Endemism in Sardinia: Evolution, ecology, and conservation in the butterfly Maniola nurag
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Citation for published version (APA):
Amsterdam: IBED, Universiteit van Amsterdam

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Endemism in Sardinia
Evolution, ecology, and conservation in the butterfly *Maniola nurag*

Andrea Grill
STELLINGEN

behorende bij het proefschrift getiteld:

Endemism in Sardinia:
evolution, ecology and conservation of the butterfly Maniola nurag

door Andrea Grill

1. *Maniola nurag* is not the result of vicariance or dispersal, but originated under sympatric conditions with *Maniola jurtina*, as a consequence of local adaptation along an environmental gradient.
   this thesis

2. The small genetic distance between *Maniola nurag* and *Maniola jurtina* suggests that divergence initiated after the desiccation of the Mediterranean sea (± 3 ma ago), associated with the abrupt climate changes at the turn from Pliocene to Pleistocene (± 3 - 1.8 ma ago).
   this thesis

3. Geographic patterns in allele frequencies, the existence of hybrizymes, and the presence of morphologically intermediate individuals in areas where the two species are sympatric, indicate that *Maniola nurag* and *Maniola jurtina* hybridize.
   this thesis

4. The $F_{st}$-values estimated for 14 *Maniola jurtina* populations in south-east England, ranging between 0.005 and 0.019 are not particularly small for Lepidoptera and surely not the result of selection.
   contra Goulson Heredity 71, 386-393 (1993)

5. In Sardinia centres of endemism generally coincide with mountainous areas.
   this thesis

6. Butterflies do not necessarily have wings.

7. Wenn zwei Dinge verschieden sind, sind sie nicht gleich; wenn aber zwei Dinge gleich sind, können sie sehr wohl verschieden sein.
   this thesis

8. The extinction of languages is at least equally tragic as the extinction of plants or animals.
   see Sutherland Nature 423, 276-279 (2003)

9. Literature increases the evolutionary fitness of humans.
   see Bernardi Poetics Today 23, 21-42 (2002)
10. Studying biology makes you aware that humans are just another species.

11. Seasoning is got to be like a butterfly’s wing touching your skin.

12. Death is the necessary condition of the season’s setting forth.
   see W.H. Auden

13. Travelling prolonges your life-time.


15. Miss God is a caramel-flowered woman.
   in collaboration with S. Breuer

16. Albania is part of Europe.


18. Als er in Amsterdam geen mensen zouden wonen, was er net genoeg plek voor 7 tijgers.

19. Home is, where you don’t have to think about what to do when you get there.
   inspired by A. Gerstenberg

20. I sardi sono piccoli, scuri e pelosi.
   after Signor Fera

21. Attraction, respect, and the capacity to continuously surprise you are the three main components of love.
   see H. Hartley Trust (1991)

22. Science should give you pleasure and satisfaction.
   see Smith. White Teeth (2000)

23. Een ijsje is een zeepaardje.
Endemism in Sardinia
Evolution, ecology, and conservation of the butterfly Maniola nurag
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ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
Prof. mr. P.F. van der Heijden
ten overstaan van een door het college voor promoties ingestelde commissie
in het openbaar te verdedigen in de Aula der Universiteit

op donderdag 4 september 2003, te 14.00 uur

door

**Andrea Grill**

geboren te Bad Ischl, Oostenrijk
Tutto è già qui
anche se non si vede
tutto è già qui
nascosto tra le pieghe
e se ci stupirà
sarà soltanto
come certe novità
che sapevamo già

(Gianmaria Testa)

...to you who wanders, flies, and loves
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Preamble

Its beginning and my origin

In one of the first classes of 'Introduction to zoology' we were asked to write a short essay on why we had chosen to study biology. I was extremely pleased about this first assignment and wrote much about a certain nut-tree in my grandfather's garden. Unfortunately, this piece of writing has been lost later on, and I do not recall a lot more from this course, except that at the exam we were asked what the handwritten numbers on the back of library books stood for. Certainly, I did not learn anything about butterflies or the rest of the animal kingdom. So, I felt, botany was the more promising tool to learn about the 'science of life' - which was what I had matriculated for - and engaged into all field-excursions the department offered, collecting lichens, mosses and dry flowers, that I pressed under piles of my heaviest books.

After the first few months I knew that I was much better with words than with numbers, and that the courses at the linguistic's institute were a lot easier for me than physics, which was my first exam at university, and the first one I failed as well. At that time, I still thought that, if only you understood things you did not have to memorize them to pass an exam (I was definitely wrong with that thought).

Despite all, the fascination and challenges of natural sciences won, and I did a degree in biology. In reality it was Rousseau's noble savage whose shadow I hoped to touch when spending as much time as possible in mountains and forests. After a while, I decided that moving beings were more fun than plants that never ran away, and got involved into zoology again. By chance, I got to work with butterflies, first in a nature conservation context, as I share the concern for the balance between nature and the built world (e.g., Crowe, 1995) - a once harmonious relationship that seems about to collapse in many parts of our modern world.

This is also how I started off with the subject of this thesis. A unique species, a little butterfly, endemic to one single island in the whole world, an island in the Mediterranean sea; a species which was possibly rare, possibly endangered, an insect, that only a few people had actually ever seen before, even fewer knew details about its life history, what it was related to, how it had come to the island. My first goal was to look at this butterfly in a nature conservation context. Was it threatened by extinction? Had bottlenecks occurred that reduced its genetic diversity and fitness? Were there human induced developments destroying its habitat? What did a nature reserve have to be like, to be beneficial for the existence and survival of this butterfly?

I began to study the dispersal abilities of 'my' butterflies, by using mark-release-recapture techniques; and I discovered that the modelling approaches used to estimate the number of butterflies present in a certain area are equivalent to the
ones used to estimate the number of words present in a book. For three years, I spent my summers writing consecutive numbers on the upper-side of butterflies' forewings, releasing them, and trying to recapture them again two days later.

After the first year, I began to feel, that the initial questions regarding nature conservation issues did not satisfy me any more. I seized to see the necessity to emphasize the man-nature duality so strongly, and rather pictured humans and their creations as a product of nature too. In that context, evolution more and more appeared to be the key to what I was interested in. 'The hidden likeness in diversity', as Jacob Bronowski put it (Crowe, 1995), the unifying links in the variety of the wild, and, ultimately, beauty, if you are willing to accept Samuel Coleridge's definition of beauty as 'unity in variety'. Questions about the origins of this butterfly, what its ancestors were, and how it evolved to be like it is, became more and more important. Why are endemics endemic? How did this particular species come to fly on this island and not another one? The more new questions came about, the more techniques were required to solve them. I wanted to look closer and closer into the structure of life, switched from field observations to molecular techniques, from allozyme markers to mitochondrial DNA, spent hours and hours looking at butterfly genitalia under the microscope, and have just started scanning hundreds of wings, in order to do morphometrics. But, as Ezra Pound said shortly but wisely: Beauty is difficult.

This is how the various, sometimes very divergent, chapters in this book came about; and it is also why, at the end of it I am left with more questions than I could ever have thought of in the beginning.

Amsterdam, 20th July, 2003

P.S.: Frankly, I never thought of science as a career. Biology has always been for me a blend of dejection and high spirits, a torture and pastime - but I never expected it to be a source of income; on the other hand, I have often dreamt of a long and exciting career as a poet, but now I am afraid I might end as an obscure curator of Lepidoptera in a great museum (contra Nabokov 1999).
Several ways of looking at a butterfly

When two things are different, they can not be equal; but when two things are equal they can still be different. Were I to announce to you the discovery of an animal which for the first two thirds of its life is a serpent, then weaving a veil of pure silk around its body, contracts itself into a motionless amphora without external mouth or limbs, and, after remaining in this state for a while without food or drink, bursts its silken box and flies into the sky like a winged bird, you would be amazed and bewildered to say the least. And you would not believe me; not if you were living at the turn of the 18th century, when the general public still considered butterflies to be the soul ("psyche") of the dead, and no relation was laid between the leaf eating 'worms' creeping along grasses in spring and the colourful 'summerbirds' floating above flowery fields later in the year.

It was then, in 1698, when from the port of Amsterdam a ship set sail for the Dutch colony of Surinam in South America. On board was the artist and pioneer entomologist Maria Sibylla Merian (1647-1717). After two-months of cramped, dirty sea voyage, she landed in tropical Paramaribo, Surinam. When she returned to the Netherlands two years later, she brought back a work which was both a significant scientific discovery and an aesthetic masterpiece. Her amply illustrated Metamorphosis Insectorum Surinamensium, published in 1705, was one of the finest examples of bookmaking of that time (Merian, reprinted 1980). She was one of the first of her era to demonstrate the metamorphosis from egg, through larva and pupa, to adult insect:

"This caterpillar has a particular smell like fruit when many different kinds are stored together. When it has reached its full size it sheds its coat or skin entirely and this it leaves hanging above it as I have illustrated. It fastens itself to a wall with its head downwards and attaches the hind part of its body as firmly as if it were glued on. In the middle of its body it spins a white thread round itself in order to stay firmly suspended. Then within half a day it turns into a date stone, in shape like a baby wrapped in swaddling clothes so that one can almost detect a human face in it.....this 'date stones' are grey, in part also green in colour. In this form they hang until April or May."

Two millenniums before Merian had published her book (Merian, 1717), the metamorphosis of insects had already been described in considerable detail by
Aristotle (384-322 B.C.) He, who is often referred to as 'the first naturalist' or 'the first biologist' (Hoyt & Schultz, 1999), created an extensive body of work that proved to be the dominant view for more than two thousand years. To my knowledge, not many other natural scientists have achieved this. Aristotle reports in considerable detail on metamorphosis, and on the discovery of a fabric that could be made from the cocoons of certain types of caterpillars. This was more than eight hundred years before the true silkworm came to Greece (D'Arcy Wentworth, 1910):

"The so-called psyche or butterfly is generated from caterpillars which grow on green leaves, chiefly on leaves of the raphanus, which some call crumbe or cabbage. At first it is less than a grain of millet; it then grows into a small grub; and in three days it is a tiny caterpillar. After this it grows on and on, and becomes quiescent and changes its shape, and is now called a chrysalis. The outer shell is hard, and the chrysalis moves if you touch it. It attaches itself by cow-web like filaments, and is unfurnished with mouth or any other apparent organ. After a little while the outer covering bursts asunder, and out flies the winged creature that we call psyche or butterfly. At first, when it is a caterpillar, it feeds and ejects excrements; but when it turns into the chrysalis it neither feeds nor ejects excrement."

In summary, three things that look different can still be the same. Metamorphosis means change in shape and structure. The body of an insect is extensively remodelled during the three stages from larva via chrysalis to adult; to such an extent that for a long time these three stages were considered to be three entirely different individuals.

Since the times of Aristotle we know that maggots change into flies, but until today, we are only beginning to understand why they do so. Similar to metamorphosis, 'evolution' means 'change'. But the change evolutionary biologists refer to is not only the change in external characters. In its narrowest and at the same time broadest definition, the one population geneticists use, evolution means 'change in the gene frequencies of a population in time'. Evolutionary biologists aim at answering the question 'why'. Why do larvae change into butterflies, and ultimately why is DNA the basis of the genetic code?

In this thesis, I try to combine the evolutionary question 'why' with the taxonomic question 'which' and the ecological question 'how'. A 'how' question seeks for proximate causes, e.g., how does a male individual manage to locate a female? A 'why' question seeks for ultimate causes, e.g., why has a species evolved the capacity to distinguish females of its own type? The aims of the study at hand are (1) to describe the diversity of endemics on the Mediterranean island of Sardinia,
with a specific focus on butterflies, (2) to investigate how an endemic species survives, and, from the patterns observed today, (3) to reconstruct why this species originally might have evolved, and finally (4) to describe ways to allow these species to coexist next to us while we use the land to build our economy. For this purpose, I start with a general overview of the variety of endemic species occurring in Sardinia, then zoom in to butterflies, and finally focus on a single species, which I will take as a model to explore the factors determining the differentiation of an endemic.

Why a butterfly?

About two millennia after Aristotle, Alfred Russel Wallace (1823-1913), who along with Charles Darwin was architect of the theory of evolution by natural selection, spent many years travelling in South America and the Malay Archipelago, supporting himself by selling specimens. His travel accounts testimony his enthusiasm for nature, in particular butterflies (Wallace, 1869).

"I have rarely enjoyed myself more than during my residence here. As I sat taking my coffee at six in the morning, rare birds would often be seen on some tree close by, when I would hastily sally out in my slippers....The next day I went again to the same shrub and succeeded in catching a female, and the day after a fine male. I found it to be as I had expected, a perfectly new and most magnificent species of Ornithoptera, and one of the most gorgeously coloured butterflies in the world. The beauty and brilliancy of this insect are indescribable, and none but a naturalist can understand the intense excitement I experienced when I at length captured it. On taking it out of my net and opening the glorious wings, my heart began to beat violently, the blood rushed to my head, and I felt much more like fainting than I have done when in apprehension of immediate death. I had a headache the rest of the day, so great was the excitement."

These few words of one of the fathers of evolutionary biology are, in a way, the explanation for why an extensive and growing body of evolutionary literature is based on research done with Lepidoptera. The ‘flying flowers’, as their Greek name πεταλοείς calls them, have always fascinated naturalists, they are beautiful to be with, rather easy to sample, and turned out to be good model organisms for investigating evolutionary questions. Obviously my own choice to focus on a butterfly, instead of a bat or a bumblebee, was based on these ample reasons.
The beginnings of nature conservation

In one of his gloomier moments, Diogenes the Cynic (4th century B.C.) said that, locked within their walls, men who first crowded into cities to escape the fury of those outside, committed every outrage against each other as if this were the sole object of their coming together (Lovejoy & Boas, 1965). This discontent of the civilised with civilisation arose from an emotional impulse to seek a life in harmony with 'nature' rather than a rational reflection. Civilisation, which means nothing else than ‘living in cities’, had appeared in Southern Mesopotamia in the late fourth millennium B.C. (Botteró, 1992). The transformation to civilization depended on the invention of agriculture, irrigation, technical development, and supervision by a literate bureaucracy (Chatwin, 1996). A ‘civilised’ is someone who lives within a literate urban civilisation. The opposite of it is a nomad following his animals from pasture to pasture. Dwelling within permanent domiciles allowed direct control over the immediate environment. Settlements allowed mankind to fix certain aspects of the continuously changing natural world and thereby provide a predictable environment. These urban human civilisations of the Old World spread outwards over all places and landscapes, until they became a serious problem for all other species sharing the same geographic space.

In our age, when the term ‘nature conservation’ has become part of everyday life, and humanity is struggling with species’ extinctions going faster than we can catalogue them, one can only look back with astonishment at the fact, that it has taken man more than two thousand years to realize, what early philosophers already seemed to intrinsically understand, that one is part of nature too, and, what is more, depends on it. By means of science and technology we now try to correct the damages industrialization and urban growth reeked. Practical technology has become the major link between humans and their environment. While today we usually divide the world into the manageable categories of the various disciplines of science and art, classical philosophy and its rebirth in the Renaissance, emphasized the interconnectedness of disciplines.

Leonardo da Vinci’s ‘universal man’ or ‘Vitruvian man’ (Figure 1) originally was not drawn to symbolize humanism in general, as it is usually viewed
today, but represents the harmony between man and nature (Crown, 1995). The
drawing is based on the assumption that the world (or cosmos) is in harmony
throughout and the human body is itself an echo of that harmony. This idea of
the interconnectedness of all things, whether man-made or natural, is yet another
variant of present days' theories on morphogenetic fields (Sheldrake, 1995),
and the superstring theory where the vibrations of a string in a 10-dimensional
space are the basis of all material and life (Boyer et al., 2003). In the ancient and
Renaissance perception, the concept of beauty was inseparable from theoretical
science and practical engineering, as well as from the basic laws governing the
universe. Human notion and the human sense of what is beautiful and what is not
were integral to that whole system.

Ancient ideas of Pythagorean parallels between musical harmony, geometry, and
the functioning of the entire universe have continued to influence scientists and
architects over the centuries. Pythagoras found from simple observation that if
two strings are set to vibrate under identical conditions, the pitch of a string will
be one octave above the other if its length is half the length of the other. If their
lengths are in a ratio of two to three, the tonal difference will be a fifth and so forth.
The Pythagoreans were confident that they had the key to nature in their hands,
and that all regularities in nature were musical. 'The music of the spheres', which
according to Pythagoras accounted for the orbits of the planets, induced Johannes
Kepler to develop his first heliocentric scheme in the early 17th century. Eventually,
he found that the shape of the orbits was elliptic rather than perfectly circular.
Nevertheless, his impulse to search for geometric principles still led him to striking
discoveries about fundamental structures of the universe.

The search for order and structure and, ultimately, beauty is not only driving
scientific discovery, but an eminent characteristic of humanity. Immanuel Kant
dismissed the view that our minds are tabulae rasaee getting filled with impressions,
sense, and cultural information, but picked up the Aristotelian view that we have
an innate drive to order the impressions of the world around us. Kant's theory
of a continuous search for order in our environment is in rough agreement with
Darwinian ideas: the mindset ensures survival in a variable and unpredictable
environment.

In medieval and classical times, building was a conscious attempt to harmonize
architecture with what man saw as being nature's own structural symmetries.
Nowadays, most other species would surely benefit if we were more aware of our
intrinsic association with the natural environment, and applied the renaissance
ideas of harmony in combination with modern technology when shaping our environment. This does not imply that our streets have to look like game trails, but suggests we should introspect and reveal the 'universal man' inside ourselves, and accept that we are also seated on a branch of the tree of life.

**Speciation**

When two things are equal, they can still be different, meaning that when two individuals look similar, they can still be genetically very different. But what are the forces that make individuals different or similar, and ultimately induce the split of one population into two or more separate entities that can in the end become separate species? (And now, I have to warn the less scientifically oriented reader, that from here onwards, things will become more and more subject specific, and you might want to consult Box I. for a first orientation in definitions of frequently used biological terms.)

Until the beginning of the 19th century, when Lamarck and Darwin developed their revolutionary ideas on the evolutionary potential of species, species were considered as fixed, non-evolving entities. Despite the great impact Darwin had on biological science, he only began to solve a few of the many questions in evolution (Darwin, 1879). In the Darwinian view, species are pictured as 'varieties', *i.e.*, groups of individuals defined by differences in morphology. Darwin and his contemporaries focused mainly on explaining the evolution of phenotypic characters, while they hardly addressed the question of how barriers to gene exchange evolve. It is the latter however, that we recognise today as the essence of speciation (Futuyma, 1998). Why are species necessary? Is it, like Dobzhansky (1937) suggested, that organisms form species because the environment presents discrete ecological niches for them to fill? Different species do usually occupy different niches and have different co-adapted gene pools. However, this does not necessarily imply that speciation is only an adaptive process. Mayr (1957, 1963) suggested that reproductive isolation can evolve after genetic changes that occur for other reasons, like geographic isolation, so that speciation is an incidental, non-adaptive consequence of the divergence of populations in allopatry.

According to the biological species concept, a species is defined as an entity that is reproductively isolated from other species. Isolating mechanisms between species can be divided into three main types: (1) premating mechanisms (*e.g.*, spatial, temporal, or ecological isolation), (2) prezygotic isolation (*e.g.*, incompatibility of
gametes), (3) postzygotic isolation (e.g., hybrid inviability or sterility).

Genetic drift, natural selection, and geographic separation are commonly accepted as the main diversifying forces (Futuyma, 1998), that can eventually lead to the fixation of alternative advantageous alleles, i.e., differentiation and, if followed by reproductive isolation, speciation. Classic examples of speciation theories include Dobzhansky’s premise that allopatry can lead to postzygotic isolation through the accumulation of genetic incompatibilities between loci in different geographically isolated populations (Dobzhansky, 1937), and Mayr’s suggestion that bottlenecks (founder events) can produce rapid differentiation (Mayr, 1954). The founder effect hypothesis has been postulated as particularly relevant for islands: speciation could take place as a result of colonisation of a small number of, or even a single gravid female(s). An oft-cited example for such an event are the endemic Hawaiian Drosophila flies, whose enormous variety of 800 species were largely explained by a founder-induced model (Carson & Templeton, 1984).

Studying the degree of variability levels and genotype distribution within a population and compare these with other populations of the same, or a closely related species, can provide insights in the evolutionary history of that population, in that it may allow us to detect historical bottlenecks, or founder effects, and quantify the amount of gene-flow between the studied populations. These genetic data are ideally combined with demographic and ecologic information (Bossart et al., 1998). Population size, mating system, sex ratio, and distribution of individuals in a certain habitat all influence the population genetic structure (Raijmann, 1996). Therefore, in this thesis, I combine population genetic techniques with ecological field data.

**Sympatric speciation**

In contrast to the allopatric model of speciation, where complete geographical isolation is the barrier to gene-flow between two populations, sympatric speciation occurs when two segments of an originally panmictic population differentiate despite continuing gene-flow. Besides these two contrasting modes of speciation, there is another model buttressed between: parapatric speciation takes place if two populations diverge along an ecological gradient, as a result of adaptation to different ecological niches although there is a contact zone between both populations. Allopatric speciation is the null hypothesis to explain species diversity (Mayr, 1942; 1982). It is intuitively more plausible, and there are many examples for speciation under allopatric conditions (especially from islands, see for example
Grant, 1998), but only few empirical examples of sympatric speciation. One of the most appealing ones is the case of Geospiza conirostris, the large cactus finch (Grant & Grant, 1989). Sympatric speciation is primarily driven by disruptive, frequency-or density dependent natural selection on resource use. It does not imply selection for pre-mating reproductive isolating mechanisms; such prezygotic isolation may evolve implicitly, as a result of selection for genes involved in assortative mating. Sympatric speciation initiates when in a group of individuals sharing the same resources, some of the group shift in resource preference (e.g., host plant or habitat). In such a situation, there is increasing competition among those individuals that are best adapted to the particular ecological niche the population is using because they are the most frequent ones (frequency-dependent-competition). Individuals most unlike the others experience the least competition and will therefore be favoured by natural selection (Pfennig & Murphy, 2002 and references therein). The result is that speciation is speeded up and the populations increasingly differentiate. Differentiation thus seems to be particularly incited, if the differences of the ecological niche the speciating groups of individuals occupy are small (Kondrashov & Kondrashov, 1999; Doebeli & Dieckmann, 2003; Tautz, 2003a).

After an initial subdivision of a population under sympatric conditions the following main phases of differentiation have been proposed (slightly modified from Tautz, 2003b):

I. Early stage of differentiation. Disruptive selection on traits that allow the use of alternative niches, coupled with an increasing degree of assortative mating. In this phase one would expect to find differently adapted types that mate assortatively. Most alleles are still shared between the populations, and gene-flow between different subpopulations still occurs, at least at genetic regions that are not involved in differential adaptation. This phase supposedly lasts less than a 100 generations.

II. Within a 1000 generations, morphotypes and assortative mating should become more pronounced. There is a strong reduction in gene-flow, and neutral alleles in the two subgroups increasingly become subject to independent drift, resulting in different frequencies of the alleles.

III. Within 10 000 generations, significant genetic differences build up. Alternative fixation and lineage sorting of neutral alleles take place. One will find new mutations that are a single mutational step away
from pre-existing alleles and can be used as diagnostic markers.

IV. Millions of generations after the initial split, a prediction of further evolution of adaptive characters is difficult. Additional adaptations are equally possible as relative stasis with respect to the initial adaptations. There is now a clear molecular distinction in allelic types and frequencies. Many population-specific alleles have evolved, that differ by multiple steps from previously existing alleles. The accumulation of many mutations has led to postzygotic isolation.

Schluter (1999) argues that ecology must influence speciation as the rate of speciation varies greatly with ecological circumstances. He summarizes evidence that young species with a sufficiently different ecology persist despite gene-flow, even if they have originated in sympathy. Further, he distinguished two hypotheses of how ecology drives speciation. The first suggests that speciation results from divergent selection stemming from the use of alternative environments and resource competition. Reproductive isolation evolves as a by-product of phenotypic differentiation and may involve reinforcement of prezygotic isolation later on. If divergent ecological selection induces mating preferences leading to reproductive isolation, sexual selection is a variant of ecological speciation. As a consequence, speciation rates are high in groups with adaptive radiation because reproductive isolation evolves most quickly when divergent selection is strongest. The second hypothesis proposes, partly overlapping with the first, that ecological processes mainly act on the viability of diverging populations. In novel environments species accumulate rapidly because more populations are able to avoid extinction for long enough to develop reproductive isolation. The absence of predators, parasites and competitors may for example lead to high population densities with reduced chance of extinction. This second idea is based on Mayr (1963) who underlined the importance of niche shift: "We see again and again that an incipient species can complete the process of speciation only if it can find a previously unoccupied niche (p. 574)."

Measuring the likeness of organisms

Allozyme markers
Allozyme markers still are one of the most efficient tools for detecting intra-specific genetic variation within populations, as well as inter-specific differentiation in closely related species. Due to its relatively low cost, the large number of loci that can be obtained, and its straightforwardness, this method is still being widely
**Box I. Glossary of frequently used biological terminology (slightly changed after Futuyma, 1998).**

**Species** = in the sense of biological species, the members of a group of populations that interbreed or potentially interbreed with each other under natural conditions. It is also a fundamental taxonomic unit, to which individual specimens are assigned, which often, but not always corresponds to the biological species.

**Gene** = the functional unit of heredity.

**Gene-flow** = The incorporation of genes into the gene pool of one population from one or more other population.

**Genetic drift** = Random changes in the frequencies of alleles or genotypes within a population.

**Bottleneck** = Severe temporary reduction in population size.

**Mutation** = An error in the replication of a nucleotide sequence, or any other alteration of the genome that is not manifested as reciprocal recombination.

**Adaptive radiation** = Evolutionary divergence of members of a single phylogenetic line into a variety of different adaptive forms; usually the taxa differ in the use of resources or habitats, and have diverged over relatively short interval of geologic time.

**Allele** = One of the several forms of the same gene, presumably differing by mutation of the DNA sequence, and capable of segregating as a unit Mendelian factor.

**Allele frequency** = The proportion of gene copies in a population which are a given allele; i.e., the probability of finding this allele when a gene is taken randomly from the population.

**Natural selection** = The differential survival and/or reproductive success of classes of entities that differ in one or more characteristics.

**Founder effect** = The principle that the founders of a new colony carry only a fraction of the total genetic variation in the source population.

**Locus** = A site on a chromosome occupied by a specific gene; more loosely, the gene itself in all its allelic states.

**Character displacement** = Refers to a pattern of geographic variation in which a character differs more between sympatric than between allopatric populations of two species; sometimes used to describe the evolutionary process of accentuation of differences between sympatric populations of two species, owing to interactions between them.

**Dispersal** = Movement of individuals or organisms to different localities; in biogeography, extension of the geographic range of a species by movement of individuals.

**Disruptive selection** = Selection in favour of two or more modal phenotypes and against those intermediate between them.

**Divergence** = The evolution of increasing difference between lineages in one or more characters.

**Ecological niche** = The combinations of all relevant environmental variables under which a species or population can persist; often also used to describe the resources a species utilizes.

**Population** = A group of conspecific organisms that occupy a more or less well defined geographic region and exhibit reproductive continuity from generation to generation; ecological and reproductive interactions among these individuals are more frequent than with other members of other populations of the same species.

**Genetic variability** = Variation in a trait within populations, measured by the variance that is due to genetic differences among individuals.

**Homozygous** = The same allele at each of the copies of a genetic locus.

**Heterozygous** = Different alleles at each of the copies of a genetic locus.

**Reinforcement** = Evolution of enhanced reproductive isolation between populations, due to natural selection for greater isolation.

**Overdominance** = The expression of two alleles in heterozygous condition of a phenotypic value for some characteristic that lies outside the range of the two corresponding homozygotes.

**Viavariance** = Separation of a continuously distributed ancestral population into separate populations, due to development of a topographic or ecological barrier.
used in Lepidoptera, so that staying with it facilitates comparisons among taxa enormously. Allozymes are electrophoretically distinguishable forms of an enzyme that are encoded by different alleles, which can be visualized with enzyme-specific staining reactions. In electrophoresis, a tissue extract or homogenate of the whole animal, as in many small insect species, is placed on a starch or cellulose acetate gel, or another medium through which proteins can move. When an electrical current is applied to the gel, the proteins move through it at a speed depending on molecule size and net electric charge. Some aminoacid substitutions can alter the net electric charge, so that variants of the same protein, encoded by different alleles, can be distinguished by their mobility. When a locus is monomorphic, all individuals exhibit the same electrophoretic mobility, when it is polymorphic, different homozygotes and heterozygotes have varying mobility, by which they can be distinguished. The banding pattern obtained on the gel can thus be interpreted to identify homozygous and heterozygous individuals, and draw conclusions about genetic polymorphism, the breeding system of individuals, population structuring, and the existence of morphologically indistinguishable species (Menken & Ulenberg, 1987; Murphy et al., 1996).

There are many statistical models to interpret genetic population structure. For allozyme data, the most important of these are the Hardy-Weinberg principle (Murphy et al., 1996 and references therein), and F-statistics (Wright, 1951; Weir & Cockerham, 1984). The Hardy-Weinberg principle states that “in the absence of selection, drift, and migration, the frequencies of alleles in a randomly mating population will maintain a stable equilibrium with genotype frequencies of $AA=p^2$, $Aa=2pq$, and $aa=q^2$, where $p$ is the frequency of the allele $A$, and $q$ is the frequency of the alternative allele $a$.” Deviations from Hardy-Weinberg equilibrium indicate that one or more of the assumptions are not met by the population.

$F$-statistics are the most commonly used indirect method to estimate gene-flow. Wright’s (1951) one locus model with two neutral alleles shows that in an island model under equilibrium conditions, the among population variance in allele frequencies is $F_{ST} = 1/(1+4Nm)$. To use allele frequency data to infer population structure requires a significant assumption, namely, that alternative alleles at a locus are selectively neutral (Kimura, 1983). Allozyme studies begin thus with neutrality as a working assumption, which thus makes the implicit assumption that evolution is mainly determined by genetic drift. This assumption holds until there is evidence for selection at a particular locus. Assuming that substitution rates in allozymes are constant over time, rough estimates of time scales for population subdivision are possible, which I will discuss below.
Measuring species' differentiation by clustering methods

Inferring the evolutionary history of a population (or species) from molecular data is based on the acceptance of a tree-like model of evolution, where ancestral characters are inherited, and the assumption that the population's evolutionary history is defined by changes in these characters (Vijverberg, 2001 and references therein). There are several methods to select the most probable evolutionary scenario among the nearly infinite set of possible phylogenies, which are either based on an algorithm that leads to the determination of a tree, or on an 'optimal criterion' that evaluates alternative phylogenies and decides which one is the 'better'. The optimal methods include the principle of parsimony, which seeks solutions that minimize the amount of evolutionary change, and likelihood methods, that estimate the actual amount of change (Swofford et al., 1996; Vijverberg, 2001). The algorithmic methods include all forms of pair-group cluster analyses, e.g. the unweighted pair-group method (UPGMA), which is based on arithmetic averaging (Sneath & Sokal, 1973), and other distance methods, e.g. neighbour-joining (Saitou & Nei, 1987). The algorithmic methods are computationally fast, but do not evaluate different trees. Consequently, the answer obtained by these methods may not be the most likely phylogeny. Algorithmic methods are therefore best used to explore the data, and find a starting tree for more thorough searches with the criterion method (Swofford et al., 1996).

In the frame of this thesis, I will rely on cluster analyses, as cluster analyses can be applied as a means of representing genetic similarity or distance data (Sneath & Sokal, 1973), this is a convenient method of exploring the allozyme data. The UPGMA method is the most commonly used clustering method and uses averages of distances within groups to determine the minimal distance between groups in building the phenogram (=tree). The tree is constructed by linking the most similar pairs of taxa, followed by successively linking more distant groups (Swofford et al., 1996). Only if the data are ultrametric, i.e., all lineages evolve at equal rates, the representation provided by the tree will be exact. It is therefore essential to keep in mind that UPGMA clusters only account for the extent of genetic similarity between groups, while the historical branching order is neglected (Swofford et al., 1996). The neighbour-joining method is conceptually related to traditional cluster analysis (Saitou & Nei, 1987), but removes the assumption that the data is ultrametric. From the original distance matrix provided, neighbour-joining constructs a modified distance matrix in which the separation between each pair of nodes is adjusted on the basis of their average divergence from all other nodes. By linking the most similar pair of nodes, an additive but unrooted tree is constructed (Swofford et al., 1996).
Estimating divergence time

In the absence of a good fossil record, as it is the case of butterflies, the only way to estimate time scales of species divergence is to infer the timescales from the amount of genetic differentiation between the species in question and its supposed ancestor. This requires the acceptance of a molecular clock hypothesis, which proposes that genes and gene products evolve at rates that are roughly constant over time and across evolutionary lineages (Arbogast et al., 2002). Although there are many controversies around this method's application (Swofford et al., 1996), molecular clocks provide at least rough, comparative estimates for evolutionary events, when there is no fossil evidence. This method has therefore substantially influenced our views on the timing of many important events in evolutionary history, in particular those related to human evolution and migration, Pleistocene speciation, and historical radiations of major groups of plants and animals (for a review see Arbogast et al., 2002). 'Local' molecular clocks, that are applied exclusively within closely related taxa or for particular genes, have been proposed to be more reliable than 'universal' clocks (Yoder & Yang, 2000). Closely related species are often expected to be similar in population size, metabolic rate, generation time, and DNA repair efficiency, which are considered the most likely sources of rate heterogeneity (Martin & Palumbi, 1993), and can consequently be expected to experience similar rates of molecular evolution. Zuckerkandl and Pauling (1965) were the first to suggest that genes and their protein products might evolve at rates constant enough to use the rates as measures of molecular divergence. Subsequent supporters of this hypothesis view molecular divergence time not as a metronome, but as a Poisson process, with regularity of the same order of magnitude as radioactive decay (Hillis et al., 1996).

It is difficult for one to find relevant data to calculate confidence limits for an allozyme (Nei's genetic, D) clock. Avise and Aquadro (1982) summarized the problem “...the major obstacle to critical tests of the electrophoretic protein clock is the almost total lack of reliable independent information about times of speciation.” Nonetheless, there has been an enormous range of estimated divergence rates for Nei's D; ranging mostly between 5 and 18 million years (Hillis et al., 1996). Obviously, with such a wide range of estimates rates, any genetic distance estimate is likely to be compatible with some geological data (Avise & Aquadro, 1982). But there have been successful attempts to calculate confidence intervals on an allozyme clock (Beerli et al., 1996), which show that although the confidence levels on the clock are fairly broad, it is possible to use the predictions to exclude some biogeographic scenarios within closely related groups of organisms.
The genus *Maniola*

Butterflies of the genus *Maniola* (Lepidoptera: Nymphalidae) are highly polymorphic. Variation within species includes variability in wing-patterns within single populations as well as in different geographic areas, and ecological variability in terms of phenology, and life history. This ecological flexibility culminates in the fact that females of *Maniola jurtina* conduct a summer-diapause in the southern areas of the Palaeartic, whereas in temperate climates they do not. Notably, three of the seven species in this group are island endemics: *Maniola chia* THOMSON 1987, from the Greek island of Chios, *Maniola cypricola* (GRAVES 1928), from Cyprus, and *Maniola nurag* (GHILIANI 1852) from Sardinia. The distribution areas of endemic and widespread species are usually mutually exclusive, except in Sardinia, where the ranges of the endemic *Maniola nurag* and the widespread *Maniola jurtina* overlap.

In Sardinia, *M. jurtina* is most abundant at sea level, but can occasionally be found up to 1000 m a.s.l. *M. nurag* flies exclusively above 500 m, and has its distributional centres around the three main mountain areas of the island (Gennargentu, Monte Limbara, and Foresta dei Sette Fratelli). At intermediate altitudes (500 - 900 m), both species are often found sympatrically (Figure 2; chapter 6).

![Figure 2](image.png)

*Figure 2.* Distribution of *Maniola jurtina* and *Maniola nurag* on Sardinia according to elevation (m a.s.l.). The white bar indicates habitats where exclusively occupied by *M. jurtina*, the grey bar shows areas where both species occur sympatrically, the black bar indicates areas where only *M. nurag* occurs.
The most widespread species in this group is *Maniola jurtina* (Linnaeus 1758). *Maniola jurtina* is distributed throughout Europe, western North Africa, parts of the Middle East, the Irish Aran Islands to the Caspian Sea, and from central Scandinavia to the Canary Islands (see chapter 8). It occurs on all Mediterranean islands (Thomson, 1987), except the Aegean Dodecanes, the island of Chios in Northern Greece where it is replaced by *M. chia* (Thomson 1987) and Cyprus, where it is replaced by the *M. cypri cola* (Graves 1928). *Maniola telmessia* (Zeller 1847) largely replaces *M. jurtina* in southern and western Turkey, although occasionally both species’ distribution areas overlap (Van Oorschot & Van den Brink, 1992; Hesselbarth et al., 1995), and flies commonly in parts of Iran, Iraq and Syria. *Maniola halicarnassus* Thomson, 1990 is recorded from the Bodrum peninsula (Turkey) and the Aegean island of Nissiros. *Maniola megala* (Oberthur 1909) flies in southern and western Turkey. All *Maniola* species are usually found below 1000 m a.s.l. (Thomson, 1973), with the exception of, *M. nurag*, which only flies in areas above 500 m and often occurs above 1000 m. In the southern alps, *M. jurtina* has occasionally been observed at higher altitudes than 1000 m as well (Higgins & Riley, 1970).

![Figure 3. A nuraghe.](image)

**The generalist: Maniola jurtina**

*Maniola jurtina*, the meadow brown, is very common in all parts of Europe, even in highly industrialized regions like Great Britain and the Netherlands. It is most
typically found on mesophile grassland, but also in light woodland and shrubland. The meadow brown is an oligophagous phytophage with larvae feeding on various kinds of grasses (Higgins & Riley, 1970; Schmitt, 1999), particularly Poa spp., and Festuca spp. Emergence time varies according to altitude and latitude, and ranges from March (Canary Islands) to July (Scandinavia). The species is protandric. Protandry is very common in butterflies, and generally in insects with high densities, sexual size dimorphism, sex ratios biased towards males, and monandry (del Castillo & Núñez-Farfán, 2002). All these characteristics, except monandry, are displayed by M. jurtina (García-Barrios, 1987).

As mentioned above, in Southern Europe, females perform an imaginal diapause during the hottest part of the summer with a concomitant delayed ovarian maturation (Verity, 1953; Dowdeswell, 1961, 1962; Scali, 1971, Masetti & Scali, 1972; Scali & Masetti, 1973). During the summer diapause, females hide in the shade of bushes or trees and remain inactive until early September when temperatures drop down below the required threshold. Such aestivation behaviour has also been described for M. telmessia (van Oorschot & van den Brink, 1992).

The summersleeper: biology and life cycle of Maniola nurag

Maniola nurag is a rare species sensu Rabinowitz (1981) in that it has a small geographic range and occupies a very specific habitat. Local populations can be large (500 - 1000 individuals), compared to other island endemics (Casula, 1999), but are still generally smaller than those of M. jurtina (see chapters 4 and 5). Maniola nurag is most probably oligophagous like M. jurtina, but with a narrower spectrum of potential host plant genera. It has been raised from Festuca morisiana (Jutzele et al., 1997), but cannot be restricted to that single plant, as we did not find F. morisiana on any of the sites where we observed the butterfly in the field. It must at least be able to feed on several plant species in the genus Festuca, maybe even other grass genera. Nevertheless, it is likely, that the endemic species is more restricted in the choice of larval host plants than its widespread relative.

The field-data presented in more detail in this thesis (chapters 5 and 6) confirm earlier anecdotal evidence in the literature, that Maniola nurag has similar life history traits as the southern populations of M. jurtina, and also exhibits the above described aestivation behaviour (Simmons, 1930). Adults are on the wing from mid May to the end of September, but most easily observed during male emergence peak at the end of May, beginning of June. Maniola nurag is clearly protandric with males arriving 1-2 weeks earlier than females. Males emerge in a shorter
period of time and in higher numbers than females, which emerge gradually and mostly only a few days before aestivation (Scali 1971, Scali and Masetti 1973). As males die before aestivation, females have to be mated in the short pre-aestivation period. Consequently, males are patrolling in search for females. This is also the reason why males are more easily detected and caught in mark-release-recapture studies (Scali 1971, Scali and Masetti 1973). Fertilization usually occurs within a few days after emergence, mostly even within the first 24 hours after eclosion of the female. Females of *M. nurag* do not start oviposition before late summer/begin autumn. The delayed ovarian maturation could be as long as 2 months in some individuals, with a mean of over one month after copulation (Garcia-Barros, 1987). Although obviously related to climatic factors, the exact mechanism that controls the maturation of the ovaries is still unknown. In Northern European populations, for example, the pre-oviposition period is only one week at most (e.g., Brakefield, 1982). After oviposition, the larvae of *M. nurag* start to feed only 2-3 weeks after they hatched, when the first autumn rains have fallen.

In *Maniola nurag* populations, we observed a slightly bimodal emergence pattern, which has earlier been observed in British *M. jurtina* populations (Dowdeswell, 1961; Thomson, 1971; Brakefield, 1982). Masetti & Scali (1972) observed the same in Tuscan populations. Thomson (1971) explained this phenomenon as a ‘temporal sub-speciation’ consisting in a bimodal pattern of appearance in both sexes. He tried to explain it with a diapause in the pupation phase of the butterfly. This explanation has received substantial and justified criticism by Garcia-Barros (1987). Following Dennis (1971) a bimodal pattern could be the result of disruptive selection against individuals emerging in the middle of the emergence period, and eventually produce total reproductive isolation of such modes. A bimodal emergence pattern would favour early emerging individuals of *M. nurag* would in years when spring is early, while late emergence would be advantageous in years with a late spring. In *M. jurtina*, Goulson (1993) did not observe any reduction of gene-flow between early and late emerging morphs of, and thus no evidence for beginning reproductive isolation. This complicated phenology behaviour is not unknown in European Satyrids (Verity, 1919), but seems especially complex in *Maniola*.

**Conservation of Maniola nurag**

Rare species are intrinsically more vulnerable to extinction than more widespread ones. A loss of fitness and increased extinction risk due to limited genetic variability has been shown for many island species (Frankenham 1997). Isolated
populations often show a reduced genetic diversity (Cassel & Tammaru 2003). Genetic diversity always decreases over time, as a natural consequence of genetic drift. Large population size counteracts drift, while small population size and inbreeding increases the level of homozygosity in the entire genome basis, which, in turn, might depress fitness as a result of the expression of partly recessive deleterious alleles (inbreeding depression) and the loss of heterozygote advantage, together with the possibility to adapt to environmental changes. Particularly island endemics are therefore often found to be limited in their evolutionary potential (Frankenham, 1997). There is no evidence in our data, that the Sardinian populations of *M. nurag* suffer from inbreeding or have historically experienced severe bottlenecks. Presently, the genetic variation in the Sardinian endemic is similarly high, as in the widespread *Maniola* species, and much greater than in other endemic Sardinian butterflies (Marchi, *et al.* 1996), so that its evolutionary potential seems intact.

Female survival over the summer diapause is a crucial parameter for the viability of *M. nurag*-populations. This ecological characteristic makes them very susceptible to human-induced change of their natural habitat (as was shown for *M. jurtina*, see Scali, 1971) and might be a special nuisance in a global warming scenario. Then imagos would possibly emerge earlier in spring but resume activity later in autumn. The consequence would be a prolonged aestivation phase that might increase the risk of female death before reproduction. It also might be that with increasing temperature individuals from intermediate altitudes move up the mountain slopes. But due to the island situation this would save them only to a limited extent, as at a certain point, they cannot go any further. Despite of showing the same life history traits in their southern populations, *M. jurtina* is, on a global scale, obviously much less prone to extinction than *M. nurag*. First, it is much more widespread and abundant and secondly does not aestivate in the Northern part of its range.

**Etymology of the epithet 'nurag'**

The Sardinian meadow brown, *M. nurag*, is named after the prehistoric buildings we find scattered across the island, the ‘nuraghi’ (Figure 3). These megalithic towers derive from a Bronze Age culture that was present in Sardinia about 3500 ago. The name ‘nuraghe’ derives from the nuragic word ‘nur’ which means ‘hollow heap’. A nuraghe (Figure 3) is made of roughly worked stones, and might stand as a single tower with a circular base, or in a complex of many towers joined together with connecting walls. The earliest form of nuraghi is dated from 1500
B.C., these were corridor nuraghi which from the outside resembled a pile of rock. More elaborate tower nuraghi have several floors with a staircase running around the interior, each floor covered with a corbelled dome, made by stacking rocks in circular courses. Over 7000 nuraghi have been preserved up to our era. Due to their well-equilibrated structure and architecture, these buildings have remained standing for more than 3000 years, although they are not cemented but rely only on well bonded and coursed stonework. Almost all of them are situated at the top of a hill or the edge of a plateau, in a position where they overview the surrounding land. This fact together with their fortress-like character indicates that the nuraghis were built for passive defence.

**Synthesis and outline**

Islands have one essential similarity to nature reserves: both are surrounded by an 'ocean' of unsuitable habitat. In this vein, island biogeography can be applied in nature conservation (Soulé, 1986). An area that was formerly part of a continuum and is then turned into an isolated reserve will show some of the patterns we find on islands, e.g., the number of species it contains will usually depend on its size (Begon et al., 1990), the number of colonizing taxa will depend on the degree of isolation etc. As centres of endemcity, islands are *a priori* in the focus of nature conservation. The mountainous areas of Sardinia have been recently designated as Prime-Butterfly-Areas (Van Swaay & Warren, 2003), in a volume compiled by joint efforts from the two most important European butterfly conservation organizations, Dutch and British Butterfly Conservation Recognition of the Prime-Butterfly-Areas by legal authorities and implementation in management policies, would not only benefit butterflies, but a number of other endemic species, simply because they occur in the same areas (see chapter 2). Although my analysis does not identify butterflies as such straightforward indicators for overall species diversity (chapter 3) as they are often proposed to be (Cleary, 2003), they can serve as attractive flagship species to raise awareness of the very particular Mediterranean community they belong to, which will ultimately benefit the entire region. Butterflies have attracted people's sympathy through the centuries, which makes them an excellent target group to stimulate attention for nature conservation. If butterflies make Sardinians and their visitors realize that their island does not only have a wonderful sea-shore, but also, landscapes with plant and animal communities, that are unique in the world, this thesis would also have a practical impact. How to incorporate Sardinian endemics in the international legal framework, and some suggestions on the establishment of nature reserves are given in chapters 4 and 5.
If we want to understand why species go extinct, we first have to understand what are the factors that initiate species differentiation and maintain their genetic diversity. Accordingly, chapters 6 and 7 give a detailed description of the ecology and genetic differentiation of the endemic satyrid butterfly *Maniola nurag*. The fact that *M. nurag* is the only endemic *Maniola* species flying in sympathy with a more widespread congeneric, *M. jurtina*, and the differential resource utilization I observed on Sardinia, gives rise to the hypothesis that the two coexisting *Maniola* species are an example of sympatric or parapatic speciation along an environmental gradient (part 3 of this thesis). Subtle niche and microhabitat differentiation could have initiated evolutionary branching, which then led to phenotypic divergence. Evolutionary branching occurs when frequency dependent selection splits a phenotypically monomorphic population into two distinct phenotypic clusters (Doebeli & Dieckmann, 2000). The genetic data suggests that the species are at a rather early stage of differentiation, comparable to the beginning of phase II of the 4 differentiation-stages proposed by Tautz (2003b) (see I. 5 of this chapter). The fact that the two species differ most conspicuously in secondary sexual characteristics is indicative of the early action of evolutionary forces on traits involved in mate recognition (Darwin, 1879), which again is evidence for a recent splitting event.

Chapter 8 illustrates morphological differences between several species in the genus *Maniola*, where I concentrate on the description of diagnostic characters in the genitalia. Here, I provide morphological evidence for the hypothesis, developed in part 3, that there are hybrids among the Sardinian individuals of *Maniola*. Finally, in chapter 9, I describe a curiosity of the genitalia structure found in a female *M. jurtina* from Amsterdam.
Part 1

DIVERSITY
II.
Endemism in Sardinia

with Paolo Casula, Roberta Lecis, Steph Menken


Love what is fanciful and rare
what from the distance of a dream steals through
what knaves condemn to death and fools can't bear.
(V. NABOKOV)
Abstract

The Tyrrhenian islands are known for their highly relictual fauna and flora and are one of the ten Mediterranean hotspots of plant diversity and endemism. There is little detailed information available on species’ biogeography, and new species are still being discovered. This chapter is the first to put together information from several groups of organisms endemic to Sardinia (viz., plants, butterflies, amphibians, lizards, and beetles), with a particular focus on butterflies and amphibians. Reviewing recent literature, we describe distributional patterns and point to centres of endemicty, which we compare with the location and extent of existing protected areas in Sardinia, in order to assess their usefulness in protecting endemic species. Further, we discuss the geological history of the Mediterranean basin and relate geophysical events to molecular-based estimates of species’ divergence times to investigate when and how Sardinian endemics came to the island and describe scenarios of speciation that might have resulted from vicariance, dispersion, and human transportation. The divergence time estimates we summarize here support, that the cladogenetic events leading to the Sardinian lineages of various taxa have occurred after the separation of the Sardo-Corsican microplate from the continental landmass and after the rotation of the Corso-Sardinian plate. Furthermore, there is evidence that the split of many Sardinian taxa has occurred after the Miocene desiccation of the Mediterranean sea (± 5 Ma ago). Areas of high endemism generally coincide with mountainous areas. The main centres of endemism in Sardinia are already included in a network of natural parks that have been proposed to become protected areas but have not yet been officially accepted as such by the Sardinian authorities. Giving them official status would be a step towards safeguarding the unique nature of Sardinia.

Introduction

Rarity and uniqueness attract people. If you got to choose a bonbon out of a box of chocolates where the majority are wrapped in red paper, but some have different colours, you could choose to take a red bonbon or take one of the rare bonbons in the other colours; most of us would probably choose a rare bonbon. If we suppose that the chocolates represent species, most naturalists would do the same. Numerous examples in recent literature show that endemism has high conservation priority (Munguira 1995; Schnittler & Ludwig 1996; Hurtrez-Boussès 1996; Gruttke et al. 1999; Cook & MacDonald 2001; Médail & Quézel 1999; Grill et al. 2002). Endemic species are often ‘specialists’ that depend on particular, and often localized resources, which makes them especially vulnerable to changes in climate,
land use, or habitat management (Munguira, 1995). Consequently, increased human pressure will induce greater losses in endemic species than in more widespread biota (Oberdorff et al. 1999). This has resulted in increased interest in detecting areas rich in endemics, which are usually termed biodiversity hotspots (Morrone & Crisci 1995; Martín et al. 2000; Myers et al. 2000). Countries or regions harbouring endemic species carry a particular conservation responsibility, as the disappearance of the species from those areas would mean their global extinction. Contrary to general belief, endemic species and regions of high endemism are poorly known even in Western Europe (Deherveng 1996). For butterflies, there have been recent efforts to overcome this lack of knowledge, and areas of high endemism and/or species-richness in Europe have been defined and pointed out to policy and decision makers as 'Prime Butterfly Areas' (Swaay and Warren 2002).

In the Mediterranean Basin, efforts to identify centres of endemcity and species richness revealed that it is one of the world’s most important regions of plant diversity, harbouring and astounding 20% of the world’s total floristic richness in only 2% of the earth’s surface (Médail & Quézel 1997; Médail & Verlaque 1997; Verlaque et al. 1997; Médail & Quézel 1999). These studies clearly underline the importance of the Mediterranean region in global conservation.

But the importance of areas of high endemcity goes beyond the straightforward aims of conservation biology. Species with restricted distributions, and taxa representing unique lineages within a species flock can be interesting model organisms to investigate the processes of differentiation, speciation, and coevolution, and often provide the key for answers to broader scale biogeographic questions. The study of islands where many endemic species and/or subspecies evolved has provided fundamental insights into the relationships between geographical patterns and biological processes for famous evolutionary biologists like Darwin, Wallace, Mayr, and Wilson (Drake et al. 2002 and references therein).

Islands often hold a large proportion of endemic species (Gómez-Campo 1985; Hurtrez-Boussès 1995; Whittaker 1997). Indeed, the Macaronesian (Madeira and the Canary Islands) and the Tyyrhenian Islands (Balearic Islands, Corsica, and Sardinia) are among the most important hotspots of endemism within Europe (Médail & Quézel 1999). Notably, the Tyyrhenian islands together with the Maritime-Ligurian Alps are known for their high relictual endemism (Médail and Quezal, 1999). Island endemics are the most vulnerable of all endemic taxa, as (1) islands are unlikely to offer refuges during ecological change, and (2) island populations are usually limited in size. Generally, all endemic taxa are potentially threatened by hybridisation, competition, predation or disease when
Table 1. Distributional patterns of Sardinian and Sardo-corsican endemics. The geographic extent of species’ distribution is shown as well as the altitudes where they occur. S=Sardinia, C=Corsica, T=other Tyrrenian islands, GS=Gennargentu-Supramonte Massif, SF=Sette Fratelli, LI=Mount Limbara, WC=West Central Sardinia, CO=Coastal areas.

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interacting with introduced taxa. An island is mostly referred to as a stretch of land surrounded by a mass of water isolating it from other land areas. By widening this definition to include areas isolated by habitat unsuitable for the taxon under consideration (Hudson 1998), analogies can be drawn for land patches or refuges that have become isolated through Quaternary ice-age events, causing populations to differentiate in allopatry. Pleistocene glaciations probably induced insular speciation sensu lato. Hence, real islands like the Mediterranean island of Sardinia, provide 'laboratories' in natura for the study of evolutionary questions (Caccone et al. 1994; Salomon 2001).

Objectives
As is true for Europe in general (Médail and Quezal, 1999), particularly in Sardinia endemic species have not yet been studied comprehensively. There is little detailed information on species’ distribution and biogeography, and new species are still being discovered, (Rota 1992; Gentili et al. 1998; Sabella et al. 1998; Selvi 1998; Mossa et al. 1999; Bacchetta et al. 2000). Here we review published data from several groups of plants, butterflies, amphibians, lizards and beetles, endemic to Sardinia in order to define centres of endemicity and see if these overlap with the existing protected areas in Sardinia. As butterflies and amphibians are the focus of our own research we particularly focus on them. Further, we explore the factors that may have incited Sardinian species to diverge from their continental ancestors or sister species.

1. Distributional patterns of endemic species
The Tyrrhenian islands belong to the ten Mediterranean hotspots of plant diversity and endemism defined by Médail and Quétal (1997), where plant richness is >2000 species per 15 000 km² and at least 10% of the species are narrow endemics. This high richness is primarily due to palaeogeographical and historical factors (Pons & Quétal 1985; Verlaque et al. 1997). In the following section we give some examples of distributional and ecological patterns of endemicity in Sardinia (summarized in Table 1) and indicate the main centres of endemicity on the island. When we speak of endemics, we consider two different groups: species exclusively endemic to Sardinia and species endemic to Sardinia and Corsica or more Tyrrhenian islands.

Plants
_Echium anchusoides_ (Boraginaceae) was only recently described (Bacchetta et al. 2000), and is endemic to the main siliceous massifs of Sardinia, which are situated
in the mountainous zones of the island. The same is true for the Sardinian oak, *Quercus ichnusa* (Fagaceae) (Mossa et al. 1999), the Sardo-Corsican thyme, *Thymus herba-barona* (Lamiaceae), the shrub *Santolina insularis* (Compositae), and the perennials *Glechoma sardoa*, and *Lamium corsicum* (Lamiaceae). *Glechoma sardoa* and *L. corsicum* are both endemic to Sardinia and Corsica (Brotzu 1998). In contrast, the Sardo-Corsican endemic plants *Vinca sardoa* (Apocynaceae) and *Ornithogalum biflorum* (Hyacinthaceae) can be found at all altitudes in a variety of habitats, including road margins and the edges of cultivated fields (Sacchetti et al. 1999; Brotzu 1998). The perennial herb *Anchusa crispa* (Boraginaceae) occurs in open herbaceous vegetation on low-lying sand dunes (Quilini et al. 2001).

**Butterflies**

Endemic Lepidoptera are usually found at altitudes above 500 metres (Cobolli et al. 1995; Biermann 1998; Kleinekuhle 1999). Only three of the 14 Sardo-Corsican endemics are observed at equal frequencies in coastal (sea-level) and mountainous habitats (Kleinekuhle 1999; Grill 2002). One of the 14 species, *Hipparchia aristaeus aristaeus* (Nymphalidae), which is usually known from mountainous areas, has also been recorded on the coast (Cobolli et al. 1995). These coastal localities, however, are not its main distributional centre. The distribution areas of Sardinian endemics are often strictly related to the composition of the underlying substrate. *Lysandra coridon gennargenti* (Lycaenidae), for example, strictly follows the distributional pattern of its host plant, *Hippocrepis comosa* (Fabaceae), which is typically found on calcareous grounds and as a consequence, the butterfly is restricted to calcareous outcrops in the ‘Barbagia di Seulo’ and the ‘Supramonte di Orgosolo’ mountains. In Sardinia *H. comosa* is most probably the only food plant used by *L. coridon gennargenti*, as the plants used by the continental European populations of *L. coridon*, viz., *Coronilla spec.* and *Astragalus glaucus* (Fabaceae), do not occur in Sardinia. *Hippocrepis comosa* is rather common on other Tyrrhenian islands and the Mediterranean mainland. Individuals of *H. comosa* in Sardinia, however, are much more fragile than those found conspecific the continent, with island populations restricted to mountainous areas whereas on the Italian mainland they are found from 0-2900 m a.s.l. (Pignatti 1982). Recognising the Sardinian form as a distinct endemic taxon could therefore be appropriate. The Sardinian blue, *Pseudophilotes barbagiae* (Lycaenidae), is exclusively dependent on *Thymus herba-barona*, the above mentioned Sardo-Corsican endemic thyme species, which grows between 1000 and 2000 m a.s.l (Pignatti 1982); its distribution is thus restricted to a few slopes in the Barbagiae and the Gennargentu mountains, and mount Limbara. *L. coridon gennargenti* and *P. barbagiae* are among the rarest butterfly species of Europe.
The Sardinian meadow brown, *Maniola nurag* (Satyridae), has its distributional centres around the three main mountainous areas of the island. The endemic hesperid *Spialia sertorius therapne* has been observed on the Gennargentu, Limbara, and Sette Fratelli mountains (Cobolli et al. 1995).

**Amphibians**

The Sardinian mountain newt, *Euproctus platycephalus* (Salamandridae) appears to occur predominantly in the three main mountain ranges: Gennargentu-Supramonte in central Sardinia, Sette Fratelli in the south-east, and Mount Limbara in the north (Lecis 2002). In this respect it is similar to most endemic butterfly species. The distribution of this endemic newt covers approximately the eastern side of the island, with very few unconfirmed records in the western areas. The genus *Speleomantes* represented on the island by four species of cave salamanders, occurs in limestone caves and humid rocky substrates: *S. supramontis* in Supramonte, *S. flavus* in Monte Albo, and *S. genei* and *S. imperialis* in the south east and in the south west. The Sardinian tree frog *Hyla sarda*, endemic to Corsica, Sardinia, and the Tuscany archipelago, inhabits lowlands and temporary waters all over the island and is quite common, locally abundant, but probably declining (Colomo 1999). The Sardinian painted frog, *Discoglossus sardus*, classified as vulnerable, is usually found in stagnant or slow moving waters and described as widespread (Colomo 1999). Its distribution area covers Corsica and Proquerolles, Port Cros, and the French Hyères archipelago.

**Lizards**

*Archaeolacerta bedriagae* (Lacertidae), the Bedriaga's rock lizard, a Sardo-Corsican endemic, seems to occur in areas of Limbara, Marghine, Monte Albo, and Gennargentu, generally in the north and centre of Sardinia (Colomo 1999). The insular endemic lacertid lizards, *Algyroides fitzingeri* and *Podarcis tiliguerta*, are described as widespread and common at different altitudes, from sea-level to the mountains in both Corsica and Sardinia (Arnold 2003; Delaugerre & Cheylan 1992). Two subspecies, *Podarcis tiliguerta ranzii* and *Podarcis tiliguerta toro*, are both restricted to one little circum-sardinian island in the north (Molarotto near Olbia) and in the south west (Toro near Sant'Antioco).

**Beetles**

The two Sardinian genera of obligate cave-dwelling beetles, *Ovobathysciola* and *Patriziella*, are obviously dependent on cave environments (Caccone & Sbordoni 2001). Recent observations indicate that *Ovobathysciola majori* and *Patriziella sardoa*
inhabit numerous caves from sea level to 1000 m elevation in the karst areas of the Supramonte massif (north-east Sardinia) whereas *Ovobathysciola gestroi* is found in the Gennargentu massif. *Ovobathysciola graffitii* and *Patriziella nuragica* have only been found in north-western Sardinia, near Sassari. *Speonomus lostiai* inhabits a few caves in west-central Sardinia (Caccone & Sbordoni 2001).

2. Speciation molecular divergence, and geological history

Insular speciation usually results from the differentiation between populations settled on the island, and the continental population from which they were isolated (Jacquard 1974; Hudson 1998; Salomon 2001). Diamond (1977) describes three successive stages that can be considered as prerequisites for insular speciation: colonization, settlement, and genetic divergence. Speciation as a consequence of geographical isolation is termed allopatric or geographical speciation. Sympatric speciation results from isolating mechanisms without the involvement of a physical barrier to gene-flow. Parapatric speciation takes place when two divergent species have disjunct geographical distributions but there is a contact zone between them, where gene-flow is possible.

Estimates of separation times can vary greatly among different genes, and even portions of one particular gene. There is, however, a general consensus that if rates are compared between closely related species for the same DNA region, sequences are very likely to display a clock-like behaviour (Caccone & Sbordoni 2001). The existence of well-dated geological events, as is the case for the islands in this study, is a great advantage when trying to calibrate molecular rates in species whose distributions have probably been shaped as a result of these events.

The geological and geophysical history

The geological evolution of the Mediterranean region is characterized by the relatively rapid opening of several back-arc basins, generally floored by oceanic crust, within the framework of the Africa-Eurasia collision and Alpine orogenesis (Speranza et al. 2002; Blondel & Aronson 1999 and references therein). In the western Mediterranean, the Liguro-Provensal Basin, a triangular sea located between the Provencal-Catalan coasts and the Corsica-Sardinia block, opened during the Oligo-Miocene. Basin spreading and the simultaneous eastward migration of the Alpine belt and Corsica-Sardinia-Calabria blocks were probably driven by the eastward retreat of a Ionian/Adriatic slab passively sinking into the mantle (Malinverno and Ryan 1986). Since the middle-late Miocene, further roll-back of
the same slab caused spreading of the Tyrrhenian Sea, the southeastward drift of the Calabrian block, and the orogenesis of the Apennines (Patacca et al. 1990). The Liguro-Provencal spreading took place simultaneously with the eastward drift of the Corsica-Sardinia block, which rotated at least 30° counter clockwise (e.g., Van der Voo 1993, Speranza et al. 2002 and references therein).

Palaeomagnetic investigations carried out in the 1970’s on Oligo-Miocene volcanics of Sardinia suggested that the island was separated from the continental landmass about 33 Ma ago, turned by 35° clockwise up to 21-20.5 Ma, and then rotated 30° counter clockwise in a few million years (De Jong et al. 1969, 1973; Edel, 1979, 1980). Since then, the end of the rotation, fixed at 19 Ma by Montigny et al. (1981) has been subject to controversy (Edel et al. 2001; Speranza et al. 2002 and references therein). New palaeomagnetic and Ar/Ar results support a begin of the rotation around 21-20.5 Ma and an end of the rotation at 18-17.5 (Edel et al. 2001; Deino et al. 2001). But there is growing evidence that the rotation did not end before 16 Ma and started after 19 Ma (Speranza et al. 2002). The question of coupling or de-coupling between Corsica and Sardinia during drifting was resolved by Vigliotti et al. (1990), who showed that after the Permian the two islands rotated as one block. During a period from 18.35 to 17.5 Ma the marine transgression occurred (Edel et al. 2001). At the same time a NE-SW shortening, interpreted as resulting from the collision of the Sardo-Corsican block with Apulia, affected parts of the island (Letouzey et al. 1982). Speranza et al. (2002) propose that at 16-19 Ma, the lithosphere of a ‘palaeo-

Figure 1. Time scales of geophysical events in relation to speciation time estimates inferred from molecular data.
### Table 2. Examples of species pairs: genetic differences between closely related species/subspecies based on allozyme markers and mtDNA.

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<tr>
<th>Species</th>
<th>Podarcis murrellii (Italian mainland)</th>
<th>Lysiandra cordon appennina (Central Italy)</th>
<th>Lysiandra cordon alcestisina (Central Spain)</th>
<th>Maniola jurtina (Austria, France, Spain)</th>
<th>Ovobathysciola graffitti (Sardinia)</th>
<th>Antilochamus buoni (Pyrenees, Spain)</th>
<th>Speonomus delarouzei (Pyrenees, Spain)</th>
<th>Speonomus brocchi (Pyrenees, Spain)</th>
<th>Speonomus hygrophilus (Pyrenees, Spain)</th>
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<td><em>Speonomus lostia</em> (Sardinia)</td>
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<td>Caconn &amp; Sbordoni 2001, Evolution</td>
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<td>Micheaux <em>et al.</em> 1996, Heredity</td>
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* Nei's genetic distance based on allozyme markers  
** based on mtDNA
Table 3. Divergence time estimates of closely related species in millions of years (Ma) or years (a).

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<tr>
<th>Species</th>
<th>Podarcis spp.</th>
<th>Euproctus montanus (Corsica)</th>
<th>Lysandra coridon appenina (Italy)</th>
<th>Lysandra coridon caelestissima (Spain)</th>
<th>Mapiola jurtina (Austria, France, Spain)</th>
<th>Ovobathysciola graffiti (Sardinia)</th>
<th>Patriziella sardou (Sardinia)</th>
<th>Apodemus syltaticus (France, Belgium)</th>
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<td><strong>Lizards</strong></td>
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<td>Lysandra coridon gennargenti</td>
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<td>Maniola nurag (Sardinia)</td>
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<td>Ovobathysciola majori (Sardinia)</td>
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<td>Ovobathysciola gestroi (Sardinia)</td>
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Ionian' oceanic corridor east of Sardinia sunk in the mantle causing a trench retreat and the Liguro-Provencal spreading. Faster subduction beneath Sardinia than beneath Corsica, due to the heterogeneous nature of the subducting plate, has been put forward as a plausible reason to explain the triangular geometry of the Liguro-Provencal Basin and the counter clockwise rotation of Sardo-Corsica. About 5 million years ago, the Mediterranean sea was almost entirely desiccated, creating connections of dry land between Sardinia and northern Italy and southern France. Sea level oscillations creating land-bridges between Sardinia and Corsica continued from Miocene until well into Pleistocene (5.7 - 0.23 Ma). (Arias et al. 1980; Cita 1976). In the Quaternary, Sardinia could have been in contact with the mainland via Elba as the sea level was up to 120 meters lower than today. During the late glacial maximum, 20 000 years ago, Sardinia was connected with Corsica.

**Lizards and amphibians**

Lanza (1983) hypothesized that the split among the Sardinian lizards, *Algyroides fitzingeri*, *Archaeolacerta bedriagae*, and *Podarcis tiliguerta* from mainland relatives is related to Premiocenic or Messinian age, while *P. sicula cettii*, could have diverged during the Pleistocene (Lanza 1983). Oliviero (1998) gives preliminary estimates of divergence times based on DNA sequences for *P. tiliguerta* (13 Ma) and *P. sicula cetti* (7 Ma), suggesting a Messinian age for both species. Ancient taxa such as *Euproctus platycephalus* and *Speleomantes genei* probably originated from ancestors already present on the Sardo-Corsican microplate prior to its detachment from the continent (Lanza 1989). Other ancient taxa such as three of the four Sardinian *Speleomantes* (*S. flavus*, *S. imperialis*, *S. supramontis*) are more closely related to the continental *Speleomantes ambrosii* (Lanza 1989; Lanza 1995), indicating that the ancestor of those three species arrived in Sardinia about 5 Ma ago from the Apennines. Jackmann et al. (1997), however, infer a close relationship of *S. flaurus*, *S. supramontis*, and *S. genei*, whereas the continental *S. italicus* would be less closely related, and give evidence for a monophyletic origin of the Sardinian *Speleomantes* group.

Caccone and co-workers (1994, 1997) used the split between the Pyrenees and the Corso-Sardinian plate and the separation of Corsica from Sardinia to calibrate mitochondrial rDNA evolutionary rates in newts of the genus *Euproctus*, which comprises three species (with distributions restricted to Corsica, Sardinia, or the Pyrenees). These genetic investigations confirmed records of morphological, anatomical and karyological studies (Bucci-Innocenti et al. 1978; Delaguerrre & Cheylan 1992): Corsican and Sardinian newts are more closely related to each other than to the Pyrenean newt (Table 2, 3). This is a sound consequence of a
previous speciation event, dated around 29 Ma ago, while the two insular species probably started diverging in Sardinia between 9 and 15 Ma ago (Caccone et al. 1994) (Figure 1).

**Butterflies and beetles**

Recently, Caccone & Sbordoni (2001) used COI divergence rates to estimate the time of isolation of cave beetles of the genera *Ovobathysciola*, *Patriziella*, and *Speonomus* to estimate the time of isolation for endemic Sardinian taxa based on COI divergence rates. They estimated divergence times of 16-10 Ma among the three *Ovobathysciola* species (*O. grafitti*, *O. majori*, *O. gestroi*), 6.3-4.6 Ma for the split between *O. grafitti* and the two *Patriziella* species, and 4.5 to 3.7 Ma for the split between *P. sarda* and *P. nuragica*, all endemic to Sardinia (Table 2, 3). The species assemblage of cave-dwelling beetles in Sardinia might be explained as the result of vicariance (Caccone & Sbordoni 2001). This assemblage probably began its diversification in a first step due to the separation of Sardinia from the continental landmass, and in a second step after the dramatic changes brought about by sea level oscillations (up to 1000 meters) in the middle and late Miocene (16-5 Ma), which separated northern Sardinia from central and southern Sardinia (Steininger & Rögl 1984). These changes might have enhanced the isolation of the ancestral forest-dwelling populations of cave beetles, which then retreated to the wet habitats in the karstic caves of Sardinia. Also the climate changes in the Pliocene (5-2 Ma ago), when the climate switched to cooler and drier conditions, forcing warm and humid subtropical forests to gradually change into a savanna-like vegetation (LaGreca 1998), could have forced some populations into the humid environment of caves where they became isolated from their ancestral forest dwelling populations (Caccone & Sbordoni 2001).

In Lepidoptera, the first expansion events vigorously influencing the European fauna occurred in the Pliocene, when Near Eastern and Balcanic species started

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**Figure 2.** Existing network of protected areas in Sardinia. Centres of endemicity are indicated with rectangles.
to invade Europe (10-1 Ma ago). Before the Pliocene, the European fauna was predominantly tropical (Leestmann 1965; Kleinekühle 1999). The migration route probably went from Central Asia, to the Near East, and Greece, which at this point was still connected to Tunisia, and from there via Sardinia and Corsica to Tuscany (Leestmann 1965; Kleinekühle 1999). During the last glacial maximum, Sardinia was much less affected than Corsica, so that thermophilic species survived in Sardinia, while cold-adapted species could persist in Corsica (Kleinekühle 1999). This might have enhanced differentiation of a number of endemic species, which during the post-glacial warming retreated to higher altitudes. Similarly, the endemic taxa of the genus Erebia in the Alpine region and Scandinavia have been suggested to be due to differentiation in glacial refugia (Roos & Arnscheid 1979; Kleinekühle 1999).

Sardinian examples of endemics which most likely evolved as a result of ice-age events are Aglais urticae ichnusa (Nymphalidae) and Hipparchia aristaevus aristaevus (Satyridae), both endemic to Sardo-Corsica, and related to Aglais urtica and Hipparchia semele (Satyridae) (Kleinekühle 1999). The latter two species are long-distance dispersers, but probably orientate their routes on determined features of the landscape and appear to be reluctant to cross large sea-areas, explaining why they never re-invaded Sardinia or Corsica. Other Sardinian species most probably originate from Central Asia. According to Leestmanns (1965), Argynnis elisa (Nymphalidae), Hipparchia neomiris, Coenonympha corinna (Satyridae), and Papilio hospiton (Nymphalidae) originated from the Asiatic species Argynnis clara, Hipparchia digna, Asiatic taxa of the genus Coenonympha and Papilio sikimensis respectively. More recent studies however, conclude a closer relationship between Papilio machaon sahareae and Papilio hospiton (Pierron 1990, 1992).

For the Sardinian Blue, Lysandra coridon gennargenti, there is genetic and morphological evidence that it is specifically different from the continental Lysandra coridon (Marchi et al. 1996; Jutzel et al. 2002). Marchi et al. (1996) suggest an allopatric speciation event. They found evidence for an absence of gene-flow to the continental populations, indicated by the presence of alternative fixed alleles at several enzymatic loci (aat, gpi and pgm) and significant differences in allele frequencies at other loci, distinguishing the Sardinian population from L. c. apennina and L. caelestissima. The genetic differentiation of L. c. gennargenti, measured using Wright’s Fst-values and Nei’s genetic distances (0.337-0.434), indicate that the Sardinian or populations evolved along an independent lineage, facilitated by isolation and the strict dependence of the butterflies on specific biotopes. Marchi et al. (1996) also found a reduction of genetic variation (Polymorphism = 17.6 %,
Heterozygosity = 0.024) with respect to the continental populations (P > 52 %, $H$ greater than or equal to 0.170). Values of Nei’s genetic distances (Table 2) between the Sardinian subspecie and the populations of continental Italy, are higher than those found between geographically isolated populations of *L. coridon* from continental Italy, and comparable to or even higher than distance-levels found between other endemic taxa that are considered separate species. Our own data on genetic differentiation between *Maniola jurtina* and *Maniola nurag* based on allozyme markers (Grill *et al.* unpublished work), for example shows smaller genetic distances (0.065-0.089) although these two butterflies are considered to be different species (Table 2). Genetic distances between the two *Maniola* species are smaller, which suggests that they probably diverged in more recent times than *L. c. gemmargenti*. However, these numbers are only indicative of the degree of differentiation, and there is no general rule for the relationship between genetic distance and taxonomic status (Menken & Ulenberg 1987; Orr 2001).

**Maniola nurag as an example for ecologically induced speciation?**

*Maniola jurtina*, the meadow brown butterfly, has been shown to be closely related to *M. nurag* in allozyme-genetic analyses (Thomson, 1987; Grill *et al.* 2003). The two species are phenotypically similar but nevertheless can usually be distinguished by their wing patterns. However, there is some overlap for individuals flying late in the season, and in exceptional cases, genital preparation might be the only way for determination. Both species fly in Sardinia but have only minor overlap in distribution area and flight period. *Maniola nurag* emerges a couple of weeks after *M. jurtina*, and has only been found above 500 m.s.l. whereas *M. jurtina* is most abundant at sea level but can occasionally be observed up to 1000 m.s.l. (Grill 2001). Adults of *M. nurag* are on the wing from May to September depending on altitude and local weather conditions, *M. jurtina* flies in Sardinia from late April to June. At lower altitudes *nurag* females aestivate during the hottest part of the summer (Grill, 2001; Kleinekühle, 1999; Tolman & Lewington, 1997). A similar aestivation behaviour has been observed in Southern populations of the pan-european species *Maniola jurtina* (Scali & Masetti 1973). *Maniola nurag* is probably better adapted to the particular conditions in the Sardinian mountains, with extremely dry and hot conditions during Mediterranean summers, and large temperature oscillations between day and night. Body size is smaller than in *M. jurtina*, the body is more compact and darker, and the upper side of both fore- and hind-wings are brighter in the endemic species. UV-photographs of wing-patterns do not reveal any differences between the two species. In both, the eyespot pupil is bright and visible, as generally observed in satyrids (S. Bryant, pers. comm.). Differentiation between
the two species could be related to larval-food-plant choice. The larvae of *M. jurtina* feed on a wide range of grass species including *Poa pratensis*, *Festuca rubra*, *Festuca arundinacea*, *Agrostis stolonifera*, *Agrostis canina*, *Bromus erectus*, *Brachypodium pinnatum*, *Holcus lanatus*, *Avenula pubescens*, and *Anthoxanthum odoratum* (Tolman & Lewington 1997). The island endemic is probably more specialized in its diet, perhaps feeding on grasses that flower relatively late in spring, and so offer oviposition sites that are still green when most other vegetation is already dry.

**Ecological and evolutionary isolation in Euproctus platycephalus**

The three species in the genus *Euproctus*, *E. platycephalus* (Sardinia), *E. asper* (Pyrenees), and *E. montanus* (Corsica) share various morphological, reproductive, and ecological traits, such as the presence of a sixth toe on the male hind legs, the mating behaviour (males actively search for females and hold them, curving body and tail in order to manipulate their spermatophores into the female cloaca in an almost real amplexus), and the typical habitat (although the Sardinian *E. platycephalus* seems to occur at lower altitudes than the other two). All three species life in streams, springs, pools or small lakes in mountainous areas. However, the Pyrenees, the Corsican and the Sardinian mountain ranges differ in geology and climate, so that apparently similar sites, might actually be very different as a result of differing environmental conditions. Pyrenean and Corsican mountains are on average higher than Sardinian ridges, and a large part of the area is still covered by *Pinus* and *Quercus* forests. In Sardinia, centuries of deforestation, stock breeding, and fires have gradually changed landscape and microclimate, especially in the centre and south of the island (mountains of Gennargentu and Sette Fratelli), where the largest part of the land is currently covered by Mediterranean ‘macchie’.

In the Gennargentu mountain system, typical landscape consists of bare or bushy slopes with *Alnus glutinosa* creating gallery forests along water courses. During the last decennia, a reduction of rainfall has reduced stream flows and connectivity between rivers (Regione Sardegna 2000). This led to a fragmentation of the habitat of the Sardinian *E. platycephalus* and might be the reason, why this species has become rarer during the last years.

**Mammals**

Sadly enough, in Sardinia as well as in Corsica most indigenous land mammals have disappeared. Human activities brought about the extinction of most of the autochthonous mammalian fauna and the gradual introduction of more than 25 taxa which constitute the present wild and domestic fauna. Such a complete turnover has also been recorded on other Mediterranean islands. These extinctions
include Prolagus sardus (Lagomorpha, Leporidae), known from subfossil remains found on Corsica, Sardinia, and adjacent small islands (Vigne 1992). Prolagus could possibly have reached Sardinia during the desiccation of the Mediterranean during the Miocene (Schule 1993). Its origin seems to be in Mongolia from where its ancestors reached Corsica and then Sardinia. Skeletal remains indicate that Prolagus was still present on Corsica and Sardinia less than 2000 years ago (Vigne 1992). The final report of a living population was made in 1774 by F. Cetti, who observed, “giant rats whose burrows are so abundant that one might think the surface of the soil had been recently turned over by pigs” on the island of Tavolara of north-eastern Sardinia. It probably attained a length of 200-250 mm but must have undergone rapid evolutionary changes following the arrival of humans on Corsica and Sardinia about 9 000 years ago (Vigne 1992). These modifications include an increase in the size of the skull but a reduction of the postcranial skeleton. In Neolithic times Prolagus was an important part of human’s diet in Sardinia, testified by the great amount of skeletons found in human-inhabited caves, like the Grotta di Corbeddu near Oliena. While it apparently survived longer than other presently extinct, mice-like, insectivorous mammals of the Mediterranean islands (Nesiotites, Tyrhenicola, Rhagamys), this human predation finally seems to have caused its extinction. Another species that became probably extinct due to human influence is the giant deer Megaceros. All extant wild ungulates on the Mediterranean islands are feral domestic animals, or continental game introduced during the Neolithic or later, none of them has Pleistocene ancestors (Schule 1993).

The largest mammal on the island is Cervus elaphus corsicanus, the Sardinian form of the European deer. It is smaller, darker and more delicately built than continental deer and restricted to three main regions, viz., Capoterra, Sette Fratelli forest, and the World Wildlife Fund park of Monte Arcosu. The Sardinian deer is protected by regional legislation and is a target species in the Italian ‘Natura 2000’ network.

Most of the other mammals presently living on the island have been introduced by man, albeit perhaps hundreds of years ago (Blonde l & Vigne 1994; Micheaux et al. 1996). Also the origin of the Tyrrenian form of the woodmouse, Apodemus sylvaticus milleri, was anthropogenic. Allozyme data suggest that all the Tyrrenian woodmice and those of peninsular Italy have a common origin but differ from the North-Western subspecies, A. sylvaticus sylvaticus. The Tyrrenian mice are well isolated from those living on the western edge of the Alpine chain, including the eastern Pyrenean beech forest. They invaded the islands via the route of Etruria to Elba and Corsica. This hypothesis is in agreement with archaeological evidence of relations between island and mainland populations of Neolithic humans (Klein
According to this theory, woodmice would have colonized the islands as 'lifters' on human boats. The island-specific alleles of Corsican and Elban Apodemus are completely absent in Sardinian mice. This indicates that Sardinia was invaded directly from Italy without the detour across Elba and Corsica (Micheaux et al. 1996).

3. Reflections on conservation issues in butterflies and salamanders

Island species and particularly endemics, are intrinsically more vulnerable to extinction than more widespread species. Habitat destruction, and/or competition with newly introduced species may have severe effects on islands' biodiversity. Low genetic variability, resulting from inbreeding or genetic drift has often been reported to decrease species' fitness, and consequently make them more vulnerable (Keller et al. 2002). Hybridisation and consequent genetic assimilation might be additional threats.

A well known example where natural hybridisation is frequent are the butterflies Papilio machaon and Papilio hospiton (Aubert et al. 1996; 1997). Laboratory crosses show that hybrids are not sterile. However, genetic assimilation does not seem to be a threat for P. hospiton as developmental perturbations impair the viability of further hybrid progenies.

A recent assessment of endemic Sardinian butterflies suggests that the lycaenids, Pseudophilotes barbagiae and Lysandra coridon gennargenti, are the only two butterfly species classified as 'vulnerable' according to the IUCN threat categories (Grill et al. 2002). However, the two more conspicuous but probably less threatened species Papilio hospiton and Argynnis elisa, are listed in Appendix II of the Bern Convention, which since 1988, legislates for the protection of invertebrates at a European level. Papilio hospiton is also listed in Annex II of the European Habitats Directive (Council Directive 92/43/EEC).

Maniola nurag could become vulnerable as a result of its complicated life-history. Oviposition only takes place after a female diapause during the hottest part of the summer. Female butterflies need large amounts of nectar before laying their eggs, so the timing of oviposition is probably related to the availability of thistles as high quality nectar resources (D. Jutzeler, pers. comm.). As a consequence, female survival over the summer diapause is a crucial factor for the viability of Maniola nurag populations. This particular ecological characteristic makes the species very susceptible to human-induced change of their habitat (as shown for M. jurtina in
Scali, 1971) and might become a particular concern if the climate warms. Under a warmer climate scenarios, imagoes would emerge earlier in spring but resume activity later in autumn. The consequence would be a prolonged aestivation phase that would increase the risk of female death before oviposition. Another effect could be that with increasing temperature, individuals from intermediate altitudes move higher up the mountain slopes. But due to the island situation these areas would only serve as a limited refuge. Although M. jurtina performs the same female diapause in its southern European populations, this species is much less vulnerable than M. nurag on a global scale. First, M. jurtina is much more widespread and abundant, and second, it does not aestivate in the northern part of its range (Scali 1971, Tolman & Lewington 1997).

As for amphibians, the Sardinian newt is the only species in the genus Euproctus for which there is high conservation concern: it is classified by the IUCN as a critically endangered species due to deterioration of its freshwater stream habitat (IUCN 2000). Recent studies have pointed out a contraction in the mountainous range inhabited by the species, as newt populations are no longer present in some localities that some years ago were still occupied (Lecis 2002). E. platycephalus is fully aquatic life, and the severe droughts in Sardinia during the last decades (Regione Sardegna 2000) might have caused a reduction and fragmentation of newt habitat and consequent isolation and decline of populations. Other threats to this endemic urodele might be the introduction of brown trout (Salmo trutta) in Sardinian water courses, illegal fishing methods, anthropogenic disturbance and water pollution. Long term detailed field surveys would be required to investigate the actual extent of population decline. In the genus Speleomantes, all four Sardinian species are rare and threatened by extinction, but none of them is officially included in any nature conservation document.

4. Conclusions and perspectives

Although this overview is far from being complete, we think that it points to some general distributional and ecological patterns of Sardinian endemics and how they evolved. Divergence time estimates from various sources suggest that the cladogenetic splits leading to the Sardinian lineages have occurred well after the separation of the Sardo-Corsican plate from the continent. Many taxa seem to be younger than the marine regressions in Miocene (5 Ma), or even result from the severe climatic changes during the latest ice-age (Figure 2).
In Sardinia, areas of high endemism generally coincide with mountains (Table 1, Figure 2). For butterflies, areas of maximum endemism also coincide with areas of maximum species richness (Biermann 1998; Kleinekuhle 1999). This is probably due to the high proportion of endemics among the entire biotic community of Sardinia (25% for butterflies) (Kleinekuhle 1999). Butterfly species richness reaches a maximum in the Gennargentu massive and decreases from East to West and towards the lowlands, and reaches its minimum at the coast. The patterns of endemity in Sardinia seem to be in agreement with what has been shown for the Iberian Peninsula (Martin et al. 2000) and the general European pattern (Balletto 1995). But as indicated in those studies, it is expected that each taxonomic group of Sardinian species follows a different pattern related to its individual ecological characteristics or dispersal ability. As no estimates of total numbers of endemic species among other organismal groups are available for Sardinia, these conclusions remain first guesses, and it might as well be that Sardinian mountain massifs generally have higher species richness and endemic species just follow this pattern. Considering that most developmental efforts of the tourism industry are concentrated in coastal areas, the concentration of endemic species at higher altitudes might help protect them from the negative effects of increased human pressure. Nevertheless, giving those areas extra protection status is surely not superfluous. There are several protected areas in Sardinia (such as National and Regional Parks, WWF Oasis, Marine reserves, and sites designated as ‘Relevant Natural Areas’), however, many political and economical problems need to be resolved before the protection of these areas can be implemented. Given the high number of endemic species in Sardinia, it is necessary that designated reserves and parks do not only exist on paper but implement conservation and management of the island’s unique habitats and species in practice. This requires increasing the awareness of local people, promotion of field surveys and publication of updated atlases.

Three main centers of endemism, namely the Limbara, Gennargentu, and Settefratelli mountains are already included in a network of natural parks that have been proposed to become protected areas but have not yet been officially accepted as such by the Sardinian authorities (Figure 2). Giving them equal status as the already established marine reserves in Villasimius, Asinara, and the Maddalena-islands would be a further step to safeguard the unique nature of Sardinia.
Acknowledgments
We thank Johann Genser, Geology Department, Univ. Salzburg, Austria, for his advice regarding the geological part of this chapter, Gabriel Nève and Emmanuel Cosson, Laboratoire Systématique Evolutif, Univ. de Provence, Marseille, France, and Steven Weiss, Univ. Graz, Austria, for valuable comments on the manuscript. The Austrian Academy of Science provided a DOC grant financing large parts of this research.
III.
Butterfly, spider, and plant communities in different land-use types in Sardinia, Italy

with Barbara Knoflach, Daniel Cleary, and Vasiliki Kati
Abstract
Butterfly, spider, and plant species richness and diversity was investigated in five different land-use types in Sardinia. In 16 one-hectare plots we measured a set of 15 environmental variables to detect the most important factors determining patterns of variation in species richness, particularly endemicity. The studied land-use types encompassed homogeneous and heterogeneous shrublands, shrublands with tree-overstorey, Quercus forest and agricultural land. A total of 30 butterfly species, among which 10 endemics, and 50 spider (morpho)species, were recorded. Butterfly and spider community composition differed according to land-use type. The main environmental factors determining diversity patterns in butterflies were the presence of flowers and trees. Spiders reacted mainly to habitat heterogeneity and land-use type. The number of endemic butterfly species per treatment increased with total species richness and altitude. Butterfly and spider richness did not co-vary across the five land-use types. Butterflies were, however, positively associated with plant species richness and elevation, whereas spiders were not. Conclusively, butterflies did not appear to be good indicators for spider diversity and species richness at the studied sites.

Keywords: butterflies, spiders, endemic, diversity gradients, community ecology, habitat management, Mediterranean shrublands, conservation

Introduction
Endemic species are often found on islands, as isolation is conducive to speciation (Grant, 1998). Consequently, the search for the causes of global patterns of endemism implicitly regards the origin of species diversity, still one of the most challenging and least understood issues we are presently dealing with in biological science. Contrary to general belief, endemic species and patterns of endemism are insufficiently known, even in well-studied Western Europe (Deharveng, 1996; Jansson, 2003). This also holds true for well understood taxa like mammals; only last year a new endemic bat species was described from Sardinia (Muccedda et al., 2002).

Endemism and extinction are closely coupled (IUCN, 2001): the more endemic species occur in an area, the more vulnerable this particular area is, as extinctions cannot be compensated from elsewhere. Reports since 1600 show that a majority of extinctions in various groups of organisms, from invertebrates to mammals, and plants, were insular taxa (Frankham 1997). The major cause of species' extinctions on islands in the past 50 000 years were human activities (Olson, 1989). In our study region Sardinia, which is among the European hotspots of biodiversity and
endemism (Médail & Quézel, 1999), human activities brought about the extinction of most of the autochthonous mammalian fauna and the gradual introduction of more than 25 mammal species, which constitute the present wild and domestic fauna. Such a turnover has also been recorded on other Mediterranean islands (Vigne, 1992). These extinctions include the endemic rabbit *Prolagus sardus* (Lagomorpha, Leporidae), known from subfossil remains found on Corsica, Sardinia, and adjacent small islands, and a number of mice-like, insectivorous mammals (*e.g.*, *Nesiotes*, *Tyrhenicola*, *Rhiaganyss*), and the giant deer, *Megaceros* (see Vigne, 1992). *Prolagus* was an important part of neolithic human's diet, as testified by the great amount of skeletons found in human-inhabited caves, such as the Grotta di Corbeddu near Oliena, and is an early example of human induced extinction of an island species.

Today, however, human induced threats are of a very different kind. Sardinia has become a popular tourist destination, entailing an increased exploitation of the coastal areas. Afforestation programmes and frequent large fires are threatening the natural diversity of the island's interior (Grill et al., 2002); the introduction of fish has been reported to seriously threaten endemic amphibians (Lecis & Norris, 2003). The island is not only known for its high proportion of endemic species, *e.g.*, 300 out of 2500 plant species are endemics (Casula, pers. comm.), and 14 out of 56 butterfly species are endemics (Grill et al., 2002). Sardinia is also one of Europe's last reserves of virgin evergreen oak forests, *Quercus ilex*, and dense Mediterranean shrublands. These Mediterranean shrub-communities with little or no tree overstorey form a unique vegetation type (Arroyo & Maranón, 1990) and have recently been proposed to be included in EU and IUCN conservation policies (Andrés & Ojeda, 2002). Their uniqueness consists in their species richness and high levels of endemism (Ojeda et al., 2000). In Sardinia, for example, the majority of the endemic butterfly species occurring on the island rely on shrub communities (Grill et al., 2002). These communities are usually associated with cultural landscapes (Webb, 1998), and have developed as the result of forest clearance followed by centuries of traditional land use.

Figure 1. Location of the study sites, and structure and size of plots.
Figure 2. Relationships between butterfly, spider, and plant species richness. \( n = \) number of species. (A) Butterflies versus plants. (B) Spiders versus plants. (C) Butterflies versus spiders.
Figure 3. (A) Total number of butterfly and spider species recorded per land-use type and Shannon diversity index ($H$). (B) Total number of endemic butterfly species recorded in each land-use type. LS=low shrub, QU=Quercus forest, GS=grassland+shrubland, ST=shrubland+trees, AG=agricultural land.
as burning, cutting, and livestock grazing) (Pungetti, 1995). Our era’s increased human-induced pressure could, however, damage them severely. In order to plan sustainable land-use, it is necessary to understand why a species occurs in a particular habitat and not in another. If we understand the niche characteristics associated with the occurrence and abundance of a species in an area, we might have a key to better understand the reasons why species go extinct, when these niche characteristics change.

In this paper, we investigate the habitat ecology of Sardinian butterflies, with the aim of extending the knowledge on the habitat association of endemic species. To compare butterflies’ habitat associations to a group with potentially different habitat requirements, spiders are used as a predatory, non-flying counterpart to plant-eating, flying butterflies. The diversity of both groups is analysed in relation to plant diversity, with the general aim of detecting environmental variables that determine patterns of variation in species richness, particularly endemism.

The following three questions are addressed: (I) Which are the main environmental factors that determine the structure and composition of the butterfly and spider communities? (II) How are butterfly, spider, and plant diversity associated? (III) Do different land-use practices influence species richness and the presence of endemics?

Methods

Study area
The study sites are situated in South-East and Central Sardinia, Italy (Figure 1). They are predominated by Mediterranean shrubland, with patches of dense shrub and trees, similar to the type of communities described in Andrés & Ojeda (2002). Common plant species are: Cistus salvifolius, Cistus monspeliensis, Arbutus unedo, Euphorbia dendroides, Asphodelus aestivus, Rubus spp., Erica arborea, Phillyrea latifolia, Olea europea, and Quercus spp. Altitudes of the study sites range from 80 to 950 m a.s.l. The climate is Mediterranean with an arid hot summer season, cool winters, and little rainfall.

Plot design and sampling
Butterfly and spider species richness and community composition, were related to plant diversity, and 15 environmental variables. These were measured in 16, 100x100 m plots that were selected to be situated in five different land-use types:
three types of extensively grazed shrubland, low shrub (LS), grassland with low shrub (GS), high shrubs with grass and trees (ST), protected Quercus ilex-forest (QU), and agricultural land (AG) with Eucalyptus spec. stands (Table 1). Plots were assigned to the categories 'homogeneous' and 'heterogeneous' according to their apparent structural plant diversity. Each plot was georeferenced with a handheld GPS device (Garmin 12XL). All field-sampling was conducted in May-June 2001.

For butterfly sampling, 10 observation hours were spent in each plot, during daytime. The plot was crossed repeatedly on foot from one end to the other at a steady pace, which was only interrupted to note down butterflies. Butterflies were mostly identified on the wing, or caught with a hand held net and released immediately after identification. Spiders were sampled with a sweep-net, taking 3 x 20 sweeps at each plot. As a consequence, our sampling method detected only spiders sitting in the vegetation at an easily-reachable height; tree-canopies and ground-dwelling species were probably not sampled. A number of spider species were only present as juveniles in this period of the year, they could not be identified further than to genus or family level. Such individuals were included as morpho-species. In butterflies, a few early, or very late flying species could have been missed, as sampling time was restricted to a particular time of year.

Each of the 16 large plots was divided into subplots of 20x20 m size (Figure 1). In each such subplot, the following 15 environmental variables were measured in 12 non-adjacent, systematically selected subplots: 1) altitude, 2) slope (measured with a clinometer), cover of: 3) herb, 4) moss, 5) fern, 6) grass, 7) rock, 8) bare-ground, 9) trees <10m, 10) trees >10m, 11) shrubs <2.5m, and 12) shrubs <0.5m (visually estimated as the percentage of the plot surface), 13) count of flowerheads (from classes with <10, >10, >20, or >50), 14) moisture (xeric, mesic), 15) %-cover of most common plant species.

Analyses
Butterfly and spider diversity were estimated in terms of species richness (S) and Shannon diversity index (H). The Shannon diversity index (Spellerberg & Fedor, 2003) is:

$$H = -\sum_{i=1}^{n} p_i \ln p_i$$

where $p_i$ is the proportion of individuals that the $i$th species contributes to the total in the sample. This simple measure to characterize a community depends on
both species richness and the evenness (equitability) with which individuals are distributed among the species.

In order to assess the main environmental factors that determine butterfly species diversity and community composition, we used the Canonical Correspondence Analysis (CCA) option from the program CANOCO (Ter Braak, 1986; Ter Braak & Smilauer, 1998). CCA extracts the major gradients in the data that can be accounted for by the measured variables. The position of a species in the resulting plot indicates the characteristics of the ecological optima for this species; its abundance or probability of occurrence will decrease with distance from its species point (McGarigal et al., 2000). In the end, this allows to classify the landscape into probability or abundance surfaces for each butterfly. Samples with sample size \( n < 4 \) were not included in the CCA analysis because the presence of rare species in any given plot is often dictated by chance (Lesica & Cooper, 1998). All species abundances were \( \log_{10}(x+1) \) transformed. A forward selection procedure using a Monte Carlo permutation test with 1000 iterations was used to select the most significant \( (P < 0.05) \) environmental variables that explained variation in community structure (Ter Braak & Verdonschot, 1995).

**Figure 4.** Mean species richness in plants, butterflies and spiders in five different land-use types. LS=low shrub, QU=Quercus forest, GS=grass+shrubland, ST=shrubland+trees, AG=agricultural land.
associations between plant, butterfly, and spider species richness were tested with Pearson correlations (two-tailed), as was the association between species richness and the elevation (meters a.s.l.) of a sampling site. The significance of differences between the sites was assayed with Student’s t-tests.

The ecological structure of the spider community was analysed by (1) Jaccard’s similarity index (Sokal & Sneath, 1963) for all pairs of individuals based on the habitat they were found in, (2) the resulting similarity matrix was factored and ordinated by Principal Coordinate analysis (PCA) using the program NTSYS 1.80 (Rohlf, 1993).

**Results**

At the 16 sites sampled, we recorded a total of 30 butterfly species belonging to four different families (Table 2), and 50 (morpho) species of spiders (24 identified to species level) from 13 families (Table 3). Ten butterfly species were endemics. All, except two of these also occur on Corsica, and 20 (i.e., all non-endemics) occur on Sicily (Higgins & Riley, 1970). No endemic spider species were found. Fifteen of the 24 spider
identified to species-level are on the species list of Sicily (Pesarini, 1989; Platnick, 2003), and 17 are on the Corsican list (Canard, 1989). Several spider species are distributionally restricted to southern Europe, viz., Micrommata ligurina, Monaeses paradoxus, Tmarus piochardi, and Cyclosa insulana.

**Association between butterflies, spiders and plants**

Pearson correlations between butterfly, spider, and plant species richness indicated that among these three groups only butterflies and plants are significantly, positively associated, and co-vary across the five land-use types that were investigated ($r=0.691$, $p=0.003$) (Figure 2). Spider species richness was not related to plant richness ($r=-0.205$, $p=0.446$) or butterfly richness ($r=-0.030$, $p=0.912$).

**Diversity**

Butterfly species diversity was highest in the ‘GS’-sites as indicated by a Shannon’s diversity index value of $H=7$, and lowest in the ‘HS’-sites ($H=2$); spider species richness was highest in the agricultural-land ($H=5$), and lowest in the forest ($H=1$). For both animal groups, species diversity was equally high in the ‘ST’-sites ($H=5$) (Figure 3). Butterfly diversity increased across the five land-use types as follows:
low shrubland < agricultural land < Quercus-forest < shrubland with trees < shrubland with grass. The number of endemic species per land-use type increased with total species richness (Table 2). For spiders, species-richness increased across land-use types in the following order: Quercus-forest < low-shrubs < shrubland-with-grass < shrubland-with-trees < agricultural-land. Mean species richness did not differ significantly between the different land use types for any of the three groups (Figure 4).

**Habitat association**

Flowerhead-abundance and % tree-cover appeared as the first two axes of a CCA analysis, and explained 55% of the variation in butterfly abundance (Figure 5). Axis 1 (41%) was determined by the abundance of flower heads, Axis 2 (14%) by tree abundance. Three of the four endemic species included in the CCA analysis, viz. Maniola jurtica, Aglais urticae ichnusa, and Lasiommata megera paramegera, were positively associated with the presence of flower-heads. Another flower-associated species was the lycaenid Lycaena phlaeas. Lycaenids are known to fly preferably in rather open habitats, such as flowery and grassy meadows. This preference was also shown for Lycaena ottomana in Greece (Grill & Cleary, 2003). Lasiommata megera paramegera is usually found in diverse, sometimes grassy, sometimes
woody habitats, but appeared here at the extreme end of the flower axis. The two nymphalids *Maniola jurtina* and *Pararge aegeria* were placed on the extreme end of the tree-axis.

The endemic *Maniola nurag* was more associated with the presence of flowers than *M. jurtina*, but less so with trees. *Celastrina argiolus* is similarly associated with trees as *M. jurtina*. Next on the flower-axis was *Aglais urticae ichnusa*. *Charaxes jasius*, *Pieris brassicae*, *Colias croea*, *Artogeia rapae*, and *Polyommatus icarus* were placed in the centre, and had a moderate association with both axes. *Gonepteryx cleopatra*, *Coenonympha corinna*, and *Pyronia cecilia* were at the far end of the flower axis and showed no particular association with either flowers or trees. Subsequent axes were related to topographical variability, and included range in elevation and the proportion of rock-cover. The slope of a site did not result as a determinant factor for butterfly species composition.

### Table 1. The 16 plots used for butterfly, spider, and plant sampling. Given are land-use type, vegetation structure, management, geographical position, and altitude in meters a.s.l.

<table>
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<th>Landuse-type</th>
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<th>Management</th>
<th>Easting</th>
<th>Northing</th>
<th>Altitude (m)</th>
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</tr>
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<td>homogenous</td>
<td>extensive grazing</td>
<td>32S 0534101</td>
<td>UTM 4347359</td>
<td>674</td>
</tr>
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<td>heterogenous</td>
<td>extensive grazing</td>
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</tr>
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</tr>
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<td>extensive grazing</td>
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<td>670</td>
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<td>extensive grazing</td>
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<td>UTM 4344433</td>
<td>722</td>
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<td>UTM 4346011</td>
<td>924</td>
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Table 2. Butterfly species observed in different land-use types in Sardinia from May to June 2001. Species present on the neighbouring islands, Sicily and Corsica, are indicated as well as the type of larval food plant each species uses according to Carter & Heagreaves (1987). G=grasses, H=herbes, S=shrubs, T=trees, LS=low shrubs, QU=Quercus forest, GS=grass+shrubland, ST=shrubland+trees, and AG=agricultural land. Endemics are underlined.

<table>
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<td>LS</td>
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<td>GS</td>
<td>ST</td>
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<tr>
<td>Nymphalidae</td>
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<td></td>
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<td>Aglais urticae icky HÜBNER 1824</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
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<td>x</td>
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<td>Lycaenidae</td>
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<td>Hesperiidae</td>
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<td>14</td>
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<td>19</td>
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<tr>
<td>Total number of endemics</td>
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<td>1</td>
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Table 3. Spider species observed in different land-use types in Sardinia from May to June 2001. Species present on the lists of the neighbouring islands, Sicily and Corisca, are indicated according to Pesarini (1994) and Canard (1989). G= grasses, H=herbs, S=shrubs, T=trees, LS=low shrubs, QU=Quercus forest, GS=grass+shrubland, ST=shrubland+trees, and AG=agricultural land.

<table>
<thead>
<tr>
<th>Arachnidia (morpho) species list</th>
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<th>Sicily</th>
<th>Corsica</th>
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<td></td>
<td>LS</td>
<td>QU</td>
<td>GS</td>
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<td><strong>Araneidae</strong></td>
<td></td>
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<tr>
<td>Araniella spp.</td>
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<tr>
<td>Araniella cucurbitina (CLERCK 1757)</td>
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<tr>
<td>Argiope lobata (PALLAS 1772)</td>
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<tr>
<td>Cyclosia insulana (COSTA 1834)</td>
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<tr>
<td>Mangora acalyptra (WALCKENAER 1802)</td>
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<tr>
<td>Mangora spec.</td>
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<tr>
<td>Neoscona adianta (WALCKENAER 1802)</td>
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<td>Zilla dioïda (WALCKENAER 1802)</td>
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<tr>
<td>Zygiella spp.</td>
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<tr>
<td><strong>Clubionidae</strong></td>
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<tr>
<td>Clubionidae spp.</td>
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<tr>
<td>Cheiracanthium spp.</td>
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<tr>
<td>Cheiracanthium stridatum SIMON 1878</td>
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<td>x</td>
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<tr>
<td><strong>Corinnidae</strong></td>
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<td>Trachelae spp.</td>
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<tr>
<td><strong>Dictynidae</strong></td>
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<td><strong>Sparassidae</strong></td>
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<tr>
<td>Micrommata ligurina (C.L. KOCH 1845)</td>
<td>x</td>
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<td>Micrommata ligurina (C.L. KOCH 1845)</td>
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<tr>
<td>Linyphiidae</td>
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<tr>
<td>Erigoninae spp.</td>
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<tr>
<td>Linyphiidae spp.</td>
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<td>Leptophantes menges KULCZYNSKI 1887</td>
<td>x</td>
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<td>Meinometa spp.</td>
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<td>Lycosidae spp.</td>
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<td>Oxyopidae</td>
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<tr>
<td>Oxyopes spp.</td>
<td>x</td>
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<tr>
<td>Oxyopes cf. nigerpalpis KULCZYNSKI 1891</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Oxyopes heteropthalmus LATREILLE 1804</td>
<td>x</td>
<td>x</td>
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<tr>
<td><strong>Philodromidae</strong></td>
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<td>Philodromus spp. (aureus - group)</td>
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<tr>
<td>Philodromus spp.</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>Philodromus levius SIMON 1875</td>
<td>x</td>
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<tr>
<td>Thanatus spp.</td>
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</table>

68
Spider communities were ordinated into three main groups by the first two axes of the PCA (Figure 6). The first axis (explaining 20% of the variation) showed land-use as an important factor influencing the community composition of spiders, the second axis (14%) reflected the influence of habitat structure on spider communities and went from homogeneous to heterogeneous habitats. The spider communities in shrubland sites were clearly set apart, as well as the agricultural-communities, with in the centre species that occurred in several different habitats, without any clear association to one or another habitat type (Figure 6). Some species’ only occurred at the agricultural site (e.g., Thyene imperialis), others to low shrub sites (e.g., Salticus cf. propinquus), or to the high shrub sites (e.g., Cheiracanthium striolatum). None of the spider species was restricted to grass-shrub or forest sites.

These same two gradients, habitat heterogeneity and land-use, were also reflected in the number of endemic butterflies per land-use type: the ‘GS’-sites and the ‘ST’-
sites contained most of the observed endemic butterflies (GS=7, ST=7; see Table 2), whereas the less heterogeneous and more human influenced 'LS'- and 'AG'-sites contained much fewer endemic butterfly species (LS=0, QU=1, AG=2).

Species richness was significantly, negatively correlated with the elevation of a sampling site for spiders (r = -0.519, p=0.039), and positively, but not significantly correlated for butterflies (r=0.409, p=0.118) (Figure 7), but not correlated for plants (r=0.175, p=0.516).

Discussion

(1) Environmental factors shaping the butterfly community
Butterfly diversity is determined mainly by vegetation composition and structure, and to a lesser extent by topography. Firstly, there is a highly significant, positive relationship between butterfly and plant species richness, and secondly, flower-head-abundance and tree-cover are the two principal environmental variables explaining variation in butterfly species composition. These first two first axes of the CCA do not only reflect the butterflies' association with nectar sources ('flower-heads') and shade ('trees'), but also represent a structural gradient from sites with only a single vegetational layer (=shrubs) and no over- or understorey, through sites with two layers (=grass and shrubs), to sites with three layers (=grass, shrubs, and trees). In the Mediterranean, butterflies often seek shelter in the shade of bushes or trees during the hottest part of the day, a kind of behaviour, that we particularly observed in the satyrids *M. jurtina, M. nurag*, and *P. aegeria*. The two ecological gradients represented by the two axes could reflect the differing requirements between larvae and adults. CCA-1 seems related to the adults' ecological requirements, *i.e.*, nectar sources, whereas the second axis, CCA-2 appears related to larval requirements, *i.e.*, food plants. Seventeen of the butterfly species we analysed feed on herbs (50%), nine on grass (26%), four on shrubs (12%), and another four on trees (12%). These numbers include two polyphagous species whose larvae feed on herbs, shrubs, and trees, *viz.*, *Polygonum c-album* and *Celastrina argiolus* (Table 2). The endemics are either grass- or herb-eating species.

The complexity of plant architecture, *i.e.*, vegetation heterogeneity and height, has been reported as an important factor determining Lepidoptera diversity (Haysom & Coulson, 1998). We indeed found butterfly diversity to increase with the heterogeneity of a site, an association that is strongest for endemics.
Elevation was also found to be positively related to species richness, but correlations were not significant. Considering, that seven of the nine endemic butterfly species were restricted to mountainous areas (> 500 m) (Grill et al., 2003), we expected to find a stronger correlation. Possibly, the lack of significance is due to small sample sizes. Differences in elevation have been shown to be related to diversity in a wide range of taxa (Hawkins & Porter, 2003 and references therein, Sutherland, 2003), including papilionid butterflies in North America (Kerr et al., 1998). Rosenzweig (1995) proposed, that the association between species diversity and elevation reflects a relationship between topographic variation and the number of habitats in an area which is also known to be positively correlated with species richness. However, recent evidence from Californian butterflies suggests that range in elevation is more than just a surrogate for plant diversity and habitat heterogeneity, as the number of habitat types found in an area, was not significantly associated with butterfly richness (Hawkins & Porter, 2003).

One of the factors that is often proposed as determining distributional patterns in butterflies, is temperature (Bryant et al., 2002 and references therein). In Sardinia, temperature differences between lowland and mountainous areas are possibly responsible for a large part of the differences in endemism richness we find between different areas. On a larger scale, the amplitude of temperature oscillations a given region experienced since the last glacial maximum (Milankovitch oscillations) seems to be a good predictor of endemism in mammals, birds, reptiles, and vascular plants (Jansson, 2003). Although we have some evidence that in Sardinia endemism increases with elevation (Grill et al., 2003), much additional work is required to understand the relationship between endemism richness and elevation.

(2) Association of butterfly and spider diversity
Spider species richness does not appear to be associated with butterfly or plant species richness. In two of the five land-use types (low-shrub and agricultural-land) spider diversity was inversely related to butterfly diversity. An inverse relationship to butterflies also appeared in the relationship with elevation, which was significantly negative for spiders, but positive for butterflies (Figure 7). The reason for these inverse relationships could lie in the distinct ecology of the two groups: predatory spiders do not directly rely on plants as food-resources, but only indirectly, i.e., if they prey on plant-eating organisms. For the spiders we sampled, butterflies do not appear to be important prey organisms. Another major difference between these two taxonomic groups is that butterflies have
changing habitat requirements in different developmental stages, while spiders rely on similar resources regardless if they are juveniles or adults. An important conclusion here is, that for the areas studied, butterflies are rather bad indicators for the diversity of the spider guild investigated, and vice versa.

Habitat heterogeneity and land-use type seem to have a different effect on spider diversity than they have on butterfly diversity. An explanation for this could be that what is experienced as homogeneous by butterflies and humans is not homogeneous for spiders. Consequently, spiders might experience human induced disturbance at a different spatial scale than butterflies, and therefore be more resistant to it. Nevertheless, spiders cluster into distinct groups of species that seem determined by gradients of land-use type and habitat heterogeneity. These two factors were also important in shaping the butterfly communities of the different study sites, as discussed above.

(3) Do different land-use practices influence species richness and the presence of endemics?
The Sardinian fauna of spiders encompasses at least 27-29 endemics (Wunderlich, 1995). The absence of endemic spider species in our samples, is probably due to methodological restrictions of the sampling method, and does not imply that the land-use types we studied, are unsuitable for endemic spiders. While sweep-nets were used for the present study, pitfall traps have been shown to be a quantitatively more efficient collection method (Zingerle, 1999), and might have provided samples with higher numbers of individuals, increasing the chance to detect rare endemic species.

Endemic butterflies occurred primarily in heterogeneous land-use types. The homogeneous low shrubland did not contain a single endemic butterfly species. The two land-use types, where we observed most of the endemics, were shrublands (‘GS’- and ‘ST’-sites). These types of mountain shrub- and grasslands are known to hold the largest amount of butterfly diversity in Mediterranean landscapes (Munguira, 1995). In Sardinia, these shrublands resulted from transformation of former oak forests, such as Quercus ilex and Q. pubescens, through long-term low-level anthropogenic influence (Pungetti, 1995). Long-term continuation of traditional land use is therefore essentially enhancing butterfly diversity in that it prevents shrub- and grasslands from reverting into secondary forests (Grill et al., 2002). Pine- or eucalypt-afforestation sites were not within the scope of this study, and the issue of afforestation has only been touched marginally. Yet, from the observations at the agricultural site, which was partly situated in an Eucalyptus...
plantation, we anticipate that afforestation will have negative effects on species diversity and endemism in Sardinia. Detailed studies on the effects of reforestation in other regions of the Western Mediterranean have documented that pine plantations result in a loss of the local fauna (Díaz et al., 1998; Romero-Alcaraz & Ávila, 2000) and flora (Chiarucci & Decominichis, 1995). What was planted in order to make the heathlands profitable, protect the soil from erosion, and ‘improve’ the physiognomy of the landscape, turned out to have adverse effects on the diversity of plants and animals (Andrés & Ojeca, 2002). Similar effects have been reported for the South African ‘fynbos’ (Richardson, 1998). Paradoxically, in Sardinia, Eucalyptus plantations have been reported as the sites with the highest soil erosion (Vacca et al., 2000). Positive effects of traditional land-use have been reported specifically for the endemic butterfly P. hospiton (Aubert et al., 1996). The importance of grazing to maintain diversity is not restricted to butterflies, and has recently been found for Orthoptera in Greece (Kati et al., 2003), and for Auchenorrhyncha, Heteroptera, Coleoptera, and Hymenoptera in Germany (Kruess & Tscharntke, 2002). The data we present from Sardinia show congruent results for Arachnida: spider diversity was comparably high in the ungrazed agricultural-land and in the extensively grazed high- and low shrubland, but much lower at the ungrazed forest sites. Obviously, overgrazing will negatively affect insect abundance, but cessation of grazing is also not desirable (compare also Munguira et al., 1997; Kruess & Tscharntke, 2002).

Among the ten endemic butterfly species recorded in this study, one, namely Papilio hospiton, is on Appendix II of the European Habitat Directive (Anonymous 1992), and on the Red List of the IUCN (IUCN, 2002); another endemic butterfly, Pseudophilotes bargabiae, has recently been classified as ‘Vulnerable’ and proposed to be included into the Habitat Directive and the Red List of the IUCN; two more species, M. nurag, and Spialia sertorius therapne have recently been assigned the status ‘Near threatened’ according to the IUCN threat criteria (Grill et al. 2002). Pseudophilotes bargabiae and M. nurag are entirely limited to Sardinia, while the other endemic butterfly species also occur on Corsica and a few other islands of the Tyrrhenian archipelago. Like the majority of the endemics, they are restricted to mountain areas.
Acknowledgements

We thank Konrad Thaler for help with species identification and manuscript review. For comments and discussions we are grateful to Steph B. J. Menken and Léon E. L. Raijmann.
Part 2

CONSERVATION
IV.
Applying the IUCN threat categories to island endemics: Sardinian butterflies (Italy)

with Roberto Crnjar, Paolo Casula, and Steph B. J. Menken

Abstract

European nature conservation documents often reflect the charisma of a species rather than its actual degree of threat. The assessment of the threat status of 14 endemic Sardinian butterfly species underlines that European nature legislation documents are incomplete. *Pseudophilotes barbagiae* and *Lysandra coridon gennargenti* (Lycaenidae) are identified as globally Vulnerable and are therefore proposed to be added to the Red Data Book of European butterflies as species of global conservation concern. A threat factor analysis identifies risks towards butterflies in Sardinia arising from increasing human activities. It is shown how the quantitative information used by the IUCN criteria together with a qualitative assessment of human induced threat factors could be combined to an objective standardized assessment that can be used also when only data on present distribution is available. Threats to Sardinian butterflies are highlighted and conservation measures proposed. The inclusion of *Pseudophilotes barbagiae* and *Lysandra coridon gennargenti* in Annex II and IV of the European Habitat Directive, and Appendix II of the Bern Convention is strongly recommended.

Keywords: IUCN criteria; Sardinian butterflies; *Pseudophilotes barbagiae*; *Lysandra coridon gennargenti*; butterfly conservation; European Habitat Directive; Bern Convention

Introduction

Endemism per se has high conservation priority (Munguira, 1995; Schnittler & Ludwig, 1996; Gruttke et al., 1999). The often extremely restricted range of endemic species gives countries or regions they inhabit a particular conservation responsibility, as disappearance from that area means their global extinction. The dependence on particular resources makes them especially vulnerable to changes in land use or habitat management (Van Swaay & Warren, 1999), even small alterations could lead to extinction (Munguira, 1995). Recognising the importance of endemic species, though, is only useful with an adequate legal framework. Red lists are one of the most effective tools available to conservationists for focusing attention on species of conservation concern (Collar, 1996; Maes & Van Swaay, 1997; Gärdenfors et al., 1999). With the introduction of the international IUCN Red List criteria (IUCN, 1994; Mace & Stuart, 1994), objective quantitative criteria have replaced assessments based on 'best professional judgement'. Nevertheless, Red lists often reflect the charisma of a species rather than its actual degree of threat and preference is given to more conspicuous and better studied taxa when establishing nature protection laws (Anonymous, 1992; Council of Europe, 1979). Considering that nine out of ten known animal species are invertebrates and eight of those are insects (Kudrna, 1986; Kerr, 1997), it is striking that only approximately 20% of the
species listed in European Nature conservation documents are insects, whereas 70% are vertebrates. Among the insect species listed on the Bern Convention is *Papilio hospiton*, the only swallowtail endemic to Europe. It is, however, generally common in its range and other authors suggest its colourful appearance as the motive for protecting it (Mikkola, 1991; Aubert et al., 1996). Priorities in European nature conservation legislation (Council of Europe, 1979; Anonymous, 1992), thus, do not seem to present a realistic view of the actual levels of threat, partly because they were established before the new IUCN criteria were published. Efforts to protect a species which is not endangered divert attention and conservation measures from much more threatened but less prestigious species. Therefore, objective standard procedures are required in order to identify a species’ true threat status and to facilitate comparisons across countries and regions (e.g., Maes & Van Swaay, 1997; Warren et al., 1997). At present, however, different techniques are still being used for compiling national Red Lists. Since the first draft guidelines for applying the IUCN Red List Categories at the national level were issued in 1995 (IUCN, 1995), discussions and improvement have continued (Regan et al., 2000), and the IUCN criteria are slowly finding their way into national and regional lists (e.g., Schnitller et al., 1994; Warren et al., 1997; Gärdenfors et al., 1999, Holzinger et al., 1999).

Recently, the IUCN criteria which were originally defined with regard to large vertebrates, have been revised for use in butterflies, and an updated Red Data Book of European butterflies was published (Van Swaay et al., 1997; Van Swaay & Warren, 1999) that considerably improved on the former version (Heath, 1981). The main differences in the revised version regard time scale and range decline. Data on trends in butterfly populations are mostly available over a period of 25 years rather than the 10 year period proposed by the IUCN. The data available for butterflies in Europe is mostly based on distributions whereas the IUCN use range declines. Thus, in the revised criteria, population decline over the last 10 years was replaced with a distribution decline over a 25 year period. The IUCN criteria C. and D. which deal with population estimates in numbers of individuals or numbers of mature individuals were not used for butterflies as absolute numbers are not relevant for insects. Extinction probabilities can not be calculated with the data available on European butterflies. Consequently, the IUCN criterion E. regarding probability of extinction within 10 years had to be left out for butterflies (Table 1). Despite these changes, for most species (i.e., particularly in South-Eastern Europe, European part of Russia) the data available were not precise enough and if available, local specialists had to be consulted for more detailed information (Van Swaay & Warren, 1999). This again contradicts our premise to use objective
standardized procedures and brings us back to the start: for regions, where no specialists are available, species are not assessed properly and their threat status is truly underestimated. This reveals a deficiency of the IUCN criteria, namely, that threats are not explicitly considered as a criterion but only implicitly in projected declines for which we almost never have enough data. Already if there is an absence of data for two of the three main parameters (i.e., decline, number of locations or fluctuations) threat could be underestimated. To overcome these problems, we suggest to include a fourth parameter that is easy to measure and extremely relevant for butterflies: human induced threat factors. Threat factor analyses have already been used by Warren et al. (1997) and Van Swaay & Warren (1999) for butterflies following the threat categories originally defined for birds by Batten et al. (1990). But they have never been used to validate and if necessary correct the results obtained with the IUCN criteria.

Therefore, along with the re-assessment of Sardinian butterflies we try to show how these threat factors could be implemented in the IUCN protocols. We believe that this would provide a crucial improvement and make their practical use in conservation issues more obvious. Then, we also point to conservation measures and propose amendments to the main European nature legislation documents.

Consequently, the three main topics covered in this paper are:

(I) an assessment of extinction risk of all 14 butterfly species endemic to Sardinia and/or the other Thyrrenian islands (Corsica, Elba, Capraia, Giglio, and Montecristo) following the IUCN Red List categories;

(II) an analysis of possible threat factors to Sardinian species and priorities for butterfly conservation in Sardinia;

(III) additions to the European Red Book of Butterflies, the Bern Convention, and European Habitat Directive.

**Methods**

**The species**
This analysis treats the 14 butterfly species and subspecies listed in Biemann (1998) and Kleinekuhle (1999) as restricted to one or more of the Thyrrenian islands (Sardinia, Corsica, Elba, Capraia, Giglio, and Montecristo): Papilionidae:
Papilio hospiton; Nymphalidae: Argynnis elisa, Argynnis paphia immaculata, Aglais urticae ichnusa; Pieridae: Euchloe insularis and Coenonympha corinna; Satyridae: Hipparchia aristaeus aristaeus, Hipparchia neomiris, Maniola nurag, and Lasiommata megera paramegaera; Lycaenidae: Plebejus idas bellieri, Pseudophilotes barbagiae, and Lysandra coridon gennargenti; Hesperidae: Spialia sertoria therapne. There is dispute about the species status of Argynnis paphia immaculata, Hipparchia aristaeus aristaeus, Lasiommata megera paramegaera, Plebejus idas bellieri, and Polyommatus coridon gennargenti. Some authors consider them subspecies (Verity, 1940; Karsholt & Razowski, 1996; Tolman & Lewington, 1997), other authors present evidence of their genetic and morphological differences to the nominate forms and support their species status (e.g., Jutzeler, 1998; Marchi et al., 1996). In the Red Data Book of European Butterflies these five species were not assessed as separate species. As we support their species status and we wish to stress their importance for nature conservation on the regional scale we treat them here as true species. Only three species, Maniola nurag, Pseudophilotes barbagiae, and Polyommatus coridon gennargenti are actually restricted to Sardinia.

For species that also occur on other Tyrrhenian islands the threat analysis covers only the Sardinian part of their populations. We do, though, consider the conspecific populations from the other islands when estimating distribution area and number of locations.

The data to estimate the size of the distribution areas of the species derive from ‘best professional judgment’, field observations in Sardinia during the last 10 years and records from the private collections, as well as from the literature (Jutzeler et al., 1997; Kleinekuhle, 1999; Leigheb et al., 2000). Distribution area is calculated as the sum of all areas contained within the shortest continuous imaginary boundary around clusters of all the known, inferred or projected locations of the species. Clusters are defined as all locations that are within 10 km of a neighbouring location.

**Applying the IUCN Red List criteria**

The IUCN Red List Criteria provide a useful framework to establish conservation priorities for species with a very restricted range like island endemics. Information on distribution area, number of locations and fluctuations are rather easy to collect in a few field seasons and allow an objective assessment of a species’ global threat status. Following Gardenfors et al. (1999), the Criteria can be used straightforwardly, provided the isolation of the regional populations from conspecific populations outside the region is complete, as the extinction risk of
such an isolated population is identical to that of an endemic taxon. This isolation applies to an island like Sardinia and assessment of threat status was executed accordingly.

To assess Sardinian species' threat status, generally criterion B of the IUCN Criteria (Table 1) which uses distribution area, number of locations, and fluctuations proved most suitable with the data available. For species with an area of occupancy of less than 100 km², or occurring on less than five locations, sub-criterion D2 of the original criteria, which was not included into the revised version, could be used. None of the species in our analysis fit this criterion. But it can surely be very useful when assessing species with very restricted distribution areas and little available data, and ought to be included into the revised criteria (Van Swaay & Warren, 1999). In the subcategory 2 of LOWER RISK also the original IUCN definition was used as we did not have enough information on decrease and present abundance to use the Van Swaay & Warren (1999) criteria.

Threat factor analysis

This analysis is based on the categories defined first for birds by Batten et al. (1990), and then used for butterflies by Warren et al. (1997) and Van Swaay & Warren (1999). For other regions threat factors could be extended according to the local situation.

Potential threat factors to Sardinian butterflies:

1. Agricultural intensification
2. Felling/destruction of woodlands
3. Abandonment/change of woodland management (replanting with conifers or eucalypts)
4. Overgrazing
5. Fire
6. Collecting
7. Building development (construction of roads, housing etc.)
8. Coastal development
9. Climatic change
10. Recreational activities, disturbance
11. Isolation and fragmentation of habitat
12. Chemical pollution (includes herbicides, pesticides, and microbiological agents)
13. Desertification (lowering of ground water level)

For each threat factor, one of three classes (high, medium, and low) is assigned as
Table 1. Criteria used to establish the threat status of butterflies in the Red Data book of European Butterflies (Van Swaay & Warren, 1999) compared to the original IUCN criteria (Table after Van Swaay & Warren, 1999). The proposed changes to include threat factors are printed in bold.

CRITICALLY ENDANGERED (CR)
A. Population reduction of at least 80% over the last 10 years.
B. Extent of occurrence less than 100 km² and two of the following:
   1. severely fragmented or known to exist at only a single location;
   2. continuing decline;
   3. extreme fluctuations.
C. Population estimates less than 250 mature individuals and a strong decrease.
D. Population estimates less than 50 individuals.
E. Probability of extinction at least 50% within 10 years.

ENDANGERED (EN)
A. Population reduction of at least 50% over the last 10 years.
B. Extent of occurrence less than 5000 km² and two of the following:
   1. severely fragmented or known to exist at no more than five locations;
   2. continuing decline;
   3. extreme fluctuations.
C. Population estimates less than 2500 mature individuals and a decrease.
D. Population estimate less than 250 individuals.
E. Probability of extinction at least 20% within 20 years.

VULNERABLE
A. Population reduction of at least 20% over the last 10 years.
B. Extent of occurrence less than 20,000 km² and two of the following:
   1. severely fragmented or known to exist at no more than ten locations;
   2. continuing decline;
   3. extreme fluctuations.
C. Population estimates less than 10,000 mature individuals and a decrease.
D. Population very small or restricted in the form of either of the following:
   1. Population estimate less than 1000 individuals.
   2. Population is characterized by an acute restriction in its area of occupancy (<100 km²) or in the number of locations (<5).
E. Probability of extinction at least 10% within 100 years.

LOWER RISK
Three subcategories:
1. Conservation dependent. Taxa on the focus of conservation programmes, the cessation of which would result in qualification for one of the threatened categories above a period of five years.
2. Near threatened. Taxa not qualifying for Conservation dependent but close to qualifying for vulnerable.
3. Least concern. Taxa not qualifying for Conservation dependent or Near threatened.

DATA DEFICIENT
There is inadequate information to make an assessment of extinction risk based on distribution or population status.

CRITICALLY ENDANGERED (CR)
A. Decrease in distribution of at least 80% over the last 25 years.
B. Present distribution less than 100 km² and two of the following:
   1. severely fragmented or known to exist at only a single location;
   2. continuing decline;
   3. extreme fluctuations.
Or
C. Present distribution less than 100 km² and
   4. >50% of threat factors high or
   >80% medium.
D. Not relevant.
E. With the material available this criterion cannot be used.

ENDANGERED (EN)
A. Decrease in distribution of 50-80% over the last 25 years.
B. Present distribution less than 5000 km² and two of the following:
   1. Severely fragmented or known to exist at no more than five locations;
   2. continuing decline;
   3. extreme fluctuations.
Or
C. Present distribution less than 5000 km² and
   4. >50% of threat factors high or
   >80% medium.
D. Not relevant.
E. With the material available this criterion cannot be used.

VULNERABLE
A. Decrease in distribution of 20-50% over the last 25 years.
B. Present distribution less than 20,000 km² and two of the following:
   1. Severely fragmented or known to exist at no more than ten locations;
   2. continuing decline;
   3. extreme fluctuations.
Or
C. Present distribution less than 20,000 km² and
   4. >50% of threat factors high or
   >80% medium.
D. Not relevant.
E. With the material available this criterion cannot be used.

LOWER RISK
Three subcategories:
1. Conservation dependent. This criterion will not be used in this context.
2. Near threatened. Decrease of more than 15% correlated with present abundance less than 1%.
Or
3. >1 threat factor high or
   >50% medium
   3. Least concern. All taxa not satisfying one of the upper categories. They are not listed in the list of threatened species.

DATA DEFICIENT
There is no data available about abundance or trend during the last 25 years.
The threat factor is

(a) **High**, when extinction or considerable reduction in range or number of populations (e.g., >20% in 25 years) is likely, if the factor continues at the present intensity without intervention.

(b) **Medium**, when local contractions in range or small reductions in number of populations (e.g., 10-20% in 25 years) make the factor likely to become ‘high’ if it continues to operate without alteration.

(c) **Low**, when it is a secondary factor which might be worsening threats from other factors. Any factor having a (potentially) measurable adverse effect at the population level or restraining a species from achieving its full potential range falls into this class.

**Combining the IUCN Red List Criteria with the Threat factors**

If butterfly species have to be assessed in a short period of time, e.g. only a single field season, Present distribution can be established rather easily. The other three parameters (i.e., number of locations, fluctuations and decline) necessary to use criterion B require a longer period of observation. In case there is no data available for at least two of the three, following the Criteria as they are now, would underestimate threat status or not allow estimates at all. For this case we suggest to include human induced threat factors as a fourth parameter, which in combination with distribution area would assess the species’ threat status (Table 1). This would allow an objective standardized assessment of threat status after only a single field season and tie the quantitative information used by the IUCN criteria together with a qualitative assessment of real threats.

If at least 50% of a species’ threat factors are **high** or at least 80% of the threat factors **medium**, combined with a restricted distribution, the species would be classified threatened (Table 1). Is at least one threat factor **high** or 50% **medium**, the species would be NEAR THREATENED.

**Identifying species of conservation concern**

The aim of this assessment is to identify species that are of conservation concern following the concept developed by Tucker & Heath (1994) for birds, which was recently adapted for butterflies (Van Swaay & Warren, 1999). These species are called Species of European Conservation Concern (SPECs) and are devided into four categories depending on their Threat Status (assessed with the IUCN Criteria) and the proportion of their geographical range in Europe. SPEC categories
(slightly modified after Van Swaay & Warren, 1999):

**SPEC 1**: Species of global conservation concern because they are restricted to Europe and considered globally threatened.

**SPEC 2**: Species whose global distribution is concentrated in Europe and that are considered threatened in Europe.

**SPEC 3**: Species whose global distribution is not concentrated in Europe but that are considered threatened in Europe.

**SPEC 4**:
- **4a**: Species whose global distribution is restricted to Europe but that are not considered threatened.
- **4b**: Species whose global distribution is concentrated in Europe but that are not considered threatened.

### Results

**Extinction risk and conservation concern**

*Pseudophilotes barbagiae* and *Lysandra coridon gennargentii* fulfil the criteria for the category **Vulnerable** (see Table 1) through their restricted distribution area (< 5000 km²) and the small number of locations (5-10) combined with fluctuations (Table 2). This makes them sensitive to becoming **Endangered** or even **Critically Endangered** in a very short period of time due to the effects of human activities or stochastic events. *Argynnis elisa*, *Plebejus idas bellieri*, *Spiralia sertorius*, and *Maniola nurag* qualify for the **Lower Risk** subcategory **Near Threatened**, due to their restricted distribution areas (5000-20 000 km²). The remainder of the species are **Least Concern** (Table 2). Consequently, *Pseudophilotes barbagiae* and *Lysandra coridon gennargentii* are species of highest European conservation concern (SPEC 1) because they are endemic to Europe and threatened, the other 12 species are SPEC 4a as they are endemic to Europe but not considered threatened. A validation of the results using human induced threat factors does not reveal any changes or necessary corrections in the assessment, as in Sardinia none of the factors was **High** and only two of them, 'fire' and 'collecting', were **Medium** (Table 3). It has to be underlined, though, that in other, more industrialized regions (*i.e.*, North/Central Europe or North America) where human pressure is higher, the situation could be entirely different.
**Human induced threat factors**

The most important threats are abandonment and change in woodland management, overgrazing, and fire (Table 3). Deforestation or replanting with conifers and eucalypts can also have devastating effects on butterflies. Grazing has an important influence on the landscape in Sardinia and is difficult to control as animals usually roam freely. According to our observations, presently, it does not seem to have severe consequences on butterflies. Much less use is made of pesticides and fertilizers than in most Central and North European countries where they reduced populations sizes of many butterfly species and decreased the number of species in many areas (Erhardt, 1995; Warren et al., 1997). Grassland management normally relies on traditional land uses (Casula, unpublished). If other factors already influence populations' size and fitness negatively, this could worsen the situation. Fire is an important factor in Mediterranean ecosystems and has multiple causes (Paraskevopoulos et al., 1994; Heras et al., 1995). Burning practices are sometimes used by shepherds for pasture renovation or initiated by carelessness and sometimes indeed on purpose for edification speculations. Fire protection measurements to prevent uncontrolled burning of large areas of land have already been implemented in Sardinia.

Generally, habitat loss, including fragmentation and isolation of patches, can still be considered a minor threat (Table 3). The extremely restricted occurrence of *Pseudophilotes barbagiae* for example is not thought to be due to loss of appropriate habitat, but to very particular ecological requirements of the butterfly. The larvae feed exclusively on flowers of *Thymus herba-barona* (Leigheb et al., 2000), a Corsican-Sardinian endemic, growing between 1000 m and 2000 m a.s.l. (Pignatti, 1982). The adult flight period is restricted to the last weeks of May and the larvae are myrmecophilous.

Threats from building development are also comparatively small (Table 3), although they could become very destructive if road construction were intensified.

Climate change is a potential threat to some species but may only operate on the long term. There is uncertainty about the response of butterflies to what seems to be a global warming trend (Dennis & Shreeve, 1991; Dennis, 1993; Elmes & Free, 1994, Parmesan et al., 1999). On the northern hemisphere increased temperatures may induce northward shifts in species' distribution. Generally, high mountainous species are thought to be the first victims (Boggs & Murphy, 1997). Southern European species may remain less affected as they are better adapted to very high temperatures as well as rapid changes in temperature (e.g. big differences between day and night temperatures).
Table 2. List of Sardinian butterflies based on records from the last decade (1990-2000). Given are the species’ range (S = Sardinia, C = Corsica, E = Elba, T = other Tyrrenian islands), their estimated distribution area in km², and the threat category assigned by us according to the IUCN criteria as opposed to the threat category assigned in the European Red List. The species assessed differently in our list are marked with an asterisk.

<table>
<thead>
<tr>
<th>Species</th>
<th>Range</th>
<th>Distribution (km²)</th>
<th>Number Locations</th>
<th>Fluctuations</th>
<th>Sardinian butterflies</th>
<th>European Red List</th>
<th>SPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilionidae</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papilio hospiton</td>
<td>SC</td>
<td>&gt; 20 000</td>
<td>&gt; 10</td>
<td>No</td>
<td>Least Concern</td>
<td>Least Concern</td>
<td>4a</td>
</tr>
<tr>
<td>Nymphalidae</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aglais urticae ictus*</td>
<td>SCE</td>
<td>&gt; 20 000</td>
<td>&gt; 10</td>
<td>Yes</td>
<td>Least Concern</td>
<td>Not assessed</td>
<td>4a</td>
</tr>
<tr>
<td>Argynnis elisa*</td>
<td>SC</td>
<td>5 000 - 20 000</td>
<td>&gt; 10</td>
<td>No</td>
<td>Near</td>
<td>Least Concern</td>
<td>4a</td>
</tr>
<tr>
<td>Argynnis paphia immaculata*</td>
<td>SCT</td>
<td>&gt; 20 000</td>
<td>&gt; 10</td>
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<td>Threatened</td>
<td>Not assessed</td>
<td>4a</td>
</tr>
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<td>Pieridae</td>
<td></td>
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</tr>
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<td>SC</td>
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<td>&gt; 10</td>
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<td>Least Concern</td>
<td>Least Concern</td>
<td>4a</td>
</tr>
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</tr>
<tr>
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<td>&gt; 10</td>
<td>No</td>
<td>Least Concern</td>
<td>Least Concern</td>
<td>4a</td>
</tr>
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<td>&gt; 10</td>
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<td>Least Concern</td>
<td>Least Concern</td>
<td>4a</td>
</tr>
<tr>
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<td>&gt; 10</td>
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<td>Least Concern</td>
<td>Least Concern</td>
<td>4a</td>
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<td>Maniola nurag*</td>
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<td>4a</td>
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<td>4a</td>
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<tr>
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<td>Near</td>
<td>Not assessed</td>
<td>4a</td>
</tr>
<tr>
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<td>S</td>
<td>&lt; 5 000</td>
<td>5-10</td>
<td>Yes</td>
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<td>Not assessed</td>
<td>1</td>
</tr>
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<td>5-10</td>
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</tr>
<tr>
<td>Spialia sericarius therape*</td>
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<td>5 000 - 20 000</td>
<td>&gt; 10</td>
<td>Yes</td>
<td>Near</td>
<td>Not assessed</td>
<td>4a</td>
</tr>
</tbody>
</table>

This table provides a comprehensive overview of the distribution and threat status of various species of butterflies in Sardinia, Corsica, Elba, and other Tyrrenian islands. The IUCN category and the European Red List category are compared, highlighting discrepancies in assessment.
Table 3. Potential threat factors to Sardinian butterflies. Categories extended from Van Swaay & Warren (1999). Threats 1, 7, 8, 9, and 10 are not relevant for Sardinia at present.

<table>
<thead>
<tr>
<th>Species</th>
<th>2</th>
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<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
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<tr>
<td>Aglais urticae icheus</td>
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<tr>
<td>Lycenidae</td>
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<td>L</td>
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</tr>
<tr>
<td>Lysandra coridon gemlargenti</td>
<td>L</td>
<td>L</td>
<td>M</td>
<td>M</td>
<td>L</td>
<td>L</td>
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</tr>
<tr>
<td>Pseudophilotes barbagiae</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>M</td>
<td>L</td>
<td>L</td>
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</tr>
<tr>
<td>Hesperidae</td>
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<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
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</tr>
</tbody>
</table>

Key to grades of threat: M (Medium) = medium threat; L (Low) = low threat (The original categories include H (High) = severe threat which is not yet relevant for Sardinian species)

Collecting is, unfortunately, still a threat factor in Sardinia. Endemic species trade well on the international market and attract many collectors, especially in Sardinia where legislative restrictions on butterfly collecting are not effectively enforced (Casula & Crnjar, pers. comm. 15. Sept. 2000). For species with a very restricted range like *Pseudophilotes barbagiae* and *Lysandra coridon gennargenti*, the capture of even some hundred individuals in consecutive years can have considerable effects on species viability.

**Discussion**

**Ammendments to the Red Data Book of European Butterflies**

Only three Sardinian species, viz., *Papilio hospiton*, *Argynnis elisa*, and *Hipparchia neomiris*, were assessed comprehensively in the Red Data Book of European Butterflies. For the other 6 species VanSwaay & Warren (1999) used low quality data concerning Sardinian butterflies and for 5 species only the nominate continental form was assessed and Tyrrhenian subspecies were not included (see Table 2). Our analysis reveals that *Pseudophilotes barbagiae* belongs to the category **Vulnerable** and thus SPEC 1, contrasting the present classification in the Red Data Book as **Not Threatened** and SPEC 4a status. *Pseudophilotes barbagiae* is a specialist restricted in resources and habitat use (Leigheb et al., 2000). Its particular ecology and very restricted range make this butterfly extremely sensitive to human activities or stochastic events. The classification as **Vulnerable** on the global scale seems thus more than justified. *Lysandra coridon gennargenti* is not assessed separately in Van Swaay & Warren (1999). We follow recent studies showing evidence for its status as a separate species (Marchi et al., 1996; Casula, 1999), and accordingly treat it as such. In our analysis it qualifies for the category **Vulnerable.** *Maniola nurag*, *Plebejus idas bellieri*, *Spialia sertorius therapne*, and *Argynnis elisa* were assigned the status **Near Threatened.** Their larval host plant choice being restricted to only one particular species or genus of plants (*Maniola nurag: Festuca* spp. (Grill, 2001, Jutzeler et al., 1997); *Argynnis elisa: endemic subspecies of Viola* spp.: *Viola corsica* resp. *Viola corsica* ssp. *limbara; Plebejus idas bellieri: Genista corsica, G. salzmannii* (Kleinekuhle, 1999); *Spialia sertorius therapne: Sanguisorba magnolii* (Tolman & Lewington, 1997)), combined with their restricted distribution area justifies this classification. The five species assigned to the category LEAST CONCERN were classified similarly in Van Swaay & Warren (1999).
Toward butterfly conservation in Sardinia

In Mediterranean countries, mountain grasslands, and shrublands are the richest habitats for butterflies. These habitat types result from long-term low level anthropogenic interference in otherwise natural areas (Munguira, 1995). In Sardinia such grass- and shrublands resulted from transformation of former oak forests, such as Quercus ilex and Q. pubescens. Stock grazing prevents those habitats from reverting to secondary forests. Although overgrazing may restrict the distribution of some butterfly species, a cessation of grazing is not going to favour their conservation either (compare also Munguira et al., 1997; Nelson & Epstein, 1998; Weiss, 1999; Wettstein & Schmid, 1999). As also stated by other authors (Aubert et al., 1996), long-term continuation of traditional land use is essential for the maintenance of butterfly diversity in the Mediterranean region. In particular Papilio hospiton, Argynnis elisa, and Pseudophilotes barbagiae would be negatively affected by changes in mountain grassland management. Plantations of Pinus spp. and Eucalyptus spp. may threaten the heathlands of higher altitudes destroying the habitats of endemics like Pseudophilotes barbagiae and Lysandra coridon gennargenti, that have small distribution areas. Similar problems with reforestation are found in other countries of southern Europe, such as the Sierra Nevada and Sierra de los Filabres in Spain or the Idhi and Dhiki Mountains in Crete as well as Corsica and Elba (Munguira, 1995). Besides causing a loss of characteristic plant communities (Kudrna, 1986; Munguira, 1995), Pinus plantations are also extremely vulnerable to fires. Eucalyptus is normally planted in swampy ground and semi-saline areas (Blamey & Grey-Wilson, 1993), but planted in mountainous regions it has devastating effects on the soil (Vacca et al., 2000). In arid environments like the mediterranean eucalypts decrease the ground water level and cause a loss of vegetative ground cover through their secondary plant compounds, which may lead to erosion. Here, reforestation with indigenous tree species, like Quercus ilex or Q. pubescens is recommended.

Nature reserves and conservation

All species treated in this paper have their main populations within the boundaries of the planned ‘Parco Nazionale del Gennargentu e Golfo di Orosei’ in the Gennargentu mountains of central Sardinia. Another national park to be set up, ‘Parco Naturale Sette Fratelli – Monte Genis’, includes the region around the Sarrabus mountains in the South-East of the island. Here as well, at least eight of the 14 endemic butterfly species occur within the borders of the planned park whose official establishment would thus surely enhance conservation action favourable for butterflies. At present, though, regional authorities are still
preventing the official ratification of nature reserves in the Gennargentu area. This is due to political mechanisms which go beyond the scope of this paper. Successful introduction and maintenance of conservation measures requires the support of the local population. Only then can conservation efforts and nature reserves fulfil their goals. Effectively directed subsidies would be a means to prevent people from abandoning traditional forms of agriculture or other activities with adverse effects on butterfly conservation and guard Sardinia from destructive development.

**Sardinian endemics in the Bern Convention and European Habitat Directive**

Two of the species assessed here, namely *Papilio hospiton* and *Argynnis elisa*, are already listed in Appendix II of the Bern Convention, which since 1988 legislates for the protection of invertebrates at the European level. *Papilio hospiton* also appears in Annex II of the European Habitat Directive (Council Directive 92/43/EEC). They both do not qualify as **Vulnerable** in our assessment, or in that of the Red Data book of European butterflies which is again evidence for the preference given to more conspicuous and better studied taxa when establishing legislative conservation priorities. With regard to the extremely localized occurrence of *Pseudophilotes barbagiae* and *Lysandra coridon gennargenti* and the particular biology of these two species, it is strongly recommended to include them and their habitats in European and national legislation and protection plans. Considering the suggested amendments in other recent studies (Šumpich & Hlaváč, 2000; Kati & Willemse, 2001) which propose the inclusion of *Macrothele calpeiana* (Arachnidae), *Chorthippus lacustris*, and *Paranocarodes chopardii* (Orthoptera) into the European Habitat Directive, a general update of European nature legislation with respect to insects seems necessary.

**Acknowledgments**

This study was funded by a doctorate grant from the Austrian Academy of Science. Chris van Swaay, Daniel F. R. Cleary, and an anonymous referee are thanked for comments on earlier versions of this manuscript. We thank Nimrod Epstein for advice and discussions, and are particularly grateful to Helen M. Regan and Léon E. L. Raijmann for insightful suggestions and encouragement.
V.

Conservation in Mediterranean nature reserves:
Conservation parameters of the endemic Sardinian butterfly
*Maniola nurag* (Lepidoptera, Satyridae)

Naturschutz und Landschaftsplanung 33, 227-232 (2001)
**Zusammenfassung**


**Summary**

Endemic species are a major focus in conservation biology in Europe. The Mediterranean is the richest area in Europe in terms of endemism, but also the least well studied. This paper summarises the results of a six-month field study on the endemic Sardinian butterfly *Maniola nurag*. It presents a method to define population structure and dynamics of the species at the regional scale. Metapopulation structure is inferred from mark-release-recapture experiments and the minimum size and structure of a protected area for effective butterfly conservation is determined. Habitat characteristics and information on ecology are reported. Landscape management parameters that will enhance conservation of this endemic species are suggested, as well as practical ways to increase public awareness for conservation measurements in Southern Europe.

**Introduction**

Past efforts to conserve threatened species by maintaining local nature reserves have not always succeeded (e.g., J.A. Thomas, 1989; C.D. Thomas, 1995). Nevertheless, national parks and nature reserves can also protect butterflies (Grill & Kati, 2000). In Sardinia all endemic and rare butterfly species occur in areas intended to become nature reserves but that are not yet officially declared such. This is a great advantage with respect to other areas in Mediterranean Europe like, for example Spain, where only two of the nine national parks have endangered butterflies in their boundaries. In Greece the most important areas for endemic or endangered butterflies are not represented in the ten national parks (65 000 ha) and in continental Italy, where most of the relevant sites are not included among the five existing national parks (271 400 ha), the situation is similar (Munguira, 1995). The Mediterranean is the richest area of Europe in terms of endemism. Only a
few endemic European butterfly species are restricted to countries outside the Mediterranean (Munguira, 1995). The importance of endemic species for nature conservation in Europe is evident: their disappearance from the restricted area where they occur would imply their total extinction. Besides, being generally products of very particular (a)biotic conditions, they are indicators of extremely rare communities whose conservation is a main focus in conservation biology.

How often and how far organisms move imposes a scale on the environment. Highly mobile individuals integrate habitat changes over broader scales than do more sedentary ones. A species' potential mobility thus determines the scale at which populations respond to habitat changes (Hanski & Gilpin, 1997). If a conservation strategy is to be efficient, a detailed knowledge of population structure on different spatial scales is necessary (May, 1994). The dispersal

Figure 1. A typical habitat of *Maniola nurag* - Monte Fumai in central Sardinia.

ability of a species is of crucial importance for the long-term persistence of its populations and has to be considered when establishing a nature reserve (Hanski, 1991; Warren, 1992; Stacey *et al.*, 1997; Mousson *et al.*, 1999). As a predictive tool of population survival in a fragmented landscape (Gilpin & Hanski, 1991; Hanski & Gilpin, 1997) the metapopulation concept as a 'population of populations' (Hanski, 1999) has been developed. In such a system, each local population has its own probability of extinction and (re)colonization. Within the landscape occupied patches are connected by migration. The question whether several populations are to be considered as a single metapopulation is decisive when proposing a conservation strategy: should management emphasize a habitat network, or the
conservation of each individual population? A nature reserve is only useful when it covers a bigger area than what is used by the organisms to be protected for local and regional movements during their lifetime (May, 1994).

In this paper (I) the population structure of *Maniola nurag* is assessed at the landscape scale, (II) population structure and dynamics of the species at the regional scale are inferred, (III) habitat characteristics reported, and (IV) a background for landscape management favourable for the conservation of this endemic species is suggested.

**Methods**

**The species**

The Sardinian Meadow Brown (*Maniola nurag*) is endemic to Sardinia; it is a univoltine species, with adults flying from May to mid September depending on altitude and local weather conditions (Figure 1). At lower altitudes females aestivate during the hottest part of the summer (Tolman & Lewington 1997; Kleinekuhle, 1999). According to Kleinekuhle, 1999 and Jutzel et al., 1997 larval host plants are grasses including *Festuca morisiana*. Adults were observed using different nectar sources including *Cistus monspeliensis* and thistles. The species is quite widespread over the island and usually observed in altitudes above 500 m. It occurs on grassy, flowery places amongst bushes and rocks. Figure 2 shows a typical habitat of the butterfly. *Maniola nurag* resembles *M. jurtina*, the widespread Meadow Brown to which it is supposed to be closely related.

**Study areas**

The study was carried out in Sardinia, Italy. Six sites ranging in elevation from 500 to 1100 m were surveyed from May to September 2000 (Figure 3). The locations were chosen at different altitudes according to the occurrence of the target species in stable populations and in such a way that levels of altitude differed among sites. Localities can be divided into two sets of sites: Monte Novo, Monte Fumai and Pira è Onni are situated in the centre of the island in the Gennargentu region (9°25'/40°5'), surrounded by Sardinia’s highest elevations; Femmina morta, Monte Eccas, Nuraghe Sa Fraigada in the south-west in the Sette Fratelli forest (9°25'/39°15'). The sites were at least 1 km apart; the greatest distance between two sites (north – south) was 100 km. Air-line distance to the coast was ca. 20 km for the northern sites and 10 km for the southern sites.
Mark-release-recapture analysis
To investigate dispersal between habitat patches and estimate population sizes, mark-release-recapture (MRR) analyses were conducted in one population at “Femmina morta”. This site (250 x 250 m) is situated on a plateau ca. 600 m above sea level (Figure 4) and was studied in May/June 2000 on the following capture days: May 25, 29, and 30; June 1, 3, 4, 8, 11, 14, 16, 18, and 19. Butterflies were caught with a hand held net and marked individually with a consecutive number on the ventral surface of the hind-wing using a thin point permanent marker pen (Figure 5). Each individual was released at the point capture immediately after marking that handling took less than one minute. The patch where butterflies were marked and the location of all subsequent recaptures were recorded as well as wing wear (estimated on an arbitrary scale from 0 = perfect condition, fresh individual to 3 = severe wing damage, old individual), sex, time, weather, and behaviour (fly, rest, mate, court, or feed). That way the ‘encounter history’ of each individual and its movements was established. Each sampling day was divided into three sub-sample periods of one hour each, to permit a later analysis of the data using the Robust-Design method (Nichols, 1992). The robust design method uses a combination of open and closed population models within the same study (Nichols, 1992). It includes secondary sampling periods within each of primary periods. The time interval between successive secondary periods within a primary period is short. Consecutive primary periods are separated by relatively long intervals. In this study it is used to estimate the number of individuals active at a given sampling occasion.

Vegetation surveys and habitat description
At all sites, general surveys of vegetation characteristics were undertaken to
provide a background against which to view and interpret the butterfly data (Sutherland & Hill, 1995). Predominant species of ground, shrub, and tree layer were noted as well as the categories bare ground, rocks, and grass. On the Femmina Morta site, transect counts of flower heads (Cistus monspeliensis) (Figure 4) were carried out to estimate the abundance of potential nectar sources. On six days (June 8, 11, 13, 14, 16, and 19) corresponding to peak-flowering period of Cistus the abundance of flower heads was counted within fifteen 1-m²-frames in a patch occupied by M. nurag and an unoccupied patch. Percent cover of predominant vegetation species and vertical vegetation structure were measured using a thin stick (= point-square) of two-meter length, that was lowered vertically into the vegetation until it touched the ground (Sutherland & Hill, 1995). The stick was marked in 10 cm intervals. The number of touches per plant-species along the length of the square pin, e.g., Arbutus unedo 1 touch at 0-10 cm, 4 touches at 50-60, 3 at 110-120 etc, were recorded. That way 150 point-quadrates were sampled all over the MRR site, using a random-stratified distribution for sample selection.

**Data analysis**

Estimates of population size in the 2000 season and daily population sizes at different sampling occasions were made from MRR data using Jolly-Seber models for open populations (Schwarz & Arnason, 1996) with the POPAN 5 program (Arnason & Schwarz, 1995; Arnason et al., 1998) which proved to provide reliable results in similar studies with Maniola jurtina (Munguira & Thomas, 1992), Parnassius appollo (Meglez et al., 1998), and Boloria aquilionaris (Mousson et al., 1999). Survival was allowed to vary with time, probabilities of capture were set equal for all capture sessions. The number of individuals active at a sampling occasion were estimated using the Robust Design procedure provided by the software MARK (White & Burnham, 1999).

Percent-cover of plant species at the MRR study area was calculated as
where \( t_{sp} \) is the presence of a species at a point-square and \( T_{nt} \) the total of point-squares sampled.

To estimate the degree of similarity between sites in terms of their dominant plant species, Sorensen Similarity Index (\( S \)) was calculated as

\[
S = \frac{2a}{b+c}
\]

where \( a \) is the number of species common to both samples, \( b \) the number of species in sample 1, and \( c \) the number of species in sample 2 (Krebs, 1989; Southwood, 1996).

**Results**

**Demographic parameters**

A total of 221 individuals (190 males and 31 females) were marked at the FM site. The total of capture events carried out is 345 for males and 42 for females. From the 75 males recaptured after marking, 36 were recaptured once, 23 twice and 16 at least three times. As females provided only nine recapture events demographic parameters could be estimated only for males. For males a total population size of 488 individuals was calculated. Estimates of daily population size (Jolly Seber Model) and individuals active per sampling occasion (Robust Design) at the MRR site are shown in Figure 6. No estimates could be made for the first and the last sampling occasion. The decrease in population size and activity in the middle of the sampling period is most likely due to rainy weather conditions on the days before
creating unfavourable conditions for butterflies. The longest interval between two
capture events was 20 days for males, for female imagoes that aestivate potential
life-time is supposed to be longer.

**Flight period and aestivation**

As frequently observed in butterflies (Wiklund & Fagerström, 1977; Mousson *et
al.*, 1999), protandry was evident. Adult males were observed at all sites by May
20th. Single females could be observed by May 25th at altitudes below 700 m. In
the beginning of June females appeared scattered at all sites. Adult butterflies’

![Graph](image1)

**Figure 5.** Age structure of *Maniola nurag* butterflies: observations of wing wear
per sampling occasion. 0 = perfect conditions, fresh individual, 1 = undamaged
wings but not fresh, 2 = slightly damaged wings, 3 = severe wing damage, old
individual.

![Map](image2)

**Figure 6.** Habitat patches investigated at the MRR site; shaded areas represent the
presence of bushes, lines are farm roads and interrupted lines footpaths; circle size
indicates the proportion of individuals observed, big circles are ‘key patches’ preferred by
the butterfly (Scale 1:4 000).
age structure derived from wing-wear is shown in Figure 7. By June 16th all individuals observed already had used wings and males had disappeared from the lower sites. By June 25th no butterflies were observed at the lower sites, whereas at the Monte Novo, Monte Fumai, and Pira e Onni sites sporadic females were observed continuously until the beginning of September. They did not fly as much as males but tended to rest in the shade under bushes for the major part of the day. Aestivation behaviour seems to be dependent on altitude. At lower altitudes females aestivate and reappear towards the end of August to deposit eggs. At higher altitudes the flight period continues uninterruptedly from June to August.

![Figure 7. Proportions of individuals flying indicated distances at the MRR site.](image)

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<tr>
<th></th>
<th>PO</th>
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<th>MN</th>
<th>MF</th>
<th>ME</th>
<th>NSF</th>
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Table 1. Sorenson Similarity Indices of the vegetation recorded at the different study sites. PO = Pirra e Onni; FM = Femmina Morta; MN = Monte Novo; MF = Monte Fumai; ME = Monte Eccas; NSF = Nuraghe sa Fraigada.
Table 2. Predominant plant species at different study sites in comparison; FM = Femmina Morta, MN = Monte Novo, MF = Monte Fumai, PO = Pira e Onni, ME = Monte Eccas, NSF = Nuraghe Su Fraigada.

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<th>ME</th>
<th>NSF</th>
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**Adult nectar resources**

Adult butterflies of both sexes were observed nectaring on plant species that were flowering during their flight period. At the southern site this was *Cistus monspeliensis* in May/June (Figure 4) and *Atractylis gummifera* in August, whereas at the northern sites besides those two species, *Carlina corymbosa* was visited. No significant differences in abundance of nectar sources were found between habitat patches occupied by *M. nurag* and unoccupied patches.
Dispersal
At the MRR site males moved actively between all habitat patches but preferred certain patches more than others (Figure 8). Those were mainly shaded bush edges where vegetation remains green longer than in open areas. Maximum distance moved by marked individuals was at least 210 m. Mean flight distance (N=221) between two capture events was 60 m. The proportions of individuals flying certain distances are shown in Figure 9. Small farm roads did not appear to be barriers to the dispersal of individuals. Of the individuals recaptured 15.7% had stayed in the patch of original capture, 66.3% were captured in at least two different patches, 18.1% of the individuals were recaptured in at least three different patches. There was no relationship between distance and the time between two consecutive recaptures (data not shown).

Habitat characteristics
All six study sites showed high similarities in vegetation composition (Table 1). *Asphodelus* spp., *Helichrysum stoechas*, *Carthamus lanatus*, *Cephalanthera rubra*, and *Cistus* spp. were common to all sites. Predominant plant species at the various study sites are shown in Table 2. At the MRR site grass, *Cistus* spp., *Asphodelus* spp. and *Arbutus unedo* were the dominant features. Other species, bare ground or rocks covered less than five percent of the surface (Figure 10). Vegetation thickness diminished with distance from the ground (Figure 11).
Discussion

Like many other butterfly species (Thomas & Hanski, 1997) M. nurag population structures show a hierarchy of scales and satisfy the requirements for a metapopulation sensu Hanski et al. (1995): (1) habitat patches support local breeding populations, (2) no single population is large enough to ensure long-term survival, (3) patches are not too isolated to prevent recolonization, (4) local dynamics are sufficiently asynchronous to make simultaneous extinction of all populations unlikely. The three southern populations in the Sette Fratelli area are to be considered as one metapopulation with occasional migration between the sites. Also the Monte Novo and Monte Fumai sites are thought to be part of one metapopulation network. Subpopulations might be separated by patches of unsuitable habitat but remain connected by migrants.

Long term persistence of local populations

With estimated daily population sizes at peak flight period not exceeding 150 pairs the long-term persistence of local populations clearly depends on extinction - recolonisation processes in a metapopulation network (Harrison & Talor, 1997). A single population would have no chance of long term survival. Many studies have confirmed the prediction that the expected lifetime of a population increases with its current size (e.g., Hanski, 1999) genetic variation. The minimum viable population size is an estimate of the minimum number of individuals in a population which is required to assure a good chance of surviving for some relatively long period of time, for instance 95% chance of survival for at least 100 years (Soulé, 1980, 1987; Lande, 1988). For metapopulations consisting of small and hence extinction-prone (Harrison & Talor, 1997; Mousson et al. 1999) local populations like those of M. nurag, an analogous concept of minimum viable metapopulation size may be defined as the minimum number of interacting local populations necessary for a long-term persistence (Harrison & Talor, 1997). I expect that for M. nurag this would be a network of three populations. To exactly predict the minimum number of local populations required for the persistence of the metapopulation, genetic population structure analysis combined with the collection of further empirical field data is being conducted.

Risk of local extinction

The garrigue-type landscape used by M. nurag is a very dynamic system and undergoes severe seasonal changes. In winter, temperatures in mountainous areas of Sardinia can reach levels below 0°C whereas in summer they rise up to 60°C ground temperature. After spring precipitations the grassland dries
out entirely until autumn rains bring back some humidity. Overgrazing by free roaming herds of sheep, goats, and horses can cause severe damage to vegetation. All Mediterranean landscapes are prone to fires. This environmental instability might easily eliminate local butterfly populations which are extremely sensitive to weather, and other disturbances.

**Dispersal ability**

The MRR survey at the Femmina Morta site showed that local mobility is high and covers distances of more than 200 m. Considering that mark-recapture experiments underestimate movement distances (Shreeve, 1995; Dennis & Shreeve, 1996), *M. nurag* is supposedly capable of moving away from its locality in excess of 500 m. Evidence from its sister species *M. jurtina* which has similar population structures shows that those butterflies are able to move in access of 330 m across unsuitable habitat (Munguira & Thomas, 1992). This indicates that also species which are thought to be rather sedentary will move considerable distances in search for new habitat (Shreeve, 1995). Records of butterflies in places clearly not suitable for breeding, such as inner cities, further support the view that individuals of even the most sedentary species occasionally move relatively long distances (Dennis & Shreeve, 1996). To have clear evidence for the butterfly’s dispersal ability, female movement, in particular, will have to be studied in detail.

**Small-scale habitat requirements and local dynamics**

*Maniola nurag* has a restricted distribution, but is abundant where it occurs. The butterfly fits thus well into the type of rare species described in the classification of Rabinowitz *et al.* (1986) and Gaston (1994). Considering that in Sardinia the type of habitat required by this butterfly is largely available, the species occupies only a small fraction of the potential habitat. This is thought to be due to particular requirements with respect to microclimatic conditions and vegetation structure. Stable metapopulations can only persist at sites providing a certain level of small-scale habitat heterogeneity. Though basically a grassland species with larvae feeding on different species of *Festuca* spp., all sites where *M. nurag* was observed contained bushes or shrubs. These structures in the vegetation provide shade during the hottest part of the day as well as shelter from predators. Heat, wind, draught, and humidity act differently on caterpillars, pupae, and adults in sheltered patches than in open areas (Hanski *et al*., 1995; Mousson *et al.* (1999). Shelter provided by bushes and trees, for example, can prevent local extinctions in case of extreme climatic conditions. Towards the end of the flight period, when vegetation had already become very dry the number of occupied patches at the
Femmina Morta site decreased and butterflies withdrew to those patches that provided more shade and comparatively fresher vegetation.

Towards an effective butterfly conservation
The main conclusion of this study is that *Maniola nurag* populations in Sardinia form metapopulation networks whose long-term persistence requires ‘connectivity’ between local populations. An efficient conservation strategy for such species should rather emphasize the management of a habitat network than the conservation of local populations. Stepping-stones must be provided to support long-distance (re)colonization processes. Additionally, the minimum amount of suitable habitat necessary for metapopulation persistence has to be considered. Areas to protect *nurag*-type species would have to cover at least 500 ha for each population in order to be bigger than what is required by the organisms for interpopulation movements. Given the metapopulation structure, it would thus mean areas of about 2000 ha. The maintenance of habitats containing the vegetation characteristics necessary for *M. nurag* (see Table 2, Figures 10 and 11) is crucial.

Management strategies favourable towards conservation of butterflies in the nature reserves to be constructed in Sardinia should include the maintenance of low density livestock grazing with controlled numbers of herds as well as extensive agriculture. Traditional land use techniques generally proved favourable for butterfly conservation in a similar study. Reforestation with non-native tree species should be stopped immediately as it destroys habitats valuable not only for butterflies but also for many other native species. Small and quiet farm roads did not appear to be barriers against dispersal. The problem of roads is more relevant for nature reserves and protected areas in North and Central Europe. A big advantage of Sardinia is that in the areas designated to become nature reserves, roads are much smaller, less well maintained, and except in summer time, much less frequented.

Wide busy roads can form barriers to dispersal (Mader, 1984; Munguira & Thomas, 1992) and many individuals are killed on windscreens (Munguira & Thomas, 1992). In a study on 23 British butterfly species Munguira & Thomas (1992) found that the mortalities of butterflies caused by vehicles were insignificant compared to those caused by natural factors: 0.6 percent of adults of species from closed populations, and 7 % of those from open populations were killed by cars. This is at least an order of magnitude lower than the proportion of young butterflies killed by predators or parasitoids (Dempster, 1984). For already endangered species, however, the effects could be more devastating. Mobile and migratory species are generally more
vulnerable to traffic (Munguira & Thomas, 1992).
It has to be pointed out, that nature conservation management in South Mediterranean areas has to be quite different from the situations we know from North-Central Europe, where public awareness for conservation measurements is much higher. As already stated by other authors (Munguira, 1995), it is evident that poorer countries are less interested in conservation. On the other hand, these are the areas in Europe where natural habitats are usually still in good condition, so the need for protected areas is less evident. Here, good information would be necessary, hand in hand with proper national and international funding (e.g. from the European Community), as subsidies are the only way to encourage traditional land uses where they are no longer profitable, and the best way to prevent a shift to activities that might pose problems for rare species.

Acknowledgements
This work was supported by a doctorate grant of the Austrian Academy of Science. Gabriel Nève and Steph Menken constantly supported this study. Roberto Crjnar and Paolo Casula provided help in selection of the field sites. Kees Nagelkerke gave valuable advice. Sally Capper, David Harrington, John Sherba, Hank Dutt and Joan Jeanrenaud linguistically improved earlier versions of this manuscript. Raoul Schrott shall be thanked for technical assistance.
Part 3

ECOLOGY AND EVOLUTION
VI.
The evolutionary perspective of ecological differences: the endemic *Maniola nurag* and the widespread *Maniola jurtina*

with Nicolas Schtickzelle, Daniel Cleary, Gabriel Nève, and Steph Menken
Abstract
Recently refined evolutionary theories have highlighted that ecological interactions and environmental gradients can play a major role in speciation processes. This paper summarizes a three-year-field study, where ecological differences between two congeneric species are used to explore the environmental factors that determine their spatial distribution. We then infer the processes that might have initiated their divergence. The butterfly Maniola nurag is endemic to the Mediterranean island Sardinia, while M. jurtina is widespread all over Europe. In Sardinia, the two species are locally sympatric. The endemic Maniola nurag is restricted to areas above 500 m, as opposed to Maniola jurtina which has its largest populations at sea level. Mark-release-recapture experiments on both species were combined with measures of environmental variables in fifteen 100x100 m plots, established in areas of potential habitat for the butterflies. Constrained linear models were parameterised from mark-recapture data to estimate individual (survival and capture probability) and population parameters (population size and recruitment). Results reveal the two species’ similarities in demography, movement patterns, life history and behaviour. Population sizes during the flight season follow a parabolic distribution. Survival probability between sampling events decrease towards the end of the flight period. Quantifiable differences include population size, adult phenology and habitat parameters. There is an altitudinal gradient in emergence time and flight period. Long-distance movements larger than 1.5 km were observed regularly, suggesting a substantial amount of gene-flow between populations. Multivariate analyses revealed four main environmental gradients determined by vegetation cover and structure. Each species is correlated with a different gradient. Our results show that, when sympatric, the two species respond to subtle differences in microhabitat-structure, which might originally have induced their divergence.

Keywords: butterflies - environmental gradients - mark-release-recapture - demography - aestivation - speciation - Nymphalidae

Introduction
At the foot of a ladder in the ‘Hell’ panel of Hieronymous Bosch’s “Garden of Earthly Delights” painted around 1485, a bird-like creature with butterfly wings clings to one of the rungs. The wings are of a female Meadow Brown butterfly, Maniola jurtina (Lepidoptera, Nymphalidae) (L.). This is probably the earliest illustration of M. jurtina, which was scientifically described only about three centuries later. Another two centuries later, M. jurtina was proposed as an interesting model organism for the study of sympatric speciation, because of the large amount of morphological variation this species exhibits in different geographic areas as well as within single populations (Ford, 1945; Dowdeswell, 1961; Thomson, 1973; Brakefield, 1979;
Numerous studies have further demonstrated that population structure, dynamics, and ecology of *M. jurtina* are highly variable depending on the specific habitat occupied (Dowdeswell, 1981; Shreeve, 1989; Goulson, 1993 and references therein). Time of emergence is well adapted to the environmental conditions of a particular site, and in the southern areas of the Palaearctic females perform an imaginal diapause (aestivation) during the hottest part of the summer, a life history trait that is paralleled by a delayed ovarian maturation (Verity, 1953; Scali, 1971, 1973; Masetti & Scali, 1972; Garcia-Barros, 1987).

In spite of its widespread distribution in the Western Palaearctic, *M. jurtina* has not yet been used to investigate evolutionary questions, particularly field studies comparing *M. jurtina* with other congeneric species, are still entirely missing. But exactly the investigation of ecological differences between closely related species is likely to lead us to an understanding of the processes that incite differentiation and speciation. There is increasing theoretical evidence, that ecological contact may often be the driving force for speciation, and environmental gradients, like altitude and temperature, can play a major role in speciation processes (Schluter, 1999; Doebeli & Dieckmann, 2000, 2003; Ogden & Thorpe, 2002).

Consequently, field studies exploring ecological differentiation under sympatric conditions are frequently asked for, but rarely put into practise (Schluter, 1999; Doebeli & Dieckmann, 2003).

In Sardinia, the distribution area of the pan-european *M. jurtina* overlaps with the range of an endemic congeneric, *Maniola nurag* (GHILIANI), whereas usually in butterflies the distribution areas of widespread species and their endemic relatives are disjunct (Dennis *et al.*, 2000). *Maniola nurag* has been considered a close relative to *M. jurtina* since the time of its description (Simmons, 1930; Thomson, 1987; Jutzeler *et al.*, 1997; Grill *et al.*, 2003). It is restricted to the mountainous areas of the island, while *M. jurtina* is most abundant at sea level, but their distribution areas overlap at intermediate altitudes. This situation provides a laboratory *in natura* to study the plausibility of sympatric speciation along environmental gradients, a scenario for which growing theoretical evidence has been presented recently (Doebeli & Dieckmann, 2003).

In contrast to allopatric models of speciation, where complete geographic isolation is the barrier to gene-flow between the differentiating populations, sympatric speciation occurs when segments of a panmictic population differentiate from each other despite high initial gene-flow (Bush, 1969). Parapatic speciation takes place if continuous populations with substantial gene-flow between them diverge
(Futuyma & Mayer, 1980), e.g., as a result of adaptation to different ecological niches. Allopatric speciation through vicariance or dispersal, is commonly agreed to be the prevalent mode of speciation in animals (Mayr, 1963; Futuyma & Mayer, 1980; Tautz, 2003). It is intuitively more plausible, and there are many examples for speciation events under apparently allopatric systems (especially from islands, see for example Grant, 1998 and references therein), but only very few empirical examples of sympatric speciation. Frequently cited are the case of Geospiza conirostris, the large cactus finch (Grant & Grant, 1989) or Rhagoletis pomonella, the apple maggot fly (Feder et al., 1993). Sympatric speciation is primarily driven by disruptive, frequency- or density dependent natural selection on resource use. It may initiate when in a group of individuals sharing the same resources, some of them shift in resource preference (e.g., host plant or habitat). In such a situation, there is increasing competition among those individuals that are best adapted to the particular ecological niche the population is using, because they are the most abundant ones (frequency-dependent-competition). The individuals most unlike the others experience the least competition and will therefore be favoured by inverse frequency-dependent natural selection (Pfennig & Murphy, 2002 and references therein). Selection will enhance mating preference and act to reinforce reproductive isolation (Dobzhansky & Pavlovsky, 1957; Kondrashov & Kondrashov, 1999). The result is that speciation is speeded. Differentiation thus seems to be particularly incited, if the differences of the ecological niche the diverging groups of individuals occupy are small (Doebeli & Dieckmann, 2003).

In this paper, we compare Sardinian populations of M. nurag with those of M. jurtina. During a three-year field study, we collected ecological data of both species, examining phenology, demography (survival, sex-ratio, recruitment, population size, residence time), life history, and behaviour, as well as habitat characteristics (vegetation, topography), in order to answer the following questions:

(I) Do M. nurag and M. jurtina differ in (a) phenology, (b) demography, (c) movement and/or dispersal ability, (d) behaviour?

(II) Which are the ecological parameters determining the occurrence of a M. nurag or M. jurtina population in an area?

(III) Do they occupy different ecological niches along an environmental gradient?

We discuss these field data on the ecological differences between the Sardinian populations of M. nurag and M. jurtina in the context of a sympatric speciation scenario, as an alternative to the more common hypothesis that island endemics
result from vicariance or dispersal.

Materials and methods

Study organisms

Maniola species are univoltine, and protandric, with males hatching at least one week earlier than females, initially causing a male-biased sex ratio (Hesselbarth et al. 1995). Larvae of *M. nurag* have been reared on *Festuca morisiana* (Jutzeler et al., 1997). The larvae of *M. jurtina* feed on a wide range of grass species including *Poa* spp., and *Festuca* spp. (Brakefield, 1982; Schneider et al., 2003). In the final stage of their development, the larvae of *M. nurag* remain hidden close to the ground during the day, and only come out to feed at night. This behaviour is to avoid dehydration during the hot hours of the day. Adults of both species use different nectar sources, including *Cistus monspeliensis* and *Carlina corymbosa* (Grill, 2001). These two thistle species are particularly important nectar sources at the end of the summer, after the aestivation period when females deposit their eggs. Adults of *M. nurag* are on the wing from May to September depending on altitude and local weather conditions, *M. jurtina* flies in Sardinia from late April to June.

**Figure 1.** Distribution areas of *M. jurtina* and *M. nurag* and location of the populations studied in mark-release-recapture experiments. *Maniola jurtina* has its main populations at sea level (light grey area) whereas *M. nurag* is restricted to the mountainous areas of Sardinia (>500 m a.s.l.). White circles indicate the highest tops of Sardinia (>1000 m) where exclusively *M. nurag* occurs. The zone of overlap, where the two species fly sympatrically is shown in dark grey. The study sites are situated at three different altitudes: 1 = low *M. jurtina* population (50 m), 2 = intermediate site where both species fly (720 m), 3 = high altitude population of *M. nurag*.
**Study area and data collection**

The study area, situated in south-east Sardinia, Italy (Figure 1), is a degraded Mediterranean shrubland (Garrigue) with patches of dense shrub (Macchie), and both evergreen and deciduous trees. Predominant plant species include *Cistus salviifolius*, *Cistus monspeliensis*, *Arbutus unedo*, *Erica arborea*, *Euphorbia dendroides*, *Asphodelus aestivus*, and *Quercus* spp.

**Mark-release-recapture**

Two Sardinian populations of *M. nurag* and two populations of *M. jurtina* were studied by mark-release-recapture (MRR) techniques. The study sites are located at three different altitudes between sea level and 1000 m a.s.l. The first ‘low’ site is situated at ‘S. Isidoro’ (32S 0526643/UTM 4346633) at 50 m a.s.l. in an abandoned agricultural terrain, and is occupied by a *M. jurtina* population. The second ‘intermediate’ site is located at 720 m a.s.l. at ‘Sedda Su Staulis’ (32S 0534042/UTM 4344398), an open shrubland extensively grazed by goats; it is here, that both species fly sympatrically. The third ‘high’ site is at the top of the ‘Monte Eccas’ at an altitude of 925 m a.s.l. (32S 0535551/UTM 4346011), where a *M. nurag* population flies.

Adult butterflies were captured with a hand-held net (Nabokov, 2000), marked with an individual number on the underside of one hind wing using a thin-point permanent marker pen (Staedtler®Lumocolor 303F) and released immediately after marking. Handling time was less than a minute.

For each (re)capture we recorded: mark, sex, age (estimated by wing-wear on an arbitrary scale from 0 = fresh individual to 3 = worn individual with destroyed wings), date, hour, wind intensity (estimated from 0 = no wind to 3 = strong wind, trees moving heavily), GPS position (Garmin 12 XL), and behaviour. Behaviour was assigned to six categories: bask, court, feed, fly, mate, or rest. In case of an interaction between two butterflies (court, mate), we recorded if the interaction was intra- or interspecific. Movement distances were calculated from the distances between two points (recorded by GPS position) where a butterfly was captured and recaptured consecutively.

Populations were monitored in three consecutive years (2000 – 2002) during the flight period of the adults. As both *M. nurag* and *M. jurtina* are univoltine species, data sets collected in different years represent independent samples.
Ecology and habitat choice

Within the same region in south-east Sardinia, fifteen 100x100m plots were assigned randomly to a 500 ha area of potential habitat for butterflies. Sites were selected \textit{a priori} using a topographical map. In these 15 plots, the three mark-release-recapture sites were included, because the target species of our study were known to occur there, and we wanted to understand and quantify their habitat requirements. Each plot was georeferenced (GPS). Habitat structure variables were measured in relation to butterfly abundance. For butterfly sampling, 10 observation hours were spent in each plot. The plot was crossed repeatedly from one end to the other, butterflies were mostly identified on the wing, or caught with a hand held net and released immediately after identification. Butterflies were sampled across the entire plot, while habitat structure variables were sampled in single systematically placed subplots of 20 x 20 m size. Twelve such subplots were sampled in each plot. In each subplot 20 variables were measured, including: slope, presence of water, estimated cover of: trees, shrubs, rocks, herbs, moss, ferns, grass, bare ground, \textit{Cistus} spp., \textit{Asphodelus} spp., \textit{Erica} spp., \textit{Arbutus} spp., \textit{Inula} spp., \textit{Quercus} spp., flower abundance, and moisture. Water and moisture were relatively invariable among plots and were therefore not included in further analysis (variables listed in Table 3).

Analysis

Demography

Demographic parameters (recapture, survival and recruitment rates, population size) were estimated using Constrained Linear Models (CLM). CLM models are a refined version of the Jolly-Seber models (Jolly, 1965; Seber, 1965) and allow the estimation of demographic parameters on basis of MRR data. A set of candidate models is constructed (details in Schtickzelle \textit{et al}., 2002), which represent biologically plausible descriptions of the variation in demographic parameters in terms of external factors (\textit{e.g.}, individual or environmental attributes). Each model is adjusted to the data by maximum likelihood techniques (UFIT option in POPAN-5: Arnason & Schwarz, 1999), and its fit quantified and estimates for its parameters formed. From this set of candidate models, the best model is selected by means of Akaike's Information Criterion corrected for small samples (AICc: Burnham and Anderson, 1998) defined as:

$$AICc = -2\ln(L) + 2n_p + [2n_p(n_r-1)]/[n_{ef} - n_r - 1]$$

with $\ln(L)$ the log-likelihood of the model, $n_p$ the number of parameters estimated
in the model and \( n_{\text{eff}} \), the effective sample size of the data set (total number of releases: each time an individual is caught and released counts as 1, whether it is a new capture or not). AICc therefore represents a trade-off between model fit and parsimony, \( i.e., \) between bias and precision in the estimates formed by the model. The lower the AICc the better the model is supported by the data, and the better is the trade-off. As long as models differ by less than two units in AICc from the best one, they are substantially supported by the data. Among the supported models we chose the most parsimonious one, \( i.e., \) the one with the smallest number of parameters. Notation follows the ANOVA type introduced by Lebreton et al. (1992): \( \Phi \) represents the survival, \( p \) the catchability, and \( B \) the recruitment; \( \cdot \) stands for a constant effect; \( t \) stands for an effect changing with each sample occasion, but with no relation between the sample times; \( \text{lin} \) infers a linear trend to the time effect.

The low number of females captured did not allow for demographic model selection. Therefore, full CLM selection was performed only on males, and male demographic parameters obtained from the best model for each data set. For females, no estimates of population size were conducted, and only the sex ratio at capture is given (Table 1).

For all butterflies captured consecutively within the same site, residence time in the population \( (e_t) \) \( i.e., \) lifetime expectancy, since we cannot differentiate between emigration and death) on day \( t \) was calculated from daily survival estimates, as the ratio between remaining butterfly-days till the end of the flight season \( (k) \) and the number of surviving individuals \( (S) \):

\[
e_t = \frac{\sum_{i=t}^{k} S_i}{S_t}
\]

**Habitat structure**

Parameters describing major patterns of habitat structure in the different sampling sites, were assessed by Principal Component Analysis (PCA) (Statistica for Windows, 1996) on \( \log_{10}(x+1) \) transformed environmental parameters. Varimax-normalised rotation was used to maximise the variation of the squared and normalized factor loadings. Rotation is used to obtain a clear pattern of factor loadings. From the PCA we extracted the first four factors and used them to assess the main environmental gradient. Factor loadings above 0.50 were considered to have substantially contributed to separation along the axes. Species’ abundance
was then tested for association with the first four axes using a Pearson-Moment correlation.

**Spatial autocorrelation**

We tested for significant spatial autocorrelation of butterfly abundance using Moran's I with the programme R 4.0 (Casgrain and Legendre, 2001). In our analysis we present correlograms that quantify the spatial dependence of abundance at five equidistant classes based on a spatial matrix of Euclidean distances obtained from UTM-coordinates at each plot. At each distance we tested if there was significant positive or negative spatial autocorrelation (Legendre & Legendre, 1998) using a Bonferroni correction for the number of distance classes.

**Behaviour and movement**

Chi-square tests and logistic regression were used to seek out differences in behaviour and movement patterns. Spearman rank correlations were used to test the influence of (1) age of the butterfly (wingwear), and (2) wind intensity on flight distances; this non-parametric method is appropriate since the absolute value of a point is replaced by its rank, so that a few very large movement events will not disproportionately influence the correlation.

**Results**

In the three years of MRR a total of 2565 individuals were marked (Table 1). *M. jurtina* male were caught on average 1.3 times, *M. nurag* males 1.5 times, *M. jurtina* females 1.0 time, *M. nurag* females 1.2 times. Males were thus captured more often than females. These estimates include multiple captures of the same individual during one sampling occasion. In all three years, the number of females captured and marked was significantly smaller than the number of males (*M. jurtina*: \( p<0.001, \text{df}=4 \); *M. nurag*: \( p<0.0001, \text{df}=4 \)). Both species are protandric and the sex ratio at capture was male-biased at all three sites for both species for all populations in all three years (Table 1). Towards the end of the flight period the proportion of females among the captured individuals increased; during the last sampling occasion there were usually only females encountered.
Table 1. Capture events in male (m) and female (f) *M. jurtina* and *M. nurag* during the three sampling occasions (2000, 2001, and 2002). Sex ratio based on capture is strongly male biased in all populations for all years.

<table>
<thead>
<tr>
<th>species</th>
<th>year</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m</td>
<td>f</td>
<td>m</td>
</tr>
<tr>
<td><em>M. jurtina</em></td>
<td>2002</td>
<td>437</td>
<td>81</td>
<td>134</td>
</tr>
<tr>
<td><em>M. nurag</em></td>
<td>2002</td>
<td>252</td>
<td>20</td>
<td>340</td>
</tr>
<tr>
<td><em>M. jurtina</em></td>
<td>2001</td>
<td>391</td>
<td>121</td>
<td>60</td>
</tr>
<tr>
<td><em>M. nurag</em></td>
<td>2001</td>
<td>142</td>
<td>30</td>
<td>157</td>
</tr>
<tr>
<td><em>M. jurtina</em></td>
<td>2000</td>
<td>108</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><em>M. nurag</em></td>
<td>2000</td>
<td>190</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Summary statistics for the 20 environmental variables at the four PCA ordinations. See text for details.

<table>
<thead>
<tr>
<th>Environmental Variable</th>
<th>PCA-1</th>
<th>PCA-2</th>
<th>PCA-3</th>
<th>PCA-4</th>
</tr>
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<tbody>
<tr>
<td>Maximum slope</td>
<td>0.51</td>
<td>-0.21</td>
<td>0.73</td>
<td>0.26</td>
</tr>
<tr>
<td>Cover trees &gt; 10 m</td>
<td>0.86</td>
<td>0.37</td>
<td>0.07</td>
<td>0.30</td>
</tr>
<tr>
<td>Cover trees &gt; 5 m</td>
<td>0.85</td>
<td>0.38</td>
<td>0.07</td>
<td>0.31</td>
</tr>
<tr>
<td>Cover shrubs &lt; 2.5 m</td>
<td>0.04</td>
<td>0.08</td>
<td>0.21</td>
<td>0.84</td>
</tr>
<tr>
<td>Cover shrubs &lt; 0.5 m</td>
<td>-0.88</td>
<td>0.07</td>
<td>0.00</td>
<td>0.26</td>
</tr>
<tr>
<td>Cover Rocks</td>
<td>0.21</td>
<td>-0.33</td>
<td>0.82</td>
<td>0.24</td>
</tr>
<tr>
<td>Cover Herbs</td>
<td>-0.64</td>
<td>-0.15</td>
<td>-0.20</td>
<td>-0.67</td>
</tr>
<tr>
<td>Cover Moss</td>
<td>0.62</td>
<td>0.10</td>
<td>0.19</td>
<td>0.56</td>
</tr>
<tr>
<td>Cover Ferns</td>
<td>0.30</td>
<td>0.10</td>
<td>0.39</td>
<td>-0.69</td>
</tr>
<tr>
<td>Cover Grass</td>
<td>-0.26</td>
<td>-0.52</td>
<td>-0.26</td>
<td>-0.64</td>
</tr>
<tr>
<td>Cover Bare ground</td>
<td>-0.33</td>
<td>0.65</td>
<td>0.48</td>
<td>0.22</td>
</tr>
<tr>
<td>Flower Abundance</td>
<td>-0.15</td>
<td>-0.85</td>
<td>-0.22</td>
<td>-0.19</td>
</tr>
<tr>
<td>Cover <em>Cistus</em> spp.</td>
<td>-0.80</td>
<td>0.28</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Cover <em>Asphodelus</em> spp.</td>
<td>-0.49</td>
<td>-0.09</td>
<td>-0.27</td>
<td>-0.76</td>
</tr>
<tr>
<td>Cover <em>Erica</em> spp.</td>
<td>-0.20</td>
<td>0.18</td>
<td>0.64</td>
<td>0.04</td>
</tr>
<tr>
<td>Cover <em>Arbutus unedo</em></td>
<td>0.18</td>
<td>0.05</td>
<td>0.34</td>
<td>0.80</td>
</tr>
<tr>
<td>Cover <em>Inula viscosa</em></td>
<td>-0.08</td>
<td>-0.87</td>
<td>0.40</td>
<td>0.15</td>
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<tr>
<td>Cover <em>Quercus</em> spp.</td>
<td>0.82</td>
<td>0.03</td>
<td>0.01</td>
<td>0.31</td>
</tr>
<tr>
<td>Variation Explained</td>
<td>5.26</td>
<td>2.87</td>
<td>3.32</td>
<td>4.18</td>
</tr>
<tr>
<td>Proportion of Total</td>
<td>0.28</td>
<td>0.15</td>
<td>0.17</td>
<td>0.22</td>
</tr>
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</table>
Table 2. Starting and supported JS models (survival, catchability, and recruitment) for *M. jurtina* and *M. nurag*. The best model is given in bold. AIC = Akaike’s Information Criterion corrected for small samples, $n_{el}$ = number of individuals marked; $n_p$ = number of parameters. See text for notation details.

<table>
<thead>
<tr>
<th>Year</th>
<th>Population</th>
<th>Model</th>
<th>AIC</th>
<th>$n_{el}$</th>
<th>$n_p$</th>
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</thead>
<tbody>
<tr>
<td>2000</td>
<td><em>M. jurtina</em> intermediate</td>
<td>$\phi(.)p(.)B(tlin+tlin')$</td>
<td>587.57</td>
<td>132</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\phi(tlin)B(tlin+tlin')$</td>
<td>589.35</td>
<td>132</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\phi(.)p(t)B(tlin+tlin')$</td>
<td>590.28</td>
<td>132</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\phi(.)p(t)B(tlin+tlin')$</td>
<td>590.40</td>
<td>132</td>
<td>16</td>
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<tr>
<td></td>
<td></td>
<td>$\phi(tlin)p(t)B(tlin+tlin')$</td>
<td>592.95</td>
<td>132</td>
<td>17</td>
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<tr>
<td></td>
<td></td>
<td>$\phi(tlin)p(t)B(tlin+tlin')$</td>
<td>598.32</td>
<td>132</td>
<td>21</td>
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<tr>
<td></td>
<td></td>
<td>$\phi(t)B(t)$</td>
<td>599.02</td>
<td>132</td>
<td>23</td>
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<td>2000</td>
<td><em>M. nurag</em> intermediate</td>
<td>$\phi(.)p(t)B(tlin+tlin')$</td>
<td>827.96</td>
<td>222</td>
<td>14</td>
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<td></td>
<td></td>
<td>$\phi(lin)p(t)B(tlin+tlin')$</td>
<td>830.10</td>
<td>222</td>
<td>15</td>
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<td></td>
<td></td>
<td>$\phi(.)p(t)B(tlin+tlin')$</td>
<td>836.06</td>
<td>222</td>
<td>19</td>
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<td>222</td>
<td>6</td>
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<td></td>
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<td>$\phi(.)p(t)B(tlin+tlin')$</td>
<td>840.65</td>
<td>222</td>
<td>5</td>
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<tr>
<td></td>
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<td>$\phi(tlin)p(t)B(tlin+tlin')$</td>
<td>841.27</td>
<td>222</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\phi(t)B(t)$</td>
<td>843.50</td>
<td>272</td>
<td>23</td>
</tr>
<tr>
<td>2001</td>
<td><em>M. jurtina</em> intermediate</td>
<td>$\phi(.)p(t)B(t)$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>M. nurag</em> intermediate</td>
<td>$\phi(.)p(t)B(tlin+tlin')$</td>
<td>389.12</td>
<td>161</td>
<td>9</td>
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Population parameters

Phenology

Emergence time varied with altitude and local weather conditions. As commonly observed in many butterfly species, females emerged about one week later than males. In the 'low' population of *M. jurtina*, adult males started flying in the first week of May, reached their peak in the second half of May and disappeared by mid June (Figure 2a). In the 'intermediate' population, *M. jurtina* emerged in the second half of May (20 - 25) and flew until the second week of June (12-15) (Figure 2b). At sea level, the species' flight period was thus 1-2 weeks longer than at higher altitudes. Similarly, *M. nurag* males in the 'intermediate' population emerged around the 20\textsuperscript{th} May, reached their peak by the end of the month, until they disappeared during the second week of June or, in case of females, started aestivation (Figure 2c). In the 'high' population *M. nurag* reached its peak a few days later, in the first week of June (Figure 2d). Like the southern populations of *M. jurtina*, also *M. nurag* females oviposited only after the aestivation period and not immediately after mating.

Demographic models

Table 2 lists the models considered as candidates and the one selected as best among them for each data set. For two ('low' *M. jurtina* 2001 and 'high' *M. nurag* 2001) of the 10 data sets, model selection was not possible due to low abundance of recaptures, or convergence problems of the estimation algorithm for some models. Parameter estimates were then obtained using a general model, that had proven to be suitable for the data from other, comparable populations (see Table 2).

Constant survival probability over the flight season was the best model in four of the ten studied populations, while it varied with time in the four others (Table 2). For the 'low' *M. jurtina* population 2001 and the 'high' population of *M. nurag* 2002, a linear trend could be inferred; in the 'low' *M. jurtina* population and the 'intermediate' population of *M. nurag* 2002, the time effect on survival was categorical, which means that a different parameter is computed for each sampling time and there is no relation between the sampling times.

Catchability was time dependent in the best model for four populations, and constant for the four others. The real situation is most probably that, like survival, catchability varies in relation to time, due to differences in weather conditions.
Figure 2. Phenology of *M. jurtina* and *M. nurag* at the three different sites, in 2001 (= black bars) and 2002 (= striped bars). (A) *M. jurtina* low site, (B) *M. jurtina* intermediate site, (C) *M. nurag* intermediate site, (D) *M. nurag* high site. Estimates of population size based on Jolly-Seber models. Start date is 7 May (= 7.5).

and/or catch intensity; but there is probably not enough information in the data to detect such variations.

Recruitment is best modelled as a parabolic distribution in eight of ten samples (Table 2). This kind of pattern has earlier been found for other univoltine butterfly...
species (Schtickzelle et al., 2002; Baguette and Schtickzelle, 2003). In the post-aestivation no marked females could be found and as a consequence no estimates could be conducted for this period.

Population size
Estimates of total population size per year (Figure 3) showed that the population size of both species was lower in the ‘intermediate’ site where the two butterfly species co-exist, than in the ‘low’ or ‘high’ sites where the respective species occurs alone. No strong fluctuations in population size between consecutive years were observed, except for the ‘high’ population of M. nurag, which doubled from 2001 to 2002 and was then even larger than the ‘low’ M. jurtina population.

Residence time in a population
From the daily survival estimates, we calculated the residence time of an individual in a population, in relation to its emergence date. For all populations, residence time generally decreased as the butterfly emerged later in the year. It changed in a similar manner for both species at the site where they occur together, but was shorter for M. jurtina at the high altitude site. Maximum residence time for males in 2002 was 4.7 days for the ‘low’ M. jurtina population, but only 2.6 days for the ‘intermediate’ population. For M. nurag in the same year, it was 3.6 days for the ‘high’ population and 3.0 days for the ‘intermediate’ population (Figure 4).

Age structure
On the first sampling occasion of all populations in all different years, a large proportion of butterflies captured were fresh individuals. This indicates that sampling indeed started well at the beginning of the flight period. In males the proportion of age-class-3 individuals started to increase by the middle of the male flight period, around 12-14 May for M. jurtina and 27-30 May for M. nurag. In females of both species, the proportion of age-class-0 individuals was larger than in males, and in most samples fresh females were found until the end of the sampling period. In four samples, no age-class-3 females were recorded; two of them contained only age class 0-1 observations and not a single 2-3 female. A second peak of fresh males appeared about 7-10 days after the first emergence. They were smaller in size and less numerous than the first wave of emerging adults, indicating bimodal emergence pattern.
Figure 3. Total male population sizes of *M. jurtina* and *M. nurag* in three different years (2000, 2001, 2002) at the three sites in comparison. Population sizes are based on Jolly Seber estimates.

**Movement and dispersal**

There were no differences in the movement rates of the two species (Figure 5), nor did the distance moved depend on the sex of a butterfly (Wald Chi-square test: Chi-square=1.153, df=1, p=0.283). Flight distances were not significantly associated with wingwear or wind intensity: Spearman rank correlation: *M. jurtina*, wingwear: r=-0.024, p=0.781; wind: r=-0.074, p=0.496; *M. nurag*, wingwear: r=-0.073, p=0.112; wind: r=-0.040, p=0.387). Due to the absence of recaptures of females after the summer diapause, female movement in the post-aestivation period could not be quantified. Most individuals of both species showed home range behaviour in that they were observed at the same site for several days or even weeks, often using the same flower resources. In *M. nurag*, we detected three long distance movements (>500m) in 2001 and 13 in 2002, and in *M. jurtina* there were 3 (>500m) in 2002. The maximum flight distances recorded were 2116 m for *M. jurtina* and 1565 m for *M. nurag*. The majority of butterflies of both species were resighted within 100 m of the first capture point (Figure 5). There was some exchange of individuals between the 'high' and the 'intermediate' site. This includes individuals of *M. jurtina*, that were occasionally recaptured at the 'high' site, although no stable populations of this species occur at this location. No exchange was detected between the 'low' site and any of the other two sites. Notably, after the aestivation, *M. nurag* females were only resighted at 'high' site the 'intermediate' site was empty.

**Behaviour**

Most of the observed individuals were flying or resting at the moment they
Figure 4. Residence time of the two species in 2002. Lifetime expectancy of an individual was calculated from survival probability estimates obtained from mark-recapture data. (A) *M. jurtina* males at the FM site, (B) comparison of *M. nurag* (dashed line) and *M. jurtina* (full line) males at the FM sites, (C) *M. nurag* males at the S. Isidoro site.

were recorded (Figure 6). Differences in the frequency of observed behavioural categories were larger between sexes than between species. The largest difference was that males were significantly more active fliers than females. This was apparent in both species (logistic regression on 'fly' vs. 'other behaviours': Wald Chi-square test: species: Chi-square = 1.35, df = 1, p = 0.25; sex: Chi-square = 69.12, df = 1, p <
Figure 5. Histogram of distances moved by individuals of (A) M. nurag and (B) M. jurtina. The maximum flight distances recorded were 2116 m for M. jurtina and (B) 1565 m for M. nurag. (Note the different scales of Figure A and B.)

Figure 6. Frequency of observed behavioural categories summarized from all populations studied in the three years (N=10) for M. jurtina and M. nurag males (white bars) and females (black bars).

0.0001; species*sex: Chi-square = 1.81, df = 1, p = 0.18). The frequencies of non-fly behaviours differed between M. nurag and M. jurtina in males, but in females showed no significant difference (logistic regression: Wald Chi-square test: species: Chi-square = 3.71, df = 1, p = 0.05; sex: Chi-square = 0.08, df = 1, p = 0.78; species*sex: Chi-square = 8.65, df = 1, p = 0.003). Maniola nurag and M. jurtina interacted with each other, i.e., flew around each other in circles; but no interspecific mating was observed.

Habitat choice and environmental gradients

The first four axes of the PCA analysis explained 82% of the variation in our dataset (Figure 7, Table 3). The first gradient (28%) separates sites with a high shrub (particularly Cistus spp.) and herb cover and sites with high tree (particularly Quercus spp.) and moss cover. The second gradient (15%) separates plots with high grass and Inula viscosa cover and high flower abundance and plots with predominantly bare ground. The third gradient (17%) is a topographical gradient, determined by the steepness of the slope in the plot, and reaches from plots with high cover of Erica spp. and rocks to plots with a low cover of Erica spp. and rocks. The fourth gradient (22%) finally represents plots with a high shrub and
Figure 7. Regression of species’ abundance on the first four axes of a PCA analysis on environmental variables, circles represent *M. jurtina*, triangles *M. nurag*. Significant relationships are depicted with a dashed line (*M. jurtina*), solid line (*M. nurag*).

*Arbutus unedo* cover to plots with high fern, grass, and *Asphodelus* spp. cover. Both species seem to respond similarly to these environmental gradients with a positive relationship with PCA-1, a negative relationship to PCA-2, and little response to the two other gradients. There are, however, some intraspecific differences: the abundance of *M. jurtina* was significantly correlated to PCA-1 \((r = 0.616, p = 0.019)\), but for *M. nurag* the relation was not significant \((r = 0.370, p = 0.192)\). Conversely, there was a significant correlation for *M. nurag* along PCA-2 \((r = -0.707, p = 0.005)\) but not for *M. jurtina* \((r = 0.412, p = 0.143)\). Both species have a nonsignificant relationship with PCA-3 and PCA-4.

Figure 8. Microdistribution of *M. jurtina* and *M. nurag* at the intermediate altitude site where they are sympatric. Data based on GPS coordinates taken at recapture.
Species' abundances were not significantly correlated with the spatial location of the plots (p > 0.05). Moran’s I ranges close to 0, thus the abundances of neither species were dependent upon the geographic distance between sampling sites.

The results of the mark-release-recapture experiments show, that at the site where *M. nurag* and *M. jurtina* fly sympatrically, both species are similarly aggregated in the landscape (Figure 8). In an area of 500 x 500 m of potential habitat, they both gathered in a quadrate of approximately 150 x 150 m.

**Discussion**

Ecological differences between *M. nurag* and *M. jurtina* are small, but significant. Where flying sympatrically at the same altitude, both species emerge in synchrony and are similarly distributed in the landscape. However, they seem to respond to slightly distinct environmental variables, indicating subtle differences in habitat preferences. *M. nurag* abundance was significantly correlated to an environmental gradient from high grass-cover and flowerhead-abundance to bare ground, whereas *M. jurtina* abundance was significantly correlated to a successional gradient, from predominantly herb and low shrub dominated sites to tree and high shrubs dominated sites. Hence, despite the overlap in many ecological characteristics, the species seem to occupy different ecological niches.

Usually, butterflies of the genus *Maniola* are lowland species (Thomson, 1987), and although *M. jurtina* can occur up to 1500 m, it is most frequent at much lower altitudes (Higgins & Riley, 1970). The Sardinian endemic *M. nurag* is a mountain species, well adapted to the environmental conditions in the mountainous midland of Sardinia, viz., extreme aridity and heat in the summer, cold winters, and potentially large oscillations between day and night temperature. This might explain why the species has never been recorded in Corsica, while most other Sardinian butterflies are distributed over both or more Tyrrhenian islands (Kleinekuhle, 1999). A similar association with flowers as we found it in *M. nurag*, was shown for Swedish *M. jurtina* populations, where the variable ‘flower density’ was correlated to the number of residents, emigrants and immigrants (Schneider et al. 2003).

**Population parameters**

We found an altitudinal gradient in emergence time: lowland *M. jurtina* emerge first, ‘intermediate’ *M. nurag* about two weeks later, synchronously with ‘intermediate’ *M. jurtina* populations. The ‘high’ *M. nurag* emerge thus about 1
month later than the 'low' *M. jurtina* population. The flight period of the 'low' *M. jurtina* population is almost twice as long as that of the populations at higher altitudes, and the emergence curve is smoother for *M. jurtina* than for *M. nurag*. The endemic species' populations emerge very rapidly, reach the peak quickly and at the end of the pre-aestivation phase disappear almost instantaneously from one day to the next.

The population sizes we estimated varied according to altitude and species. Intra-specific competition and/or sub-optimal habitat could be important factors explaining this variation. The 'intermediate' *M. jurtina* population is significantly smaller than the 'low' population, a difference that we constantly found over the years. An explanation for this could be interspecific competition at the sites where the two butterfly species are sympatric. Given that niche differentiation between the two species is small, in coexistence, it is possible that interspecific competition for limited resources depresses the population sizes of both. Competition might also explain the shorter maximum residence time of *M. jurtina* individuals at the 'intermediate' site. If smaller population sizes indeed reflect competition, the two species must share some important resources, probably larval host plants. However, another interpretation would be, that the 'intermediate' location is a sub-optimal habitat for *M. jurtina*, so that it produces less offspring than at sea-level, where conditions are more favourable for this species. At the site where both species occur in sympatry, *M. nurag* was always found to outnumber *M. jurtina*, nevertheless population size was smaller than in the *M. nurag* population at the 'high' site. The 'intermediate' site appears therefore sub-optimal for both species. From the fact that the 'intermediate' population of *M. nurag* are larger than the one of *M. jurtina*, we conclude that the endemic is well adapted to the congeneric competitor.

The differences between males and females we found in capture probabilities have also been observed in other recent studies on butterflies (Petit et al. 2001, Schtickzelle et al. 2002, and references therein). Sex ratios biased towards males, such as we observed in both species, are expected to increase the evolutionary stable amount of protandry in the population. Models for the evolution of protandry in butterflies indicate that the maintenance of protandry depends on the assumption that females only mate once while males are capable of multiple matings (Zonneveld and Metz, 1991). These authors also point out that protandry depends on the rate of encounter between sexes and on the sex ratio. Protandry maximizes the number of unmated females which a male is likely to encounter in its lifetime (Wiklund & Fagerström, 1977; Iwasa *et al.*, 1983). The amount of
protandry is supposed to decrease if the rate of encounter between males and females decreases and the death rate increases. In our system, we found that earlier emerging males have a higher residence time than males emerging later. This might compensate for encounter probabilities, of the early emerging males.

The fact that survival probability varies in relation to time confirms what has already been discussed in similar mark-recapture-work (Petit et al. 2001, Schtickzelle et al. 2002): butterfly survival rate depends on daily variation in weather conditions as well as the climatic conditions of the particular season. The fact, that models with survival constant in relation with time turned out to be the best for some data sets, probably indicates that the recapture rate, and therefore the information contained in the data, is too low to detect time variation.

Life history
Our results confirm earlier anecdotal evidence (Simmons 1930), stating that M. nurag females aestivate during the hottest part of the summer, a behaviour that has also been described from Maniola megala, Maniola telmessia, Maniola halicarnassus and Maniola chia, all of which inhabit dry hot habitats in the south eastern part of Europe (Van Oorschot & Brink 1992). Post-aestivating M. nurag females can be found scarcely until September. In continental populations of M. jurtina a few males seem to undergo a summer diapause as well (Scali 1971), but in Sardinia exclusively females were observed in the post-aestivation period. Aestivation involves a postponement of gonad development (Scali 1971) and eggs ripen only when the first rains have fallen and grasses start to vegetate again. Eggs are then fertilized with the sperm stored a couple of weeks or even months earlier (Scali 1971). The crucial point for the persistence of a M. nurag population is thus female survival rate during aestivation. As these patterns are controlled by ecological characteristics (temperature, availability of food-plants, humidity) of the site where a population flies, the Sardinian species might be highly vulnerable to extinction when global warming becomes continues: as populations would start to emerge earlier in spring, and autumn rains would arrive later, the summer diapause would have to be much longer than it is now, and consequently increase the risk that females die before oviposition.

Behaviour
The most conspicuous difference in the behavioural classes we studied was, that males were more active fliers than females. This may be due to males actively 'patrolling' in search for females, while females, on the other hand, only undertake flights to localize nectar sources and, after aestivation, oviposition
sites, but otherwise spend most of the time resting. The interspecific differences we found for males in the non-fly behavioural classes are significant, but might be an artefact due to the larger number of observations in *M. jurtina*. In *Maniola*, males exhibit the most conspicuous secondary sexual characters (dark upper fore wing brands), which differ between species. Female choice is therefore expected to be the most important factor governing species-specific mating, and may also have been important in the development of assortative mating in the initial phase of speciation. We never encountered a copula consisting of individuals of *M. nurag* and *M. jurtina*, and as far as we know, no interspecific hybrid has ever been described between these two species. Nevertheless, hybridisation is probably possible, given the overlap in phenology of the two butterflies, and their closely relatedness (Grill *et al.*, 2003).

**Movement & dispersal**

Butterflies move through the landscape to forage, locate mates, and for oviposition. Species' mobility may vary in terms of the distance moved or in the frequency of inter-patch movements (Shreeve, 1995; Norberg *et al.*, 2002). The dispersal curves we observed in *M. jurtina* and *M. nurag* correspond to the leptokurtic distribution of movement distances, that is commonly found in dispersal data from various taxa (Nathan, 2001; Morales, 2002). They are probably the result of movement behaviour interacting with heterogeneous landscape structure. When small numbers of individuals move longer distances than the majority, population heterogeneity generates the distributions we found for our two species. Morales (2002) found that long leptokurtic tails were particularly frequent when individuals reacted to habitat boundaries, and when as species experienced the landscape as coarse-grained, i.e., consisting of relatively large, distinct patches. The pattern we found in the dispersal curves of both *Maniola* species indicate, that habitat boundaries (edge effect) and landscape heterogeneity are influencing their dispersal behaviour.

Differences in dispersal ability between the two study species are most relevant for our research question. Is one of them more willing to cross unsuitable habitat and consequently more likely to colonize new patches and extend its distribution area? Is *M. jurtina* more prone to fly longer distances because it is larger than *M. nurag* and therefore has stronger thorax muscles, which is supposedly favourable for flying longer distances (Norberg *et al.*, 2002)? We did not find significant differences in movement ability between the two species, which confirms what has been shown earlier (Conradt *et al.*, 2000, 2002): Meadow Brown butterflies have rather small, well-defined home ranges, with most of the individuals never leaving the population. Mean and maximum movement distances were similar to those
shown in similar studies on *M. jurtina* from Northern Europe (Schneider *et al.*, 2003). Considering, that the number of long distance movements that we detected probably only included a small proportion of the individuals that are actually dispersing (*e.g.*, Schneider, 2003), we conclude that there is a considerable amount of gene-flow between neighbouring, and even more distant populations, a finding that is also supported by population genetic data (Grill *et al.*, 2003). We also might speculate, that single butterflies are able to move larger distances than the 1.5 – 2 km we observed. It is possible, that there is an exchange of *M. jurtina* individuals between Sardinia and the neighbouring islands, in particular Corsica, where this species also flies. ‘Backpacking’ of butterflies on human means of transport could further result in a very low level exchange of individuals between the island and the continent.

Surprisingly, we never resighted any *M. nurag* female at the intermediate site after the aestivation period, but only at the high site, possibly indicating unidirectional uphill movement before aestivation. Although, we have no empirical evidence for such an uphill movement (as no marked females were recaptured after aestivation), it could be a female strategy to bring their offspring into more favourable habitats: at higher altitudes the vegetation stays green longer and conditions are cooler and more humid and thus better for young larvae than at intermediate altitudes. Such behaviour would support the hypothesis, that *M. nurag* originated as a real mountain species and later slowly colonized areas below 1000 m. On the other hand, it would mean that the lower habitats have to be recolonized each year, which seems unlikely.

**Habitat choice and environmental gradients**

For both species we found a significant relationship between abundance and environmental variables, but no evidence for a relationship between abundance and distance. Individuals were, however, clearly aggregated at the landscape scale. The environmental conditions of a specific site are thus more important for the presence of *M. nurag*, than its geographic location in the landscape.

Although *M. nurag* and *M. jurtina* utilize the same space in the landscape, their ecological niches seem to be differentiated, which is reflected in differences in habitat structure variables. The differences in habitat preference that the two species show in the adult stage, might be even greater in the larval stage. Larvae are much more spatially associated with their host plants than adult butterflies with their nectar sources, and oviposition sites, and we assume, that the endemic
species has a much narrower spectrum of larval host plants than the widespread species. The environmental gradient we found in the habitat use of our two target species, could make them an example for a sympatric speciation scenario, where ecological contact facilitates speciation, potentially more than spatial isolation, as has been theoretically predicted by recently published speciation models (Doebelei & Dieckmann 2003). In such a scenario, local adaptation along an environmental gradient increases the degree of frequency dependence in spatially localized ecological interactions, and hence the likelihood that these interactions generate disruptive selection. We conclude that the subtle ecological differences we found could have played an important role in the differentiation of *M. nurag* and *M. jurtina* in Sardinia.

**Acknowledgments**

We thank Paolo Salvai, Andrea Salvai, Giorgio Delogu, Robbert Erents, and Eline van Haastrecht for their indispensable assistance with field work; Paolo Casula and Roberto Crnjar for help with the selection of the study sites and many useful discussions; Vito Matera for storage of samples; Irma Wynhoff for extensive reviewing earlier versions of this manuscript; Martin Genner for critical comments. Raoul Schrott provided butterfly nets and other materials. Anna and Franz Grill are acknowledged for logistic support. The Austrian Academy of Science funded this work with a 3-year DOC grant to A. Grill.
VII.
Genetic differentiation in the island endemic *Maniola nurag* and the widespread *Maniola jurtina*

with Wil van Ginkel, Gabriel Nève, and Steph B. J. Menken
Abstract
In butterflies, the distribution areas of widespread species and their endemic relatives are usually vicariant. In Sardinia, the ranges of an endemic and a widespread Maniola species overlap, and the two species possibly hybridise. In this paper, we analyse patterns of genetic differentiation in Maniola nurag and Maniola jurtina from Sardinia by means of allozyme markers, compare them to mainland M. jurtina populations, and interpret the data with regard to the endemic species' evolutionary history. Sardinian M. nurag and M. jurtina have equally high levels of genetic variation ($H_{nurag} = 0.141-0.270; H_{jurtina} = 0.137-0.189$) as mainland M. jurtina ($H_{mainland} = 0.141-0.236$). Total genetic diversity at fifteen polymorphic loci is mostly due to within population variation ($F_{IS}$). The close relationship of the two species is illustrated by the fact, that 63 of the 76 alleles screened are shared by both species. Small genetic distance between them (Nei's $D = 0.21$) indicates that divergence initiated after the desiccation of the Mediterranean ($\pm 3$ ma ago), and was possibly associated with the abrupt climate changes at the turn from Pliocene to Pleistocene (1.8 - 3 ma). Geographic patterns in allozyme allele frequencies hint at the existence of hybrizymes, and suggest the presence of hybrids in areas where M. nurag and M. jurtina are sympatric. Island populations of neither species show signs of loss of genetic diversity, inbreeding, or bottlenecks. We propose that M. nurag did not result from vicariance or dispersal, but originated under sympatric or parapatric conditions, as a consequence of local adaptation along an environmental gradient.

Keywords: Maniola nurag, butterfly, genetic population structure, hybridisation, endemic, allozymes, inbreeding, Lepidoptera, Nymphalidae, divergence time

Introduction
A species is usually genetically structured over space and in time. Historical founder events, bottlenecks, and gene-flow are important evolutionary agents responsible for changes in the genetic structure of populations. Assessing genetic variation across geographic areas can thus provide means to trace the history of these populations and eventually the history of species (Avise, 1994; Hewitt, 1999; Schmitt & Seitz, 2002).

Several speciation models have been proposed, which are habitually characterized by the level of gene-flow between diverging populations during the initial stages of speciation (Dobzhansky, 1940). Population divergence in the presence of gene-flow was often considered to be unrealistic. However, a number of theoretical
studies have reported the plausibility of sympatric and parapatric speciation, and have shown that spatially localized interactions along environmental gradients can facilitate species’ differentiation (e.g., Kondrashov & Kondrashov, 1999; Doebeli & Dieckmann, 2000; 2003). Despite this growing theoretical evidence that ecologically driven speciation can occur, empirical studies showing examples for such speciation modes still remain scarce (Ogden & Thorpe, 2002; Scriber, 2002; Lushai et al., 2003). Earlier sympatric speciation models involved ecologically driven reproductive isolation associated with adaptation to alternative resources (niche shift), as was elegantly shown for the host races in the tephritid fly Rhagoletis pomonella (Bush, 1994) or the large cactus finch Geospiza conirostris (Grant & Grant, 1989). Recent modelling advances suggest that competition for continuously distributed resources, driven by sexual selection against intermediate phenotypes, could be the driving force for sympatric speciation (Doebeli & Dieckmann, 2003). Intermediate phenotypes procure fewer resources as a consequence of density- and frequency-dependent selection, and are selected against under disruptive selection (Turelli et al., 2001).

Hybrid zones form an ideal environment to study sympatric speciation (Arnold, 1997). A ‘hybrid zone’ sensu Arnold (1997) is a geographical area where “two populations of individuals that are distinguishable on the basis of one or more heritable characters overlap spatially and temporally and cross to form viable and at least partially fertile offspring.” In such a parapatric situation, gene-flow can slow down or even inhibit differentiation by spreading favourable alleles across the hybrid zone (Kim & Rieseberg, 1999), whereas reinforcement can cause prezygotic reproductive isolation (Turelli et al., 2001). Reinforcement intensifies mate preference (Dobzhansky & Pavlovsky, 1957; Butlin, 1995), and can lead to character displacement. With character displacement, the differences between sympatric populations of two species are accentuated as a result of reproductive or ecological interactions between them (Futuyma, 1998). Although character displacement has generally been interpreted as an evolutionary response to secondary contact, it can also evolve in situ across an environmental gradient, despite continuing gene-flow (Turelli et al., 2001). The existence of hybrid zones and steep genetic clines (Schilthuizen et al., 1999, Lushai et al., 2003) shows that selection can dominate gene-flow over small spatial scales and therefore allow for parapatric divergence. In many hybrid zones, particular allozymes called ‘hybrizymes’ (Woodruff, 1989) have been found, representing alleles that are not present or very rare in the parental taxa, and reflect novel genetic variation (Schilthuizen & Gittenberger, 1994b; Arntzen, 2001). Hybrid zones have been extensively investigated in plants, and also in animals (e.g. Barton & Hewitt, 1985; Hewitt, 1988, 1999; Schilthuizen &
Lombaerts, 1995; Arntzen, 2001; Capula, 2002) but only rarely so in Lepidoptera (Aagaard, 2002; Scriber, 2002; Lushai et al., 2003).

The distribution areas of widespread species and their endemic relatives are usually disjunct in butterflies (Dennis et al., 2000). In the genus Maniola (Lepidoptera, Nymphalalidae), however, the Sardinian endemic Maniola nurag (GHILIANI 1852) and its widespread close relative, Maniola jurtina (L. 1758), are found in sympathy and possibly hybridise (Grill et al., 2003b, 2003d). In order to find out whether the present sympatric occurrence of the two species can be best explained under the assumption of a sympatric, parapatric, or allopatric mode of speciation, we investigate the population genetic structure in a number of island populations of both species, and compare these to continental populations of M. jurtina by means of allozyme markers. As we found ecological as well as morphological support suggesting that M. nurag and M. jurtina possibly hybridise in Sardinia (Grill et al., 2003b; 2003c), we further evaluate the probability of hybrid occurrence in the Sardinian Maniola.

Allozymes are co-dominant markers and efficient to study population differentiation in Lepidoptera because of the large number of polymorphic loci (e.g., Raijmann & Menken, 2000; Nève, 2000; Schmitt & Seitz, 2002), and also provide us with a straightforward tool to detect interspecific hybridisation (Menken & Ulenber, 1987; Schilthuizen & Gittenberger, 1994b; Schilthuizen & Lombaerts 1995; Arntzen, 2001; Capula 2002); diagnostic loci differentiate between species (Hewitt, 1988; Grant & Grant, 1996; Schilthuizen et al., 1999), and consequently can reveal whether hybridisation takes place.

If the present sympatric occurrence of M. nurag and M. jurtina in Sardinia resulted from a sympatric or parapatric speciation event we would expect to find evidence for reinforcement or disruptive selection on traits that are associated with the use of alternative niches (Mayr, 1963, Bush, 1969). In an early phase of differentiation, most alleles at polymorphic loci are still shared between the populations in similar frequencies, and gene-flow between the diverging populations is large. Genetic regions that are involved in differential adaptation, however, continue to diverge through selection. In later stages of differentiation, gene-flow will be further reduced, and neutral alleles increasingly become subject to independent drift, resulting in different frequencies of such alleles in the diverging populations (Tautz, 2003b). If M. nurag evolved allopatrically, vicariant speciation would not have led to a substantial loss of genetic variation, but founder speciation would, if the number of founding individuals was small (Mayr, 1954). If M. jurtina invaded
Sardinia later on, we would similarly expect a lower level of genetic variability in the Sardinian *M. jurtina*, compared to the continental *M. jurtina* as a consequence of a founder effect. If *M. nurag* was a palaeo-endemic instead of a neo-endemic, variation levels can be high or low, depending on historical bottlenecks, speed of size reduction, and the level of variation before contraction of the distribution area.

Background

The genus *Maniola* is divided into one widespread species, *M. jurtina*, common and abundant throughout the Western Palaearctic and six largely vicariant species: *Maniola telmessia* largely replaces *M. jurtina* in southern and western Turkey, eastwards from the Bosporus, *Maniola halicarnassus* flies in the Bodrum peninsula (Turkey) and the Aegean island of Nissiros, *Maniola megala* occurs in southern Turkey and eastwards to Iran, *Maniola chia* is endemic to the Greek island of Chios, *Maniola cypricola* to Cyprus, and *M. nurag* to Sardinia.

Butterflies of the genus *Maniola* fly in diverse, open habitats, with occasional trees and shrubs. Variation within species includes pronounced wing-pattern polymorphism within populations, and ecological variability related to latitude and altitude (Shreeve, 1989; Goulson, 1993). Time of emergence is well adapted to the environmental conditions of a particular site, and in the Southern areas of the Palaearctic females perform an imaginal diapause ( aestivation ) during the hottest part of the summer, with a concomitant delayed ovarian maturation (Verity, 1953); Masetti & Scali, 1972; Garcia-Barros, 1987). Adults of *M. nurag* are on the wing from May to September depending on altitude and local weather conditions, *M. jurtina* flies in Sardinia from late April to June. Like many other butterflies, *Maniola* species are protandric, with males hatching at least one week earlier than females, causing an initial disproportionate sex ratio (Grill et al., 2003b). *Maniola nurag* is a mountain species, restricted to areas above 500 m (Grill et al., 2003b), with its main populations at 1000 m a.s.l. The main Sardinian populations of *M. jurtina* fly at sea-level, but the species can occasionally be found sympatric with *M. nurag* at higher altitudes; single individuals of *M. jurtina* are found up to 900 m, but no stable populations were observed at this altitude (Grill et al. 2003b). Both species are univoltine and overwinter as larvae. Larvae of *M. nurag* have been reared with *Festuca morisiana* (Jutzeler et al. 1997). Their actual range of host plants has not yet been investigated in the field, but probably includes other *Festuca* species, and grass species outside the genus *Festuca*. They are very evasive, and hide close to the ground during the day, while coming out to feed only at night. Host plants of
**Figure 1.** Study areas and sampling sites. White circles represent *M. jurtina*, grey circles *M. nurag*. Population numbers are explained in Table 1.

*M. jurtina* include a wide range of grass species, preferably *Poa* spp. (Higgins & Riley 1970; Hesselbarth et al. 1995), but also *F. morisiana*. Adults of both species can be observed at different nectar sources including *Cistus monspeliensis* and *Carlina corymbosa* (Grill, 2001).

The main phenotypic characteristics to differentiate *M. nurag* from the *M. jurtina* are yellow-orange areas on the upper side of the male wings. Male upper forewing brand marks are more conspicuous and female markings brighter and more sharply defined in the island endemic. Contours of both species' wings are similar, but *M. nurag* is smaller than *M. jurtina* in both sexes. Due to these slight morphological differences, *M. nurag* has been considered a close relative of *M. jurtina* since the time of its description (Simmons, 1930; Thomson, 1976; Jutzieler et al. 1997). In the locality where populations of the two species occur sympatrically, we occasionally encountered individuals that could not be clearly attributed to
either species, and showed intermediate wing patterns. Interestingly, individuals with similar intermediate wing characteristics have been identified in older material (since 1970s) of the entomological collection of the Zoological Museum of Amsterdam. As a result, the *Maniola* specimens from Sardinia can be divided in three rather than two phenotypic groups.

**Materials and methods**

**Collection of samples**

Adult individuals were collected from 10 *M. nurag* populations and from 16 *M. jurtina* populations in both Sardinia and in continental Europe during the 1999-2002 flight season (Table 1, Figure 1). Geographical distance between populations was estimated as the crow flies, and ranged from 2 to 1930 km. A total number of 406 adult butterflies have been collected. Samples were frozen in the field in liquid nitrogen, then brought to the laboratory in Amsterdam, and stored at −70 °C until they were analysed electrophoretically. Sample sizes ranged from 3 to 34 per population (Table 1). Moreover, we encountered individuals that could not be clearly attributed to either species on the basis of wing morphology. These individuals were assigned to a third group. They resembled the intermediate form in the collection of the Zoological Museum in Amsterdam, and are supposedly hybrids between *M. nurag* and *M. jurtina*.

**Allozyme electrophoresis**

From an initial screening of 22 enzyme systems, allozyme variation was assayed at 15 interpretable allozyme loci, which were polymorphic at the 99% level (in parentheses, locus-abbreviation and E.C. number): Aldolase (*aldo*, 4.1.2.13), Aspartate aminotransaminase, two loci (*aat*, 2.6.1.1), Glucose-6-phosphate dehydrogenase (*g6pdh*, 1.1.1.49), Glycerol-3-phosphate dehydrogenase (*gpd*, 1.1.1.8), Isocitrate dehydrogenase, two loci (*idh*, 1.1.1.42), Malate dehydrogenase, two loci (*mdh*, 1.1.1.37), Malic enzyme (*me*, 1.1.1.40), Mannose-6-phosphate isomerase (*mpi*, 5.3.1.8), Peptidase leu-ala (*pep-leu-ala*, 3.4.11), Phosphoglucoisomerase (*pgi*, 5.3.1.9), Phosphoglucomutase (*pgm*, 5.4.2.2), and 6-Phosphogluconic dehydrogenase (*6pgdh*, 1.1.1.44). After sexing, head and thorax of a specimen were ground by hand in 120 μl homogenizing buffer (0.1 M Tris, 0.05 M Malic acid, 0.001 M EDTA, 0.001 M MgCl2, 0.05 mM NADP). Homogenates were then centrifuged at 10,000 rpm at 4°C for 5 minutes. The clear supernatant was either stamped on cellulose acetate gels (Titan III, Helena Laboratories, Beaumont, TX, USA) following the procedure of Hebert and Beaton (1989), or soaked onto Whatman
Table 1. *Maniola nurag* and *M. jurtina* populations samples per year and per region (abbreviations for populations in brackets) and genetic summary statistics; number of individuals electrophoresed (N), mean sample size per locus (n), mean number of alleles per locus (A), percentage polymorphic loci (P), and mean observed ($H_o$) and expected ($H_e$) heterozygosity under Hardy-Weinberg equilibrium.

<table>
<thead>
<tr>
<th>Population</th>
<th>Region</th>
<th>Year</th>
<th>Altitude (m)</th>
<th>N</th>
<th>Mean sample size per locus</th>
<th>Mean number of alleles per locus</th>
<th>Percentage of loci polymorphic</th>
<th>Mean heterozygosity</th>
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</thead>
<tbody>
<tr>
<td><em>Maniola nurag</em></td>
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<tr>
<td>1 Monte Novo (MN)</td>
<td>Sardinia</td>
<td>2000</td>
<td>1000</td>
<td>30</td>
<td>24.1 (+1.3)</td>
<td>2.9 (+0.4)</td>
<td>66.7</td>
<td>0.182 (+0.43) 0.274 (+0.063)</td>
</tr>
<tr>
<td>2 Monte Fumai (MF)</td>
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<td>2000</td>
<td>910</td>
<td>9</td>
<td>5.9 (+0.7)</td>
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<td>0.270 (+0.073) 0.358 (+0.079)</td>
</tr>
<tr>
<td>3 Pir' e Onni (PO)</td>
<td>Sardinia</td>
<td>2000</td>
<td>940</td>
<td>2</td>
<td>1.8 (+0.1)</td>
<td>1.7 (+0.2)</td>
<td>60.0</td>
<td>0.267 (+0.083) 0.367 (+0.085)</td>
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<td></td>
<td></td>
<td>2001</td>
<td>22</td>
<td></td>
<td>21.2 (+0.3)</td>
<td>2.6 (+0.3)</td>
<td>60.0</td>
<td>0.177 (+0.044) 0.254 (+0.062)</td>
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<td>4 Monte Eccas (EC)</td>
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<td>66.7</td>
<td>0.200 (+0.055) 0.219 (+0.056)</td>
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<td>12</td>
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<td>0.224 (+0.057) 0.232 (+0.056)</td>
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<td>6 Sette Fratelli (SF)</td>
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<td>73.3</td>
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<td>60.0</td>
<td>0.167 (+0.052) 0.201 (+0.061)</td>
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Table 1 continued.

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<th>Mean sample size (per locus)</th>
<th>Mean number of alleles per locus</th>
<th>Percentage of loci polymorphic</th>
<th>Mean heterozygosity expected</th>
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<td>73.5</td>
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<td>80.0</td>
<td>0.182 (±0.051) 0.229 (±0.046)</td>
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<td></td>
<td>France</td>
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<td>750</td>
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<td>30.2 (±1.3)</td>
<td>3.0 (±0.3)</td>
<td>73.3</td>
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<td>1.5 (±0.2)</td>
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<td>950</td>
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<td>9.2 (±0.9)</td>
<td>2.2 (±0.3)</td>
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<td>2.2 (±0.3)</td>
<td>53.3</td>
<td>0.160 (±0.045) 0.187 (±0.050)</td>
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<td>20</td>
<td>3</td>
<td>2.9 (±0.1)</td>
<td>1.6 (±0.2)</td>
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<td>240</td>
<td>19</td>
<td>18.1 (±0.05)</td>
<td>2.5 (±0.3)</td>
<td>66.7</td>
<td>0.174 (±0.037) 0.189 (±0.035)</td>
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<td>0.139 (±0.044) 0.229 (±0.065)</td>
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3 MM chromatography paper wicks for insertion into 11% w/v starch gels following the procedure described by Menken (1982). During sample preparation and electrophoresis, specimens were kept on melting ice. *Aat, 6pgdh, gpi, pgm, pep-leu-ala, idh,* and *g6pdh* were electrophoresed on cellulose acetate gels at room temperature, the remainder of the loci on starch gels at 4°C.

Three buffer systems were used: tris citrate pH 8.0 (Selander et al., 1971) for *gpd, aconitase, mpi, me,* and *mdh;* tris glycine pH 8.4 (Hebert and Beaton, 1989) for *pgm, pep-leu-ala,* and *gpi;* and amine citrate pH 6.1 (Clayton and Tretiak, 1972) for *aat, 6pgdh, idh,* and *g6pdh.* Alleles were named in alphabetical order of increasing (anodal) migration, except for *mdh-1,* which migrated cathodally. All loci are located in the nuclear DNA, and probably inherited as autosomal genes in a Mendelian fashion. Because sample sizes were sometimes small, it was necessary to analyse a large number of (polymorphic) loci to accurately estimate genetic variability (Nei, 1978).

**Data analysis**

Loci were tested for linkage disequilibrium with GENEPOP, Version 3.3 (Raymond & Rousset, 2001), and checked for sex-linkage by hand.

**Genetic differentiation.**

To estimate genetic variation, the following parameters were calculated per population: proportion of polymorphic loci, mean effective number of alleles, and observed and expected heterozygosity. Exact tests for Hardy-Weinberg equilibrium were performed with the GENEPOP computer package, Version 3.3 (Raymond & Rousset, 2001). Probability values without bias using a Markov chain method were used following the algorithm of Guo & Thompson (1992). We further used default parameters in GENEPOP for dememorisation number, batches, and permutations for all Markov chain tests performed. Sequential Bonferroni adjustments (Rice, 1989) were applied to judge significance levels for all simultaneous tests with an initial *α* level of 0.05. GENEPOP was also used to estimate three summary statistics: *F* *s*, the correlation of genes within individuals in the entire population; *F* *s*, the correlation of genes within individuals within (sub)-population; and *F* *s*, the correlation of genes of different individuals in the same (sub)-population (Wright, 1951; definitions following Weir & Cockerham (1984). *F* *s* values were tested for departure from zero by the *χ* ² method of Workman & Niswander (1970), and inbreeding coefficients (*F* *is*) were tested for departure from zero by the test developed by Li & Horvitz (1953) using the computer program 'Theta' (Ellis 1994). *F* *ir*, *F* *is,* and *F* *is* estimates were calculated according to Weir & Cockerham's (1984)
as $F$, $f$, and $\theta$ respectively, because their method is not based on assumptions concerning numbers of populations, sample sizes, and heterozygote frequencies. A bootstrap procedure with 10000 repeats was employed to estimate the variance of the $F$-statistics (Van Dongen, 1995). Following Slatkin & Barton (1989) negative $F_{ST}$ values must be considered as 0, and indicate that mathematically, that the amount of gene-flow between the respective populations is infinite, i.e., the populations function as one panmictic unit.

Cluster analyses were conducted on Nei's genetic distances (1978) with (1) the unweighted pair-group method with arithmetic averaging (UPGMA procedure in BIOSYS 2, Swofford & Selander, 1997), and (2) the neighbour joining method (Saitou & Nei, 1987) in PHYLIP (Felsenstein, 2002). For the cluster analyses, samples with less than five individuals were left out. The data was tested for recent reductions in effective population size with the computer program BOTTLENECK (Cornuet & Luikart, 1996). This program computes for each population sample and for each locus the distribution of the gene diversity expected from the observed number of alleles, at a given sample size under the assumption of mutation-drift equilibrium. This distribution is obtained through simulating the coalescent process of $n$ genes under three possible mutation models, viz. the infinite allele model, the two-phased model of mutation, and the stepwise mutation model. This enables the computation of the average expected heterozygosity which is compared to the observed gene diversity to establish whether there is a gene diversity excess or deficit at a locus. Once all loci available in a population sample have been processed, three statistical tests are performed for each mutation model (Cornuet and Luikart, 1996), and the allele frequency distribution is established in order to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (due to recent bottlenecks which provoke a mode shift).

*Gene-flow*

Gene-flow between populations was indirectly estimated with both Wright’s $F_{ST}$ (Wright 1931) and the private allele method [a private allele is an allele found in only one subpopulation (Slatkin 1985; Barton & Slatkin 1986)]. Wright (1951) showed that $F_{ST}$ is a useful estimator of gene-flow if $N_m = 1/4[1/(1/ F_{ST})^2]$ (Wright 1951). Frequencies of alleles averaged over the number of populations in which they occur are useful to measure the spatial distribution of alleles in subdivided populations, and are relatively independent of selection and mutation rates (Slatkin 1981). The conditional average frequency of alleles found in only one (sub)population (i.e., a private allele), can be used as a quantitative estimator of
Table 2. Allele frequencies at 15 polymorphic loci in populations of *M. nurag* (n) and *M. jurtina* (j), and intermediates (i). Alleles coded alphabetically according to their relative mobility. Loci not in Hardy Weinberg equilibrium printed in bold. Populations are presented per year, study area and species and coded according to the abbreviations given in Table 1.

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Results obtained from the private allele method are reliable if $0.1 < N_m < 10$, and if the number of private alleles is $>20$ (Slatkin 1985). The relationship between gene-flow and geographical distance was tested by regressing $F_{ST}/(1 - F_{ST})$ estimates to the natural logarithm of geographic distances (Rousset, 1997). The association between genetic and geographic distance was tested with a Mantel test using 1000 random permutations to test for independence between genotype counts and location (Mantel 1967 in Raymond & Rousset 2001). Significance was evaluated with Spearman Rank correlation using GENEPOP (Raymond & Rousset 2001).

**Interspecific genetic differentiation**

The total allozyme dataset was examined for loci with alternatively fixed alleles or non-overlapping variation patterns between *M. nurag* and *M. jurtina*. To quantify spatial and temporal genetic differences, genetic similarity of individuals at the six loci that appeared most distinctive between the two species (i.e., idh-1, idh-2, pep-leu-ala, pgm, gpi, g6pdh) was analysed by a principal coordinate analysis (PCA) following the procedure in Arntzen (2001), in which the presence or absence of each allele at each locus was defined as a separate character state and was assumed to be independent (although in reality it is limited to a maximum of two scores of 1 per locus). Jaccard’s coefficient of association was chosen to represent the genetic similarity between individuals because it considers joint absences to be uninformative (Sneath & Sokal, 1973). This similarity matrix was transformed into a scalar product and subsequently factored. All these calculations were conducted with NTSYS 1.80 (Rohlf 1993).

**Results**

The results are presented in five sections describing in order, general aspects of both species’ genetic variation, temporal variability, spatial variability, genetic differentiation between the two species, and divergence time estimates.

**General aspects of genetic variation**

In total, 76 different alleles were detected at the 15 allozyme loci studied, 63 of which were shared by both species. None of the loci was found to be alternatively fixed between the two species. Neither linkage disequilibrium between loci, nor sex-linkage was detected. A number of private and low-frequency alleles were found across island and continental *M. nurag* and *M. jurtina* populations. Seven alleles were restricted to *M. nurag*, among which five were private alleles (frequencies ranged from 0.022-0.250), and six alleles were restricted to *M. jurtina*,

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among which three were private alleles (0.023-0.071); in M. jurtina one private allele belonged to the Corsican population (0.167), and two to Sardinian (0.023-0.053) populations.

Tests for Hardy-Weinberg equilibrium showed significant deviations in 21 out of 303 tests ($p<0.01$) after the significance level was corrected for experiment-wise error using the Bonferroni procedure; deviations were found at ten loci in one or more populations (Table 2). $F_{st}$-values departed significantly from zero in 13 out of 72 tests for M. nurag, and in 11 out of 73 tests for M. jurtina. $F_{is}$-values departed significantly from zero in 19 of 72 tests for M. nurag, and in 19 of 73 tests for M. jurtina (Table 3). There was no pattern to the heterozygote deficit, neither with respect to population nor to locus (compare also Table 1).

Generally, levels of genetic variation in Sardinian populations (M. jurtina, $H_o=0.137-0.189$; M. nurag, $H_o=0.141-0.270$) were comparable to the mainland M. jurtina ($H_o=0.141-0.236$). The intermediate individuals contained similar levels of genetic variation ($H_o=0.167-0.178$) (Table 1). Mean number of alleles per locus and percentages of polymorphic loci in M. nurag ($A=1.7-3.1$, $P=60-80\%$) were also comparable to M. jurtina ($A=1.5-3.0$, $P=40-80$). No significant effect of genetic bottleneck was detected in the Sardinian populations of M. nurag or M. jurtina (BOTTLENECK, $p > 0.05$).

**Temporal variability in genetic variation**

Temporal variation in heterozygosity ($H_o$), percentage polymorphic loci ($P$), and number of alleles per locus ($A$) is small in both species (Table 1). For all three seasons, total genic diversity, Weir and Cockerman’s (1984) $F_{it}$ (M. nurag: mean $F_{it} = 0.170 - 0.376$; M. jurtina: $F_{it} = 0.213 - 0.343$) in both species is mostly due to within population variation ($F_{is} = 0.163 - 0.325$) and to a lesser extent to among population variation ($F_{st}$) (Table 3). In M. nurag mean $F_{st}$ values ($=\theta$) summarized over the 15 polymorphic loci were similar in the 2000 ($0.052 \pm 0.060$) and 2001 season ($0.061 \pm 0.031$), but much lower in the 2002 season ($0.008 \pm 0.022$). In M. jurtina mean $F_{st}$ values calculated across Europe (season 2000: $0.065 \pm 0.039$), were similar as when calculated within Sardinia (season 2000: $0.065 \pm 0.090$, and season 2001: $0.050 \pm 0.048$). For the 2002 season, $F_{st}$ values could not be calculated for M. jurtina, as only one Sardinian population was analysed. $F_{st}$ values differed significantly from zero in 13 instances for M. nurag, and in 11 instances for M. jurtina. None of these deviations was consistent over loci or years (Table 3).
Spatial variability in genetic variation

$F_{ST}$ values indicate a low degree of spatial differentiation in both species. Correlations between genetic differences and geographic distance were not significant for either species at any spatial scale (Figure 2). Mantel tests show that in both species gene exchange among populations is not dependent upon their geographic location ($M. jurtina$: $p = 0.234$; $M. nurag$: $p = 0.762$).

**Figure 2.** Relationship between geographical distance and gene-flow plotted as $F_{ST}/(1-F_{ST})$ against the natural logarithm of geographic distances (in kilometers) between populations, fitting $F_{ST}/(1-F_{ST})$ to $a+b \ln(\text{distance})$. (A) $M. jurtina$ $a=0.083$, $b=0.001$, Mantel test associated $P=0.234$; (B) $M. nurag$ $a=0.090$, $b=0.004$, $P=0.762$.

Gene-flow

Levels of gene-flow were estimated using two different approaches: (1) the average number of migrants exchanged per generation among populations ($N_m$) on basis of Weir and Cockerham's (1984) $F_{ST}$, (2) Slatkin's (1985) private allele method to estimate $N_m$ (corrected for sample size). Estimates were conducted across different geographic scales, i.e., within Sardinia for $M. nurag$ and $M. jurtina$, and across Europe for $M. jurtina$. $F_{ST}$ based gene-flow ($N_m$) estimates reached similar level in both species, regardless if they were calculated across Sardinia ($N_m = 3.6 - 4.6$) or across Europe ($N_m = 3.6$) (Table 3). Private allele based estimates ranged from 0.88 - 3.13 for $M. nurag$ and from 0.88 - 1.64 for $M. jurtina$ (Table 4).

Genetic differentiation between the two species

Populations clustered into two main groups when plotted in a UPGMA phenogram, based on Nei's (1978) unbiased genetic distances ($D$) (Figure 3a). The average genetic distance between $M. nurag$ and $M. jurtina$ is $D=0.21$. The intermediate individuals split into a separate group on the basis of the first two groups. The $M. jurtina$ samples 'Sette Fratelli 2000' appeared separated, and the $M.$
Table 3. Estimates of Weir & Cockerham's F-statistics at 15 polymorphic loci summarized over populations of *M. nurag* and *M. jurtina* sampled per year, and mean estimated gene-flow level (*N_m*). Means are based on a bootstrap of 10,000 repeats and given with standard deviation (SD). Asterisks indicate significant departure from zero.

<table>
<thead>
<tr>
<th>Year</th>
<th>M. nurag 2000</th>
<th>M. jurtina 2000</th>
<th>M. jurtina 2001</th>
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<td><em>F_s</em></td>
<td><em>F_it</em></td>
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<td>0.20</td>
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<td>2003</td>
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* *p < 0.05
** *p < 0.001
*** *p < 0.0001

*M. jurtina* samples 'Monte Eccas 2001' clustered with the *M. nurag* group; both come from sites where the two species are sympatric. The neighbour-joining diagram had similar topologies as the UPGMA phenogram, and showed the same split into a *M. nurag*, a *M. jurtina* group, and an intermediate group (Figure 3b).
Table 4. $N_m$ values (corrected for small sample size) based on the private allele method (Barton & Slatkin, 1986). Mean sample size ($n$) and estimates ($N_m$) are presented for each year at different geographic scales: within Sardinia, Sardinia & Corsica, and Europe.

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<th>year</th>
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<td>8.6</td>
<td>1.27</td>
<td>Sardinia+Corsica</td>
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<td>12.3</td>
<td>0.88</td>
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<td>2001</td>
<td>9.32</td>
<td>1.42</td>
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<td>2002</td>
<td>7.85</td>
<td>1.33</td>
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Geographic patterns in allele distribution

At some loci there was a geographic pattern to the frequency distribution of alleles (Figure 4): Pgm-a was limited to M. nurag (0.029-0.083), the intermediate group (0.056-0.125), and the M. jurtina ‘Monte Eccas 2001’ population (0.083) (Figure 4a); pgm-g was restricted to four M. nurag populations (0.029 - 0.069); pep-leu-alac was found in two M. jurtina populations (0.167-0.227) and some intermediate individuals (Figure 4b); aat-1-d was restricted to four M. jurtina populations (0.071-0.167); idh-1-a, aat-1-b were limited to Sardinian populations and did not occur on the mainland. G6pdh-b was fixed or almost fixed in most Sardinian M. jurtina populations (0.977 - 1.000) and overall common in all ‘pure’ populations of this species (0.500 - 0.952), whereas it occurred in lower frequencies in M. nurag (0.250 - 0.625) but was more common in the intermediate form (0.438-0.813); g6pdh-c was rare in M. jurtina (0.045 – 0.286), more frequent in M. nurag (0.350 – 0.625), and the intermediate form had intermediate levels of this allele (0.188 – 0.438).

Hybrizymes

Other alleles showed clines in frequency from the mainland M. jurtina, over the Sardinian M. jurtina to M. nurag, with intermediate individuals at intermediate frequency levels (Figure 5; only populations with $n>5$ were included in Figure 5), and we consider them potential hybrizymes. Me-b was absent or rare in the
Figure 3. (A) UPGMA diagram of Nei’s genetic distance $D$ (Nei, 1978) for $M. \text{nurag}$ ('$N$') and $M. \text{jurtina}$ ('$J$') samples from different geographic areas and years with $n > 5$ individuals. (Cophenetic correlation = 0.88). Population’s names as in Table 1. (B) Neighbour-joining tree with the same set of samples.
pep-leu-ala

4B

pep-leu-ala

4A

pgm

- other

158
Figure 4. Allele frequencies for M. nurag and M. jurtina in Sardinian samples from 2000-2002 at three allozyme loci; (A) pgm, (B) pep-leu-ala, (C) g6pdh. Numbers refer to populations listed in Table 1. White circles represent M. jurtina, grey circles M. nurag. On the map, areas where only M. jurtina was found are white, areas where only M. nurag was found are shaded dark-grey, areas of the suggested hybrid zone are shaded in light-grey.

continental M. jurtina (0 - 0.091), slightly more frequent in the Sardinian M. jurtina (0.125), and most frequent in the intermediate group (0.389). Pgm-b was very rare or absent in all mainland populations of M. jurtina (0 - 0.091) except population ‘Amsterdam’, but considerably more common in M. nurag populations (0.188 - 0.591), with intermediate levels in the intermediate group (0.188 - 0.333). 6pgdh-c was absent or rare in M. jurtina (0 - 0.068), higher frequent in M. nurag (0.119 - 0.200) and still more frequent in the intermediates (0.125 - 0.498). 6pgdh-d was lower frequent on the continent (0.076 - 0.227) than in the Sardinian populations (0.135 - 0.571) and the intermediate group (0.498). G6pdh-a was absent in ‘pure’ M. nurag populations, but more frequent at the sympatric sites (0.714), and low frequent in the continental M. jurtina (0 - 0.214).

Similarity matrix of allozymes
Character loadings of the first- and second axis of a PCA analysis derived from individual genetic profiles at the six allozyme loci, that appeared to be most distinctive between the two species, and contained a high amount of variation (viz.,
Figure 5. Allele frequencies for *M. nurag* and *M. jurtina* samples with $n > 5$ from Sardinia and Europe in the years 2000-2002 at four allozyme loci: *me*, *pgm*, *6pdh*, and *g6pdh*.

Figure 6. Bivariate scatterplot of the character loadings from the first and the second PCA-axes derived from Jaccard’s coefficient of association of individual allozyme profiles of the 6 most differentiating loci (*idh-1*, *idh-2*, *pep-leu-ala*, *pgm*, *gpi*, *g6pdh*); included only individuals where all loci were analysed. Species membership is indicated by outline polygons: dashed line for *M. nurag*, solid line for *M. jurtina*. Individuals of the intermediate type are indicated with open circles.
*idh-1, idh-2, pep-leu-ala, pgm, gpi, and g6pdh*), described no clear clustering of data points (Figure 6). The horizontal axis (PCA-2) provides a separation into a *M. nurag* and a *M. jurtina* group, with a zone of overlap. The 'intermediate' individuals are positioned partly in this zone of overlap, partly outside the two main groups.

**Divergence times**

Assuming a clockwise behaviour of evolutionary rates for proteins (Thorpe 1982, and references therein, Marchi *et al.*, 1996), we estimated species divergence times from genetic distance values as approximately 3.6 - 1 million years ago (ma), depending on the calibration used, with Nei's *D* = 18 ma (Thorpe 1982), or *D* = 5 ma (Nei, 1972).

**Discussion**

*Maniola* *nurag* and *M. jurtina* are closely related species. This is reflected in the few species-specific alleles, and the absence of diagnostic loci. Sixty-three of the alleles we detected in our analysis were shared by both species. This genetic similarity between the two species was also underpinned by the results obtained through the cluster and PCA analyses (Figures 5 and 6). In both clustering methods, UPGMA and Neighbour-joining, the samples were separated into a *M. jurtina*, a *M. nurag* group, with the intermediate individuals apart. The separation of the 'Sette Fratelli 2000' sample from their respective conspecifics could be an artefact due to missing data at certain loci (Swofford *et al.*, 1996), as another sample from 'Sette Fratelli' from a different year clustered well within the *M. jurtina* group.

The island populations of both species have equally high genetic variation as the continental populations of *M. jurtina*. This finding is surprising, as island populations (Frankham, 1997), and isolated populations in general (Cassel & Tammaru, 2003) frequently have lower genetic variation in comparison to mainland populations (for review see: Frankham, 1997). In this review covering a variety of taxa, including mammals, birds, plants, and arthropods, Frankham (1997) found that a significant majority of island species had lower levels of genetic variation than related mainland species (80%), and that insular endemic species showed less genetic variation than related non-endemic species (89%). Such a pattern was also found in a study on another endemic Sardinian butterfly, *Polyommatus coridon gennargenti* (Lycaenidae) (Marchi *et al.*, 1996). These authors detected severe inbreeding in the endemic lycaenid, low levels of genetic variation and a low proportion of polymorphic loci (*P* = 17.6 %, *H* = 0.024) in comparison to its continental relatives (*P* = 58.8 %, *H* = 0.185). In contrast to these findings, for the
endemic Maniola nurag we found equally high levels of genetic variation ($H=0.141-0.270$) as in M. jurtina from the mainland ($H=0.141-0.236$). Why does this endemic butterfly have such a high genetic variability? Dispersal ability is an important factor increasing variation in island species (Frankham, 1997), as it increases the effective size of the population, and therefore counteracts drift and thus slows down the march to homozygosity. In a three-year field study on M. nurag (Grill et al., 2003b), it was concluded that butterflies disperse regularly over distances up to 2 km, and that there is a substantial amount of dispersal between neighbouring populations. The findings from mark-release-recapture experiments are also well in agreement with our estimates of gene-flow.

If we calculate the heterozygosity values expected for neutral alleles (Kimura & Crow, 1964) following $H=4N_e\mu/1+4N_e\mu$), assuming that the species has a total population size of 50 000 individuals on the whole island (derived from an estimated number of 300 - 1000 individuals per population as inferred in Grill et al., 2003b), we obtain a similar heterozygosity level ($H=0.09$), like we found in our data (0.141-0.270), considering that we only used polymorphic loci for the analysis.

Seven percent of the tests for Hardy-Weinberg equilibrium revealed significant deviations from equilibrium. All deviations were caused by a heterozygote deficit. Deviations were detected at several loci in different populations, but there was no apparent pattern to them, i.e., they were neither locus specific, nor did they occur consistently at all loci of a certain population. Deviations from Hardy-Weinberg equilibrium are often found in butterfly populations (Nève et al., 2000, E. Meglécz pers. comm.), and do not always have a straightforward explanation. In the studied Maniola populations, they might be due to the Wahlund-effect, i.e., pooling of different populations, which are by themselves in Hardy-Weinberg equilibrium (Wahlund 1928). Apart from the Wahlund-effect, other possible reasons for deviating from Hardy Weinberg equilibrium are recent immigration having not yet reached equilibrium, inbreeding, underdominance, sex-linkage or null alleles. Sex-linkage was not present; inbreeding can be excluded because it would influence all loci at the same time, whereas underdominance and null alleles are locus specific, and can thus also be excluded.

The intermediate form - a hybrid?
The individuals that we a priori defined as ‘intermediates’ according to wing patterns, appear in the PCA scatterplot (Figure 6) partly as a non-overlapping group outside the overlapping clusters of the ‘pure’ M. jurtina and M. nurag, and partly in the zone where both species overlap. The intermediate individuals also appear
as a separate branch in the phenograms obtained by UPGMA or Neighbour-joined clustering. Results of both analyses reflect that the allele frequency distribution in the intermediate group is distinct from *M. nurag* as well as *M. jurtina*. In the zone of overlap, together with the phenotypic ‘intermediates’, cluster a majority of the individuals from the ‘Sette Fratelli’ and ‘Monte Eccas’ populations situated in the Southern part of Sardinia.

The ‘Sette fratelli’ population has been identified as one of the sites in Sardinia, where both species occur sympatrically in stable populations, *i.e.*, are present in similar abundances every year (Grill *et al.*, 2003b). Ecological similarity of these sympatric populations suggests that hybridisation of the two species is conceivable. Genital preparations revealed no apparent anatomical pre-mating barriers prohibiting hybridisation (Grill *et al.*, 2003c). Interestingly, two individuals with intermediate wing patterns, that were found to have also intermediate genitalia characteristics (Grill *et al.*, 2003c), had a *pgm-a* allele, which was normally only found in *M. nurag*. Thus, they can not be ‘pure’ *M. jurtina*; what is more, they contained a *me-b* allele, one of the potential hybrizymes. At four other loci, we find a cline in frequency towards the intermediate individuals (Figure 5). *Me-b*, for example, is absent in most continental *M. jurtina* populations, very low frequent in the French and the Sardinian lowland populations, but very well represented in the intermediate individuals (0.389), and it is also present at the locality, where both species occur sympatrically. In *pgm-b* we observe a cline from ‘pure’ *M. jurtina* (0 - 0.091) towards Sardinian *M. jurtina*, intermediates (0.333), and finally *M. nurag*, with highest frequencies in the last mentioned (0.591). *G6pdh-c* and *g6pdh-d* show a similar cline towards intermediate individuals and the sympatric populations; and likewise does *g6pdh-a* (Figure 5). These patterns look very similar to what has been observed by Schilthuizen & Gittenberger (1994) in snails, where a so called ‘hybrizyme’ allele had a very low frequency outside the hybridzone, and peaked in its centre. Low frequency *F₁* hybrids between an endemic and a widespread butterfly species paralleled with first generation backcrosses have been detected between the Sardo-Corsican endemic *Papilio hospiton* and the holarctic *P. machaon* (Cianchi *et al.*, 2003). Based on the data discussed here, we would anticipate a similar scenario for *M. nurag* and *M. jurtina.*
Speciation

Vicariance and subsequent dispersal

In his study of British populations of *M. jurtina*, Goulson (1993) found little genetic variation, with only 4 out of 12 allozyme loci to be polymorphic; at the same four polymorphic loci, we found a considerably greater amount of variation for our samples. He further observed that in Great Britain geographically distant populations differed very little in gene frequencies. Similarly to Sardinian and Central and Western European populations, also British *M. jurtina* population structure did not follow an isolation by distance pattern. As island size generally correlates with genetic diversity (Malone et al. 2003), and England is considerably larger than Sardinia, and also closer to the continent, this result is contrary to expectation. An explanation might be, that in Britain, *M. jurtina* approaches the end of its range, and the reduced genetic variability results hereof.

This comparison with the British *M. jurtina* once more illustrates, the rather high genetic variability in Sardinian Maniola, which is also the main evidence against an allopatric, vicariant speciation event of *M. nurag* in Sardinia, followed by a later invasion of continental *M. jurtina*. If *M. jurtina* had colonized the island relatively recently, we would expect a sign of a founder-event (genetic bottleneck) in our data. As our genotype data from *M. jurtina* did not show traces of bottlenecks, we conclude that it is unlikely that the widespread species has colonized Sardinia only recently, unless the colonizing population was large, would have experienced rapid growth directly after the founder-event, or continuous immigration from the mainland has taken place.

Another, theoretical consideration against the hypothesis that *M. nurag* has differentiated in allopatry, arises from a comparison with the distribution areas of the two other island endemics in this genus: the Greek endemic *M. chia* and the Cypriote endemic *M. cypricola* have no distributional overlap with other Maniola species, although their respective islands are much closer to the mainland than Sardinia is to Italy. Phenotypically, these species are even more similar to *M. jurtina* and *M. telenessia* respectively, than *M. nurag* is to *M. jurtina*. Consequently, in these two endemics are likely to be the result of vicariance or dispersal. In *M. nurag* on the other hand, the distributional overlap with *M. jurtina*, leaves room for speculations on a sympatric mode of speciation.
Sympatric differentiation

Recently diverged taxa often differ very conspicuously in secondary sexual characteristics, which indicate the action of sexual selection on phenotypes involved in mate recognition (Darwin 1879, Shaw & Parsons 2002). This is probably also the case in *M. nurag*. However, the extent of morphological diversification does not necessarily reflect the extent of ecological radiation (Strauss 1984). In the case of *M. jurtina* and *M. nurag*, evidence from field data suggests that the two species respond to slightly different ecological gradients (Grill et al., 2003c). We assume that similar small ecological diversification could originally have initiated differentiation along an environmental cline, a scenario increasingly receives attention (Ogden & Thorpe 2002, Doebeli & Dieckmann 2003). *Maniola nurag*, which today is restricted to areas above 500 meters a.s.l., would have evolved into a mountain species, very well adapted to the particular conditions on Mediterranean mountains, with extreme aridity in summer, relatively cold winters, and extreme temperature shifts between day and night in spring and autumn. *Maniola jurtina* on the other hand remained predominantly in the lowlands. The occasional hybridisation we suspect to happen between the two species, could be either the result of secondary contact and have arisen in situ, similar to what has been suggested for *P. hospiton* (Cianchi et al., 2003), or it indicates that the speciation process between *M. nurag* and *M. jurtina* is still going on.

Relict species

During the last glacial maximum, many plant and animal species that were adapted to warmer climates retracted to Mediterranean refugia in southern Europe. At the end of the glaciation period, strong climatic fluctuations took place, with rapid switches between warm and cold periods, leading to major changes in the distribution and composition of the European flora and fauna (Hewitt 1999). Some species with a formerly larger distribution area continued to persist only as a relict species in a relatively small area. *Boloria aquilionaris* (Nymphalidae) is an arctic-alpine example for such a relict (Baguette & Schtickzelle, 2003).

Populations isolated in refugia during the last glaciation usually exhibit reductions in heterozygosity and genetic variation (Broders et al. 1999, Malone et al. 2003) as a consequence of the bottleneck they passed when populations became isolated. If *M. nurag* also was a relict species, we again would expect a reduction of genetic diversity, as a rapid severe reduction in distribution area and numbers of individuals due to climatic changes, is analogous to a severe bottleneck. In such a scenario, we might also expect low numbers of private alleles. Usually, mostly the common alleles survive in bottlenecked populations, as happened in the endemic
Polyommatus coridon gennargenti (Marchi et al., 1996). Maniola nurag on the contrary possesses a similar number of private alleles as the widespread species. Another argument against the relict-hypothesis is, that Tyrrenian relics are usually scattered over several islands (Kleinekuhle, 1999; Médail & Quézel, 1999), while M. nurag is restricted to Sardinia.

**Divergence time estimates**

Despite the continuing controversy about molecular clocks (Arbogast et al., 2002), we used a ‘sloppy’ protein-based molecular clock to obtain some rough estimates of divergence times. For the split of M. nurag from M. jurtina from a common ancestor, we estimate a divergence time, of 3.6 – 1 million years, and thus falls into the late Pliocene. These estimates happen to coincide with the major biotic turnover inferred for this period (3 – 1.8 Ma ago) (Vrba 1985, Vrba 1992); significant faunal change and increased speciation events appear to have happened 1.8 – 2.5 ma ago as a result of major climate changes toward a cooler, drier, and more variable climate. This massive species turnover has been viewed as part of the ‘turnover pulse’ hypothesis, which postulates that climate change results in brief periods of significant evolutionary change (Vrba 1985, Vrba 1992, Behrensmeyer et al., 1997).

For example, the late Pliocene radiation of hominid species, and ultimately even the emergence of the genus Homo have been attributed to such global climatic events (de Menocal 1995). In the Mediterranean region, this climate change forced warm and humid subtropical forests to gradually change into a savanna-like vegetation (LaGreca 1998). Caccone & Sbordoni (2001) attribute the differentiation of endemic Sardinian cave beetles in the genus Patriziella to Pliocene climate changes. Our own divergence time estimates for M. nurag and M. jurtina are congruent with the estimates presented by Marchi et al. (1996) for the Sardinian endemic lycaenid butterfly Polyommatus coridon gennargenti. As Marchi et al. (1996) used the same calibrations we used (i.e., Nei’s $D = 18$ ma, or $D = 5$ ma), our estimates are directly comparable with theirs. Calculations of divergence times for other Sardinian taxa (references in Grill et al., 2003a), show that the split of the Sardinian lineage has occurred after the Messinian crisis ($\pm 5$ ma), when marine regressions led to a desiccation of the Mediterranean sea (Steininger & Rögl, 1984), and after the continuing marine regressions from late Miocene to Pliocene (Arias et al. 1980; Cita 1976) that brought Sardinia in contact with continental Italy and southern France (5.7 – 2 Ma ago) (Grill et al., 2003a). During the last glacial maximum, 20 000 years ago, Sardinia was connected with Corsica for the last time. The last glacial maximum is the very period, when M. jurtina differentiated into a western and an
eastern group from two disjunct glacial refugia (Schmitt, 1999), analogous to what has been shown for the bear, U. arctos (Taberlet & Bouvet, 1994). Notably, g6pdh, where we found a potential hybrizyme, was also one of the two loci indicating ice-age induced genetic differentiation in Schmitt’s study. In Schmitt’s (1999) scenario, the Sardinian M. jurtina would belong to the western lineage. Obviously, allozymes can only provide very approximative estimations, but could be useful for relative comparisons with other Sardinian taxa. A more precise dating of the speciation event of M. nurag awaits a phylogenetic analysis based on a combination of DNA sequences and wing pattern characteristics, and would entail the verification of the sympatric speciation hypothesis we proposed here.

Acknowledgments
We thank Léon E.L. Rajmann for constant support, statistical and technical advice, and many insightful discussions; Steven Weiss for linguistic improvements; Pim Arntzen and Peter Roessingh for critical reading of an earlier version of this manuscript.
Part 4

MORPHOLOGY
VIII.
The shape of endemics:
Notes on male and female genitalia in the genus *Maniola* (Schränk, 1801), (Lepidoptera, Nymphalidae, Satyrinae)

with Rob de Vos & Jan van Arkel

Contributions to Zoology (accepted with minor modifications)
Abstract
Butterflies of the genus *Maniola* are known for their large morphological variation, at the inter- as well as intraspecific level. Given the overlap in wing-patterns, habitat selection, and geographic distribution of various *Maniola* species, genitalia morphology is sometimes the only possibility to tell specimen apart. In this paper we describe diagnostic characters to distinguish different *Maniola* species by means of their genitalia. Included is also the first detailed description and illustration of the genital apparatus of the Sardinian endemic *Maniola nurag*. Further, we describe two Sardinian individuals with intermediate characteristics between *M. nurag* and *M. jurtina*, and propose that they are hybrid forms. In the end, we shortly discuss the justification of the species status for the island endemics *M. chia* and *M. cypricola*.

Introduction

"Made up of concave and convex hills and valleys", was one of the first descriptions of the genital structure of a male Meadow Brown, *Maniola jurtina* (L.), meant to emphasize that this species' genitalia are very irregularly shaped for a Satyrid (Muschamp, 1915). Since then, geographic variation in genital morphology of the Meadow Brown has been extensively discussed (Thomson, 1973, 1976; Goulson, 1993). In recent decades, two new *Maniola* species have been described (Thomson, 1987, 1990). First, the island endemic, *Maniola chia* THOMSON, 1987, whose distribution is restricted to the Greek island of Chios. Second, *Maniola halicarnassus* THOMSON, 1990, which flies on the Bodrum peninsula (Turkey) and the Aegean island of Nissiros. *Maniola nurag* (GHIJANI, 1852) is endemic to Sardinia, and a third endemic has been described from the island of Cyprus, *Maniola cypricola* (GRAVES, 1928) (for distribution areas of species see Figure 1).

*Maniola megala* (OBERTHÜR, 1909) occurs on the Greek island Lesbos, throughout southern Turkey and in Iran. Although neighbouring islands would be in flight distance for all island endemics, the ranges of the island-*Maniola* species are well confined to the borders of the respective island. In Chios, *M. chia* entirely replaces *M. jurtina* and *Maniola telnnessia* (ZELLER, 1847), species that are commonly found on the neighbouring islands and the Turkish mainland, which is only a few kilometers distant from Chios. In Sardinia, on the other hand, *M. nurag* flies sympatrically with *M. jurtina*. Although, the latter species is usually concentrated on the coast, whereas the Sardinian endemic has its distributional centres in the mountain areas of the island (> 500
m), there is a zone of overlap at intermediate altitudes (500 - 900 m), where both species fly contemporarily at the same sites (Grill et al., 2003).

Butterflies of the genus *Maniola* are known for their large morphological variation (Figure 2), at inter- as well as intraspecific level, on both local and continental scale (Ford, 1945; Thomson, 1973). Given the overlap in wing-patterns, habitat selection, and geographic distribution of various *Maniola* species, genitalia morphology is sometimes the only possibility to tell specimens apart. What is more, genitalia shapes can also much vary within a single species (Thomson, 1973). Nevertheless, the species status of *M. chia* and *M. cypricola* has been justified mainly because of differences in the form of the male genitalia; in wing-patterns they resemble *M. jurtina* and *M. telmessia*, respectively. For the third endemic species in this genus, *M. nurag*, genitalia structure and shape has never been described and illustrated in detail as yet.

In this paper, the genital apparatus of *M. nurag* is described and illustrated for the first time. We further describe two Sardinian individuals, whose genitalia seem to be intermediate between *M. nurag* and *M. jurtina*. The genitalia morphology of these Sardinian specimens is compared to the shape and structure of the genital organs in all other *Maniola* species, except *M. megala*, as this species can be unequivocally distinguished from its congeners by its appreciably larger size, and the wing underside markings.

Ergo, the three main questions we address in this paper are:

1. Are there diagnostic characters in the genitalia of the different *Maniola* species?
2. What is the status of the Sardinian intermediate individuals in the genus *Maniola*?
3. Is species status justified for *M. chia* and *M. cypricola*?

**Material and methods**

In May 2002 we collected a series of males and females of *M. nurag* (5 males, 3 females) and *M. jurtina* (3 males, 3 females) from Sardinia (Italy), in July 2002 *M. jurtina* (3 males, 3 females) from Amsterdam (The Netherlands), and in September of the same year *M. chia* (1 male, 3 females) from Chios (Greece). These specimens were compared with specimens of *M. telmessia* (2 males, 2 females) and *M. halicarnassus* (2 males, 2 females) collected by H. van Oorschot in Turkey, present
Figure 2. Variation in wing pattern in the genus *Maniola*. All the specimens are in the collection of the Zoological Museum Amsterdam.

Figure 1. Distribution areas of six European species of the genus *Maniola*: (A) *Maniola halicarnassus*, (B) *M. telmessia*, (C) *M. nurag*, (D) *M. chia*, (E) *M. cypricola*, and (F) *M. jurtina*.

in the Zoological Museum Amsterdam. Two of the *M. nurag* we dissected could not be unequivocally attributed to *M. nurag*; according to wing-pattern they could be a light, small *M. jurtina* as well as a dark *M. nurag*. Small sample sizes are sufficient, as this study aims at a qualitative and not quantitative description of characters. The butterflies were conserved dry or frozen until preparation. Butterflies were identified using characteristics in their wing-patterns following Van Oorschot & Van den Brink (1992) and Tolman & Lewington (1997). All individuals studied are in the collection of the Zoological Museum, Amsterdam.

Dissection and photography
Prior to dissection, the abdomen of each specimen was separated from the rest of the animal and soaked in potassiumhydroxide (10%) for approximately 15 hours. The abdomen was then put into ethanol (30%), and all the soft material (fat,
content of intestine, body fluids) was squeezed out of the abdomen with a small brush. A cut was made between the 8th and the 9th segment, and the genitalia were gently pulled out, in males they were left connected to the 9th and 10th segment. Subsequently, the genitalia were thoroughly cleaned with a bird’s feather. All scales were removed in order to clearly see the structures of the genital armature.

In order to photograph the form of the signa in the bursa copulatrix, which may show important distinctive characteristics between species, the female genitalia were dyed with chlorazolblack. The dye was fixed in 95% ethanol. For handling, the genitalia were kept in 30% ethanol, as in stronger concentrations of ethanol the chitine hardens and breaks easily. For long term conservation the genitalia will be transferred to a glycerol-tube or Euparol slide. To stabilize the samples for photography, they were positioned laterally in a small drop of ethanol (30%), flattened between two glass lids and photographed under the microscope (magnification x 25).

Traditionally, mostly male genitalia are used for differentiation between species, whose separation is difficult on basis of external characters. In males, differences in structure, shape, and size of genitalia are more pronounced than in females. The general anatomy of the male genital apparatus is common to all nymphalid butterflies; diagnostic differences between species can be established in the structures we describe below.

**General structure of the male genitalia (Figure 3)**

The male genital apparatus comprises the strongly sclerotised and modified 9th and 10th abdominal segments; it consists of the aedeagus, which corresponds to the penis, and is usually located between the paired valvae, which clasp the female abdomen during copulation (Corbet & Pendlebury, 1992). The dorsal tegumen is distally joined to the uncus, from which it is sometimes separated by a strong membrane, termed fenestrulla. The strongly chitinised uncus is capable of up-and-down movement in many groups and seems to assist in grasping the female during copulation. The gnathos arises from the ventral side of the junction of tegumen and uncus. The tegumen is fused with the ring-like vinculum which is ventrally extended to form a bulbous structure, termed the saccus. The paired valvae are joined to the vinculum and consist of more or less flattened sacs, with a proximal opening through which the distal ends of the muscles which displace them are attached. The distal portion of the aedeagus contains an eversible, sac-like membranous component called vesica, sometimes equipped with chitinised spines.
Figure 3. Lateral view of the male genitalia of a *Maniola* butterfly (generalized).

Figure 4. Lateral view of the female genitalia of a *Maniola* butterfly (generalized).
or plates, called *cornuti*, whose shape can be of diagnostic significance (Corbet & Pendlebury, 1992). The *Julien organ* are transformed scent scales, which appear as rod-shaped structures at the end of the last segment of the abdomen; they are only present in a few species.

**General structure of the female genitalia (Figure 4)**
In females, the shape and structure of the *bursa copulatrix*, the *ostiium bursae*, and the *ovipositor lobes* can be of diagnostic value. In some species, the *bursa copulatrix* has sclerotised areas on the walls, termed *signa*, that may be formed like spines, teeth, or a plate, and vary from species to species. If the *bursa copulatrix* contains a flask-shaped structure, this is the male spermatophore. In this study, we eliminated the spermatophores, if present, for photography.

**Results**
In males, the main distinctive characters between *Maniola* species are the shape of the valvae, the gnathos, and the strength and size of the *Julien organ* (Table 1, Figure 5). There is also some variation in the aedeagus, but it is difficult to use this as a character, as it has a rather soft structure and changes its shape according to the angle from where you look at it. The male genital apparatus also varies in size between species. Among the individuals we studied, *M. jurtina* has the largest and *M. telmessia* the smallest genitalia. In females, we found differences in the shape of the ovipositor lobes and the length of the ductus bursae (Figure 6). In all species studied, except *M. jurtina*, the surface of the *bursa copulatrix* contains two *signa* that consist of spine-like sclerotised structures (Figure 7).

**Maniola jurtina** (Figure 5A)
Male: Gnathos markedly swollen at the base, than quickly narrowing. Valvae bigger than all other species except *M. halicarnassus*, in shape most similar to *M. nurag*, but with a characteristic curve towards the distal process; distal and dorsal process round; ventral edge different from the other *Maniola* species, most similar to *M. nurag*. *Julien organ* always clearly visible, very thick and strong, can be twice the size as in congeners.

Female: Length of ductus bursae comparable to the other species (Figure 6A); notably in none of the dissected females of this species we found *signa*, although they were clearly visible in all female individuals of the other species. The absence of these marks might be a good distinctive characteristic between *M. jurtina* and the other species in the genus *Maniola.*
Table 1. Comparative listing of characters to differentiate different *Maniola* species on the basis of the male genitalia.

<table>
<thead>
<tr>
<th>character</th>
<th><em>M. jurtina</em></th>
<th><em>M. nurig</em></th>
<th><em>M. chu</em></th>
<th><em>M. telmessia</em></th>
<th><em>M. halicarnassus</em></th>
<th><em>M. cypricola</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>gnathos</td>
<td>markedly swollen at base.</td>
<td>swollen at base, gradually narrowing</td>
<td>slightly swollen at base, less than in nurig</td>
<td>slightly swollen at base, smaller than in other <em>Maniola</em></td>
<td>thick at base, but gradually narrowing</td>
<td>swollen at base, gradually narrowing</td>
</tr>
<tr>
<td>valvae</td>
<td>larger than all other <em>Maniola</em> with characteristic curve towards dorsal process</td>
<td>smaller than in <em>jurtina</em>, larger than in <em>telemessia</em></td>
<td>larger than in <em>telemessia</em>, comparable to <em>jurtina</em> in size</td>
<td>similar to <em>chu</em></td>
<td>large, comparable to <em>jurtina</em> in size</td>
<td>small, comparable to <em>telemessia</em></td>
</tr>
<tr>
<td>dorsal process</td>
<td>round</td>
<td>round, flatter than <em>jurtina</em></td>
<td>wide and flatter than <em>jurtina</em></td>
<td>pointed</td>
<td>sharply rounded, not pointed</td>
<td>sharply rounded, considerably longer than in all others</td>
</tr>
<tr>
<td>distal process</td>
<td>round</td>
<td>clearly pointed</td>
<td>pointed</td>
<td>pointed</td>
<td>pointed</td>
<td>clearly pointed</td>
</tr>
<tr>
<td>ventral edge</td>
<td>begins flat, than curved</td>
<td>begins flat, than curved</td>
<td>curved</td>
<td>curved</td>
<td>begins flat, curves in pointed angle, than continues flat</td>
<td></td>
</tr>
<tr>
<td>Julien organ</td>
<td>thick and strong</td>
<td>more fragile than in <em>jurtina</em> stronger than in <em>telemessia</em></td>
<td>present, more fragile than in <em>jurtina</em></td>
<td>very fragile, often broken off</td>
<td>fragile, but stronger than in <em>telemessia</em></td>
<td>more fragile than in <em>jurtina</em></td>
</tr>
</tbody>
</table>

Maniola telmessia (Figure 5B)
Male: Genital apparatus clearly smaller than in all other Maniola. Gnathos similar to M. chia, slightly swollen at the base, vesica round. Valvae similar in shape to M. chia and M. halicarnassus; distal process pointed, similar to M. chia and M. nurag, dorsal process almost pointed. Ventral edge similar to M. chia and M. halicarnassus, clearly different from M. jurtina. Julien organ present but very fragile. In M. telmessia the Julien organ often brakes off, and in earlier literature it was considered to be lacking (see references in Thomson, 1973).
Female: Ductus bursae similar to M. nurag and M. chia (Figure 6B); signa clearly visible, short, pointed at the posterior end, broadening at the anterior end (Figure 7A).

Maniola nurag (Figure 5C)
Male: Gnathos substantially swollen at the base, vesica round at its extremity. Valvae considerably smaller than in M. jurtina but larger than in M. telmessia. Dorsal process clearly visible, round, flatter than in M. jurtina; distal process clearly pointed, sharper than in M. jurtina; ventral edge curved, similar to M. jurtina. Julien organ present, more fragile than in M. jurtina, but stronger than in M. telmessia.
Female: Ductus bursae similar to other Maniola species (Figure 6C); Bursa with two elongated signa, which vary considerably in length and visibility (Figure 7B).

Maniola chia (Figure 5D)
Male: Gnathos slightly swollen at the base, but less than in M. nurag, shape and size of aedeagus similar to M. nurag. Valvae larger than in M. telmessia, comparable to those of jurtina in size, but not in shape; distal process pointed similar to M. telmessia and M. nurag, dorsal process wider and flatter than in M. jurtina and M. nurag, slightly pointed. Ventral edge differently curved than in M. jurtina, similar to M. telmessia. Julien organ more delicate than in M. jurtina but thicker than in M. telmessia, comparable to M. nurag.
Female: Ductus bursae relatively short, notably shorter than in M. halicarnassus (Figure 6D); Bursa in all individuals with two crescent-formed signa, clearly visible, but shorter than in M. nurag, length comparable to the other three species (Figure 7C).

Maniola halicarnassus (Figure 5E)
Male: Gnathos thicker at the base but gradually narrowing towards the end. Valvae of similar size as in M. jurtina, but different in shape; distal process pointed, dorsal process sharply rounded but not pointed, connection between distal and...
dorsal process straighter than in other species. Ventral edge similar to telmessia.
Julien organ thinner than in *M. jurtina*, *M. nurag* and *M. chia*, but stronger than in *M. telmessia*.
Female: Ductus bursae slightly longer than in the other species (Figure 6E); signa variable, but clearly visible, pointed towards the posterior end, broadening towards the anterior end (Figure 7D).

*Maniola cypricola* (Figure 5F)
Male: Gnathos swollen at base, gradually narrowing. Valvae small, comparable to *telmessia* in size and shape, line towards distal process straight, as opposed to all other species, where it is slightly curved; distal process clearly pointed, dorsal process sharply rounded, basis considerably longer than in other *Maniola*.
Female: Ductus bursae relatively short (Figure 6F); signa clearly visible, elongated, round at the ends (Figure 7E).

Intermediate form (Figure 5G)
The two individuals with wing-patterns that seemed intermediates between *M. nurag* and *M. jurtina*, were also intermediate in genitalia structure.
Male: Gnathos markedly swollen at base, than quickly narrowing. Valvae larger than in *M. nurag*; distal process pointed like in *M. nurag*, but position like in *M. jurtina*, dorsal process slightly pointed as opposed to the other two species; ventral edge similar to *M. jurtina*. Julien organ thicker, but not as solid as in *M. jurtina*.
We were not able to locate any females of this type.

Discussion & Conclusions

(1) Are there diagnostic characters in the genitalia of the different *Maniola* species?
In females, the most unequivocal characteristic to distinguish *M. jurtina* from the other five species we studied, seems the absence of signa on the female bursa. Signa were present in all studied individuals *M. nurag*, *M. chia*, *M. cypricola*, *M. telmessia*, and *M. halicarnassus*, but absent in *M. jurtina*. However, it might be, that this characteristic is just much rarer in *M. jurtina* than in the other species, but still occasionally present (Thomson, pers. comm.). The female genitalia of the other *Maniola* species do not show diagnostic characters. As usual, the main characters to differentiate between species are in the male genital apparatus.
*Maniola jurtina* can be clearly distinguished by shape and size of the valvae and
the Julien organ. *Maniola nurag* is generally well recognisable by the form of its valvae. *Maniola telmessia* is distinctive by its smaller size, the outline of the valvae and the shape of distal and dorsal processes. In *Maniola halicarnassus* the form of the dorsal process as well as the connection between dorsal and distal process are distinctive. Diagnosis is further facilitated through the wing-patterns, which additionally to genitalia structure, differentiate this species from the other *Maniola*. More of a problem poses *M. chia*, which is very similar to *M. telmessia* in the shape of its genital apparatus, and almost indistinguishable from *M. jurtina* by its wings. Maybe it is this intermediate position between *M. telmessia* and *M. jurtina*, that can serve as a distinctive characteristic: chia = genitalia like *telmessia* plus wing-pattern of *jurtina*. But obviously, for this species more samples are necessary to obtain a better picture.

Thomson (1973) the species *M. jurtina* into three main types, the eastern, the western, and the primitive type, where in the primitive type the dorsal process has an irregular ‘fringe’, which usually extends to the distal process, in the western type the dorsal process is long with a pointed or very sharply rounded extremity or a short flat top, and in the eastern type the dorsal process is fairly short with a flat or almost flat top. Also the gnathos and the ventral edge vary among the three types. He postulated that the genital apparatus of *M. nurag* and *M. telmessia* were close to the western type he found in *M. jurtina*. At the time his publication appeared, *M. halicarnassus* and *M. chia* were not described yet, and consequently he could not discuss them at the time. According to his ideas, the primitive type individuals in the eastern range of *M. jurtina* are relict elements of the original type of *Maniola*, which were the ancestors of all *Maniola* species we know in Europe today. He found the eastern forms with the primitive valvae in mountain localities from 1500 – 2000 metres a.s.l., and concluded that the Iranian forms of *M. jurtina* are probably the oldest surviving ancestors of this genus, which he considers originally a mountain species. From Iran, he suggests, the butterflies have travelled westwards in two flows, one towards the south, the other one towards the north. Differentiation of the southern migrant groups resulted in what he calls the western type of valvae, and the northern migrants led to the eastern type. *M. telmessia* would be the result of an extreme differentiation of the western type, that had become so different from the ancestors that it resisted a reinvasion of the eastern type *M. jurtina* later on in the areas of what we call Greece and Turkey today, which is why we find *M. telmessia* and *M. jurtina* flying in sympathy in most of their ranges. Thomson (1973) further suggests that *M. nurag* is the furthest development of the *M. jurtina* ancestor. He bases this on the fact that the valvae are purely of western form, and
Figure 5. Male genital apparatus of different Maniola species in comparison. (A) *M. jurtina*, (B) *M. telmessia*, (C) *M. nurag*, (D) *M. chin*, (E) *M. halicarnassus*, (F) *M. cypricola*, and (G) intermediate form (from Sardinia).
Figure 6. Female genital apparatus of different Maniola species in comparison. (A) M. jurtina, (B) M. telnessia, (C) M. nurag, (D) M. chia, (E) M. halicarnassus, and (F) M. cypricola.
Figure 7. Signa in the female bursa copulatrix of (A) *M. telmessia*, (B) *M. nurag*, (C) *M. chia*, (D) *M. halicarnassus*, (E) *M. cypricola*. 
the fulvous of the butterfly is very extensive. Although all this reasoning is very intriguing, and indeed partly convincing, it remains in the realm of conjecture. To answer questions like that, large scale phylogenetic and phylogeographic analysis based on molecular data are indispensable.

The illustrations presented in figure 6 show that based on male genitalia shape, the six species we investigated would fall into two groups: *M. telmessia*, *M. halicarnassus*, *M. chia*, *M. cypricola* on the one hand, and *M. nurag* and *M. jurtina* on the other. This pattern corresponds well to the geographic distribution of these species: the first four are flying in the eastern Mediterranean, the latter two in the western Mediterranean. It also confirms our genetic data on the close genetic relationship of *M. nurag* and *M. jurtina* (Grill, 2001; Grill et al., 2003).

(2) What is the status of the Sardinian intermediate individuals in the genus *Maniola*?

In the two Sardinian individuals with intermediate wing-patterns also the genitalia are of intermediate form; in their contours they resemble *M. jurtina*, but they are smaller and the distal process is pointed like in *M. nurag* and *M. telmessia*. The explanations for this are twofold: (I) These two individuals are hybrids between *M. jurtina* and *M. nurag*, and therefore have intermediate wings as well as genital structure. (II) There is a third form of *Maniola* flying in Sardinia. This intermediate type, however, is clearly more similar to *M. nurag* and *M. jurtina*, than to any of the other *Maniola* species, which makes the hybrid-idea plausible. Considering the similarities in size and structure of the genitalia in *M. jurtina* and *M. nurag*, hybridisation seems physically possible. The new 'intermediate' form we found in Sardinia is another example, for the potential of the genus *Maniola* as an interesting model system to study adaptation and speciation processes.

(3) Is species status justified for *M. chia* and *M. cypricola*?

Considering the intraspecific variation in the male genital apparatus, illustrated by Thomson (1973), in *M. jurtina* from different areas in Europe, the use of genitalia structure to justify species status remains problematic. The characters we give in Table 1 provide guidelines for differentiation between different *Maniola* species, but have to be used in combination with wing characteristics and ecological data of the site where the specimen was collected. The wing-patterns of *M. chia*, for example, resemble those of *M. jurtina* (Figure 2) so closely, that these two species are indistinguishable without taking into account the geographic provenance and male genitalia structure of the specimen. Also *M. cypricola* is phenotypically
extremely similar to *M. telmessia*. On basis of the valvae, however, *M. chia* and *M. cypricola* can be clearly distinguished from the other *Maniola*. What is more, given that these two species are island endemics and therefore completely isolated from other congeneric populations, they are a genetically distinct entity. In a nature conservation context they would therefore be considered as 'evolutionary significant units', regardless if they are 'real' endemic species or not (Gärdenfors *et al.*, 1999).

**Acknowledgements**

We cordially thank Harry van Oorschot for his kind advice. George Thomson is acknowledged for his extremely valuable comments on the manuscript. Willem Hogenes is thanked for allowing to study the material in the Zoological Museum Amsterdam on which this paper was based, Sandrine Ulenberg and Steph B. J. Menken for commenting on an earlier draft of this manuscript.
IX.
An aberration in the female genitalia of *Maniola jurtina* (SCHRANK, 1801)

with Rob de Vos

submitted to Entomologische Berichten
Abstract
This note describes and illustrates an aberration observed in the genitalia of a female *Maniola jurtina* from Amsterdam (The Netherlands). The specimen had two bursae copulatrices, which both contained spermatophores. In external characters, size of genital apparatus, and shape of ovipositor lobes it corresponded to a normal specimen of the species. Signa were absent in both bursae.

Introduction
Butterflies of the genus *Maniola* are known for their large morphological variation, within single populations as well as continent wide (Ford, 1945; Thomson, 1973). Given the overlap in wing patterns, habitat selection and geographic distribution of various *Maniola* species, in certain cases, genitalia morphology is the only possibility to determine a specimen. Comparative research into the shape and structure of the genital apparatus in different *Maniola* is therefore essential to gain information on what are good characters to use as secure tools for species' identification.

While collecting individuals for a study on population genetics and genitalia variation in the genus *Maniola*, we found a particularity in a female individual of the Meadow Brown butterfly, *Maniola jurtina* (Lepidoptera: Nymphalidae, Satyrinae), collected in Amsterdam (Frankendael) in July 2002. This individual possesses two bursae copulatrices, an aberration that has never been described in *M. jurtina* before.

*Maniola jurtina* is one of the most common butterflies in the Netherlands. It can be regularly observed in the middle of Amsterdam, in most larger parks, provided that parts of the meadows are not reaped. The butterfly flies mainly from the end of June to the end of July, in areas that give the impression of wilderness, close to water-systems, on flowery meadows between bushes and trees. At the time of collection, females of *M. jurtina* were observed rather commonly, whereas males were becoming uncommon. This indicates that the emergence of the species in Holland had already begun at least a month before. Like many other butterfly species, Meadow Browns are protandric, which means that males emerge before females.

The female genitalia of a butterfly
The female genitalia are less differentiated between species than the male. Consequently, mainly the male genitalia are used for separation of species that are
difficult to determine on basis of external morphological characters. We here give a
generalized description of the genitalia structures in a female butterfly (illustrated
in figure 1). As butterflies are Ditrysia, the adult female has two genital openings.
At the extreme end of the abdomen on the 10th segment we find the opening of the
oviduct and anus, while the opening of the bursa copulatrix is situated on the ventral
side of the 8th segment. A pair of hairy, slightly sclerotised lobes, the ovipositor lobes
(or papillae anales), cover the opening of the oviduct. Their shape is characteristic of
a genus. These lobes are continued as a sclerotised rod, the posterior apophyses, and
often the tergum of the 8th segment has another pair of rods, the anterior apophyses.
The bursa copulatrix is a membranous sac which accommodates the male vesica
during copulation. It is connected with the ostium bursae, the opening of the bursa
by means of the ductus bursae, which may differ in length and width from one
species to another. In some species, the bursa copulatrix has sclerotised areas on
the walls, termed signa, that may be formed like spines, teeth, or a plate, and vary
from species to species. Sometimes the bursa copulatrix is distended through the
presence of a flask-shaped capsule. This is the male spermatophore containing the
spermatozoa. Shape and structure of the bursa copulatrix, the ostium bursae, and the
ovipositor lobes can be of diagnostic value to differentiate between species.

![Diagram of female butterfly genitalia](image)

**Figure 1.** Female genitalia of a butterfly, lateral view (generalized).

**Material and Methods**

**Dissection and photography of the M. jurtina specimen from Amsterdam**

Prior to dissection the abdomen of the specimen was separated from the rest of
the animal and soaked in Potassiumhydroxide (10%) for approximately 15 hours.
KOH dissolves the hard chitinous structures of the abdomen, so that it can be
dissected without breaking the genitalia. In order to photograph the preparation it
was dyed with chlorazolblack. To stabilize the samples for photography, they were
positioned laterally in a small drop of ethanol (30%), flattened between two glass lids and photographed under the microscope (magnification x 25).

Results

Description of the aberration

1) External characters (Figure 2): Length of forewings (from base at costa to the apex, without fringes): 22.6 mm; wings show the common pattern of a normal *M. jurtina* on upper- and underside.

2) Genitalia (Figure 3 A, B): Size of genital apparatus and shape of ovipositor lobes as to be expected in normal *M. jurtina* (e.g., Thomson, 1973). Two bursae and two ducti bursae. Size and length of first ductus bursae comparable to other individuals of the same species. Second bursa smaller and ductus bursae shorter. Both bursae normally shaped, not deformed or atrophied; signa absent in both. Both bursae contain a spermatophore.

![Figure 2](image_url)

*Figure 2.* External characters of the aberrative *M. jurtina*: wing patterns. (A) Forewing upperside, (B) forewing underside, (C) hindwing upperside, (D) hindwing underside.
Figure 3. Genitalia of a female *M. jurtina* from Amsterdam (Frankendael), the individual has two bursae copulatrixes. (A) photograph (photographer: Jan van Arkel), (B) drawing of the most important structures: two bursae with the spermatophores inside (Magnification x 50).

Conclusions

Aberrations in the genital apparatus and wing patterns are not uncommon in Lepidoptera. In the genus *Maniola*, for example, a gynandromorph *Maniola telmessia* from the Greek island of Foúrni has been described by Olivier & Coutsis (1990). That specimen was an almost bilateral gynandromorph. It had male and female parts of the genital apparatus, and left wings were entirely female, whereas the right wings were entirely male.

The *M. jurtina* specimen described in this paper possesses the normal characteristics
of a female *M. jurtina* in wing-patterns and size, only the genitalia are aberrant. To our knowledge, a female Nymphalid butterfly with two bursae copulatrices has never been documented elsewhere in the literature. Consequently, we suppose that what we observe here is indeed a very rare form of aberration.

However, such records always remain mostly of anecdotic interest. As these divergent individuals are mostly sterile, they usually have no evolutionary influence on the genetic pool of the population on the whole. Notably, in the case of the Amsterdam *M. jurtina*, both bursae contained spermatophores and were therefore presumably fertilized. It is not out of question, that the butterfly would have laid fertilized eggs and produced viable offspring, if it would not have ended up in our net.
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Summary

Evolution means ‘change’. For population geneticists evolution more specifically means ‘change in the allele frequencies of a population in time’. These changes can result in species’ differentiation and are the source of biological diversity. An understanding of the evolutionary processes creating diversity is therefore not only of theoretical interest, but may also lead to a better understanding of the reasons why species go extinct, and could in the end have a practical use in nature conservation.

In this thesis I focus on the evolution and ecology of species, that are restricted to the Mediterranean island of Sardinia, i.e., are endemic to Sardinia. Besides providing natural laboratories to study evolution, differentiation and speciation in particular, endemic species are also a major focus in conservation biology, as their disappearance from the small area they occupy would signify their total extinction. Sardinia belongs to an archipelago called the Tyrrhenian islands. These islands are one of the ten Mediterranean hotspots of diversity and endemism. In Sardinia, for example, 12% of all plants are endemic, and 14 of the 56 butterfly species occurring on the island are Tyrrhenian endemics.

I start with a general overview of endemic species in Sardinia (Part 1), zoom in on butterflies (Part 2), and finally concentrate on the Sardinian satyrid Maniola nurag (Part 3 and 4).

Quite a few early evolutionary biologists have been inspired by the great diversity in pattern and colour found on the wings of butterflies. Butterflies of the genus Maniola are highly polymorphic. Variation includes intra- as well as interspecific differences. Notably, three of the seven species in the genus are island endemics (M. nurag, M. chia, M. cypricola). Usually, endemics are distributionally separated from their widespread relatives. However, in Sardinia, an endemic (M. nurag) and a widespread Maniola species (M. jurtina) occur sympatrically, which might indicate a sympatric speciation event (speciation without an extrinsic barrier to gene-flow) as an alternative to an allopatric speciation event (speciation without gene-flow) to explain the origin of M. nurag.

The first part of this thesis deals with the question - “Which are the endemics and where do they live?” It starts with a review of recent literature describing patterns of endemity in different groups of organisms (plants, butterflies, beetles, amphibians, and lizards) endemic to Sardinia (Chapter II). I shortly summarize the geological history of the Mediterranean basin and try to relate dated geophysical events to molecular-based estimates of species’ divergence times. Areas of endemism generally coincide with mountain areas. Most endemics appear to have originated through vicariance or are relicts (palaeo-endemics) from pre-glaciation periods. Some came to the island by active dispersal, or passively by human transportation. Mediterranean shrubland communities are identified as an important habitat for endemic Sardinian species, particularly butterflies (Chapter III). Further, I compare butterfly (hebivore) species richness in different habitat types with
spider (predator) species richness, and find no significant relationship between these two groups. In the areas studied in Sardinia, butterflies are thus not such straightforward indicators of overall species diversity as they are often supposed to be. On the other hand, the number of butterfly species present in an area was positively related to overall plant species richness and elevation above sea level.

Part two deals with the conservation of Sardinian endemics. - "How to protect endemics?" Legal tools for setting nature conservation priorities are addressed as well as landscape management. The threat status of Sardinian butterflies is assessed by applying the IUCN criteria (Chapter IV). This chapter also shows how the quantitative information, as formulated in the IUCN criteria, can be combined with a qualitative assessment of human induced threat factors. This method allows an objective standardised assessment of threat, even when data are scarce. Two butterfly species, *Pseudophilotes barbagiae* and *Lysandra coridon gennargenti*, were identified as globally 'vulnerable', and their inclusion in the European Habitat Directive and the Bern Convention is strongly recommended. A mark-recapture study showed that populations of the endemic butterfly *M. nurag* form metapopulation networks whose long-term persistence requires connectivity between local populations (Chapter V). It also became apparent that areas to protect *nurag*-type species should cover at least 200 ha. I recommend traditional land-use practices as an important tool to combine nature-conservation interests with the interests of regional economy.

The third part continues questioning the origin of *M. nurag*. The two chapters in this part of the thesis take the butterfly *M. nurag* as a model to trace the evolutionary history of an island endemic, and connect present theoretical advances in evolutionary biology with population genetic data on the endemic butterfly (Chapter VI and VII). This part combines ecological field data (mark-release-recapture) with molecular genetic data (allozyme markers). Patterns of genetic diversity and population structure of Sardinian populations of *M. nurag* and *M. jurtina* are compared to mainland populations of *M. jurtina*. Results are used to determine whether allopatric or sympatric speciation best explains the origin of *M. nurag*. The small genetic distance between *M. jurtina* and *M. nurag* indicates that they are closely related. Divergence-time was estimated at 1.8 - 3 ma ago.

*M. nurag* is a mountain species, whereas *M. jurtina* also occurs at sea-level. At intermediate altitudes they meet and occasionally seem to produce hybrids. Geographic patterns in allele frequency patterns indeed hint at the presence of hybrids in areas where *M. nurag* and *M. jurtina* fly together, and also morphologically these individuals are intermediate between *M. nurag* and *M. jurtina*. From genetic and ecological data, it is concluded that *M. nurag* might not be the result of vicariance but originated under sympatric or parapatric circumstances, as a consequence of adaptation along an environmental gradient. The results underline the importance of environmental gradients in generating biodiversity. Disjunct distributions of closely related species do not
necessarily have to be the result of passive (allopatric) splitting of populations in the past, but can also follow genetic splitting within the population itself.

Part four provides a description of the genitalia structure of three endemic *Maniola* species, *M. nurag*, *M. chia*, and *M. cypricola* (Chapter VIII). It gives the first detailed description and illustration of the genitalia of *M. nurag*, and is extended to an overview of morphologic variation in the genus *Maniola*. Given the overlap in wing patterns and geographic distribution in this genus, genitalia morphology is occasional the only feature to tell species apart. Further, I describe two Sardinian individuals with intermediate characteristics between *M. nurag* and *M. jurtina*. These two specimen also appeared genetically as 'intermediates' (as explained in chapter VII). I describe a number of diagnostic characters to distinguish various *Maniola* species by means of genitalia morphology, and shortly discuss the justification of the species status for *M. chia* and *M. cypricola*. Chapter IX describes an aberration (two bursae copulatrixe) in the female genitalia of an individual of *M. jurtina* found in Amsterdam during the collection of material for the present thesis.
Zusammenfassung


Der erste Teil dieser Dissertation beschäftigt sich mit der Frage – „Welche Arten sind auf Sardinien endemisch, und wo leben sie?“. Er beginnt mit einer Übersicht rezenten Literatur und beschreibt die Ausbreitungsmuster verschiedener Gruppen sardischer Endemiten (Pflanzen, Tagfalter, Käfer, Amfibia, und Eidechsen) (Kapitel II). Ich fasse die geologische Geschichte des mediterranen Beckens kurz


Der dritte Teil beschäftigt sich mit dem evolutionären Ursprung von *M. nurag*. In den zwei Kapiteln in dieser Teil der Arbeit umfasst, benütze ich diesem endemischen Falter als Model, um die evolutionäre Geschichte eines Inselendemiten zu untersuchen, und theoretische Fortschritte der Evolutionsbiologie mit populationsgenetischen Daten über diese Art zu kombinieren (Kapitel VI und VII). In diesem Teil werden ökologischen Freilanddaten (Fang-Wiederfang), und genetische Daten (Allozyme) dazu benützt, Ökologie, Populationsstruktur und genetische Diversität von sardischen Populationen von *M. nurag* und *M. jurtina*


Samenvatting

Evolutie betekent in het algemeen ‘verandering’. Voor populatiegenetici betekent evolutie specifiek ‘verandering in de allele frequenties van een populatie in de tijd’. Deze veranderingen kunnen o.a. soortvorming ten gevolge hebben, en zijn de bron van alle biologische diversiteit. Inzicht in de evolutietypische processen die deze diversiteit doen ontstaan is dus niet slechts van theoretisch belang, maar kan ook leiden tot een beter begrip van de redenen waarom soorten uitsterven, dat uiteindelijk praktische toepassingen voor, bij voorbeeld, natuurbescherminvraagstukken kan opleveren.

In dit proefschrift richt ik me op de evolutie en ecologie van soorten, die in hun verspreidingsgebied beperkt zijn tot het mediterrane eiland Sardinië, met andere woorden, endemisch zijn voor Sardinië. Behalve dat ze een soort natuurlijke laboratoria representeren om evolutie, differentiatie en soortvorming te bestuderen, zijn endemische soorten ook een kernpunt voor natuurbescherming, omdat hun verdwijnen uit het kleine gebied waar ze voorkomen hun volledige uitsterven zou betekenen. Sardinië hoort bij een archipel die de Tyrrhenische eilanden wordt genoemd. Deze eilandengroep behoort tot de tien mediterrane hotspots voor biodiversiteit en endemisme. In Sardinië bij voorbeeld, zijn 12% van alle planten endemisch, en 14 van de 56 dagvlinders die op het eiland voorkomen zijn Tyrrehense endemen.

Beginnend met een algemeen overzicht over de endemische soorten die op Sardinië voorkomen (Deel 1), richt ik me speciaal op dagvlinders (Deel 2), en concentreer me tenslotte op een Sardische endem, het zandoogje Maniola nurag (Deel 3 en 4). Een aantal evolutiebiologen heeft zich laten inspireren door de grote variatie in patronen en kleuren op de vleugels van vlinders. Vlinders van het geslacht Maniola zijn zeer polymorf. Variatie omvat niet alleen verschillen tussen soorten, maar ook binnen een enkele populatie van een soort. Drie van de zeven soorten in het geslacht zijn eiland-endemen (M. nurag, M. chia, M. cypricola). Normaliter zijn endemen geografisch gescheiden van verwante wijdverspreide soorten (allopatrisch voorkomen). Toch komen op Sardinië een endemische (M. nurag) en een wijdverspreide Maniola soort (M. jurtina) op dezelfde plek voor (sympatrisch voorkomen). Dat zou een indicatie kunnen zijn, dat de splitsing tussen deze twee nauwverwanten soorten sympatrisch is geweest (dus zonder een geografische barrière tegen genen-uitwisseling). Dit lijkt een realistisch alternatief te zijn voor een meer algemeen aanvaard allopatrische soortvormingsproces om zodoende het ontstaan van M. nurag te verklaren.

Het eerste gedeelte van dit proefschrift gaat over de vraag - „Wat zijn de endemse soorten, en waar leven ze?” Het begint met een overzicht van de recente literatuur, en beschrijft de verspreidings-patronen van Sardische endemen uit verschillende groepen (planten, dagvlinders, kevers amphibieën, en hagedissen) (Hoofdstuk II). De geologische geschiedenis van het mediterrane bekken wordt kort samengevat, en er wordt een verband gelegd tussen gedateerde geofysische gebeurtenissen en
op moleculaire evolutie gebaseerde schattingen van de tijd sinds de afsplitting van soorten. Gebieden waar vele endemen voorkomen vallen in het algemeen samen met bergachtige gebieden. De meeste endemen blijken door vicariantie ontstaan te zijn, of ze zijn relictten (palaeo-endemen) uit pre-glaciale tijdperken. Sommigen kwamen naar het eiland door gebruik te maken van hun eigen verspreidingsmogelijkheden, anderen als gevolg van passieve verspreiding door de mens. Mediterrane struikgewasgemeenschappen (macchie en garrigue) worden geacht belangrijke habitats voor Sardse endemen te zijn, in het bijzonder voor vlinders (Hoofdstuk III). Verder vergeleek ik de dagvlinder (herbivore) soortenzicht in verschillende habitats met die van spinnen (predators), en vond geen significant verband in voorkomen tussen deze twee groepen. In de bestudeerde gebieden op Sardinië zouden vlinders dus niet direct als indicatoren van de totale soortenzicht dom gebruikt kunnen worden. Aan de andere kant was het aantal in een gebied voorkomende vlindersoorten wel positief gerelateerd met de totale plantensoortenzicht dom, en met de hoogte.

Het tweede gedeelte van het proefschrift gaat over de bescherming van Sardische endemen. "Hoe moeten endemen beschermd worden?". Wettelijke instrumenten om prioriteiten voor natuurbescherming te bepalen worden hier besproken, naast elementen van landschaps-management. De mate waarin dagvlinders worden bedreigd wordt beoordeeld met behulp van de IUCN criteria (Hoofdstuk IV). Dit hoofdstuk laat ook zien hoe de kwantitatieve informatie, zoals geformuleerd in de IUCN criteria met een kwalitatieve beoordeling van door de mens veroorzaakte bedreigingsfactoren gecombineerd kan worden. Deze methode maakt een gestandaardiseerde beoordeling van de bedreigingsstatus van een soort mogelijk, ook als de gegevens schaars zijn. Twee vlindersoorten, te weten Pseudophilotes barbariae en Lysandra coridon gennargenti, werden geïdentificeerd als wereldwijd 'kwetsbaar' ('vulnerable'), en ik raad aan, dat ze in de Europese Habitat Directive en Bern Convention worden opgenomen. Een vangst - terugvangstudie liet zien, dat populaties van de endemische vlinder M. nurag metapopulaties vormen, waarvoor op lange termijn een verbinding tussen de lokale populaties noodzakelijk is (Hoofdstuk V). Het bleek verder dat gebieden om nurag-achtige soorten te beschermen meer dan 200 ha groot zouden moeten zijn. Tenslotte stel ik voor om traditionele landbouwmethoden als een belangrijk middel in te zetten teneinde de belangen van natuurbescherming met die van de regionale economie te verenigen.

Het derde deel behandelt de evolutionaire oorsprong van M. nurag. In de twee hoofdstukken in dit gedeelte van het proefschrift dient M. nurag als model om de evolutionaire geschiedenis van een eiland-endeeem te traceren, en huidige theoretische evolutiebiologische inzichten met populatiegenetische gegevens van de endemische soort te combineren (Hoofdstuk VI and VII). Dit gedeelde combineert ecologische veldgegevens (vang - terugvangst) met genetische informatie (allozyme). Patronen van genetische diversiteit en populatiestructuur van eilandpopulaties van M. nurag en M. jurtina worden vergeleken met
vastelandpopulaties van M. jurtina. Deze gegevens worden gebruikt om te bepalen of allopatrische dan wel sympatrische soortvormingsmodellen de oorsprong van M. nurag kunnen verklaren. De kleine genetische afstand tussen de twee soorten toont aan dat ze nauw verwant zijn. Het moment van splitsing word geschat op 1.8 - 3 miljoen jaar geleden.

Maniola nurag is een bergsoort, terwijl M. jurtina ook op zee-niveau voorkomt. Op intermediaire hoogtes ontmoeten ze elkaar en lijken soms hybriden te produceren. Geografische patronen in allelfrequenties duiden inderdaad op de aanwezigheid van hybrides in gebieden waar M. nurag en M. jurtina samen voorkomen. Ook morfologisch zijn deze individuen intermediair tussen M. nurag en M. jurtina. Uit de genetische en ecologische gegevens kan worden geconcludeerd dat het onwaarschijnlijk is dat M. nurag door vicariantie is ontstaan, maar het is aannemelijker dat soortvorming onder sympatrische omstandigheden plaatsvond, als gevolg van adaptatie langs ecologische gradiënten. Deze resultaten onderstrepen de betekenis van ecologische gradiënten bij het ontstaan van biodiversiteit. Disjuncte verspreiding van nauw verwante soorten hoewel dus niet noodzakelijkerwijs het resultaat van passieve (allopatrische) splitsing van populaties in het verleden zijn, maar kan ook het gevolg zijn van een genetische opdeling binnen populaties.

Deel vier geeft een beschrijving van de genitaliënstructuur van drie endemische Maniola soorten: M. nurag, M. chia, en M. cypricola (Hoofdstuk VIII). Het houdt de eerste detaillereerde beschrijving en illustratie van het voortplantingsapparaat van M. nurag in, en wordt uitgebreid tot een overzicht van morfologische variatie in het genus Maniola. Gezien de overlap in vleugelpatronen en de geografische verspreiding in het genus, is morfologie van de genitaliën in enkele gevallen namelijk het enige kenmerk om soorten uit elkaar te houden. Verder beschrijf ik twee individuen met kenmerken die intermediair lijken tussen M. nurag en M. jurtina. Deze twee individuen zijn ook genetisch gezien 'intermediair' (zoals wordt verduidelijk in hoofdstuk VII). Een aantal kenmerken die diagnostisch zijn tussen de verschillende Maniola soorten worden gedefinieerd. De aannemelijkheid van de soortstatus voor M. chia en M. cypricola word kort behandeld. Hoofdstuk IX beschrijft een afwijking (twee bursae copulatrices) in de vrouwelijke genitaliën van een individu van M. jurtina, gevonden in Amsterdam tijdens het verzamelen van materiaal voor dit proefschrift.
Permbledhje

Evolucion do te thote “ndryshim”. Per shkencetaret te cilet studiojne gjenetiken e popullatave evolucion ne menyre te specifikuar do te thote “ndryshim te konstelacionit te frekuencave te alleles brenda nje popullate), ndryshime qe sjellin ne formimin e llojeve dhe specieve te reja brenda te njjetes popullate, te cilet jane dhe burimi i shumellojshmerise biologjike. Njohurite mbi proceset evolutive te cilet krijojne kete shumellojshmeri kane atethe jo vetem nje interes te vecante teorik por bejne te kupto me mire dhe shkaqet e zhdukes se llojeve dhe si perfundim dobine praktike qe ka mbrojtja e natyres.

Ne presantimin e kesaj tezes ujëm perqendruar mbi ekologjine e llojeve perhapja e te cilave eshte e kufizuar ne njerin prej ishujve mesdhetare Sardenja. Perverc kesaj ato prezantojne nje lloj laboratori natyror ku mund te studiohet evolucioni ndryshueshmerise dhe krijojen te cilet qendrojne ne epiqendren e vezgjimit te naturës sepse zhduka e ketyre llojeve nga nje territor i caktuara do te thote dhe zhduksen e pergjithshme te tyre. Sardenja eshte pjesa e ishujve Tirrene. Ky grup ishujsh pershhijet ne dhjeteshen e ishujve me kryesor te mesdheet persa i perket shumellojshmerise biologjike dhe endemike qe gjenen vetem ne kete zone. Per shembull reth 12% e te gjitha llojeve te bimeve ne Sardenja jane endemike, dhe 14 nga 56 llojet e fluturave te dites te cilet takohen ne ishull jane tirrenene endemike.

Duke filluar me nje pershkrim te perqgjithshem te llojeve endemike te Sardenjes (pjesa e pare), me pas pjesa e dyte perqendrohet tek fluturat e dites dhe si perfundim tek nje lloj specifik fluture qe karakterizon Sardenjen e quajtur Maniola nurag (pjesa e trete dhe e katert). Nje numer i madh evolucionbiologesh jane frymezuar ne punen e tyre nga larmija e motiveve dhe ngjyrave te flatrave te ketyre fluturave. Flatrat e spezieve te fluturave Maniola jane shume polimorfe por ndryshueshmeria nuk eshte e kufizuar vetem midis llojeve porse dhe mes variazioneve te llojeve brenda te njjetes popullate. Tre nga shtate llojet te bejne pjesi ne kete gjeti jane endemike ishulllore (Maniola cypricola, Maniola chia, Maniola nurag). Normalisht zonat e perqgjatjes se llojeve endemike jane te ndara nga pikepamja geografike nga spezieve e tjera te cilet jane shume me te shperndara geografikisht. Ne Sardenje gjenen ne te njjeten zone flutura endemike (M. nurag) dhe nje lloj tjeter qe eshte shume i perhapur me emrin (Manolia jurtina) (perqgjatje simpatrike). Ky mund te jete nje udhezim qe tregon se ndarja nga krijimi i ketyre llojeve te njgjashme eshte bere ne simpatра pra pa pasur nje barriere geografike qe te kete influencuar ne ndryshimin gjenetik te ketyre dy llojeve. Kjo duket nje alternative realiste per shpjegimin e orijines se M. nurag ne ndryshim me skenarin e krijimit te llojeve si shkak i barrierave geografike qe pengojne ne shkembinin e geneve dhe sjellin si pasoje krijimin e llojeve te reja.

Pjesa e pare e ketij disertazionit trajton pyetjen “Kush jane llojet e fluturave endemike ne Sardenje dhe ku ndeshen ato?”. Ajo fillon me nje veshtrim te perqgjithshem te nje literature bashkëkohore dhe pershkruan modelin e perqgjatjes se grupeve te ndryshme endemike te Sardenjes (bimeve, fluturave te dites, brumbujve,
amfibeve, dhe hardhuckave). Historia gjeologjike e relievit te bacin nesdhetar eshte permabledhur shkurtimisht ne nje volum ku ekspozohen percarjet midis llojeve me kalimin e kohes si pasoje e ndryshimeve gjeofizike dhe molekulare. Ne Sardenje duket se endemiket shfaqen me teper ne zonat malore. Pjesa e me madhe e llojeve endemike jane krijuar nga vikarianza ose ato jane relikte te periodhave pregglaciale. Disa syresh u perhapen ne menyre active ne ishull (shkaku natyror), ndersa disa te tje re ne menyre passive (shkaku njerezor). Si abitate kryesore per endemiket e ne menyre te vecante te fluturave te dites ne Sarenje nje rol te rendesishem ka prania e makieve ferrave dhe gemushave dhe perhajes se tyre te gjere (kap. III). Nje krahaimi e bere ne te njejeten habitat midis ndryshueshmerise se llojeve te fluturave te dites (herbivore) dhe ndryshueshmerise brenda llojeve te merimangave (predator) nuk dha ndonje rezultat sinjikativ persa e perket lidhjeve qe mund te kene keto dy grupe me njeri tjetrin. Keshlu ne zonat e studuara fluturat e dites duket se nuk jane ndonje tregues indikativ per studimin e pergjithshem te shumellojshmerise se ketyre zonave. Nga ana tjeter numri i llojeve te fluturave te ndeshura ne nje zone te caktuar lidhej me shumellojshmerine e bimeve te zones dhe me lartesine e kesaj zone nga niveli i detit.

Pjesa e dyte pershkruan mundesite e mbrojtjes se grupeve endemike ne Sardenja. "Si mund te mbrohen endemiket". Instrumentat ligjore per vendosjen e prioriteteve per mbrojtjen rigoroze te natyres me metodën e ashtuquajtuar landscape management. Me ndihmen e kriterive IUNC studiohon statusi i fluturave te Sardenjes qe kanosen nga zhduka (kap. IV). Ne kete kapitull tregohet menyra se si faktori qantitativ i formuluar ne kriteret IUNC mund te kombinohet me vleresimin kualitatit i faktoreve kercenues dhe shkatatura nga njeriu per keto lloj grupesh. Kjo lloj metode lejon standartizimin e faktoreve kercenues qe mund te sjellin ne zhduken e ketyre llojeve edhe nese konstiston shume pak informacion mbi to. Dy lloje fluturash Pseudophilotes barbagiae dhe Lysandra coridon gennargenti vleresohen boterisht si shume te prekura "vulnerable" dhe une propozoj qe keto dy lloje teuten ne listen i llojeve qe kercenohen te zhduken e qe duhen mbrojtur ne menyre rigorose te parashikuar nga direktivat e habitatave europian dhe te konventes se Bernes. Nepermjet metode se kapjes dhe rikapjes se popullatave te ndryshme dola ne perfundimin si eksistojne lidhje mes popullatave te ndryshme te M. nurag (metapopulatione) (kap.V). Si rrjedhin per mbrojtjen e llojeve te ngjashme te nurageve duhen zona te mbrojtura me superfaze jo me te vogel se 200 ha. Gjithashtu una rekomandoj ne keto zona aplikimin e metodave bujqesore tradizionale te cilat jane ne dobi jo vetem te naturres llojeve dhe speziave qe rrrezikojne te zhduken ne pergjithes i por dhe te ekonome regionale te ketyre zonave.

Pjesa e trete disertazione trajton ekologjin dhe evolucionin e zhvillimin e fluturave M. nurag. Ne dy kapitujt e kesaj pjesa per studimin e dhe kerkimin historiko-evolutiv te nje endemike te ketij ishulli une zgjodha M. nurag si dhe kombinimin e te dheneve teorike bashkekohore te zhvillimit te evolucioneve biologjike me te dhena genetike te popullatave te ketyre zonave (kap.VI). Ekologjia, strukturat
e popullatave dhe diversiteti gjenetik i popullazionve Sarde te M. nurag dhe M. jurtina jane krahasuar me popullimet fikse te M. jurtina. Gjithashtu ne studimin tim kam perdorur dy metoda, metoden ekologjike te marrejes te te dhenave nepermjjet kapijes dhe rikapijes se lllojeve ne terren dhe ate te marrjes te te dhenave gjenetike te ketyre llojeve (allozyme) ne laborator. Konkluzionet e ketyre te dhenave cojne ne interpretimin perfundimtar te krişimit dhe origjines se lllojeve te M. nurag ne forme alopatrike apo sympatrike. Ndryshimi midis ketyre dy llojeve pra M. nurag dhe M. jurtina kane fillenes ne nje hark kohor prej 1.8 deri ne 3 miliion vjetesh me pare. Maniola nurag ndeshet ne pergjithesin e lartesite e mesme dhe te larta mb 500 m, ndersa M. jurtina ndeshet me teper ne lartesite e ulta gati ne nivelet dhe detit. Ne lartesite mesatare keto dy llloje gjenden ne forme te hibrizduar. Allelfrekuenzat pasqyron ne menyre te qarte ekzistencenc e hibrizduar te ketyre dy lllojete cilat jane gershetuar me nje rata tjeteren. Gjithashtu nga pikapamja morfologjike keta dy individe jane te gershetuar ne menyre intervmjettive. Puna ime e bazuar ne te dhenat ekologjike dhe gjenetike sjell ne konkluzionin se M. nurag nuk jane krijuar nga faktoret e vikaranzes por nga proceset sympatrike dhe parapatrike dhe si pasoje e adaptimit ne nje spekter te gjate kohor te nje gradienti ekologjik. Keto te dhena perforcojn rendesine e gradientit ekologjik ne krişimin e biodiversitetit. Keshtu prezenca e ketyre llojeve te ngjashme dhe perhapja dhe ndarja brenda popullates dhe karakteristikave te vecanta brenda nje popullate nuk ka vetem shkaqe passive (ndarjeve allopatrike) por mund te jene dhe shkak i nje ndryshimeve te konstelacioneve gjenetike brenda popullates.

Fjesa e katert pershkruan strukturen e ndertimit te organeve te riprodhimit per kete nga secili ishull mora tre llloje te Maniola: M. nurag, M. chia, M. cypricola (kap VIII). Ky kapitull ilustron dhe pershkruan ne menyre te hollesishme organin riprodhues te M. nurag dhe hedh nje sy mbi variacionet morfologjike te shperndarjes se Manolias. Shpesh studimi i organeve gjenetale eshte e vetmja mundesi per njohjen e ndryshimeve midis ketyre lllojete pasi njashmeria e motiveve dhe ngjyrave te flaterave si dhe perhapja e njejte gjeografike nuk lejojen te krişimet te njohjen e ndryshimimive midis ketyre lllojete. Me vone une kam pershkruar dy individe (hibride) me tipare te ndërthurura te cilet hasen ne ishullin e Sardenjes ku dhe organi i tyre i riprodhimit ka tipare te theksuara intermediare ashtu sic eshte pershkruar hollesisht ne kapitullin e VIII-te. Pershkruhen gjithashtu tipare te vecanta te diferencimit te lllojete te ndryshme te Maniolave si dhe diskutohet shkurtimit të aresydet e statutit te lllojij per M.chia dhe M. cypricola. Kapitulli i IX-te pershkruan nje perjashtim te aparatit gjenetalfemor (dy bursae copulatrixe) te nje eksemplari fluturre te lllojjet M. jurtina, e cila u gjend ne Amsterdam gjate mbledhjes se materialeve per pergatitjen e kesaj teze.

(Translation into Albanian: Qemal Mullaj)
Acknowledgements

Everyone I want to thank here has contributed to this thesis either by discussing with me the scientific and philosophical issues that bear on evolutionary and ecological research, or by providing me with their comments on earlier drafts. Some even did both. And some did even more than that and helped me with field work.

_Thirty-one Ways of Saying Thank You_

I
Now, that the shuffle of more than three years went so much faster than it appeared it was you who trusted me first believed in the spirit and that this plan could be reared gave me a place your time the space to grow and catch the butterfly before it flew out of sight _daarvoor te danken_ Steph B. J. Menken.

II
You agreed to be my committee no matter if you came from a distant city or the room round the corner Roberto Crnjar Paul Brakefield Peter van Tienderen Fred Schram Gabriel Nève Gerard Oostermeijer Sandrine Ulenberg.

III
Dank für die Unterstützung aus der Heimat, die richtige Mischung aus Zweifel und Vertrauen, das Schauen, Bestimmen all dieser Spinnen, Konrad Thaler, Barbara Knoflach.

IV
Hans den Nijs you gave me the adress, Gerard gave me the name that's how it actually came that I am here now and not in Spain so, thank you again.

V
Your help under the Sardinian sun made hunting insects much more more fun, and who else would have carried endemic beer, with the name of a butterfly up a mountain? Doctorandi _in spe_ Robbert Erents Eline van Haastrecht Valeria Amatiello _E certo_ last but not least: _i genelli_, Dott. Andrea e Dott. Paolo Salvai.

VI

VII
O man of wings, how does it come, that you always chose a meal so much worse than mine, in the streets of L.A. all asphalt and steel, or the Hawaiian paradise? While drinking some wine and catching no birds sometimes three words. And at the end of the day your inescapable graphs. _We can see_ Clearly now, _the rain is gone_ Daniel Cleary.
VII
Nobody I know
can cut starch-gels in an equally
elegant manner,
like the jam in my Viennese Sachertorte
your buffers between the layers,
and the stainings
the topping of wipped cream.
Really, how could I ever have
scored a single gene
without your gentle introduction
into the basics of biochemistry and
how to get DNA out of a butterfly’s leg
that’s as simple as cooking an egg
says Wil van Ginkel,
my mother in the laboratory
no other word for that story.

IX
You make
the museum
with its timeless corridors
and dusty gloom
have a face
be a place,
a livingroom,
a kitchen,
and an office
together,
for all kinds of weather
never hesitated to assist
would have guided me through mist,
always had a paperclip,
some advice for a trip
Anna Achmatova in your pocket
and your heart never locked
Tatjana Das.

X
Thank you for smiles,
books,
sometimes a chat,
and much more than that,
Yde de Jong
Mario de Kluywers
Willem Hogenes
Saskia Marijnissen
Ellinor Michel
Nimrod Epstein
Martijn Lammertink
Annelies Pierrot
Rob van Soest
Rob Moolenbeek
Sandrine Ulenberg
Annie Zuiderwijk.

XI
Spirals of colours
coiled in your drawers
full of DVDs, CDs and film,
whatever you will
see
in air, forest or harbour
Jan van Arkel
Photographs it sharper.

XII
A tick of the molecular clock
always made our discussions
work,
but I don’t count that way,
nor do you,
and almost everything
is millions of grasshoppers’ heartbeats ago,
Katja Peijnenburg
Patrick Meirmans
Peter Kuperus
Martijn Egas
Hans Breeuwer
Marc Stift
Martin Genner.

XIII
The right words
written in the right moment
without further comments.
Ronald Vonk?

XIII
You sings for birds, you sing for fish,
and ambitious scientists,
read my manuscripts many afternoons
while you could also have been
drawing moons,
Steven Weiss
Vasiliki Kati.

XV
Easy to hear, harder to see
that’s what statistics were
for me
until you switched on your screens
to tell me
what it all means
Pim Arntzen
Nicolas Schtickzelle.

XVI
Grazie,
Paolo Casula
Stefania Battarino
Andrea Fera
Giorgi Delogu
Daniela Porcu
per essere 'endemici'.

XVII
Grazie
di sempre essere benvenuta,
dei canzoni tra la la,
gelati
e discussioni
staff, researchers and students of the
Dipartimento di biologia sperimentale e
fisiologia
Dell' Università di Cagliari.

XVIII
Access to a -70° freezer
indispensable to let enzymes survive
when butterflies died,
was given by
Annalisa Marchi
Dipartimento di Genetica
Università di Cagliari,
and
Stefan Galler
Institut für Zoologie
Universität Salzburg.

XIX
How many degrees is the angle
in which butterflies
hold their wings
on Corsican meadows?
How to photograph the smile of a moth?
And is this really what I thought?
Many things you taught me.
Thank you for your enthusiasm for
the scaled beings with wings
David Jutzeler
Rob de Vos
Harry van Oorschot.

XX
Your Australian butterfly nets,
which came as an emergency package
across the sea,
when the basic equipment of
Lepidopterology
was stolen by i ladri,
captured (and released)
thousands of butterflies,
while you remained
invisible, in the shadow
of a grass, drinking a glas
of snow, and writing
about another island
than this,
Raoul Schrott.

XXI
Fascinating that one
can capture Maniola with a T-Shirt,
and send a post-card from China
addressed to an
Institute of Higher Butterfly Studies,
which actually arrives,
Hans Genser.

XXII
'De vlinderstichting' was the birthplace of
my knowledge on butterflies,
Chris van Swaay and Irma Wynhoff altijd
met Rat und Tat zur Stelle für Flügeltiere
und andere seltene Arten.

XXIII
Interdisciplinary advisors,
Jaap van Ginckel
Alan Berg
Annette Gerstenberg
Yanna Tsanidaki
Andrea Schmid
Lotte Knijn, Ruud Knijn
Marco Knijn
Mona Geitzenauer
Sabine Breuer
Doris Hochaspöck
Angelika Winter
Thomas Anzböck
Joan Jeanrenaud
David Harrington
John Sherba
Hank Dutt
Jennifer Culp
Alexander Balanescu
Khalid Seydo.

XXIV
O you have your indispensable share
in the making of this scientist.
And did not despair
crawling through shrubs and Rubus bushes
to catch only bugs, not even a hare.
One entire day of the three you were free
you dedicated to butter and fly
who else would that buy
but Qemal Mullaj.
(Faleminderit per gjith fluturat
qe me ke kap dhe
lsptsráp
dhe per te keqen e henes tate)
two years.
You drove me to
trains, busses, airplanes, and ferries,
about 350 times a year.
You gave me books,
and sometimes a beer,
and the freedom to decide.

XXVIII
In memoriam Heinz Pammesberger who
generously sponsored the printing and binding
of this thesis.

XXIX
Jo Oma, iatzi is soweit, und's diandl is endlich
gross wuan und fertig mitn studium, und des
kint sicha duach deine hoizknechtnocka, dass i
so guat nodenga ho kina.
Opa, nua du woast wia oile pflonzn hoassn.
My grandmother, the cook,
no need of a book,
my grandfather, the gardener,
who knows all plants' scientific names.

XXX
The moth is a moth is a moth is a moth.
That's not what you wrote.
How beautiful science and life can be
that's what you showed me.
You gave me raspberries,
strawberries and cherries
of just the right colour and taste,
and made every allel e number
find its place
before the summer.

Een dugong is een zoogdier
flying in a green light.
Léon E.

XXXI
The green and white at the tops
of the mountains behind your back-yard
were the things you showed me first,
The sun, you said, is the law of all things,
if someone catches her wings,
he will forever have the brightest flowers.
You gave me your back,
your arms and neck,
the way I walk,
and the meadows of Helleborus niger in the
forest,
my ancestors better known as 'da Pfandl Opa',
('de Pfandl Oma'),
A. & A. Pammesberger.
Grazie a te ho una barca da scrivere
ho un treno da perdere
e un’invito all’Hotel Supramonte dove ho visto la neve

(Fabrizio de André)
CURRICULUM VITAE

Andrea Grill was born in 1975. She completed secondary school at the 'Bundesgymnasium Bad Ischl' (Austria) in June 1993. In October of the same year, she started studying linguistics and literature (Italian, Spanish, and modern Greek) and biology at the 'Paris Lodron Universität Salzburg'. In 1994/1995, she pursued her studies at the 'Università degli studi di Perugia' (Italy) with courses on early and contemporary Italian literature, translation, and marine biology. The following years of her studies were characterized by frequent travelling, where she was particularly fascinated by the Balkans and Central America.

In 1997, she got involved with butterflies for the first time, while working with Chris van Swaay from 'Dutch Butterfly Conservation' (Wageningen, The Netherlands) on the establishment of a Red List for European butterflies. Subsequently, she conducted a research project in north-eastern Greece, 'Butterfly diversity and conservation in Dadia Nature Reserve', under the supervision of Dr. John R. Haslett (University of Salzburg) and Dr. Despoina Vokou (Aristoteles University of Thessaloniki), and in June 1999 she graduated with a 'Magister' degree in biology/ ecology at the University of Salzburg. In the same year, she obtained a Bachelor degree in Italian language. In the summer of 1999 Andrea worked as a field-assistant in the 'Hohe Tauern Nationalpark' on a project entitled 'Flies as keystone species in alpine ecosystems'. During the following months she started working for the 'Open University' in Salzburg, teaching Italian for beginners. Teaching of languages and translations have continued to be side-steps of her scientific career as a biologist ever since.

In December 1999 she got the opportunity to perform an internship at the European Parliament in the frame of a 'Ramon y Cajal' scholarship, and worked until March 2000 at the Directorate General for Research, Department Scientific and Technological Options Assessment, in Luxemburg.

In the meantime, she had been awarded a DOC grant from the Austrian Academy of Science to conduct the PhD research project on endemic Sardinian butterflies she had proposed. Under the supervision of Prof. Dr. Steph B. J. Menken she began to work as an externally funded AIO (= assistant in training) at the 'Evolutionary Biology' group of the Institute for Systematics and Population Biology (presently Institute for Biodiversity and Ecosystem Dynamics), and conducted the research which culminated in this doctorate thesis.

During the years 2000 – 2003 she lived and worked in Sardinia during the summer months, and in Amsterdam during the winter. In Sardinia, she collaborated with the 'Dipartimento di Biologia Sperimentale' of the 'Università di Cagliari', under the supervision of Prof. Dr. Roberto Crnjar. The period of her PhD research was marked by collaborations with the 'Laboratoire Systématique Evolutive', Université de Provence (France) and the 'Institut für Zoologie und Limnologie', Universität Innsbruck (Austria). To the latter institution she was officially affiliated to until February 2003, with Dr. Konrad Thaler as her Austrian supervisor. Besides conducting her own research, she supervised several students' graduate projects at the University of Amsterdam as well as the University of Cagliari.

Apart from biology, literature and languages have always remained her great passions, resulting in a range of literary publications e.g., in 'Literatur und Kritik'.

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Publications

(publications that form part of this thesis are omitted)


