

# **Phylogeography and Conservation of Postglacial Relicts**

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BfN - German Federal Agency for Nature Conservation

CRP - Centre de Recherche Public Gabriel Lippmann

IUCN - World Conservation Union

Ministère de l'environnement Luxembourg

SNL - Société des naturalistes luxembourgeois

University Lüneburg

University Trier





## Welcome in Luxemburg...

History meets future and the transgression of disciplines

In summer 2006 we started different research studies on species, which occur in small and isolated populations. We chose different butterfly species and plants to study their phylogeography and recent population status with different molecular techniques. We had the impression that some of our model organisms are declining faster than we could gain any information about their ecological demands. With our analysis of the phylogeography of the species as background we try to explain their reactions to the recent climatic and land use changes during the last decades. We were fascinated by this coming together of different disciplines and approaches, which are crucial to explain the genetic constitution and future scenarios of species.

When we surfed through the internet looking for similar studies to ours and comparing them with our results, we discovered many papers, describing phenomena like species phylogeographic history, range shifts, historical and recent habitat fragmentation, genetic variability, drift and depressions combined with organism's fitness. These studies had one aspect in common: most of the model organisms exist as relicts showing a wider distribution area in former times (e.g. before or during the last ice age) in comparison to the recent distribution, which has been shrinking due to restriction processes (biotic/abiotic parameters) to mostly small and isolated population remnants.

Such species are today of main interest for conservation biology and population genetics. Therefore, we decided to organise an international symposium for bringing together different disciplines, working on the same topic: relict species. For the better understanding of these species we decided to start in the past with the species' history and meet the future in conservation concepts. This journey through thousands of years we will pass together in transgressing different disciplines, starting with phylogeography, biogeography, conservation biology, population biology, population genetics, ecology, climatology and conservation politics.

With this multidisciplinary agenda we try to gain a better understanding of relict species and their protection. We invited scientists and political makers to participate in this discussion process and welcome you beyond the heading "Phylogeography and Conservation of Postglacial Relicts". A first extract of ideas is fixed in this little abstract book, and will be later published in detail in a book with the same title – which we hope to become a guide for scientists, politicians and students, interested in relict species.

Luxembourg, October 2007

Jan Habel and Marc Meyer



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# **Background and aims of the symposium**



## **Background and aims of the symposium**

### **Convener**

National Museum of Natural History Luxembourg (MNHN)

### **Partners**

BfN – German Federal Agency for Nature Conservation

IUCN – World Conservation Union

CRP – Centre de Recherche Public Gabriel Lippmann

Ministère de l'environnement Luxembourg

Universität Lüneburg

Universität Trier

SNL – Société des naturalistes luxembourgeois

### **With the support of**

FNR - Fonds national de la recherche Luxembourg

MNHN Luxembourg – Musée national d'histoire naturelle Luxembourg

Ministère de la culture, de l'enseignement supérieur et de la recherche

Luxembourg

### **Of interest to**

- Scientists with the main focus on phylogeography and biogeography of the Western Palaearctis
- Scientists involved in conservation ecology/biology and genetics
- Managers from public organisations and freelancers charged with conservation concepts
- Representatives of NGOs, revealing in the protection of endangered species
- Students

### **Theme**

Sustainable development and the conservation of biological diversity have become a major topic for science and politics since the Rio Convention in 1992. One of the key objectives of the convention is the conservation of inter- and intraspecific variability across the globe. Current diversity patterns are strongly influenced by both historic effects such as ice ages, and recent effects such as land management. Of particular importance in this context is the understanding and conservation of postglacial relict species, which give key information on the distribution of genetic diversity in today's world. Therefore, the proposed meeting will focus on relict species in the context of

biodiversity protection. Relict populations usually suffer more severely from environmental stochasticity than non-relict populations. This situation has recently changed to the worse due to anthropogenic landuse changes resulting in increasing fragmentation and decreasing habitat sizes. As a consequence, the numbers and sizes of many relict populations have decreased as well, often combined with the loss of genetic diversity and reduced individual fitness. To stop and finally reverse this development, numerous conservation efforts have been introduced, for example the Habitats and Birds Directives in the European Union.

This meeting will provide a platform for scientists to discuss the phylogeography of species and their conservation management. Population biology and phylogeography are crucial for the conservation of relict species and their genetic diversity. The meeting provides a unique opportunity in bringing together representatives from natural sciences and applied conservation management. This combination will enable participants to develop new ideas on how to manage these endangered populations of major importance.

#### **Organisation and scientific committee (alphabetic order)**

Thorsten Assmann (Universität Lüneburg)  
Petra Dieker (Universität Münster)  
Jan Habel (MNHN Luxembourg)  
Joachim Kosuch (Universität Trier)  
Marc Meyer (MNHN Luxembourg)  
Christian Ries (MNHN Luxembourg, SNL Luxembourg)  
Thomas Schmitt (Universität Trier)  
Tanja Walisch (MNHN Luxembourg)

#### **Conference venue**

The conference location is the Abbey of Neumünster called 'Centre culturel de rencontre Abbaye de Neumünster' (CCRN). It is located in the UNESCO World heritage area in the Alzette valley overlooked by the old town of Luxembourg. It includes three buildings of which the oldest goes back to the XVIIth century, surrounding a vast square. The rich past of this historic building and the multicultural vocation of Luxembourg are major topics of the CCRN. Meetings, debates, creations, spectacles, conferences, seminars, exhibitions and receptions are organized to promote the dialogue of cultures. With European networks and in partnership with many cultural institutions from different backgrounds, the CCRN favours the exchange of experiences and contributes to transnational cooperations between actors of different cultural origins.

The CCRN is close to the Museum of Natural History at the heart of Luxembourg-city

### **Accommodation**

Each participant has to book him-/herself an accommodation (except for the people presenting an oral keynote-communication). A list of hotels in Luxembourg will be proposed by the organizer (see accommodation on our website [www.symposium.lu/relicts](http://www.symposium.lu/relicts)). Early booking is recommended.

### **Registration**

For registration please use the registration form on the conference homepage and send it electronically.

### **Costs**

Reduced fees will apply to students and retired persons of all member states and to scientist from new member states of the European Union. The conference fee will be according to the time of registration; deadline for early booking is the 1st July 2007.

	early booking	late booking
Full price	65 €	75 €
Reduced price	30 €	35 €

This fee includes admittance to conference sessions, conference documents including abstract volume, welcome party, lunch and coffee breaks.

### **Payment**

Before you pay, send your registration form. Please pay the conference fee in Euro and free of charge by transfer to account n°

IBAN LU54 1111 1329 0010 0000 of the  
TRESORERIE DE L'ETAT CPTÉ MUSEE NAT D'HIST: NATURELLE at  
Bank 'P&T Luxembourg, L-1090 Luxembourg',  
BIC code 'CCPLLULL'.

Please use the conference abbreviation (relicts 2007) as the payment identification.

### **Currency**

The Luxembourg monetary unit is the Euro (EUR, €).

### **Cancellation**

Cancellations have to be sent in writing to the conference secretariat via Email (janchristianhabel@gmx.de). Your payment will be refunded (minus bank costs of the transfer).

### **Secretariat contact**

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# Programme



## Programme

### Thursday, 18.10.2007

14.00h, opening conference office in the CCRN

14.00h, all participants of the Symposium have free entrance for the MNHN-Luxembourg

Time	Section	Topic	Referent	Chairman
18.45		Address of welcome	Jan Habel, Tanja Walisch	Jan Habel
19.00-20.00		<b>Phylogeography – a tool for the understanding of relict species' history</b>	<b>John Avise (Keynote)</b>	
20.00-23.30		Welcome party in the MNHN	Marc Meyer	

### Friday, 19.10.2007

Time	Section	Topic	Referent	Chairman
9.00	Continental species	<b>Extra-Mediterranean refugia, post-glacial vegetation history and area dynamics in Eastern Europe</b>	<b>Zoltan Varga (Keynote)</b>	Guy Colling
9.45		Relicts and speciation in neotropical butterflies	James Mallet	
10.05		The nematode genus <i>Pristionchus</i> as case model for biogeography, biodiversity and species interaction	Matthias Herrmann	
10.25		Conservation and management of the habitats of two relict butterflies in the Belgian Ardennes: <i>Proclissiana eunomia</i> and <i>Lycaena helle</i>	Philippe Goffart, Camille Turlure	
10.45		Coffee break & poster session		
11.15		The Book of Invasions: Phylogeography of pygmy shrews ( <i>Sorex minutus</i> ) in Ireland and Europe	Alan McDevitt, Victor Rambau, Tom J. Hayden, Glenn Yannic, Jeremy B. Searle	James Mallet
11.35		Living on the northern margin - effects on genetics and morphology in two <i>Coenonympha</i> butterflies	Anna Lundhagen, Thomas Schmitt, Joachim Besold, Niklas Wahlberg, Sören Nylin, Toomas Tammaru	

11.55		Genetic admixture of palearctic and nearctic burbot lineages in northern North America	Kathryn Elmer, Matthias Sanetra, Axel Meyer		
12.15		Molecular and phytosociological insight into postglacial relicts <i>Lathyrus pannonicus</i> and <i>Oxytropis pilosa</i>	Matthias Schlee, Guido Grimm, Markus Göker, Wilhelm Sauer, Vera Hemleben		
12.35		lunch			
14.30	Mediterranean species	<b>Phylogeographic patterns within the Mediterranean region</b>	<b>Michael Veith (Keynote)</b>	Zoltan Varga	
15.15		Glacial relicts and post-glacial invasions of Central European reptiles	Ulrich Joger		
15.35		Cave beetles as postglacial relicts	Ruxandra Bucur		
15.55		The origin of the tortoises ( <i>Testudo marginata</i> ) introduced by man in Sardinia	Mélanie Perez, Marie-Catherine Boisselier, Josie Lambourdière, S. Soubzmaigne, Sarah Samadi, Roger Bour		
16.15		Coffee break & poster session			
16.45		Diversification in the Mediterranean: A phylogeographic analysis of the genus <i>Oedipoda</i> , Latreille 1829	Axel Hochkirch, Yvonne Görzig		Diethart Matthies
17.05		Mammal relict populations in temperate and tropical regions: their origin, their historical demography and their role on the postglacial recolonisations	Johan Michaux		
18.25 - 19.05	Alp. s.	<b>Tracing range shifts in the southern European mountain ranges: examples from arctic-alpine plants and from <i>Androsace</i> sect. <i>Aretia</i> (Primulaceae)</b>	<b>Peter Schönswetter (Keynote)</b>		

### Saturday, 20.10.2007

Time	Section	Topic	Referent	Chairman
8.30		Book-meeting: concept, instructions, discussion	Jan Habel, Thorsten Assmann	
9.00	Alpine Species	<b>Biogeography and relict status of alpine species</b>	<b>Thomas Schmitt (Keynote)</b>	Alfried Vogler
9.45		Multiple glacial refugiums of unwinged ground beetles in Europe – molecular data support classical phylogeographic models	Claudia Drees, Jan Habel, Werner Härdtle, Andrea Matern, Goddert von Oheimb, Thomas Reimann, Thorsten Assmann	

10.05		Oviposition electivity in two co-occurring blue butterflies ( <i>Polyommatus eros</i> and <i>P. escheri</i> ) in subalpine pastures of the Spanish Pyrenees	Thomas Fartmann	
10.25		Relicts of the cold past – arctic-alpine disjunct arthropods in Europe	Christoph Muster	
10.45		Coffee break & poster session		
11.15		Comparative phylogeography of two montane leaf beetles in Europe	Patrick Mardulyn	John Avise
11.35		What factors limit the habitat selection by alpine relic butterflies' Genus <i>Erebia</i> in Sudetan Mts. (Czech Republic and Poland)	Martin Konvicka, Thomas Schmitt, Jiri Benes	
11.55		Phylogeographic analyses of a boreal-temperate ectomycorrhizal basidiomycete, <i>Amanita muscaria</i> , suggest multiple forest refugia in Alaska during the Last Glacial Maximum	József Geml	
12.15		<b>ESUs, MUs and species concepts – how many species do we need?</b>	<b>Alfried Vogler (Keynote)</b>	
13.00		lunch break		
14.30	Conservation Biology	<b>The intriguing state of some postglacial relict plant populations reaching the northern border of their geographical range – conservation concepts</b>	<b>Jan Cerovsky (Keynote)</b>	Michael Veith
15.15		Conservation genetics and phylogeography of the threatened Corsican and Barbary red deer ( <i>Cervus elaphus corsicanus</i> and <i>C. e. barbarus</i> )	Frank Zachos	
15.35		Conservation of the grey bush cricket <i>Platycleis albopunctata</i> (Orthoptera: Tettigoniidae) under differing habitat conditions: implications from an individual-based model	Eva Maria Griebeler	
15.55		Differentiation between and within refugia; What does the genetics of relict species tell us about their conservation?	Gabriel Nève	
16.15		Coffee break & poster session		
16.45			Linking genetics and ecology: reconstructing the history of relict populations of <i>Carabus variolosus</i>	Andrea Matern, Konjev Desender, Claudia Drees, Eva Gaublomme, Alfried Vogler, Thorsten Assmann
17.05		A new method for the estimate of the size of small isolated and threatened animal populations: the case of the relict <i>Triturus alpestris inexpectatus</i> in southern Italy	Alain Dubois, Annemarie Ohler	
17.25		break		
18.00 - 19.00	Panel Discussion	<b>Conserving relict species for generations – under the impact of climate change</b>	<b>Jan Cerovsky James Mallet John Avise Markus Quante</b>	Thorsten Assmann
19.00 - 19.05		Parting words	Jan Habel	



# **Abstracts (oral communications)**





## Extra-Mediterranean refuges, post-glacial vegetation history and area dynamics in Eastern Europe

Zoltán Varga

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Key words: Refugial vs. invasion fauna, extra-Mediterranean refuges, “non-analogue” Upper Pleistocene assemblages, postglacial expansions, refugial vs. peripheric isolates, relict species

The European fauna was traditionally subdivided into a “*holothermic*” refugial and a “*holopsychric*” invasion type. The former type was differentiated according to the secondary subdivision of the large Mediterranean refugial area. This view was strongly confirmed and modulated by the recent molecular results and re-formulated as repetitive patterns of the generalised “*paradigms*” of core areas and tracks of post-glacial re-population. The second type was considered for a long time as result of a “Siberian” invasion, despite of the evidences which have revealed the taxonomical differentiation of northeastern “boreal” and southern European montane populations. Recently, growing evidence from fossil records and genetic research is starting to suggest that the traditional paradigm became questionable that the arboreal refugia were restricted to southern Europe and in particular the Mediterranean peninsulas (Balkan, Italian and Iberian) during the full-glacials. Despite the “individualistic” migration behavior, the varied ecological requirements and heterogeneous modes of dispersal, a significant part of the temperate woody flora shows a largely congruent pattern with the genetically most unique populations located in southern and central Italy, Corsica, and the Balkan peninsula, although they extend into northern Italy, Croatia, and Romania, i.e., to higher latitudes. Oppositely, taxa with a more boreal distribution do not coincide with the overall pattern which can be explained by the survival of more northern populations of these species during the last glaciations. From a vegetation point of view, the early establishment of forest taxa was observed e.g. in several areas of the Eastern Carpathians during the early part of the last deglaciation, which is not surprising because several of these species were present in southwestern and eastern marginal areas of the Carpathian basin during the last glacial maximum. The earlier “tree-less tundra” models for Europe north of the transverse mountain ranges of the Pyrenees, Alps and Carpathians have repeatedly been questioned by surveys of the Late Pleistocene Mammalian fauna, as well, which have pointed out that the carrying capacity adequate to feed the herds of large herbivores demands a rather productive environment, like the cold-continental meadow steppes in southern Siberia



and northern Mongolia. The presence of northern temperate refugia also supports to explain the “non-analogue” assemblages of small mammals discovered from the Late Pleistocene of unglaciated areas of eastern Central Europe. The mixture of species today typifying the tundra, steppe and even semi-desert habitats (e.g. lemmings, marmots, ground squirrels, jerboas, etc.) seems to include also species from deciduous woodland. Extra-Mediterranean core areas were identified also in widely dispersed cool-tolerant frogs and reptiles, as *Rana arvalis*, *Zootoca vivipara*, *Vipera berus*, etc. Some of these core areas had been at least near to the Carpathians and/or marginal areas of the Carpathian basin. The close geological and also faunal connection of the Carpathians suggests the existence of highly dynamic contacts with mountains of the Balkan peninsula during the climatic fluctuations of the Upper Pleistocene. These connections show a highly contrasting picture compared with the refugia of the Iberian and Appenin peninsula which were much more sheltered by glaciated mountains of Pyrenées and Alps, respectively. The Carpathian basin exhibit also numerous important characters concerning its post-glacial re-population. The organisation of community-complexes of the Pannonian forest-steppe connected by habitat ecotones resulted in the overlap of several different faunal types, e.g. Mediterranean, Balkanic, Siberian, Ponto-Caspian, Ponto-Pannonian, Turano-Eremic and Xeromontane elements. The Eastern and Southern Carpathians, together with the mountains of Western Transylvania, can be considered as core areas of survival and autochthonous evolution in some invertebrate groups with limited mobility. In mobile insect groups only peripherally isolated sibling species/subspecies have been evolved, which display manifold biogeographic connections, e.g. to the Balkan peninsula, Asia minor or Southern Russia. The distribution of the Mediterranean-Manchurian bicentric faunal elements, connected to the Ponto-Caspian waterway-system, shows long-distance disjunctions from the eastern Asiatic taxa. Gallery forests of Illyrian and Pannonian type and wetlands accompanying the large rivers of the Pannonian lowland have served as corridors of the northwards expansion of these species. The post-glacial re-population of the Carpathian basin from different directions has been supported by Illyrian vs. Dacian vicarious pairs of sister species/subspecies. Cases of vicarious boreo-Eurasian (“Siberian”)-Mediterranean montane sibling taxa have also been established.

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## Relicts and speciation in neotropical butterflies

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Keywords: Pleistocene refugiums, speciation, phylogeography

In the 1960s-1970s, the refugium theory of tropical biogeography was used to explain the paradox between (1) great species diversity in contiguous rainforest of the South American tropics, (2) the prevailing view of speciation in allopatry. Today's phylogeographic studies are refining this view and incorporating non-allopatric speciation via ecological specialization into the mix of theories.

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## The nematode genus *Pristionchus* as case model for biogeography, biodiversity and species interaction

Matthias Herrmann

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Keywords: Biodiversity, Phylogeography, cryptic species, species interaction

Our lab has established the nematode *Pristionchus pacificus* (Nematoda: Diplogastridae) as a satellite organism for comparison with *Caenorhabditis elegans*. The focus of the studies was first on comparative developmental and cell biological topics. As we then wanted to investigate not only macroevolution but also microevolution of the genus *Pristionchus* the need to get more strains of *Pristionchus pacificus* and also other species of the genus increased. But as the few strains we had at the beginning of our studies had been found in randomly taken soil samples, ecology and biology of the nematode in nature had remained unknown for many years. Knowing that many other genera of the family show an association with insects we wanted to start a long-term, large-scale project to examine the biology of the genus *Pristionchus*. In the last three years we sampled insects in Europe, America, Asia and Africa and could show a strong association with beetles. Many beetles of the superfamily Scarabaeoidea plus the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) host *Pristionchus* species. The nematodes sit on the beetles in larval stage (so called dauer larva) without harming their host. Only after the insects' death the nematodes grow adult and start reproducing, feeding on the bacteria and fungi degrading the insect. Twenty two different *Pristionchus* species have been identified so far. As a morphological species determination is difficult in most and even impossible in some cases we set up molecular identification by sequencing the small subunit of the ribosomal RNA (SSU) followed by mating test with reference strains. Cryptic *Pristionchus* species and the fact that we found some species on more than one continent makes the whole genus an important model system for speciation, biodiversity and species invasion.

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## Conservation and management of the habitats of two relict butterflies in the Belgian Ardennes: *Procllossiana eunomia* and *Lycaena helle*

Philippe Goffart<sup>1</sup>, Camille Turlure<sup>2</sup>

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Keywords: Habitat management, species conservation, butterfly, mowing, grazing

Habitat preservation and restoration are cornerstones of species' conservation and the main challenge for nature managers. To preserve species, lots of preventive measures or restoration projects are in progress in Europe. But, to improve the effectiveness of this undertaking, it would be highly desirable to: (1) identify precisely species' habitat requirements, (2) develop means to maintain or restore habitats, (3) assess effects of diverse habitat management methods on population dynamics and (4) find way(s) to evaluate and minimize their potential negative impacts. Here we focus on two relict butterfly species, the violet copper, *Lycaena helle*, and the bog fritillary butterfly, *Procllossiana eunomia*. Both species inhabit alluvial humid grasslands and peatbogs in the Belgian Ardennes, and share the same hostplant, the bistort, *Polygonum bistorta*.

Field studies and experiments carried out at several sites showed that:

- (1) The two species have slightly different habitat requirements. *L. helle* thrives in the neighbourhood of forest edges and *P. eunomia* reproduce preferentially at places with big grass tussocks and a high density of hostplants. This can be better understood and described with reference to the resources used by the species in the ecological space and time (Dennis, Shreeve & Van Dyck 2003).
- (2) Natural afforestation of these species habitats is prevented by mowing (traditional way of management) or extensive grazing with cattles or ponies.
- (3) Mowing can strongly affect population dynamics, especially those of *P. eunomia* due to the removal of tussocks on which caterpillars live (Goffart et al. 2001). However, autumn mowing seems to spare *L. helle* populations. This management method should be applied in long-term rotation, to preserve sufficient refugium areas every year for these two species.
- (4) When intensive, grazing has also a negative effect on both species, particularly on hostplant availability and tussocks preservation



(Schtickzelle, Turlure & Baguette 2007). But, if used with caution (stocking rate < 0,2 LU/ha/year ; late summer grazing preferentially) and only on large sites (> 10 ha), it can successfully preserve butterfly populations and their habitats.

Conservation of such relict butterflies in our present landscapes will be discussed in the light of these findings.

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## The Book of Invasions: Phylogeography of pygmy shrews (*Sorex minutus*) in Ireland and Europe

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Keywords: colonisation, Ireland, mtDNA, Y chromosome, microsatellites, refugia

Whether or not the pygmy shrew is a native species to Ireland has been the subject of much debate. If not native, from where and by what means did it colonise Ireland? In order to answer these questions, an 800bp fragment of the control region of the maternally inherited mitochondrial genome and four introns of the paternally inherited Y chromosome (3kb) have been sequenced from pygmy shrews throughout Ireland, Britain and Europe. This study is also incorporating the use of five polymorphic microsatellite loci (biparental markers) to compare genetic diversity and possible gene flow between Ireland and Britain. According to mtDNA sequence data, the majority of Britain and central/northern Europe appears to have been colonised from a glacial refugium in central Europe while Ireland was colonised from northern Iberia/southern or central France. Like other recent studies (eg. bank vole), this highlights the importance of glacial refugia other than the Mediterranean peninsulas for postglacial colonisation of Europe by small mammals. However, according to the Y chromosome sequence data, Irish, British, French, Italian, Swiss and Spanish pygmy shrew populations are grouped together in the same Clade. This points to a bias in male-female dispersal and colonisation in pygmy shrew populations. Microsatellite data also suggests that Ireland is a subset of the British population as all alleles found in Ireland are also found in Britain, with no alleles unique to Ireland. By using a multi-marker approach, we have revealed that the colonisation of Ireland by the pygmy shrew is more complex than previously thought. Although the Irish population belongs to the Iberian/French lineage, our data suggests the possibility that Ireland was colonised from its nearest landmass, Britain. It is likely that Ireland was colonised by human-mediated introduction rather than natural overland colonisation. Even though the pygmy shrew was probably introduced to Ireland, the fact that it is quite common (it is Ireland's only shrew species) and a representative of the



Iberian/French genetic lineage make it a high priority for conservation in a European context. This study also highlights the importance of using multiple genetic markers when inferring the phylogeographic structure and colonisation history of species.

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## Living on the northern range margin: effects on genetics and morphology in two *Coenonympha* butterflies

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Scandinavia is to some extent inhabited by species that are locally red-listed but globally widespread. Some of these Scandinavian populations are considered relicts from the temperature peak about 5000 years ago while others appear to have their natural distribution limit in this region. One argument why conservation resources should be spent on these peripheral populations of globally non-threatened species is that they may be genetically unique and carry potential for future speciation events. We were interested to study to what extent this argument is supported and have focused on two closely related butterflies, *Coenonympha hero* and *C. arcania*, which both have their northern range margin in south central Scandinavia and Estonia. The Scandinavian populations are considered isolated from the main distribution range. We analysed variability in allozymes, microsatellites, mtDNA sequences and wing morphology to evaluate the uniqueness of these populations. Genetic and morphologic patterns were compared with the patterns found in populations from a central part and a peripheral but not isolated part of the species distributions. The results are compared between these two species, and data from similar studies, to evaluate the generality of the patterns found. The results will be discussed in the light of both long and short term events and processes.

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## Genetic admixture of palearctic and nearctic burbot lineages in northern North America

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Keywords: admixture, population genetics, freshwater fish, holartic

The burbot (*Lota lota*) has one of the largest distributions of any freshwater fish, encompassing the entire holarctic of Asia, Europe, and North America. Thus, in the zoogeographic history of this species dramatic and relatively recent range contraction and expansion has occurred, caused by glaciation across the north during the Pleistocene. Historically, two subspecies have been recognized and these correspond to two phylogenetically distinct nearctic and palearctic lineages that meet in northern Canada. Within the North American burbot lineage, three genetic clades are proposed to have originated in the Pacific, Missourian, and Mississippian glacial refugia. In this study, we characterize the distribution of genetic diversity and quantify gene flow in burbot collected from across the species range in North America using 11 species-specific microsatellite loci. Phylogeographic lineages and glacial refugia are determined using mtDNA control region sequence data and microsatellite genotype profiles. We use Bayesian and coalescent analytical methods to assess the degree of admixture of the palearctic and nearctic burbot lineages in contact zones. Our findings about the post-glacial dynamics of the burbot across North America are discussed in the context of historical separation and contemporary population genetic patterns.

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## Molecular and phytosociological insight into postglacial relicts *Lathyrus pannonicus* and *Oxytropis pilosa*

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Keywords: *Lathyrus pannonicus*; *Oxytropis pilosa*; nrDNA; low-level taxonomy; postglacial migration

*Lathyrus pannonicus* and *Oxytropis pilosa* are two rare and relict species in Europe that presumably originated in Siberia, Russia, and are basically adapted to xerothermic environments. The species were investigated by phytosociological and molecular methods to obtain insight in their ecological needs and to increase knowledge of conservation needs of the present refugiums. Distribution areas inhabited by *L. pannonicus* and *O. pilosa* are similar, but the former has undergone an ecological shift in the Pannonian area and could therefore exceed its territory to more humid habitats in Western Europe and Mediterranean regions. In *L. pannonicus* six subspecies and many intermediate forms can be recognized. *Oxytropis pilosa*, which is ecologically and morphologically homogeneous, could on the other hand establish populations also in the Alps, Scandinavia (including seashore of Gotland), and the Baltic. Intraspecific and intraindividual genetic variability can be detected by cloning and sequencing of a large number of nrDNA spacer sequences (internal transcribed spacers, ITS1 and ITS2, and 5' external transcribed spacer, 5' ETS). The spacers of the nrDNA are structured in a way that allows to trace back population dynamics and migration pathways: They are biparentally inherited, part of a multicopy gene region, and are undergoing (partly incomplete) concerted evolution as well as frequent intragenomic recombination. The ecologically and morphologically conserved *O. pilosa* is separated into two general sequence types, an Eastern and a Western type with a transition zone in Central Europe. Moreover, at outermost areas additional unique mutations can be distinguished to characterise relict populations and/or terminal migration points. *Lathyrus pannonicus* shows a higher genetic diversification due to the ecological shift from xerothermic to even moist habitats, and a broader spectrum of biogeographic glacial refugiums and (re-)migrations between these regions. The original xerothermic ecotype covers the whole species' range from Siberia to Southern Spain, with the *L. pannonicus* subsp.



*multijugus* and *L. ledebouri* complex in the Altai, and *L. pannonicus* subsp. *collinus* westward from the Crimea Peninsula via the Pannonian area to Southern France and Southern Spain. The derived moisture ecotype (subsp. *pannonicus*) developed in the Pannonian area and postglacial relics of an intermediate ecotype in Istria (subsp. *varius*) are closely linked to this ecotype which finally extended to Italy and via the Sea Alps and Loire Valley (subsp. *asphodeloides*) to Atlantic Spain (subsp. *longestipulatus*). Ecologically and phylobiogeographically induced sequence signals are partly independent of each other but agree with the phytosociological-ecological and taxonomic setting of analysed populations. Using sequences obtained by cloning of PCR products of the nrDNA spacers in such a context has a high potential aside their traditional application to compute phylogenetic trees of low hierarchy. Such a degree of variability cannot be handled with standard phylogenetic approaches. We therefore used phylogenetic networks in combination with novel distance functions based on morphological and molecular data. The large dataset (> 300 sequences for *L. pannonicus*; > 200 sequences for *O. pilosa*) fills the gap between sequence-based phylogenies and genetic analyses at population level. According to our data, postglacial migration pathways but also nunatak-like outlast led to these chorological high disjunctions of the present populations. Conservation efforts must hastily be improved so that the disjunct populations are protected to retain their natural genetic diversity. Networking concepts will fail and are not necessarily desirable as speciation is in progress. As a result of phytosociological surveys, conservation efforts need to include more intensive cultivation of the habitats; the preference lies on a management system, which emphasizes more on a grazing than on a cutting regime. Disturbances and opening of the soil are recommended for these pioneers that in some cases escape to margins of trails in nature protection areas and occasionally form mass populations in pastures grazed by sheep, cattle, and even horses.

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## **What do we really know about the evolution of Mediterranean amphibian communities?**

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It is sound to assume that the evolution of species restricted to classical Mediterranean centers of endemism was triggered by late Pliocene and Pleistocene glaciations. Most recent evidence comes from the application of molecular markers and the calibration of a molecular clock. However, discordance between species and gene trees, lack of rate constancy among lineages, alternative or even wrong calibration points, as well as phylogenetic and historical uncertainties may seize our illusion that reconstructing a time frame for the evolution of species is possible. Using Mediterranean amphibians as a model I will outline strategies of how a nifying evolutionary scenario may even so be achieved at community level. This will finally allow us to better assess the impact of glacial cycles on the evolution of Mediterranean amphibians.

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## Glacial relicts and post-glacial invasions of Central European reptiles

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Keywords: phylogeography, mitochondrial DNA, reptiles, relicts

A phylogeographic analysis of 7 species complexes of European reptiles was executed using mitochondrial DNA and ISSR genomic fingerprinting. While mitochondrial genes (here mainly cytochrome b sequences) enabled us to draw conclusions about phylogeographic history and differentiation, the additional application of bisexually inherited markers was helpful to determine which stage had been reached in speciation. As species with similar distribution patterns in southern and central Europe had been selected, matching phylogeographic branching patterns could be used to draw some general conclusions:

- 1) Highest genetic diversity among the analyzed species complexes was observed in their representatives in southern Europe, the Near East and the Caucasus. Obviously the differentiation took place in the Pleistocene refugia.
- 2) The species complexes were found in different stages of speciation. In some cases, cryptic species could be detected at the southern edge of distribution.
- 3) Evidence for multiple range retractions and expansions, which were postulated for the times of Pleistocene climatic oscillations, could be found in the Balcans, but in Central Europe their traces have been wiped out. Only the Holocene invasion has left imprints in the genomes from this area.
- 4) The Balcans and the Pontic region were the origins of active invaders into Central and Northern Europe.
- 5) Groups from the Iberic and Apennine peninsulas rarely conquered other regions. This limitation can be attributed to the barrier function of the Pyrenees and the Alps.

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## Cave species as postglacial relicts

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Keywords: troglobites, underground speciation, relictual model

Underground ecosystems are, through geological periods, buffered from profound climatic modifications and thus, have retained a broad diversity of living relicts, the great majority of which being represented by terrestrial, troglobitic species, with a reduced mobility. The most widely accepted model for the evolution of terrestrial troglobites in temperate latitudes is the climatic-relict model, also called the relictual or Pleistocene-effect model. According to this model, genetic isolation and accelerated speciation was driven by geographic isolation of pre-adapted animal groups during glacial-interglacial periods in Pleistocene. The interruption of gene exchange between epigeal and hypogean populations and the extinction of surface populations favoured the speciation process of underground faunal groups.

The authors mention the example of cave beetles (Leiodidae Cholevinae Leptodirini) as a model group for studies of past climatic changes as postglacial relicts. The species complex *Speonomus delarouzei* colonizes the eastern part of the Pyrenees. Juberthie (1988) e.g., describes the scenario of speciation in this species complex during three glacial-interglacial episodes. Important contributions for clarifying the process of colonization and speciation in the underground environment by cave beetles come from ecological, ethological (Moldovan, 1997), geological data and, lately, from the application of the modern techniques of molecular genetics (Bucur et al., 2003). Because of the high degree of endemism of this group (many of the species being restricted to singular caves), and their susceptibility to both human activities and minor changes of the subterranean microclimate, cave fauna, in general, and especially the cave beetles, are highly recommended to be subject of intensive conservation research.

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## The origin of the tortoises (*Testudo marginata*) introduced by man in Sardinia

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Keywords: tortoise, introduction, genetic markers, Sardinia

*Testudo marginata*, the largest European tortoise, is indigenous in Greece and in the South of Albania. Populations of these tortoises have been introduced in Sardinia. According to the bibliographic data, this introduction took place either during the Etruscan period or at the end of the 19th century. We studied the origin of these introduced populations and the mechanism of these introductions using polymorphic and specific genetic markers, microsatellites loci, and tools for analysis of population genetic data.

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## Diversification in the Mediterranean: A phylogeographic analysis of the genus *Oedipoda*, Latreille 1829

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The Mediterranean region belongs to the European hotspots of biodiversity and endemism. The band-winged grasshopper genus *Oedipoda* consists of 23 species distributed from Europe and northern Africa to Central Asia, ten of which have a Mediterranean distribution. We sequenced two mitochondrial (12s rRNA, ND1-16s rRNA) and one nuclear (ITS2) gene fragments of 87 individuals from twelve species to infer a phylogeny for the West-Palaearctic species. Our results revealed a basal subdivision of the genus in two major groups (*germanica*-group and *caerulescens*-group). The resolution of the *germanica*-group was relatively low and the taxonomical assignment was sometimes in conflict with our phylogeny. This was particularly true for *O. germanica*, *O. fuscocincta* and *O. coerulea*, indicating recent gene flow among these parapatric species. A potential hybrid zone might be found in the Pyrenees. *O. germanica* specimens from Italy and Greece were genetically distinct, indicating separate refugia for these populations. The Cyprian endemic *O. venusta* branched off as a sister species to the Anatolian *O. aurea*, both represented a basal branch of the *germanica*-group. Within the *caerulescens*-group, *O. himalayana* and *O. fedtshonki* (both from Kashmir) represented the basal group, followed by the eastern Anatolian species *O. schochi*. The two sister species *O. charpentieri* and *O. miniata* turned out to contain three distinct lineages, with *O. charpentieri* occurring in the western Mediterranean, *O. miniata* in the eastern Mediterranean and a third lineage in the central part (Tunisia, Balearic Islands, Sardinia). A former hypothesis of hybridization on the Balearic Islands could not be confirmed. *O. canariensis*, an endemic species to the Canary Islands, contained three separate lineages for Tenerife, La Gomera and Gran Canaria/Fuerteventura. However, the genetic distances between these clades were relatively low. *O. caerulescens* is the most widespread species, which had only a low degree of intraspecific differentiation. The specimens from France to Russia turned out to be genetically rather homogenous. Only two separate lineages were found, one in northern Africa and another one on the Iberian Peninsula. Our results indicate that the current taxonomy of *Oedipoda* partly conflicts with the phylogenetic inference. The three species *O. germanica*, *O. coerulea* and *O. fuscocincta* should be studied in more detail to test the hypothesis of recent hybridization. Sequencing of a more variable nuclear marker than ITS might

help to unravel this problem. The Central Mediterranean lineage of the *charpentieri-miniata* complex should be studied morphologically to test, whether it represents a species.

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## **Mammal relict populations in temperate and tropical regions: their origin, their historical demography and their role on the postglacial recolonisations**

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Since several years, the explosion of phylogeographic studies in Europe but also in other regions in the world evidenced the presence of particular populations or species, genetically highly divergent to the other ones and often distributed in restricted areas. The aim of our presentation will be to present several examples of “relict” populations in different mammal species living in Western Europe (Genera *Apodemus*, *Myodes*, *Erinaceus*, *Crocidura*..) and South East Asia (*Laonastes aenigmamus*). On the basis of their genetic structure, we will propose different hypotheses explaining their origin, their demographic histories and their importance for the mammal biodiversity. On the conservation point of view, our genetic data associated to informations about the recent human disturbances will allow to estimate their chance of long term survival and to propose applied conservation measures.

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## Tracing range shifts in the southern European mountain ranges: examples from arctic-alpine plants and from *Androsace* sect. *Aretia* (*Primulaceae*)

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What does an organism make a relict? The answer to this question may be not straightforward and is certainly multi-faceted, but one aspect certainly is that relicts are regarded as testimonies of past range shifts that can help to shed light on the genesis of extant distribution areas. In my presentation I will consequently focus on range shifts and illustrate this with phylogeographic case-studies from arctic-alpine plants as well as from several species of *Androsace* (*Primulaceae*) distributed throughout the southern European mountain ranges.

A considerable number of plant species of predominantly arctic distribution grow in non-Arctic mountain ranges as well, a distribution type referred to as arctic-alpine. There are strong differences between taxa regarding the extent of their distribution area outside the Arctic. While some arctic-alpine plants occur frequently in many mountain ranges of the northern hemisphere, others are very rare and sometimes only a few populations outside of the Arctic are known. In my presentation I will unravel the immigration history of five rare arctic-alpine model taxa (*Carex atrofusca*, *Carex bigelowii*, *Juncus biglumis*, *Minuartia biflora*, *Ranunculus pygmaeus*) into the Alps, applying molecular methods (fingerprinting and sequencing). I will focus on the circumpolar phylogeographical pattern, the source areas for the colonisation of the Alps, the mode of origin of the Alpine populations (single vs. multiple introductions) and the phylogeographical structure within the Alps. As some of the rare arctic-alpine plants in the Alps are critically endangered, I will also touch on conservation strategies.

Due to historical climatic factors, and the scattered distribution of mountain ranges, alpine plants in Europe often have distributions which are on some scale disjunct, either between mountain ranges, or within a larger mountain range. Simple geographical arguments have often been used to suggest that current patterns of disjunction match the former locations of refugia during the ice ages. While this may hold true for narrow endemic taxa, found only in one small area, it need not be so for more widely distributed taxa. We studied intraspecific genetic variation in *Androsace* section *Aretia* to probe the causes of patchy distributions in alpine plants. This European-centred group contains species with disjunctions at all ranges from a few tens of kilometres to several hundred, but which are otherwise similar in floral syndrome and apparent dispersal abilities. Our results show that, even if the



current disjunct distributions coincide with potential glacial refugia, more recent dispersal between areas is a better explanation in many cases, and that other biological factors such as hybridisation, extinction and cryptic speciation can further disrupt our ability to draw conclusions simply from cartographical data.

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## Biogeography and relict status of alpine species

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Keywords: Alps, mountains, phylogeography, ice ages, endemism

Alpine, arctic and arctic-alpine species have often been seen as relicts of the widespread floras and faunas of the periglacial belts of the ice ages. However, recent research shows that the biogeography of these species is not as simplistic as formerly often assumed. Therefore, this presentation gives an overview on the biogeography of this heterogeneous group of species giving examples of distribution patterns and genetic structures in the four main groups: (i) endemics of the Alps with strongly restricted and with broader distributions, (ii) alpine disjunct species with perialpine disjunctions and with a wide western Palearctic distribution, (iii) oro-Mediterranean species and (iv) arctic-alpine disjunct species. These data are reflected in the light of the relict character of populations of these species in the past and present.

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## Multiple glacial refugia of unwinged ground beetles in Europe? Molecular data support classical phylogeographic models

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Keywords: massifs de refugium, Holdhaus-line, multiple refugia, postglacial expansion, Carabidae

Since the 1930s several European zoologists have developed scenarios for glacial refugia and postglacial expansions. These scenarios were mainly based on studies of the morphological differentiation and the distribution patterns of insect species, especially of those with a low power of dispersal. Rensch, Holdhaus, Reinig, and especially Lindroth were important authors in this context. Holdhaus recognised that the distribution of blind euedaphic and troglobitic beetles is restricted to a well definable area in Europe, south of a line connecting Bordeaux, Lyon, the southern Alps, the Carpathians and the Black Sea. This so-called "Holdhaus line" is defined by the northern distribution limit of beetles with the lowest known power of dispersal. Only two small areas with blind beetles are known north of this line (Jura, north-eastern Alps). South of this line, many endemic animal and plant species are occurring. These areas are called "Massifs de Refugium" since Chodat & Pampanini (1902). If highly specialised endemic plant and animal species were able to survive the glacial period(s) in southern France and northern Italy, also other species which are currently more widely distributed in Europe had the chance to survive in these "Massifs de Refugium". Molecular analyses of populations of the ground beetle genus *Carabus* prove the existence of these postulated refugium areas. Genetic differentiation of *C. auronitens*, for example, is more pronounced in areas south of the "Holdhaus line" than in areas north of it where this species was not able to survive owing to its habitat selection. Furthermore, the differentiation gives good evidence for multiple refugia. A comparison between populations south and north of the "Holdhaus line" offers the opportunity to verify the „allele elimination hypothesis“ first published by Reinig (1938) as a possible consequence of postglacial expansions.



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## Relicts of the cold past – arctic-alpine disjunct arthropods in Europe

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Keywords: arctic-alpine distribution, cold-adapted fauna, climate warming, comparative phylogeography, genetic diversity, mitochondrial divergence

The cold-adapted fauna is assumed to experience area regression in warmer periods, such as the Postglacial. The most striking consequence is the arctic-alpine range disjunction, which is congruently found in multiple non-related taxa. Their current high altitude and latitude populations are relicts of formerly wider distributions in the periglacial tundra belt. Especially the remaining narrow areas in low and southern mountain ranges seem susceptible to anthropogenic global warming. In a comparative phylogeographic study on arctic-alpine distributions in Europe, we investigated the biogeographic history and population genetic consequences in three codistributed taxa, wolf spiders of the *Pardosa saltuaria* group, the ground beetle *Nebria rufescens* and the butterfly *Erebia pandrose*. Analysis of the mitochondrial ND1 gene revealed a general divergence pattern. All populations north of the glacial permafrost limits form a single clade of shallow divergence (<1%), suggesting population connectivity in the late Pleistocene. On the other hand, the Balkan populations show signs of old vicariance prior to the last glaciations in all three taxa. The relationships of the Pyrenean populations are inconsistent, they belong to the northern clade in *Nebria* beetles, while they are as distinct as the Balkan ones in *Pardosa* and *Erebia*. Although similar area relationships suggest a common biogeographic history, we observed striking differences in sequence divergence among the investigated taxa. The mean Kimura 2 parameter distance was 2.1% in the spiders, 1.2% in the beetles, but only 0.2% in the codistributed butterflies. Therefore, ND1 was used to analyse within-population diversity only in *Pardosa* spiders. As expected, we found a reduced genetic diversity in small populations of the northern clade (Bohemian Forest, Giant Mountains) as well as in the southern high mountains (Pyrenees, Balkans). However, not all hypotheses were confirmed. An inference of population sizes by a Bayesian coalescence approach resulted in lower size estimates of ancestral populations at glacial times compared to modern populations from Scandinavia and the Alps. Thus, the overall impact of climate warming on cold-adapted species must not necessarily be as negative as regularly assumed.

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## Comparative phylogeography of two montane leaf beetles in Europe

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Keywords: Phylogeography, Chrysomelidae, genetic variation, montane habitat, population differentiation

Using mitochondrial and nuclear DNA fragments, we compare the distribution of genetic variation of two leaf beetle species adapted to montane and arctic habitats across most of their geographic range. From this comparison, we try to gain insights into their history of glacial and postglacial differentiation and expansion.

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## What factors limit the habitat selection by alpine relict butterflies? Genus *Erebia* in Sudetan Mts. (Czech Republic and Poland)

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Keywords: colonisation, mountain habitats, *Erebia epiphron*,

Insular “middle mountains” of the Hercynian chain are poor on alpine insect species due to small areas overtopping the timberline. There are two relict butterflies native to Jeseník Mts. (max. 1495 m), NW Czech Republic, each traditionally classified as a endemic subspecies: *Erebia sudetica sudetica* (Staudinger, 1851), a species protected by EU Habitat Directive, and *E. epiphron silesiana* Meyer & Dür, 1852. Whereas *E. epiphron silesiana* is restricted to summit Nardus-dominated grasslands and never enters continuous mountain forests, *E. sudetica sudetica* inhabits damp locations at the timberline plus open wet sites within mountains forests, descending to ca 700 m. Whereas *E. epiphron silesiana* forms continuous populations, the distribution of *E. sudetica sudetica* conforms to a metapopulation model, with local colonies interconnected by dispersal. Being restricted to relatively low mountains, these two species, but particularly *epiphron*, are thought to be particularly threatened by warming climate. However, their locally restricted distribution might be an outcome of limited range of available habitats, rather than some ecophysiological constraints. A non-native population of *E. epiphron* in Krkonose Mts. (max. 1620 m), transferred there from the Jeseník Mts. in the 1930s, descends to ca 1100 m, colonising large clearings opened up during forest die-backs in the 1980s, and also hay meadows within the forest belt. There is behavioural and molecular evidence that *E. epiphron* cannot disperse through continuous forest. In contrast, recent monitoring of Jeseník *E. sudetica* reveals that similarly to Krkonose *E. epiphron*, but unlike Jeseník *E. epiphron*, *E. sudetica* colonises woodland clearings and margins of forest roads. We interpret this so that the clearings and other open structures are situated too far from the “natural” treeless habitats in Jeseník, but closely adjoin them in Krkonose. The more distant openings are within colonisation limits of *E. sudetica*, species adapted to living near woodland structures and to disperse through them, but not of *E. epiphron*. Given that open treeless conditions in upper mountain belt represent the key to survival of both species, it is no longer permissible to strive for dense high productivity woodlands in altitudes near the timberline. Instead, natural disturbances such as avalanches, windfalls and insect pest outbreaks, should be viewed as factors supporting the relict species, and local



mimicking of their effects should be actively applied if natural disturbances cease to operate. Effects of past efforts to keep as much forest area as possible, including deliberate planting of *Pinus mugo*, should be actively reversed. The unanswered questions regarding habitat limits of high mountain *Erebia* species critical for predicting their fates under warming climate include their ecophysiological requirements, such as cold hardiness, diapause initiation and possible high-temperature thresholds for survival. Additionally, only molecular markers can answer whether the colonies of *E. sudetica* descending below the forest belt in the Jeseník Mts. have originated from regional alpine or piedmont populations.

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## Phylogeographic analyses of a boreal-temperate ectomycorrhizal basidiomycete, *Amanita muscaria*, suggest multiple forest refugia in Alaska during the Last Glacial Maximum

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Keywords: Beringia, beta-tubulin, fungi, elongation factor, ITS, rDNA

Beringia, including Alaska and north-eastern Siberia, has long been a focal point for biogeographical research in a wide range of plant and animal taxa. First, due to its diverse landscape and climate and the fact that much of the region remained ice-free during glacial maxima, Beringia served as a refugium for arctic flora and fauna. Second, during much of the Tertiary and the Quaternary periods, Beringia was the major land connection between Asia and North America and provided migration routes to a wide variety of organisms. Whether boreal forest refugia existed in Beringia during the Last Glacial Maximum (LGM) is a major, as yet unanswered question in quaternary science. Although earlier pollen-based biome reconstructions suggest that all of Beringia was covered by arctic tundra, increasing amount of plant molecular data and the most recent pollen data support the theory that boreal forest existed in small refugia in Alaska. From a general point of view, the importance of host trees in the distribution of ectomycorrhizal (ECM) fungi has been repeatedly noted. Despite the ecological importance of ectomycorrhizal (ECM) fungi, very little is known about their phylogeography. To test the hypothesis of LGM forest refugia in Alaska, we analyzed molecular data from *Amanita muscaria*, an ECM fungus native to temperate and boreal forest regions of the Northern Hemisphere. Our phylogeographic analyses based on multi-locus DNA sequence data suggest the existence of three distinct clades in Alaska. One of them, corresponding to *A. muscaria* var. *regalis*, is associated with subalpine habitats, while the other two (the “North American” and “Eurasian” clades) are strictly boreal-temperate. Haplotypes from Interior Alaska form unique groups within the forest-dwelling clades, while haplotypes from Southeast Alaska and the Pacific Northwest form a group endemic to the coastal rainforests. These results suggest genetic isolation preceding the LGM and support the hypothesis of glacial forest refugia in Interior Alaska and along the coast of Southeast Alaska and British Columbia.

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## **ESUs, MUs and species concepts – how many species do we need?**

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The Evolutionarily Significant Unit (ESU) and Management Unit (MU) were the answer of the 1990's to the question of 'what to conserve'. As molecular markers are applied more broadly, the ESU concept in its various guises can now be utilised to assess entire lineages and communities. This is a step up from the single-taxon applications of earlier studies establishing conservation status of particular populations. This talk will focus on the pattern versus process based definitions of ESU and what we can learn from the distribution of sequence variation in multi-species analyses. Specifically, what are the factors contributing to the formation of ESUs, and can we make predictions about how many ESUs there are in a lineage? Are relictual populations a major source of ESU formation?

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## The intriguing state of some postglacial relict plant populations reaching the north-western border of their geographic range – Conservation concepts

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Key words: postglacial relict plants, distribution limits, doubts about indigenoussness, conservation problems

The message of the presentation is based on three case studies on three plant species, all of them occurring in the Czech Republic.

1. The Dog's-tooth Violet, *Erythronium dnes-canis* L., from the Lily Family (*Liliaceae*) has been first recorded in 1828 in Central Bohemia, approximately 20 km S of the Czech capital Prague. The locality of a meridional species is an extremely isolated one, several hundreds kilometres distant from the border of the plant's continuous South European range. The nice early spring flower has become one of the most popular plants of the Czechia's flora. Doubts have been, however, expressed about its indigenoussness in the site. There are no direct proofs, but it looks very clear the plant being a native relict from the Middle Holocene. The locality in Central Bohemia has been much larger than the recently remaining four microlocalities containing over 1,000 specimens. In the early years of the 20th century, thousands of flowers were collected and sold at the Prague florist markets. The habitat of *Erythronium dnes-canis* are light deciduous woods. This had also been the case in the Bohemian site: the former forest management by coppicing had been very favourable for the plant. A strong decline started with modifying the woods into spruce plantations. Now the largest microlocality is protected as a National Nature Monument with a special management.

2. The Siberian Leopard-plant, *Ligularia sibirica* (L.) Cass., from the Daisy Family (*Asteraceae*) has been discovered in North Bohemia in 1814. It still thrives there in several rather numerous populations. The plant on the NW limit of its geographical distribution (with the core range in the European Russia, Siberia, China and Japan) is in the Czech Republic evidently a relict from a past continuous distribution in the early postglacial period (Praeboreal). Nevertheless, also in this case hypotheses had been expressed about its alien origin; some of them rather ridiculous (seeds brought in from Siberia with hay to feed the Cossack horses during the Napoleonic Wars!). The habitat of the plant is wet grasslands with a high groundwater level. To protect the plant efficiently, the sites, mostly protected, have to be managed – mainly by maintaining a high enough water level, mowing the meadows, and preventing a self-sowing of trees as well as spreading of invasive plant species. Not long ago, a new occurrence of

*Ligularia sibirica* has been discovered in South Bohemia – and immediately doubts concerning its autochthony have emerged.

3. The Pelt Fern, *Notholaena marantae* (L.) Desv., has been known since 1858 from South Moravia, where it grows on dry sunny serpentinite rocks in a deep river valley. This has been – until recently – the northernmost station of that species. In this case there is no doubt, one of the most remarkable ferns of the Czech flora, is a relict from some warm postglacial period. The area called Mohelno with many other rare and threatened plant as well as animal species is protected as a National Nature Reserve. It is now subject to a special monitoring because of some serious environmental impacts accomplished in the close surroundings: launching a nuclear power plant and constructing two river dams with their lakes. A microclimatic change has probably already been demonstrated by an increasing succession of trees suppressing the rock flora and vegetation, the rare fern in the first place. A management was executed, in particular the unwanted trees and scrub removed. It was good that the administration of the nuclear power plant shared the funding of the management. Quite recently a sensational discovery has been made in the Bohemian Karst SW of Prague. The Czech Republic has now a new northernmost occurrence of *Notholaena marantae*. The species was considered, at least at the N border of its range, to be an obligatory serpentiniophyte. This is not the case in the South, but neither is the case in the Bohemian Karst, the fern occurring there on different rocks, nevertheless with a similar chemical composition (picrite). The site is a very small one and has been discovered only due to a very detailed search in the framework of the Nature 2000 mapping.

The population is by all means spontaneous and most probably native.

From the above case studies conclusions can be derived, which are very characteristic for relict plant species, their populations, habitats and conservation – not only in the Czech Republic:

1. Attractive relict plant species are very popular among broad general public because of their even somehow mysterious uniqueness. Newspaper articles, TV programmes present them to people like detective stories, nature trails lead tourists to their stations. Thus those species are becoming a tool of promotion the knowledge and conservation of plants.

2. Curiously enough, relict plant species, particularly those occurring in isolated localities far from their core ranges, awake – even among botanists themselves – a suspicion about their autochthony sometimes even aimed at discrediting their scientific and conservation values. Fortunately, such efforts do not bring many negative effects.

3. With the advancing detailed survey and research, in Europe particularly in the framework of the Nature 2000, some quite astonishing, until now unknown occurrences of relict species are being discovered.

4. Relict plant species have survived in certain environments – habitats, which for ages have escaped deteriorating human impacts: this, however, ceased to be valid in the past two centuries and during several past decades in particular. Therefore the relict species are priority objects of interest for nature conservation. Their occurrence has been a reason for the



establishment of many protected areas of national or even international interest.

5. In a countryside dominated by humans and their heavy environmental impacts, the habitats of relict plant species have to be protected especially carefully, and – as a response to both human-made and spontaneous processes – mostly have to be specifically managed to enable the relicts to survive. This need is now being growingly recognized by state authorities and non governmental bodies, both nationally and internationally. One of the new encouraging features is a support from the business sector.

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## Conservation genetics and phylogeography of the threatened Corsican and Barbary red deer (*Cervus elaphus corsicanus* and *C. e. barbarus*)

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Keywords: red deer, Sardinia, Corsica, Tunisia, conservation

The two red deer subspecies *Cervus elaphus corsicanus* (Corsican red deer, endemic to the Tyrrhenian islands of Sardinia and Corsica) and *C. e. barbarus* (Barbary red deer, presently confined to a small area along the Tunisian-Algerian border) are classified by the IUCN as “Endangered” and “Lower risk (near threatened)”, respectively (until recently, the Barbary red deer's status was “Vulnerable”). Both subspecies underwent severe bottlenecks in the 20th century. The Tunisian population possibly comprised merely 7 animals in the early 1960s. Since then, reintroductions from a reserve at El Feidja have been carried out. The Corsican population of *C. e. corsicanus* became extinct around 1970, but was refounded with effectively 13 Sardinian animals in the 1980s and 1990s. We have analysed genetic variability and differentiation in these two subspecies (Sardinia: n = 29, Corsica: n = 10, Tunisia: n = 30) at 8-13 polymorphic microsatellite loci and sequences of the mitochondrial control region. Apart from gaining insight into the genetic status of these threatened populations we particularly addressed the following issues: (1) Are the different Tunisian stocks already differentiated, or does Tunisia still harbour a genetically homogeneous population? (2) Is there any (incipient) genetic differentiation between the newly founded Corsican population and its population of origin from Sardinia / are there signs of a founder effect in Corsican red deer? (3) By comparing our data with those from other European red deer populations we wanted to shed further light on the phylogeography of *C. e. corsicanus* because it is still unclear whether these deer are derived from European mainland or from North-African Barbary red deer. Likewise, it is still an open question if they were introduced to the Tyrrhenian islands by humans or if they colonised these islands naturally following a sea-level transgression during a glacial maximum. Our analyses yielded very low genetic variability for *C. e. corsicanus*. The Barbary red deer also showed low variability values in a European comparison, but diversity was not as low as might have been feared in the light of the population's history. Analyses of molecular variance, assignment tests and factorial correspondence analysis did not yield any signs of differentiation among the Tunisian subgroups studied, whereas there was clear evidence of a differentiation between the Sardinian and the

Corsican red deer population. The phylogeographic analysis showed close relationships between *C. e. corsicanus* to autochthonous Italian mainland red deer at nuclear loci, again suggesting that a natural colonisation of the Tyrrhenian islands seems possible. The results presented are discussed with respect to their implications for the conservation and management of these rare deer taxa.

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## **Conservation of the grey bush cricket *Platycleis albopunctata* (Orthoptera: Tettigoniidae) under differing habitat conditions: implications from an individual-based model.**

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Keywords: thermophile species, differences in population dynamics, extinction risk, MVP, day-degree model

The grey bush cricket *Platycleis albopunctata* (Orthoptera: Tettigoniidae) is a thermophile species that is distributed throughout Europe. In Germany, it mostly inhabits semi-arid grasslands and it is considered threatened due to the loss of its natural habitat. Field studies on this species have shown that temperature conditions of inhabited grasslands differ strongly (Mesobrometum, Xerobrometum). It has been suggested that such differences in habitat conditions influence population dynamics of the species and that this in turn may impact its extinction risk (Gottschalk et al. 2003).

In order to better understand the mechanisms which determine the vulnerability of the bush cricket in habitats with different temperature profiles, we developed a stochastic individual based model (Griebeler & Gottschalk 2000a,b). In this model, population dynamics is implemented as a day-degree model. It is based on the assumption that individuals of a population daily accumulate temperatures during their development. The model distinguishes three life-stages: eggs, larvae and adults. During simulation, the temperature sum input received by an egg or larva is updated daily. Both stages pass to the next stage if their temperature sum inputs exceed specific limits. The reproductive output of adults also depends on temperature. For each life-stage a mortality rate is additionally assumed.

To test the hypothesis of thermal conditions generating differing population dynamics and differences in the species' extinction risk I applied this model to the populations studied by Gottschalk et al. (2003). These authors monitored two populations inhabiting grasslands situated in Baden-Württemberg, Southern Germany which had strongly different temperature profiles over a period of ten years (Gottschalk et al. 2003). For both populations, annual population sizes calculated by the model were in overall agreement with field estimates. The model resembled natural phenologies of larvae and adults that had been recorded over two years. In the warmer grassland, the population size remained relatively constant over the ten years, while the other showed strong fluctuations in size. For both populations, MVP (Minimum Viable Population size) estimates calculated by

the model were highly different due to differences in thermal conditions of habitats. A second simulation study in which I systematically varied mean annual temperature in a habitat corroborated that the extinction risk of the bush cricket increases with degrading thermal conditions of habitats. Consequently, for thermophile species MVP estimates found in literature should be interpreted carefully.

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## **Differentiation between and within refugia: What does the genetics of relict species tell us about their conservation?**

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Keywords: refugia, genetic differentiation, conservation

Within refugium zones and areas recolonized since the Würm glacial period, genetic isolation of the populations depends on the distribution of the habitats and on the dispersal capabilities of the relevant species.

Resulting population structure may show different patterns according to these processes: from widespread species showing low genetic differentiation over very large distances to species showing high genetic variability in some mountain refugia. In several cases, species show high variation between refugia while showing low differentiation in other areas. Such cases clearly show the importance of refugium zones for the conservation of the genetic make up of these species.

In an era of global warming processes, the future of many such cases poses considerable challenges.

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## Linking genetics and ecology: reconstructing the history of relic populations of *Carabus variolosus*

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Keywords: population genetics, gene flow, *Carabus variolosus*

The flightless European beetle *Carabus variolosus* is listed as a species of public interest in the EU Habitat and Species Directive. This expresses the member states' commitment to securing the long-term survival of this rare and threatened habitat specialist of headwater areas and swamps in woodlands. Knowledge of its genetic composition and phylogeography is of major importance for the implementation of conservation measures. Analysis of genetic variation at both nuclear (16 allozyme loci) and mitochondrial markers (CO1) was conducted on individuals sampled from 12 populations (plus 3 outgroup populations for mtDNA), covering a large part of the species' western distribution range from the northern and western edge (Germany and France) to South Slovenia. Significant genetic diversion and restriction of gene flow was revealed. An overall  $F_{ST}$  value of 0.465 and poor accordance between geographical and genetic distance suggest a high degree of isolation of the island-like distributed populations, even between neighbouring populations within drainages. Postglacial colonization processes are not reflected in allozyme variability; rather, stochastic effects have caused a seemingly random distribution of genetic variants. It is likely that interpopulation differentiation was accelerated by small population sizes. Our findings were supplemented with results from a mark-recapture study at two relic populations and are discussed in the light of the past history of the species and the concept of conservation units.

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## **A new method for the estimate of the size of small isolated and threatened animal populations: the case of the relict *Triturus alpestris inexpectatus* in southern Italy**

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Keywords: small population, population size, mark-recapture

The size of an animal population can be rather accurately estimated by a mark-recapture method, but this method may have a negative impact on survivorship of marked specimens. This should be avoided especially in the case of small endangered populations. We here propose a new method for such an estimate that can be used in the case of habitats shared by several species, among which one only is particularly endangered. The population size of a non-endangered species in this habitat may be estimated by random collecting and mark-recapture method, whereas specimens of the endangered species are just randomly collected along with them, but not marked. Proportion of marked versus unmarked specimens allows estimating population size of the unmarked species. The efficiency of this method is illustrated with a case study of a mixed population of *Triturus carnifex* and the endangered *Triturus alpestris inexpectatus* in a small lake of southern Italy.

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# **Abstracts (poster presentations)**





## Mitochondrial DNA differentiation in the relict Baltic and Arctic *Saduria entomon* L. (Crustacea: Isopoda) populations

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Keywords: *Saduria entomon*, glacial relict, mtDNA, phylogeography

In the brackish Baltic Sea, the isopod *Saduria entomon* is a representative of the glacial relict crustaceans that have their main distribution in northern seas. The age of *Saduria entomon* range discontinuity: the Baltic - sub-Arctic and Arctic waters is ca. 10 000 years. The Baltic populations of this species were subjected to specific, drastic environmental changes periodically very unfavourable in comparison with those experienced by populations from the main range. The levels of genetic divergence among geographically distant *Saduria entomon* populations were studied from mitochondrial cytochrome c oxidase subunit I/II (CO I/II) gene sequences. The results showed that *Saduria entomon* populations within the Baltic, White and Barents Sea basins are genetically unique. The evidence is detected not only for strong phylogeographic structure but also for lack of gene flow as no mtDNA haplotype within populations is shared across distant localities. The geographical heterogeneity of haplotype frequencies within North-eastern Europe suggests that populations have themselves been isolated by physical barriers (the Baltic-Arctic) and other factors that limit gene flow and promote genetic drift. According to the data presented in this study all populations belong to the same evolutionary lineage.

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## **Population genetic structure of the caddisfly *Rhyacophila pubescens*, PICTET 1834. – First results: Populations north of the Alps**

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Keywords: Trichoptera, *Rhyacophila pubescens*, mt COI, population genetic structure, niche specificity

In this study we analyse the genetic population structure of the caddisfly *Rhyacophila pubescens*, a montane species which exhibits an insular distribution pattern due to its strong binding to unimpaired calc-sinter and tufa streams and thus to mountain ranges with calcareous geology. Due to its restricted range and high niche specificity, the species is considered “vulnerable” in the Red List in Germany. To date, we have analysed sequence data (mt COI) of 146 individuals from 29 sites north of the Alps. Our results show that there are 22 haplotypes one of which is central in the haplotype network, occurs in all regions and seems to be ancestral to others. We hypothesize that the species has recently (re-)colonized the area north of the Alps, probably from a southern refugium. Furthermore, in almost every mountain range we analysed, recent differentiation processes are detected and endemic haplotypes are found. We therefore conclude limited extant gene flow and isolation between populations. This indicates high levels of microendemic diversity in the species and highlights the need to conserve existing populations of *R. pubescens* to maintain its genetic diversity.

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## Genetic variation of the putative postglacial relict *Saxifraga rosacea* subsp. *sponhemica* in Europe

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We studied the genetic population structure of the rare plant *Saxifraga rosacea* ssp. *sponhemica*, a putative postglacial relict which occurs in naturally isolated populations on rocks and scree slopes in Europe. In each of 34 populations situated in the main distribution areas in the Luxembourg Oesling, the Belgian Ardennes, the German Mid-Rhine region, the French Jura and the Czech Bohemian Karst, we sampled fourteen plants along transects. To test for genetic structuring within the populations, more than one transect was used in four large populations. There was strong genetic differentiation (RAPD patterns) among regions (15% of total variation), among populations within regions (21%) and between transects within populations (11%, all  $P < 0.001$ ). The strong genetic variation among populations indicates that gene flow is low. The significant genetic differentiation between transects within populations implies little gene flow even within populations. Possible explanations are pollinator behaviour and restricted dispersal of the seeds. Genetic differentiation among all 38 transects (pairwise PhiST) was related to geographic distance (Mantel test,  $r = 0.539$ ,  $P < 0.0001$ ). The positive correlation between population size and molecular diversity measures like the expected heterozygosity and the Shannon Index ( $P < 0.05$ ), suggest loss of genetic variation though drift in small populations. The populations in the French Jura were less genetically diverse than the populations located in the other regions (t-test;  $P < 0.001$ ). The Jura populations form a disjunct cluster at the south-western edge of the distributional range of *S. rosacea* ssp. *sponhemica*. Their lower levels of genetic diversity are probably due to more pronounced isolation, reduced gene flow or founder effects in the peripheral populations.

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## **Conservation and phylogeography of the endangered lesser horseshoe bat (*Rhinolophus hipposideros*)**

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During the last glacial maximum (~ 20,000 years ago), much of Northern Europe was covered in vast ice sheets. Few animals survived in situ in pockets that were ice-free. Most populations either went extinct or migrated south to areas known as glacial refugia. After the ice-age many areas were recolonised. Most phylogeographic studies based in Europe did not include samples from Ireland. The origins of Ireland's populations are largely unknown, as are the means by which they arrived. Studies which have included Ireland have revealed a variety of colonization routes. This finding has implications for conservation management plans.

Bats comprise over a quarter of Ireland's terrestrial mammal species. All but one are in the family Vespertilionidae. The remaining bat (*Rhinolophus hipposideros*) is in the horseshoe family, Rhinolophidae. Though this bat has a wide geographic range in Europe, its populations are contracting and becoming extinct. Ireland has one of the largest population's of this species. However, the phylogenetic relationship of Ireland's population and the remaining European colonies is unknown.

Samples are being obtained from Ireland and Europe, from which mitochondrial and nuclear DNA will be analysed.

From these data, a preliminary population genetic phylogeny has been generated. Ecological and acoustic data are being collected to support the findings.

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## Within-population genetic structure in the common ash (*Fraxinus excelsior L.*) assessed from microsatellite data

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Spatial genetic structure was analysed with five highly polymorphic microsatellite loci in a Romanian population of common ash (*Fraxinus excelsior L.*), a wind-pollinated and wind-dispersed tree species occurring in mixed deciduous forests over almost all of Europe. Contributions of seed and pollen dispersal to total gene flow were investigated by analysing the pattern of decrease in kinship coefficients among pairs of individuals with geographical distance and comparing it with simulation results. Plots of kinship against the logarithm of distance were decomposed into a slope and a shape component. Simulations showed that the slope is informative about the global level of gene flow, in agreement with theoretical expectations, whereas the shape component was correlated with the relative importance of seed vs. pollen dispersal. Hence, our results indicate that insights into the relative contributions of seed and pollen dispersal to overall gene flow can be gained from details of the pattern of spatial genetic structure at biparentally inherited loci. In common ash, the slope provided an estimate of total gene dispersal in terms of Wright's neighbourhood size of  $N_b = 519$  individuals. No precise estimate of seed vs. pollen flow could be obtained from the shape because of the stochasticity inherent to the data, but the parameter combinations that best fitted the data indicated restricted seed flow (mean dispersal seed distance  $<14$  m), and moderate pollen flow, (mean dispersal pollen distance  $>70$  m  $<nd <140$  m).

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## High genetic diversity and differentiation of an endangered boreal relict species in Central Europe: A mirror of better times?

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**Keywords:** *Lycaena helle*, butterfly, conservation genetics, microsatellites, climate change, fragmentation, habitat directive, wetlands

Rising temperatures and agricultural intensification during the last few decades have caused a strong decline of moist and cool sites of nutrient-poor grasslands, which have therefore become more and more scarce. This fragmentation has resulted in a reduction of the abundance of species demanding these specific ecological conditions accompanied by the reduction of gene flow. Thus, the natural restriction to montane areas has been enforced by recent habitat deterioration. To test the effects of the recent habitat fragmentation of these habitats on a local and regional scale, we selected the endangered butterfly *Lycaena helle*, which formerly had a wider distribution, but recently has become reduced to some disjunct patches in Central Europe. We analysed five polymorphic microsatellite loci for 238 individuals sampled at 15 different sites. Our study sites in Germany, Luxembourg and Belgium are geographically split into two regions: the Ardennes/Eifel complex and the Westerwald, but three regional population clusters can be distinguished. A high genetic diversity was detected in all local populations and is in some contrast to general assumptions of survival strategies of specialised and sedentary species. This high allelic richness might be a relict of an anciently higher abundance and wider distribution. The strong genetic differentiation among the Ardennes, the Eifel and the Westerwald ( $F_{CT}$ : 8.4%) supports the assumption that these three distinct geographical regions represent three different genetic units. The local genetic differentiations within these regions underline natural and anthropogenic habitat fragmentation. While ongoing gene flow seems to exist among the Eifel populations (mirrored by their low  $F_{ST}$  value), a high genetic differentiation in the Ardennes and the Westerwald indicates a disruption of the former most probably interconnected populations. Our genetic data obtained on different spatial scales show the genetic



consequence of a decline of population network systems and the necessity of conservation measures at the metapopulation level.

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## Diploid relict species of Asteraceae subtribe Hieraciinae in evolutionary and phylogeographic context

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Keywords: *Hieracium*, *Andryala*, *Pilosella*, molecular phylogeny, hybridization, refugiums

The Hieraciinae are, according to recent results (Mol. Phylogen. Evol. 42 (2007) 347-361), composed of the genera *Hieracium*, *Pilosella*, *Hispidella* and *Andryala*. While the monotypic *Hispidella*, all species of *Andryala* and the American *Hieracium* subgen. *Chionoracium* are only known as diploid, *Hieracium* and *Pilosella* are notorious for their complex taxonomic structure due to a mixture of sexual and different apomictic reproductive pathways, ancient or recent hybridization, and diploid or various polyploid cytotypes. The difficulties species identification and delimitation pose for non-specialists, the existence of different taxonomic concepts, and insufficient knowledge about geographical distribution, genetic variability and population dynamics have thwarted their proper treatment in Red Data books and thus the conservation of particular species. Our work is aimed at a better understanding of species relationships and patterns of genetic variability in order to identify refugiums that have played a role in the speciation of these groups and to provide a better basis for their taxonomic evaluation and improved treatment in floras and field guides. *Pilosella* chloroplast haplotypes form two major groups with no correlation to morphology or species relationships while haplotypes of *Andryala* species are derived from one of the *Pilosella* haplotypes. This can be explained by two ancient hybridization events predating most of the speciation observed in the *Hieraciinae*: one between the ancestor of *Hieracium* and partly differentiated *Pilosella*, and a subsequent one between this introgressed *Pilosella* lineage and the ancestor of all *Andryala* species investigated so far. Distribution areas and species numbers of *Pilosella* species belonging to one or the other haplotype group as well as the extinction of intermediate haplotypes suggest their differentiation in different glacial refugiums. Relict species in *Pilosella* comprise *P. argyrocoma*, a rather rare species with disjunct distribution on the Iberian Peninsula, and *P. vahlii*, an endemic species of the high mountains of central Spain (IUCN Red List of Threatened Plants 1997, both as *Hieracium*); *P. breviscapa*, an endemic species of the E Pyrenees; and *P. alpicola* with an extremely disjunct distribution in the Alps, the Carpathians and the Balkan mountains which is generally indicative of a relict situation. Some populations of *P. castellana* in the mountains of



Spain may as well have relict character. From an evolutionary point of view, these relict species are occurring in all four major lineages of *Pilosella* as revealed by ITS sequencing. All of them show derivatives of the original chloroplast haplotype except *P. alpicola*, whose introgressed chloroplast corresponds to its more eastern distribution. *Andryala* comprises two relict species, *A. levitomentosa* (IUCN RL of Threatened Plants 1997, Romanian RL), a highly endangered species occurring as a single population of 200-300 plants in the Romanian Carpathians far apart from the current distribution area of the genus, and *A. agardhii* (Lista Roja de la Flora Vascular Española 2000) which is confined to the Sierra Nevada (Spain) and Moyen Atlas (Morocco). Both form old lineages separated from other species of this genus which today has its main distribution in North Africa, the Iberian Peninsula and Macaronesia. In *Hieracium*, several partly diploid species/microspecies that are closely related and most likely of relict character occur at the foot of the Pyrenees, e.g. *H. gouani*, *H. recoderi*, and *H. cordifolium*; the latter appears to be of hybrid origin involving a Pyrenean taxon and a species from a different clade. *Hieracium stelligerum* is only known from ca 15 localities on limestone rocks in S France and can also be supposed to be a relict species. Probably the most endangered species of relict character occurs in Sicily: *H. lucidum* (IUCN RL of Threatened Species 2006) is only known from a single population consisting of very few plants on north facing limestone cliffs of Monte Gallo near Palermo. In SE Europe, geographical ranges of diploid hawkweeds coincide well with principal refugial areas. This is the case with the recently described local endemics *H. petrovae* and *H. kittanae* from the central Rhodopy Mts. in S Bulgaria and with some populations of *H. waldsteinii* s.l. from Montenegro. *Hieracium pojoritense*, a diploid species, is endemic to calcareous mountains in NE Romania. Besides these, some populations of otherwise geographically more widespread species might be considered as relict ones, e.g. widespread diploid populations of the otherwise triploid *H. alpinum* in the E and S Carpathians, and rather rare diploid populations of *H. prenanthoides* in the westernmost part of the Alps. While not all diploid relict species of this genus have been studied yet, also some polyploids of relict character may exist.

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## Evolutionary history of two newt subspecies inferred from multiple sequence markers

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Keywords: *Triturus vulgaris*, phylogeography, multilocus analysis, population parameters

Information on the patterns of genetic differentiation between closely related species and divergent populations within species is essential for understanding of microevolutionary processes, as well as for conservation strategies which require information about genetic subdivision and levels of genetic variation in different parts of the species range. Because of incomplete lineage sorting and stochasticity of the coalescent process inferences about the historical processes and estimates of demographic parameters should be based on many unlinked loci. We analyzed the sequence variation of mitochondrial DNA (mtDNA) and eight nuclear markers in two subspecies of the smooth newt: *Triturus vulgaris kosswigi* and *T. v. vulgaris*. Both groups inhabit the area of western Turkey which is the one of the regions of glacial refugia for the *Triturus* species. However, whereas *T.v.kosswigi* distribution range is restricted to the southern coast of the Black Sea near Bosphorus, *T.v.vulgaris* has broader distribution in Europe including both formerly glaciated and unglaciated regions. The earlier mtDNA study estimated their divergence at 2.8-3.8 Myr. To infer demographic history, patterns of gene flow and past hybridization we studied sequence variation in mtDNA five nuclear introns, one single copy non-coding nuclear marker and two nuclear genes. A suite of phylogeographic methods including phylogenetic analyses, Nested Clade Analysis and isolation with migration models of population divergence were used to assess genetic structure of populations, estimate historical population size changes and infer patterns of gene flow within and between subspecies. We found substantial genetic structure, evidence for demographic stability in *T. v. vulgaris* and for demographic decline in *T. v. kosswigi*. Surprisingly, long-term effective population sizes of both subspecies have been similar. MtDNA introgression from *T. v. vulgaris* to *T. v. kosswigi* is restricted geographically to the east of Bosphorus. We found no shared haplotypes between subspecies among nuclear genes with an exception of one gene.

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## Reticulate evolution and phylogeographical structure of *Heliosperma pusillum sensu lato* (Sileneae, Caryophyllaceae)

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Keywords: Silene, Balkan Peninsula

*Heliosperma pusillum sensu lato* is one of the three main evolutionary lineages within *Heliosperma*, a genus distributed in southern European mountains. The other two lineages, *H. alpestre* and *H. macranthum* are endemics of eastern Alps and Prokletije Mts/Komovi Mts. in Montenegro and Albania, respectively, whereas *H. pusillum* s.l. ranges from the Sierra Cantabrica in Spain over the Pyrenees, Alps and the Balkan Peninsula to the Carpathians. We have used sequences of the nuclear low-copy gene RPB2 from the RNA Polymerase gene family and the chloroplast spacer psbE-petG as well as Amplified Fragment Length Polymorphism (AFLP) markers to examine phylogenetic and biogeographical patterns within *H. pusillum* s. l., which has its main diversity on the Balkan Peninsula. Traditional morphology-based taxonomic concepts of the *H. pusillum* group are not supported by molecular data. The pattern indicated by molecular markers is complex. The psbE-petG sequences indicate a deep split within the *H. pusillum*-group, and two main geographical groups are found, western and eastern, the borderline between them being River Drina (the border between Bosnia and Herzegovina/Serbia and Montenegro). Some further geographical groups (e.g. Carpathians) are also supported. RPB2 sequences indicate a more complex pattern. The deep cpDNA split between the eastern and western groups is not supported. There are rather several clades resolved, some of them geographically well defined, whereas others group accessions from geographically remote areas (Pyrennes-Alps-Dinaric Mountains). Several RPB2 accessions exhibit nucleotide polymorphism (allelic variation), which is indicative of gene flow/hybridisation among the populations, supported also by ITS data. AFLP markers have not been scored yet, but will hopefully be available at the time of the conference.

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## Populations of *Rhyacophila aquitanica* (McLachlan 1879) in potential high-mountain speciation centers: signs of isolation

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Keywords: Isolation of populations, Trichoptera, Micromorphology

*Rhyacophila aquitanica* is a European high-mountain species, inhabiting cold, fast-running streams of the Carpathians, Massif Central, Vosges, Schwarzwald and the Alps. Present populations of *R. aquitanica* are separated in different European mountain ranges. These populations are suitable to demonstrate the existence of extra-Mediterranean high-mountain refugia of dinodal species as these were probably able to survive the cold periods of the Pleistocene in the vicinity of ice sheets, with a relatively short vertical migration, in contrast to boreal and arboreal species. Divergence among *R. aquitanica* populations is investigated with classic morphological and molecular methods. This work presents the results of morphological measurements on *R. aquitanica* specimens originating from the whole European distribution range of the species. Multivariate analysis of the measurement data clearly shows the separation of the eastern and western European populations (these were recognized as different subspecies by Botoaneanu in 1995) and presents a certain level of morphologic divergence among Carpathian populations. The increased level of divergence among the populations of *R. aquitanica* may be the effect of long-term isolation in the vicinity of present distribution ranges. These areas may prove to be very important in the preservation of genetic diversity of *R. aquitanica* and other species sharing similar ecological needs.

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## Distribution and Status of some relict insect species in Lithuania

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Keywords: Odonata, Lepidoptera, Coleoptera, Lithuania, raised bog

A small part of the Lithuanian landscape (3,8 %) is covered with raised bogs and transition mires, and fens. Raised bogs make up only 22% of the total wetland area. Wetland habitats are very vulnerable as they are subject to changes in hydrological regime and the overgrowth process. Bog fires are controversially disputed. Some environmentalists consider them as means to improve the habitat status, though there are examples (the bogs of Pravirðulis and Aduðiðkis) showing that a more intensive overgrowth with birch and other deciduous trees takes place after fire and a number of relict species become extinct. Long-term investigations show that the majority of relict insect species in Lithuania inhabit raised bogs of a different size. Wetlands of a different type constitute a larger part of the area of four strict nature reserves: Þuvintas, Èepkeliai, Kamanos and Vieðvilè. These reserves are a haven for most post-glacial relict insect species in Lithuania. The Red Data Book of Lithuania contains insect species that are found in raised bog habitats only: *Coenagrion johansonii* (Wallengren), *Nehalennia speciosa* (Charpentier), *Aeshna crenata* (Hagen) (Odonata), *Agonum ericeti* (Panzer), *Carabus nitens* (Linnaeus) (Coleoptera), *Macaria carbonaria* (Clerck), *Boloria frigga* (Thunberg), *Oeneis jutta* (Hübner) (Lepidoptera). Moth and butterflies complexes found in Lithuanian bogs reveal the quality and stability of these habitats. Several specific insect species - *Prolita sexpunctella* (Fabricius), *Altenia perspersella* (Wocke), *Chionodes viduella* (Fabricius) (Lepidoptera, Gelechiidae), *Buckleria paludum* (Zeller) (Lepidoptera, Pterophoridae), *Boloria eunomia* (Esper), *B. aquilonaris* (Stichel) (Lepidoptera, Nymphalidae), etc. - form specific raised bogs complexes. The main complexes of wetland insect species are rather evenly distributed over Lithuanian bogs. A significantly lower number of relict species is found in bogs in the western part of the country. The species *Oeneis jutta*, one of the main indicator showing a good status of a raised bog habitat, is not found there. Wetlands in south-eastern and northern parts of Lithuania are the richest in specific insect species. The species *Boloria frigga* are found only in Èepkeliai Strict Nature Reserve and *Chionodes viduella* - in Mũðos Tyrelis mire, respectively. Raised bog lakes are important habitats for the protected beetle species *Dytiscus latissimus* (Linnaeus) and *Graphoderus bilineatus* (De Geer), and dragonfly species *Coenagrion johansonii*, *Aeshna crenata*,



*Leucorrhinia pectoralis* (Charpentier) and *L. albifrons* (Burmeister). In most cases, a large number of the Lithuanian population of these species is found on the lakes with an area of only several hectares in the south-eastern part of the country. The status of the dragonfly species of the genus *Leucorrhinia* appears to be the best among the protected relict insect in Lithuania. Although they are most numerous found in wetland habitats, but due to their plasticity they form close populations near beaver dams and other suitable places. In most bogs, *Agonum ericeti* is one of the most numerous beetle species. The species *Oeneis jutta* in suitable habitats make up populations from several tens to several hundreds of individuals and is detected in raised bogs of a different size in the country. Lately, new data has been collected on the distribution of insect complexes in raised bogs and their needs for habitat quality.

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## Does inbreeding have an effect on leaf fluctuating asymmetry in the rare *Saxifraga rosacea* subsp. *sponhemica*?

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*Saxifraga rosacea* subsp. *sponhemica* is a rare plant growing on scree slopes and rock faces of Central and Eastern Europe. Currently, the great majority of populations are small and isolated, but the plant was probably more widespread during the last glaciations. Small and isolated populations are known to be especially susceptible to inbreeding and genetic drift. To study how seriously inbreeding affected developmental stability in this plant, we measured fluctuating asymmetry, an indicator of development instability, for several leaf characters of plants inbred to various degrees and grown in a common garden. The plants were offspring from plants of a natural Luxembourg population which had been subjected to different pollination treatments: flowers had either been bagged and left to spontaneously self pollinate, open pollinated, hand-pollinated with pollen from the same plant (selfed), with pollen from a different plant of the same population or with pollen from a different population. A mixed model ANOVA testing the effect of leaf side, measurement error and of directional asymmetry and antisymmetry indicated that two of the leaf traits studied showed real fluctuating asymmetry. Orthogonal contrasts testing for the effect of the different pollination treatments on these two FA indices revealed that both spontaneous and hand-selfing significantly increased the fluctuating asymmetry of leaves, measured as the absolute value of the mean difference between the distances from the left and right leaf margins to the midrib, in the F1 generation ( $F=5.32$ ,  $P<0.05$ ). These results suggest that the developmental stability of plants of *S. rosacea* subsp. *sponhemica* is negatively affected by inbreeding. Conservation strategies should therefore take into account both the size and level of isolation of populations to avoid enhanced inbreeding.

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## Morphological and allozyme variation of *Silene nutans* (Caryophyllaceae) at the north-western margin of its distribution range

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*Silene nutans* L. (Caryophyllaceae) is an insect-pollinated, long-lived perennial herb occurring on rock outcrops in dry, thermophilous grasslands, open forests and forest edges. It shows a continental distribution range extending from north-western Europe to central Siberia and the South-Caucasus. At the western and northern margins of its European range, it shows a disjunct and scattered distribution. The study of allozyme and morphological (leaf shape, fruit size) variation of range-margin populations, from Belgium, France, Sweden and NFinland reveals divergence among populations. On the one hand, the populations in Belgium are differentiated into two parapatric edaphic ecotypes, calcicolous and silicicolous. These ecotypes, which occur in different, contiguous but not overlapping ecological niches, show contrasted leaf shape and fruit size, and can be distinguished into two different gene pools based on allozymes. In France, there is no indication of ecotypic differentiation but two geographical groups, peripheral and central, could be distinguished based on allozymes and fruit size. The peripheral populations were genetically closely related to the Belgian silicicolous ecotype. The central populations were closer to the Belgian calcicolous ecotype, but yet significantly differentiated for allozyme and morphological traits. This suggests an ancient differentiation and different migration histories for the Belgian ecotypes. Belgium may constitute a contact zone between the two groups, and adaptive radiation may have occurred owing to geological heterogeneity (presence of calcareous and siliceous bedrocks). They may also represent unique genetic patterns for the species and may be of great value from a conservation point of view. In Sweden and NFinland, two groups can be identified based on allozyme and leaf shape patterns, suggesting two major postglacial immigration routes, from south and east, and a contact zone with admixed populations in central Sweden. Moreover, these populations differ in morphological traits (leaf shape, fruit size) from the western populations. In conclusion, the results on allozyme and morphological variation at the north-western margin of its

distribution range suggest a quite complex pattern of the geographic structuring of genetic variation of *S. nutans*, likely shaped by past migration history but also by ecological specialization and recent processes. The phylogeographical study of the European populations of *S. nutans* using cytoplasmic DNA markers (mtDNA and cpDNA) currently in progress (see the poster of S. Le Cadre et al.) will certainly contribute to elucidate the relative contributions of historical and recent processes (e.g. selection) to present-day genetic structure and to define potential Evolutionarily Significant Units (ESU).

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## The genetic heritage, future conservation and preservation of the red deer (*Cervus elaphus*) in County Kerry, Ireland.

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Keywords: red deer, phylogenetics, Ireland, conservation

Although red deer were native to Ireland both prior to and after the last glacial maximum, there are no authenticated red deer remains in Irish archaeological assemblages between 11,790 and 4,190 years ago. The Killarney National Park (KNP) population is believed to be the only wild herd originating from the native stock, which colonised the island during the Woodgrange interstadial (13,000 to 11,000 years ago); it is possible that this population was in fact introduced during the Neolithic or even within the last 500 years. Historical Irish written records document recent (from the 1600s) anthropogenic mediated introductions of red deer throughout many counties of the island of Ireland from a variety of different sources including deer from Scotland, England, Denmark and Germany.

Investigations of the KNP red deer could determine conclusively the origins of this population, and thus test the hypothesis that these deer are the only truly native herd remaining in Ireland. Up until this study, the phylogeographical relationship between the KNP red deer with Western European red deer populations has been unknown, as indeed the relationship of this population to other Irish red deer populations.

Extractions of mitochondrial DNA and ancient DNA from collected tissues have been used in conjunction with craniometric data from adult deer skulls. Ancient DNA extractions from archaeological remains sourced from Ireland, England and the Western façade of Continental Europe will be performed, and additionally, modern tissue samples of red deer from other Irish populations and those in Western European countries have been compared to the KNP deer. Suitable multivariate statistical analyses will be performed on these data. Overall results and comparisons between craniometric data and genetic data will be suitably assessed. At the very least the position of Irish red deer within the diversity seen across Western Europe will be determined from this study. Moreover, these results will have important

implications for the future management and conservation of this species in Kerry, which prior to 2005 it was illegal to shoot red deer in County Kerry (Wildlife Acts 1976, 2000).

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## Habitat requirements and population dynamics of a relict butterfly species: *Boloria aquilonaris*

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Keywords: *Boloria aquilonaris*, butterfly, peat bog, conservation

The cranberry butterfly *Boloria aquilonaris* is a relict butterfly species from acid peat bogs and damp heaths, where its host plant *Vaccinium oxycoccos* occurs. The species is widespread and abundant in northern Europe, but Belgian populations are highly fragmented (27 stations in the Ardennes and 2 stations in the Lorraine). The goals of our study are to: (1) identify precisely the ecological needs of both caterpillars and adults, (2) compare resources distribution and analyze to what extent it differs between populations of the two sites (Fange de Pisserotte & Troufferies de Libin), (3) assess and compare population sizes through time and space, (4) evaluate population viabilities, and (5) formulate conservation guidelines for this endangered species. Field studies and experiments carried out during several years showed that: (1) Larvae thrive only on sphagnum hills covered by the cranberry, probably because hills act as a buffer for daily temperature fluctuations; (2) Females daily move between laying site (sphagnum hills covered by cranberry) and feeding sites (places with abundant flowers of mainly *Potentilla palustris*, *Narthecium ossifragum* and *Cirsium palustre*). Spatial distribution (agglomeration or dissemination) of these two kind of sites is correlated with life history traits (thorax and wing sizes). (3) Natural fluctuations of population sizes are observed but this can not be explained by caterpillar parasitism, contrary to what we found for other Nymphalidae species. (4) Belgian populations' persistence is different according to stations, but in a landscape view the species viability is not guaranteed because of isolation, climatic, stochastic and genetic factors. (5) To preserve this species in Belgium, we might restore habitats by restricting reforestation on stations, by cutting spruce plantations surrounding the peat bogs and re-establishing hydrologic regime.

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## **Preliminary comparison of the population structure of the two montane Trichoptera *H. tenuis* (NAVAS 1932) and *D. discolor* (RAMBUR, 1842).**

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Keywords: Trichoptera, montane, mtCOI, population structure

In this study we compared mitochondrial sequence data (COI) to infer the population structure of the two montane caddisflies *Hydropsyche tenuis* and *Drusus discolor*. The two species represent different models of montane aquatic insects: *D. discolor* is restricted to elevations above 600m, *Hydropsyche tenuis* is limited to the same mountain ranges in central Europe but inhabits much lower elevations. Thus the two represent different models for montane insects. Our study aims to examine whether conservation status and threat to climate warming varies between occupants of different niches with similar distribution patterns. To determine the basic population structure of both species conventional population genetics analyses were applied to mitochondrial sequence data. We collected and sampled 121 specimens of *H. tenuis* from 29 sites in 10 different regions of the central European highlands and 138 individuals of *D. discolor* from 40 sites in 11 different regions. Nine unique haplotypes were identified for *H. tenuis* and 34 haplotypes for *D. discolor*. The maximum difference between all haplotypes is for *H. tenuis* 0.8% (4 bp) and for *D. discolor* 4.0% (20 bp). We observed haplotype overlap between geographic regions for both species.

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