work in this case as the fundamental assumptions (see above) of the incidence function model are violated. In dynamic patch networks, the presence of a species may be a reflection of the history of the surrounding landscape and extinctions can be deterministic rather than stochastic.

Since the modelling approach to metapopulations inhabiting dynamic patch networks is still undeveloped, we analyzed the dynamics of *E. aurinia* in its patch network in a somewhat ad hoc manner (**V**). We manually adjusted the parameter values of the incidence function model to give an average incidence, similar to that observed in the field, in a dynamic landscape that we attempted to make as realistic as possible. With this approach we discovered that the meadows are essential to the survival of *E. aurinia* in SE Finland. Our study (**V**) highlights the need for a theoretical framework to be developed for the study of dynamic populations in a dynamic landscape. This is especially important for butterflies as most endangered species inhabit early successional habitats (Thomas 1993). Indeed, several melitaeines are known to inhabit early successional habitat, e. g. *M. diamina* (**IV**), *M. athalia* (Warren 1991), *E. gillettii* (Williams 1988) and *E. maturna* (Wahlberg 1999).

It appears that at least for *Melitaea* and *Euphydryas* species a patchy population structure is ubiquitous. This is not an intrinsic feature of the butterflies, however, but is largely dependent on the distribution of the larval host plants in the landscape. Whether all suitable habitat patches are occupied or whether the species in question exists in a stochastic balance between extinctions and colonizations depends on the spatial configuration of the patches and on their number. The probable situation is that in the central parts of a species' distribution, suitable habitat patches are numerous and relatively close to each other. As one moves towards the edge of a species' range, the patch network becomes less dense and the occurrence of a species becomes more dependent on metapopulation processes (Thomas et al. 1998). Some rare or endangered species may be entirely dependent on metapopulation processes, as is the case with *M. diamina* in Finland (**IV**).

Movements of individuals

Movements of melitaeine butterflies have been much investigated using mark-recapture studies, beginning with the seminal work of Ehrlich (1961, 1965). Most studies have found melitaeines to be fairly sedentary, regardless of how common or rare they are (Ehrlich 1965; Schrier et al. 1976; Cullenward et al. 1979; Brown and Ehrlich 1980; Warren 1987; Hanski et al. 1994; Vogel 1996; Munguira et al. 1997). Due to this sedentary behaviour, populations of the butterflies have been referred to as "closed populations" (Thomas 1984; Warren 1992). A comparison between *E. editha* and *Erebia epipsodea*, a butterfly with an "open population structure", showed that the former moved much less and shorter distances than the latter (Brussard and Ehrlich 1970).

Hanski et al. (1994) found that the division of butterfly population structures into open and closed based on movements was unsatisfactory. They proposed that population structure should be based on whether suitable habitats are discrete entities in a matrix of unsuitable habitat and on the magnitude of movements of the adult butterflies. Discrete habitat patches of similar size and infrequent movements between patches leads to a classical metapopulation, while diffuse habitat and

vagile behaviour leads to a large panmictic population. The sedentary behaviour of melitaeines predisposes them to a classical metapopulation structure.

Yet, despite their sedentary behaviour, movements of over a kilometer have been recorded in all studied species (Ehrlich et al. 1975; Hanski et al. 1994; Hanski and Kuussaari 1995; **III**). Movements of this magnitude are however rare and it has been questioned whether they contribute at all to gene flow between populations (Ehrlich et al. 1975). Ehrlich et al. based their scepticism on one metapopulation of *E. editha*, where offspring of migrants have a very low probability of survival. This is because migration generally happens later in the flight season and larvae of later egg batches are faced with the senescence of the host plant before they have reached diapause size.

The unimportance of long distance migrants cannot be generalized to other *E. editha* populations, much less to all melitaeine species. In most species the occassional long-distance migrant tends to lead to less genetic differentiation in local populations. This has been observed in many melitaeines (Brussard and Vawter 1975; McKechnie et al. 1975; Vawter and Brussard 1975; Brussard et al. 1989; Debinski 1994; Johannesen et al. 1996). Long-distance migration has also important impacts on metapopulation dynamics. For instance, *M. cinxia* has been observed to colonize empty patches up to 5 km from the nearest occupied patches (Hanski 1999). Long-distance migration can help stabilize metapopulation dynamics.

What factors affect the emigration of a butterfly individual? The quality of the habitat patch is evidently an important factor (White and Levin 1981; Murphy and White 1984; Thomas and Singer 1987; Kuussaari et al. 1996). Butterflies tend to leave patches that have less nectar sources (Kuussaari et al. 1996) and do not have the preferred host plant (Thomas and Singer 1987). Also temporal variation in habitat quality affects butterfly movements. Butterflies were more likely to emigrate from habitat patches that were more susceptible to drought in dry years (White and Levin 1981; Murphy and White 1984).

Other factors affecting emigration are the area of habitat patches, the quality of the patch boundary, the density of the local population and the size of the butterfly individual (Kuussaari et al. 1996). To grossly simplify the case, large butterflies tend to leave small patches with open boundaries and low density of other individuals. A negatively density-dependent emigration rate has been found in *E. editha* (Gilbert and Singer 1973), *E. chalcedona* (Brown and Ehrlich 1980) and *M. cinxia* (Kuussaari et al. 1996). The effect of the size of a butterfly has only been studied in *M. cinxia*, where it was found that larger females were more likely to emigrate than small ones (Kuussaari et al. 1996).

Factors affecting immigration have not been studied in great detail. Kuussaari et al. (1996) found that butterflies were more likely to immigrate into larger patches close to existing populations. A similar pattern has been found in the surveys of the *M. cinxia* metapopulation, as larger and less isolated patches are more likely to be colonized (Hanski et al. 1995a). These effects of patch area and connectivity have been found in other species of butterfly as well (Thomas and Hanski 1997).

One major factor affecting the evolution of the propensity of an individual to emigrate has until recently eluded researchers. This factor is mortality during migration. If mortality during migration is high, selection pressures should be for sedentary behaviour; where as if mortality during migration is low, there should be no barriers to wide ranging migration behaviour. It is not known just how evolutionarily labile the propensity to migrate is. Melitaeines appear to be a remarkably homogenous group when it comes to migration behaviour. Is this because all studied species happen to inhabit similar patch networks or is there a phylogenetic component to the propensity to migrate?

The best way to approach this question is to compare different species. Previous comparisons between species have been hindered by *ad hoc* methods of analyzing mark-recapture data that are difficult to compare. Recently a new model (the VM model) has been

developed for the purpose of analyzing mark-recapture data from several patches (Hanski et al. 2000). The parameters of this model describe daily survival probabilities within a habitat patch and during migration, emigration propensity, the scaling of patch area to emigration and immigration and the effect of distance on migration. The parameter values obtained with the VM model are comparable. Using the VM model, we found that five species of melitaeines in Finland are more similar to each other than any is to other unrelated species (**III**). A closer look at the five species reveals some variation at a finer level. For instance *E. aurinia* tends to move further than *E. maturna* and *M. cinxia* females have a greater propensity to emigrate than all the other species and sexes. The parameter values we estimated for each species and sex predicted that about 10–20% of migration events failed.

Our study shows that while there is no phylogenetic component at the level of the five species, there may be phylogenetic constraints on the magnitude of migration distances in the group as a whole (**III**). Melitaeines are known to be rather sedentary and indeed there are no records of melitaeines migrating very long distances, unlike for species in the closely related Nymphalini (such as *Vanessa atalanta*, *Cynthia cardui* and *Inachis io*). A more extensive comparative study is needed to discover whether there are limits to the migration behaviour of melitaeines. Such a study is now possible as the VM model makes different studies more comparable.

Conclusions

I have attempted to show in this thesis how knowledge of phylogeny can be used to elucidate patterns of evolution in a group of species. The melitaeines are highly suitable for comparative studies. They are a relatively small group of species (ca. 250 species) that are relatively similar in many respects, yet vary in interesting ways in other respects. Previously melitaeine species have been studied in isolation and some results would be better understood if a phylogenetic perspective would be adopted. Now that a reasonable phylogenetical hypothesis is available for the group (**I**), the population biology of these species can be studied at even greater depth.

The evolution of host plant use in the melitaeines lends itself readily to comparative analyses, because the basic data are available for a wide range of species (**II**). Though individual species may show extreme lability in the use of host plant species (e.g. Radtkey and Singer 1995), the group as a whole appears to be mainly restricted to plants containing iridoids. The butterflies can be seen to be labile in host plant use at a small phylogenetic scale, while being conservative at a large phylogenetic scale. The current evidence points to three major independent colonizations of plants without iridoids within the melitaeines. Almost all the host plants without iridoids belong to Acanthaceae and Asteraceae, which are closely related to the other host plant families, suggesting that there may be a possiblity for preadaptation to some other compound(s) common to all (or some) of the host plant families.

Comparing the population structures and movements of different species can greatly advance our understanding of species inhabiting highly fragmented landscapes. Our studies (**III, IV, V**) represent only an indication of this potential. By taking history into account one can infer the lability and limits of traits associated with movements of individuals in their landscape. This kind of information is relevant to conservation. Finding that the movement abilities are free to evolve within certain limits in a group of related species would lead to different conclusions than finding that different clades have their own distinct limits within the broader limits for the whole group. The former finding would suggest that species are able to respond quickly to changes in the landscape, while the latter would suggest that species are more constrained by phylogeny and may respond to changes in the landscape by going extinct. We were able to study so few species that no definite conclusions can be drawn, but there do

appear to be limits to movement ability within which melitaeines evolve (**III**).

Two factors may explain the predisposition of melitaeines to exhibit a metapopulation structure, phylogenetic constraints on the range of movement abilities in melitaeines and the patchy nature of their habitats. If there truly are phylogenetic constraints, knowledge of the configuration of the patch network may be enough to predict the occurrence of a melitaeine species in that network (**IV**). Common species would thus inhabit a dense network of habitat patches, while rare species would live in sparse patch networks. The magnitude of "dense" and "sparse" would be similar in all species. Once again our studies represent just the beginning. A comprehensive research program in comparative metapopulation biology would be needed to test the above hypothesis.

Our study on *E. aurinia* (**V**) highlights the need to consider other characteristics of the habitat patch network rather than just the area and location of each patch. The host plants of most temperate melitaeines are small herbs that tend to be found in early successional habitats. This suggests that historically the evolution of melitaeine movement abilities has been influenced by dynamic landscapes. The study of the dynamics of melitaeine metapopulations in dynamic landscapes is in its infancy and our study along with that of Warren (1991) serve to bring attention to this phenomenon. It is not possible at this moment to say anything about the general implications of dynamic landscapes on the evolution of melitaeines.

Challenges for the future

The above paragraphs bring forth areas of research that would confirm, strengthen or possibly refute the results obtained so far. To reiterate, the metapopulation structures of many species should continue to be studied especially with reference to the movement abilities of each species in their respective landscapes. More attention should be paid to the dynamics of the landscape itself and the influence of this on the evolution of melitaeines. Many aspects of melitaeine life history show interesting variation that could be profitably studied by applying the comparative approach.

Variation in larval group size in the melitaeines promises to be a rich area for comparative studies. All melitaeine species that have been studied lay their eggs in batches. Some species lay their eggs in large batches of over 300 eggs while others lay eggs in batches of less than 10 eggs. Factors affecting the evolution of clutch size in different species have yet to be analyzed. There are certainly complex interactions between larvae and the biotic and abiotic environment. For some species larval host plant defences may be a crucial selective factor, in others thermoregulation may be important and in yet others avoidance of parasitoids may play an important role. The relationship between web-spinning and group size is unknown at the moment. It may be that in species for which a web is important, a larger group size is advantageous, as it lowers the per capita cost of spinning the web. A correlation between web spinning ability and egg batch size would support this hypothesis.

The coevolution of melitaeines and their parasitoids is another potential topic for comparative studies. All recorded parasitoids are apparently specialists of melitaeines. Some species are, however, generalists within the melitaeines, while others are specialists on only one melitaeine species. Parasitoids may be a selective factor on the behaviour of butterfly larvae. It may be that melitaeine species that live solitarily as larvae do not have highly specialized parasitoids as the host larvae are difficult for the parasitoids to find. On the other hand, extreme specialization by parasitoids may set the stage for stepwise coevolution between the parasitoids and their hosts. A phylogeny of both groups is necessary to investigate this possibility.

Finally, with a reliable phylogeny of the tribe Melitaeini available, this group of butterflies has the potential to become a model group of insects in evolutionary and popula-

tion biology. Much is already known about many species, and placing this knowledge into a historical perspective can help us understand many aspects of evolution of life history traits.

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