Comparative phylogeography of two co-distributed arctic-alpine freshwater insect species in Europe

Dissertation zur Erlangung des Grades

Doktor der Naturwissenschaften

am Fachbereich Biologie

der Johannes Gutenberg-Universität in Mainz

Kathrin Theißinger-Theobald

geboren am 27. Juni 1980 in Neustadt an der Weinstrasse

Mainz, im Mai 2011

Dekan: [redacted]

Erster Berichterstatter: [redacted]

Zweiter Berichterstatter: [redacted]

Tag der mündlichen Prüfung: 26.08.2011

This thesis is based on the following five research articles, which are presented as chapters 1 to 5 and referred to in the summary results and the concluding discussion by their respective numbers:

(1) Theissinger K, [redacted], [redacted], [redacted], [redacted], [redacted] (*in press*) Molecular data and species distribution models reveal the Pleistocene history of the mayfly *Ameletus inopinatus* (Ephemeroptera: Siphlonuridae). *Freshwater Biology*

(2) Theissinger K, [redacted], [redacted], [redacted], [redacted], [redacted], [redacted] (*submitted*) Postglacial disjunct distribution in an arctic-alpine freshwater insect (*Arcynopteryx compacta*, Plecoptera, Perlodidae).

(3) [redacted], Theissinger K, [redacted], [redacted], [redacted], [redacted], [redacted], [redacted] (2011) Modelling range shifts and assessing genetic diversity distribution of the montane aquatic mayfly *Ameletus inopinatus* in Europe under climate change scenarios. *Conservation Genetics,* 12, 503-515.

(4) Theissinger K, [redacted], [redacted], [redacted], [redacted] (2008) Isolation and characterization of 10 higly polymorphic di- and trinucleotide microsatellite markers in the mayfly *Ameletus inopinatus* (Ephemeroptera: Siphlonuridae). *Molecular Ecology Resources*, 8, 1285–1287.

(5) Theissinger K, [redacted], [redacted], [redacted] (2009) Isolation and characterization of 11 polymorphic trinucleotide microsatellite markers in the stonefly *Arcynopteryx compacta* (Plecoptera: Perlodidae). *Molecular Ecology Resources*, 9, 357–359.

CONTENT

ABSTRACT

The distribution pattern of European arctic-alpine disjunct species is of growing interest among biogeographers due to the arising variety of inferred demographic histories. In this thesis I used the co-distributed mayfly *Ameletus inopinatus* and the stonefly *Arcynopteryx compacta* as model species to investigate the European Pleistocene and Holocene history of stream-inhabiting arctic-alpine aquatic insects **(1, 2)**. I used last glacial maximum (LGM) species distribution models (SDM) to derive hypotheses on the glacial survival during the LGM and the recolonization of Fennoscandia: 1) both species potentially survived glacial cycles in periglacial, extra Mediterranean refugia, and 2) postglacial recolonization of Fennoscandia originated from these refugia. I tested these hypotheses using mitochondrial sequence (mtCOI) and species specific microsatellite data. Additionally, I used future SDM to predict the impact of climate change induced range shifts and habitat loss on the overall genetic diversity of the endangered mayfly *A. inopinatus* **(3)**.

I observed old lineages, deep splits, and almost complete lineage sorting of mtCOI sequences between mountain ranges. These results support the hypothesis that both *A. compacta* and *A. inopinatus* persisted in multiple periglacial extra-Mediterranean refugia in Central Europe during the LGM. However, the recolonization of Fennoscandia was very different between the two study species. For *A. inopinatus* I found strong differentiation between the Fennoscandian and all other populations in sequence and microsatellite data, indicating that Fennoscandia was recolonized from an extra European refugium **(1)**. High mtCOI genetic structure within Fennoscandia supports a recolonization of multiple lineages from independent refugia. However, this structure was not apparent in the microsatellite data,

consistent with secondary contact without sexual incompability. In contrast, *A. compacta* exhibited low genetic structure and shared mtCOI haplotypes among Fennoscandia and the Black Forest, suggesting a shared Pleistocene refugium in the periglacial tundrabelt **(2)**. Again, there is incongruence with the microsatellite data, which could be explained with ancestral polymorphism or female-biased dispersal. Future SDM projects major regional habitat loss for *A. inopinatus*, particularly in Central European mountain ranges **(3)**. By relating these range shifts to my population genetic results, I identified conservation units primarily in Eastern Europe, that if preserved would maintain high levels of the present-day genetic diversity of *A. inopinatus* and continue to provide long-term suitable habitat under future climate warming scenarios.

In this thesis I show that despite similar present day distributions the underlying demographic histories of the study species are vastly different, which might be due to differing dispersal capabilities and niche plasticity. I present genetic, climatic, and ecological data that can be used to prioritize conservation efforts for cold-adapted freshwater insects in light of future climate change. Overall, this thesis provides a next step in filling the knowledge gap regarding molecular studies of the arctic-alpine invertebrate fauna. However, there is continued need to explore the phenomenon of arctic-alpine disjunctions to help understand the processes of range expansion, regression, and lineage diversification in Europe’s high latitude and high altitude biota.

GENERAL INTRODUCTION

During the Pleistocene, 2,588,000 to 12,000 years before present, the Earth experienced repeated climatic oscillations, which resulted in numerous range expansions and regressions in most taxa (Hewitt 2000, Malicky 2000). The Last Glacial Maximum (LGM) was a key event in the distribution history of most European species and represents the opposite climatic extreme from the present interglacial climate (Hewitt 2004). Only 18,000 – 21,000 years ago (Beebee & Rowe 2008) the landscape and climate of the Eurasian continent were dramatically different as compared to the present day: glaciers covered almost half of Europe; the Alps, Pyrenees, and Carpathians were also ice covered; climatic conditions were significantly drier and colder (Hewitt 2004). The lowered sea levels exposed the Beringian land bridge that connected the North American and the Siberian land masses (Hopkins et al. 1982). The distribution of species during the LGM was also quite different from the present distributions. Due to decreasing temperatures and approaching glaciers most species experienced severe reduction and fragmentation of ranges, resulting in more or less isolated refugial populations (Hewitt 1996, 2004). During this cold phase temperate taxa often became restricted to refuge populations in more southern ranges. In Europe these refuge regions were often located on the Mediterranean peninsulas (Taberlet et al. 1998), but also in Central Europe (Schmitt et al. 2010). Typically, refuge populations can be associated with multiple genetic lineages and higher genetic diversity compared to more recently established populations (Hewitt 2000). Based on distributions of genes and genotypes across landscapes as well as by using intraspecific molecular phylogeographic analyses it is thus possible to make inferences about where such refugia were located (Avise

2000). After the retreat of the ice sheet about ten thousand years ago the northern habitats became suitable again and species recolonized these areas from single or multiple refugia (Avise 2000, Hewitt 2000). Such recolonization events were often influenced by migration barriers, such as the Alps or the Pyrenees, which hindered some taxa to disperse from the Mediterranean refuge areas to occupy their historical ranges (Taberlet et al. 1998). In Europe, Fennoscandia was the last region to become free of ice (Lundqvist & Mejdahl, 1995). Consequently, it was recolonized more recently than southern regions. The short time since recolonization of the North has resulted in genetically more homogenous populations than in the more temperate regions (Pamilo & Savolainen 1999). However, a high degree of genetic differentiation between populations can also occur due to temporarily reduced population sizes and an increased power of genetic drift (Gyllensten 1985; Mikko & Andersson 1995).

ARCTIC-ALPINE DISTRIBUTIONS

Arctic-alpine distribution ranges comprise the arctic as well as the alpine belt of more southern mountain ranges (De Lattin 1967). Typically, arctic-alpine distributions are large and often encompass the entire Northern Hemisphere (circumpolar distribution; De Lattin 1967). Current distribution patterns of species adapted to high altitudes and latitudes are often strongly disjunct (De Lattin 1967), with continuous distribution in the North and patchy “sky island” distribution at high elevations of southern mountain ranges. Schmitt (2007) proposes two general biogeographical arctic-alpine distribution patterns. I) interglacial disjunct, i.e. disconnected distribution during glacial phases, thus resulting in comparably deeper/older genetic splits. II) postglacial disjunct, i.e. widely distributed species throughout the glacial, with interruption of gene flow only during recent climate warming, resulting in relatively young genetic

splits. Until the 1960s it was consensus that such disjunction cannot be explained without *in situ* survival during the glaciations (the “nunatak hypothesis”); the alternative “*tabula rasa* hypothesis” of postglacial immigration was regarded to be only of historical interest (Brochmann et al. 2003). Nowadays it is recognized that the high latitude and altitude habitats were covered by ice during the glaciations, and thus all present areas must have been recolonized (Muster & Berendonk 2006). The populations from southern disjunct parts of the distribution areas of arctic-alpine arthropods can be regarded as relicts, which originated at very different time scales (Schmitt et al. 2010). Many isolated sky island populations are small and widely separated from their distribution centre (Varga and Schmitt 2008).

The traditional concept for arctic-alpine distributed species of large continuous distributions in the periglacial tundrabelt during the glaciations (i.e. postglacial disjunction *sensu* Schmitt 2007) was first proclaimed by Holdhaus (1954) and De Lattin (1967). Cold-adapted species, such as those found in arctic-alpine environments, were more successful and widespread during the glaciations and experienced bottlenecks and range contractions during interglacials (de Lattin 1967, Hewitt 2004). Refugial hypotheses and models for understanding processes of postglacial colonization are mainly derived from studies on temperate taxa (Schmitt et al. 2010). Many latitudinally shifting species experienced extensive postglacial expansions, resulting in signatures of rapid population growth that date to the end of the LGM approximately 10,000 years ago (Galbreath et al. 2009). However, arctic-alpine species did not necessarily respond to climate change through large-scale latitudinal shifts (Galbreath et al. 2009). In contrast, in such cold-adapted species glacial histories were rather dominated by elevation shifts and should therefore show signs of population expansion coincident with the rise of the LGM, followed by

population decline during the Holocene as climate warming caused an upslope shift (Galbreath et al. 2009).

PHYLOGEOGRAPHY IN FRESHWATER SPECIES

Species responses to climate change vary for organisms depending on their life history traits, e.g. level of cold-tolerance and dispersal capabilities (Deffontaine et al. 2005, Pinceel et al. 2005, Schönswetter et al. 2005). Several species may have survived glacial periods in small pockets of suitable habitat within the periglacial region (e.g. reviewed by Stewart & Lister 2001, European perch: Nesbø et al. 1999, eastern chipmunk: Rowe et al. 2004, bank vole: Deffontaine et al. 2005, a terrestrial slug: Pinceel et al. 2005, alpine plants: Schönswetter et al. 2005). While phylogeographic studies in Europe have in the past focussed primarily on terrestrial plants and animals, aquatic invertebrates have recently been given increasing attention (Pauls et al. 2006 & 2009, Brändle et al. 2007, Bálint 2008, Engelhardt et al. 2008, Benke et al. 2009, Lehrian et al. 2009 and 2010). Aquatic species generally exhibit a wide range of life histories that differ from those of terrestrial species because their physical environment is fundamentally different (Danks 2007). Hence, they are expected to show varying responses to past climate change. This is especially true for cold-tolerant stream-inhabiting species, since permanently running water cannot cool much below 0°C. Thus, cold-tolerant aquatic organisms like montane aquatic insects would have been subject to much less severe temperature decreases than their terrestrial counterparts (Malicky 1983, Pauls et al. 2006). Although their present-day distribution suggests that montane stream-dwelling insects may form part of the arboreal biome, their response to climate change might have been vastly different. In contrast to most of the arboreal species, the survival of montane aquatic insects in the Central European periglacial and in extra-

Mediterranean mountain systems seems to be a rule, rather than an exception. Studying the intraspecific genetic variation of cold-tolerant aquatic species may provide important additions to our understanding of the location of Pleistocene refugia.

IN FOCUS: THE STUDY SPECIES

The mayfly *Ameletus inopinatus* EATON 1887 (Ephemeroptera: Siphlonuridae, Figure 1) inhabits mountain ranges of higher elevation (> 600 m asl) on the British Isles and in Central Europe. In northern Eurasia, it is known from Scandinavia to Western Siberia, where it occurs also at lower altitudes. The larvae can be found in marsh lands, slow running creeks, grassy lake shores, and also in slow running branches of torrential rivers. It is considered a representative of the Eurasian, boreo-montane biome-type (Haybach 2003). Jacob (1979) assumes the species to have occurred in the ice free areas north of the Alps during the LGM due to its boreo-montane distribution. Haybach (2003), on the other hand, argues that the species' absence from the Alps and the Pyrenees indicates that *A. inopinatus* was not distributed in Europe during the last ice age. He suggests the species is a recent (postglacial) colonizer of Central Europe, with refugial areas situated in Eastern Europe.

The stonefly *Arcynopteryx compacta* MCLACHLAN 1872 (Perlodidae, Figure 1) exhibits the typical arctic-alpine disjunction sensu DeLattin (1967), with restriction to the highest elevations in the southern European mountains (sky island populations), although it is presently absent from the Central Alps (Illies 1955), and a continuous holarctic distribution (Lillehammer 1974). *A. compacta* is a comparatively large predator and highly adapted to crenal and epirhithral regions (Graf et al. 1995). At high latitudes it is occasionally also found on lake shores (Lillehammer 1974). The

species is presumably very restricted in its dispersal capabilities: all adult males and females of some populations are brachypterous, and stoneflies in general are poor fliers (Malmqvist 2000). It is considered a typical representative of the boreo-alpine montane biome-type, which survived Pleistocene glaciations in the central European periglacial plains and postglacially retreated to high altitudes and latitudes (Illies 1955). The species current Central European populations seem to represent glacial relicts that either continue to inhabit their pre-glacial distribution areas (interglacial disjunct) or represent small relict populations of the species glacial distribution (postglacially disjunct).

Figure 1 Larval and adult habitus as well as preferred stream types of the study organisms.

SAMPLING DESIGN

As many and in particular arctic-alpine species inhabit large and complex ranges, it is essential for phylogeographical research to take into account the entire range of a widespread species to sufficiently clarify its evolutionary history (Bunje 2005). In this thesis I compiled complete and consistent datasets across Europe, containing samples of all mountain ranges inhabited by *A. compacta* **(2)** and nearly all major distribution areas for *A. inopinatus* **(1)** (Figure 2). Larval and adult specimens of the target species were collected in the summer months of 2007 – 2009, using water nets and manually searching in preferred microhabitats. I generated sequence data from the "DNA barcode" region (Hebert et al. 2003) of the mtCOI gene, producing a 620 base pair fragment. I used eight (*A. inopinatus*) and six (*A. compacta*) species-specific microsatellite primer pairs **(4, 5)** to genotype the sampled individuals. In total, For *A. inopinatus* / *A. compacta* I sequenced 264 / 334 and genotyped 389 / 366 individuals from ten / eight mountain ranges and 32 / 46 sampling sites across Europe **(1, 2)**.

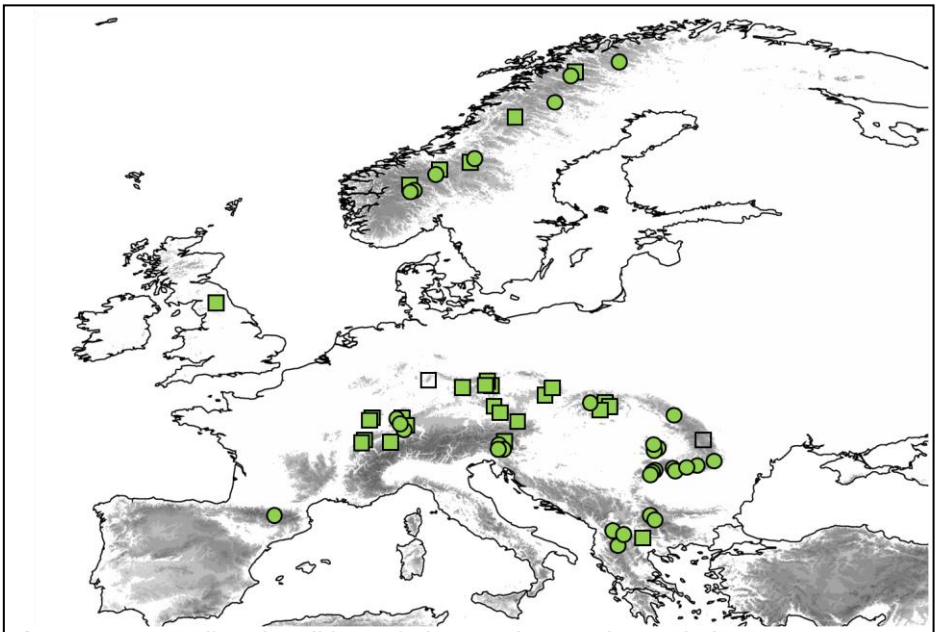


Figure 2 Sampling localities of the study species. Circles: *A. compacta*; Squares: *A. inopinatus*; Squares without filling: mountain range inhabited by *A. inopinatus* without sampling success.

MOLECULAR MARKERS

In the past twenty years most phylogeographic studies of animals have focussed on mitochondrial (mt) sequence data (see Beheregaray 2008 for review). The popularity of mtDNA in phylogenetic and phylogeographic studies of animals can be explained by its maternal, clonal inheritance, its relatively rapid rate of base substitution, the relative ease with which it can be isolated and analysed (Palumbi 1996, Roderick 1996, Avise 2000) and most importantly because it allows interpreting data in a historic framework (Roderick 1996, Avise 2000, 2004). It is thus still a marker of choice for phylogeographic inferences (Zink & Barraclough 2008). However, when studying evolutionary young areas the mutation rate of mtDNA might not be high enough to resolve population genetic structure or recently diverged phylogeographic lineages. Moreover, it reflects the history of effectively only one locus, and thus, erroneous population genetic inferences are easily drawn (Pamilo & Nei 1988). Due to the maternal inheritance, mt sequence data can also lead to biased results in case of sex-dependent dispersal.

Therefore I used a second, independent marker set. Microsatellites are biparentally inherited nuclear markers and have become widely used in population genetic studies (Avise 2004). The main disadvantage of using microsatellite markers was once the time spent on primer development (Ashley and Dow 1994). Using new approaches, such as the poly repeat enrichment protocol (Glenn and Schable 2005), primer development is on the scale of weeks rather than months. I applied the enrichment protocol to successfully develop a set of polymorphic microsatellite loci for *A. inopinatus* **(4)** and *A. compacta* **(5)**, with kind support of Dr. Kevin Feldheim from the Field Museum in Chicago (USA).

With the advances in genetic analyses, microsatellites have become valuable markers for phylogeographic studies, especially by providing indirect measures of

gene flow and migration rates (e.g. Davis et al. 2006, Scott et al. 2005). However, there are also a few drawbacks with microsatellites that should be taken into consideration. The first relates to the high mutation rate (10^{-2} – 10^{-6} per locus per generation) that under the stepwise mutation model may lead to allele size homoplasy (Estoup et al. 1995), i.e. two alleles may be identical in size without being identical by descent. This in turn may lead to an underestimation of genetic diversity and the population differentiation in a phylogenetic or population genetic study. Another problem concerns non-amplifying alleles induced by mutations on the PCR binding sequence. The presence of such null alleles may cause serious miss-assignments at the individual level and overestimate the rate of homozygosity in populations, causing deviations from Hardy-Weinberg equilibrium (Hoffman & Amos 2005).

SPECIES DISTRIBUTION MODELLING (SDM)

Phylogeography has benefitted from recent advances in biostatistical analysis and GIS-based techniques, which now allow a more powerful investigation of the geographic components of genetic variation (Richards et al. 2007). SDM projects ecological niche models onto a model of past climatic conditions to predict the historic distribution of a species and thus explore the degree to which the distribution has changed (Graham et al. 2004). Phylogeography and SDM thus both investigate biogeographical patterns by studying spatial-geographic variation across a species distribution range. Hence, paleodistribution models can provide invaluable information about past population associations that could have led to the current genetic variation structure (Richards et al. 2007). The combined use of genetic and biostatistical techniques can provide a powerful tool to detect historical underlying events (Richards et al. 2007), such as dispersal barriers, migratory pathways,

potential dispersal corridors, as well as the localization of putative Pleistocene refugia. SDM is especially useful for reconstructing the paleodistribution of species for which fossil data are not available (Carstens & Richards 2007), as is the case for most insects. Richards et al. (2007) argue that the use of SDM to develop alternative phylogeographic hypotheses, when coupled with genetic approaches to testing them, can profoundly improve phylogeographic research, and several recent studies have successfully applied this approach (e.g. Knowles et al. 2007, Shepard & Burbrink 2008). Waltari et al. (2007) investigated if SDM and phylogeographical techniques lead to concordant predictions of Pleistocene refugia of 20 different species (mammals, reptiles and birds). They found that 14 of 20 species examined had significant agreement between the two reconstructions of historic biogeography. SDM can thus be used as a framework for phylogeographical hypothesis testing.

I generated potential distribution maps for present **(1, 2, 3)**, LGM **(1, 2)**, and future **(3)** climate data with the program MaxEnt (Phillips et al. 2006), which has been utilized extensively to model species distributions for many ecological, evolutionary, and conservation applications (see Elith et al. 2011 for review). MaxEnt utilizes a maximum entropy approach to make predictions from incomplete information. It uses presence-only records and contrasts them with pseudo-absence data sampled from the remainder of the study area. Despite new modeling methods that use several algorithms to average the distribution range of a species, such as e.g. BIOMOD, I chose to use this maximum entropy approach because it has proven good performance with presence-only data, as reviewed in Elith et al. (2011).

Presence-only data for both species arose from my own collections **(1, 2)** and international databases. The availability of presence-absence data is often limited for species and/or regions, whereas global presence records are available for many species in museum databases. The use of presence-only data in SDM, instead of

modeling with presence-absence data, has caused discussions about the sorts of species distributions, i.e. potential versus realized niche (Elith et al. 2011). A fundamental limitation of presence-only data is that sample selection bias has a much stronger effect on presence-only models than on presence-absence models (Phillips et al. 2009). However, Elith et al. (2011) show that presence-only data can be used to model the same ecological relationships as with presence-absence data.

The climate information I used for the SDM came from the WorldClim database (www.worldclim.org), which contains sets of various global terrestrial climate layers. Bioclimatic variables are derived from monthly temperature and rainfall values in order to generate more biologically meaningful variables (Hijmans et al. 2005). The use of significantly correlated climatic variables can hinder the interpretation of the contribution of single variables to the occurrence probability of the species (Phillips et al. 2006). I therefore used only those climatic variables that were uncorrelated (correlation coefficient < 0.5) and most likely to influence the occurrence of the study species (BIO1 = Annual Mean Temperature; BIO12 = Annual Precipitation; and BIO15 = Precipitation Seasonality).

RESEARCH OBJECTIVES

In this thesis I examined the distribution patterns of two co-distributed taxa to investigate the European Pleistocene and Holocene history of arctic-alpine freshwater insects **(1, 2)**. I used SDM to derive hypotheses on the glacial survival of both species during the LGM. In particular, I aimed to answer questions regarding the population histories of Central Europe and the recolonization histories of Fennoscandia. Based on SDM results I hypothesized that both species could have survived glacial cycles in periglacial, extra-Mediterranean refugia. Concerning the necessary recolonization of previously glaciated northern habitats I hypothesized that

both species postglacially recolonized Fennoscandia from extra-Mediterranean refugia in Central Europe following the retreat of the ice sheet. I tested these hypotheses using two genetic data sets: sequence data of the mitochondrial cytochrome oxidase I (mtCOI) gene, and species specific microsatellites **(4, 5)**. The combination of SDM and two different types of molecular data provides advantages in localizing Pleistocene refugia and in reconstructing the demographic history of populations. I discuss the respective results of **(1)** and **(2)** in a comparative framework to further dissolve the processes and mechanisms of range expansion, regression, and lineage diversification in Europe's high latitude and high altitude biota. Additionally, I used future SDM to predict the impact of climate change induced range shifts and habitat loss on the overall genetic diversity of the endangered mayfly *A. inopinatus* **(3)**. Based on microsatellite data I examined the population structure to identify genetic hotspots for *A. inopinatus* across its European range. I incorporated the population genetic results and the SDM to estimate the future range of *A. inopinatus* and to evaluate how these genetic hotspots should be considered for conservation.

CHAPTER 1

Molecular data and species distribution models reveal the Pleistocene history of the mayfly *Ameletus inopinatus* (Ephemeroptera: Siphlonuridae)

ABSTRACT

We investigated the Pleistocene and Holocene history of the rare mayfly *Ameletus inopinatus* (Ephemeroptera: Siphlonuridae) in Europe. We used *A. inopinatus* as a model species to explore the phylogeography of montane cold-tolerant aquatic insects with arctic-alpine distributions. Using species distribution models we developed hypotheses of the species demographic history in Central Europe and the recolonization history of Fennoscandia. We tested the hypotheses using mitochondrial cytochrome oxidase I (mtCOI) sequence data and compared our genetic results with previously generated microsatellite data to explore genetic diversity distributions of *A. inopinatus*. We observed old lineages, deep splits, and almost complete lineage sorting of mtCOI sequences among mountain ranges. These results support a periglacial survival, i.e. persistence in the periphery of Pleistocene glaciers in Central Europe. A strong differentiation between the Fennoscandian and all other populations was prevalent, indicating that Fennoscandia was recolonized from a refugium not covered in our sampling. High degrees of population genetic structure within the northern samples suggest that Fennoscandia was recolonized by more than one lineage. However, this structure was not apparent in the microsatellite data, consistent with secondary contact without sexual incompability, or sex-biased dispersal. Our demographic analyses indicate that 1) the separation of northern and Central European lineages occurred during the early

Pleistocene; 2) Central European populations have persisted independently throughout the Pleistocene; and 3) the species extended its range about 150 thousand years ago, which most probably coincided with one of the major Pleistocene glaciations. Our study presents an integrative assessment of the phylogeography of an arctic-alpine aquatic insect using species distribution modelling as well as both mtDNA sequence and nuclear microsatellite data.

INTRODUCTION

Aquatic species exhibit different life histories than terrestrial species because their physical environment is fundamentally different (Danks 2007). Hence, aquatic species are expected to differ in their response to past climate change from terrestrial species. This is especially true for cold-tolerant stream-inhabiting species, since permanently running water cannot cool much below 0°C. Thus, cold-tolerant aquatic organisms would have been subject to much less severe temperature decreases than their terrestrial counterparts (Malicky 1983, Pauls et al. 2006). The life history of most aquatic insects includes a lotic and a terrestrial phase. For cold-tolerant montane stream-dwelling insects, present-day distributions suggest that they may form part of the arboreal or boreo-montane biome, indicating that they follow the historical distribution pattern of terrestrial species. However, their response to climate change might have been vastly different because the lotic stage usually constitutes by far the longest developmental stage. In fact, recent phylogeographic studies of montane aquatic insects indicate that survival in the Central European periglacial, i.e. in the periphery of Pleistocene glaciers, and in extra-Mediterranean mountain systems, seems to be a rule, rather than an exception (e.g. Pauls et al. 2006, Bálint 2008, Lehrian et al. 2010), and thus contradict the classical terrestrial Mediterranean refugia scenarios (e.g. Hewitt 2004, Schmitt 2007).

To date no phylogeographic studies of aquatic insects have addressed the origin of post-glacial arctic-alpine disjunct distributions. Arctic-alpine disjunct distributions are unique to the European and Eurasian regions, and are characterized by disjunction between high elevation ranges (sky islands) in the southern mountain ranges and continuous boreal habitats in the North (De Lattin 1967). We use *Ameletus inopinatus* Eaton 1887 (Ephemeroptera: Siphlonuridae) as a model species to explore the Pleistocene history of montane cold-tolerant aquatic insects with arctic-alpine distributions. *Ameletus inopinatus* inhabits mountain ranges of higher elevation (> 600 m asl) on the British Isles and in Central Europe, but is absent in the Central Alps. In northern Eurasia, it is known from Scandinavia to Western Siberia, where it also occurs at lower altitudes and occasionally in the shallow littoral zone of lakes. It is considered a representative of the Eurasian, boreo-montane biome-type (Haybach 2003). Jacob (1979) assumed the species occurred in the ice free areas north of the Alps during the last glacial maximum (LGM; 20ka bp) because of its boreo-montane distribution. Haybach (2003) on the other hand argued that the species' absence from the Alps and the Pyrenees indicates that *A. inopinatus* was not distributed in Europe during the last ice age, but is a recent (post-glacial) colonizer of Central Europe from refugial areas in Eastern Europe. Neither Haybach's nor Jacob's scenarios attempted to judge the arctic-alpine disjunction of *A. inopinatus*. We analysed the two scenarios and the recolonization of Fennoscandia by generating a historical species distribution model (SDM). SDM has become a popular tool in modern phylogeography, especially for reconstructing the paleodistribution of species for which fossil data are not available (Carstens & Richards 2007), as is the case for most insects. Richards et al. (2007) suggest that the coupled use of SDM to develop alternative phylogeographic hypotheses that can be tested with genetic approaches can profoundly improve phylogeographic research. Several recent studies have

successfully applied this approach (e.g. Knowles et al. 2007, Waltari et al. 2007, Shepard & Burbrink 2008, Jakob et al. 2009, Buckley et al. 2010).

However, applying SDM approaches to freshwater species is challenging, as aquatic species are bound to the water column and may thus seem more influenced by local environmental and microhabitat conditions than macroecological parameters, such as climate variables. As for most lotic systems the abiotic factors of headwater mountain streams and alpine lakes are largely determined by air temperature and precipitation, but also by riparian vegetation and geology. While some species may be adapted or restricted to certain geological features (e.g. Engelhardt et al. 2008), many species occur in a variety of geological settings, and their presence is strongly determined by water velocity, concentration of dissolved oxygen, substrate quality, and organic matter inputs (Danks 2007, Graf et al. 2008, 2009). Some studies on mountain dwelling freshwater species additionally use slope derived from digital elevation models as a proxy for oxygen saturation (e.g. Bálint et al. submitted). However, slope does not seem to be a relevant predictor for *A. inopinatus* since it occurs in rather slow running creeks and lakes with week current. Leathwick et al. (2008) successfully applied SDM to examine the relationship between diadromy and dispersal ability in New Zealand's freshwater fish fauna. They constructed functionally relevant predictors such as estimates of catchment-driven variability in local flow, and access to and from the sea for migratory species. Environmental predictors were derived from an extensive GIS database describing New Zealand's river and stream network. However, such hydrological data sets, or continuous data sets on substrate or organic matter inputs are usually not available at a larger scale (Cordellier & Pfenninger 2009). It would also be difficult to project such data into other time periods. Nevertheless, SDM have been widely used to examine distribution ranges of freshwater species (Elith & Leathwick 2009). Regarding the

examination of historical processes that have shaped the present-day distribution of freshwater limpets, Cordellier & Pfenninger (2008, 2009) showed that the climate envelope of the study species were well described through climatic variables solely based on air temperature and precipitation. Accordingly, in this study we use SDM solely based on climatic variables to predict the current and past distribution range of the study species.

We generated potential distribution maps of *A. inopinatus* in the present and during the LGM using the program MAXENT 3.1.1. (Phillips et al. 2006) (Fig. 1.1, see Appendix for method details). The maps show suitable LGM climate conditions for *A. inopinatus* within Central Europe and on the three Mediterranean peninsulas (Fig. 1.1, see Appendix for result details). Based on the SDM we derived two hypotheses pertaining to the population history in Central Europe and about the recolonization of Fennoscandia, i.e. the Scandinavian highlands, after the LGM. The hypotheses were tested with mitochondrial (mtDNA) sequence data. SDM projects large expanses of suitable (but disconnected) habitat in Central and Western Europe during the LGM, but limited suitable areas for eastern refugia (Fig. 1.1B, Appendix). We thus hypothesize that populations of *A. inopinatus* persisted in the periglacial of Central Europe during the LGM (Hypothesis 1). Under this scenario, *A. inopinatus* was either widespread during the periglacial and is currently in regression, or persisted in suitable highland habitats throughout the LGM. In either case we would expect distinct mtCOI lineages or strong genetic differentiation among mountain ranges in Central and Western Europe. In the current warm climate the species is presumably regressive and/or potentially undergoing a bottleneck. We thus expect analyses of demographic history to show no signs of recent or current expansion and limited genetic diversity in currently inhabited regions. Based on the LGM SDM projections we further hypothesize that suitable regions in Central and Western Europe are

potential sources for the northward recolonization of Fennoscandia (Hypothesis 2). If Fennoscandia was recolonized from Western or Central Europe we would expect low genetic differentiation between Fennoscandia and these populations, one phylogenetic Fennoscandia lineage, and very low genetic diversity as a result of a founder effect. Alternatively, more than one region could have served as source populations for the recolonization of Fennoscandia. In this case we would expect multiple phylogenetic Fennoscandian lineages that share haplotypes with the source regions (i.e. Central Europe, British Isles, Eastern Europe or a combination of these) and comparatively higher genetic diversity of Fennoscandian populations due to intermixing of divergent lineages. Additionally, we compare our genetic results with microsatellite data from Taubmann et al. (2011), who recently analysed the genetic diversity distributions and future genetic diversity losses under climate change scenarios, and their conservation implications for this mayfly species.

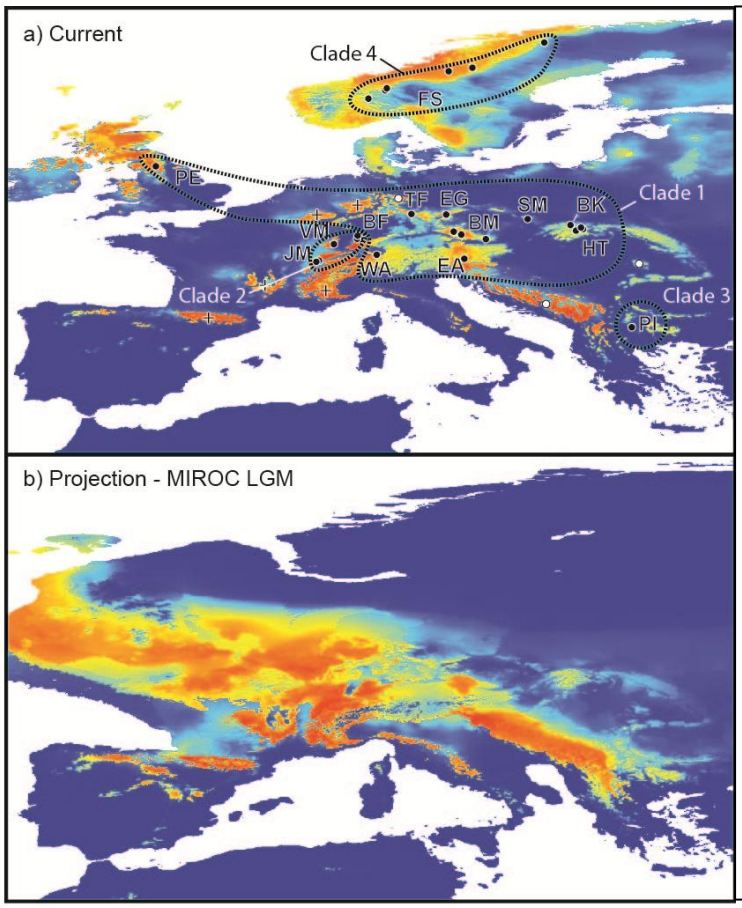


Figure 1.1 SDM for *A. inopinatus* based on three bioclimatic variables and 88 presence locality points, as calculated with Maxent 3.3.0. Warmer colours indicate regions with better climatic conditions for species occurrence. **a**: current distribution. Labels (black dots) indicate sampled mountain ranges (west to east): PE, Pennines; JM, Jura Mountains; VM, Vosges Mountains; BF, Black Forest; FS, Fennoscandia; WA, West Alps; TF, Thuringian Forest; EG, Erzgebirge; BM, Bohemian Massif; EA, East Alps; SM, Sudety Mountains; BK, Beskides; HT, High Tatra; PI, Pirin Mountains. White dots indicate regions where the species occurs but samples are lacking. + indicate regions with over-projections ("false positives"). Dotted lines indicate the inferred four main clades based on mtCOI sequence data. **b**: paleodistribution at the LGM (20000 ybp) based on the MIROC model.

METHODS

Field work

We collected *A. inopinatus* larvae at higher elevation (>620 m asl in Central Europe; >470 m asl in Fennoscandia) in marsh lands, slow running creeks, larger highland streams, and grassy lake shores, using hand nets to sweep submerged macrophytes and grasses. *Ameletus inopinatus* is characterized by a typical brush on the maxillae. We identified specimens under a field microscope to ensure correct identification. Each specimen was stored in individual tubes with 96% EtOH in cooler boxes. Upon returning to the lab we stored specimens in fresh 96% EtOH at –20°C until DNA extraction.

Laboratory work

We used DNEasy tissue kits (QIAGEN, Hilden, Germany) to extract whole genomic DNA following the manufacturer's protocol. After removing the gut, DNA was extracted from the abdomen. Abdomens were lysed in ATL tissue lysis buffer and Proteinase K at 55°C. Cleared abdomens were placed in individual tubes together with the torso and head as vouchers. All vouchers were labelled and stored at 8°C in 96% EtOH in the Senckenberg Museum (SMF129 – SMF182). We eluted final DNA extracts in ddH_2O and stored them at 8°C until polymerase chain reaction (PCR) was complete. DNA extracts were stored long term at –80°C and linked to the voucher specimens in the Senckenberg Museum.

We used the primers LCO1490 (5´ GGT CAA CAA ATC ATA AAG ATA TTG G 3´) and HCO2198 (5´ TAA ACT TCA GGG TGA CCA AAA AAT CA 3´) (Folmer et al. 1994) to produce a 658 base pair fragment. PCR was performed using PuReTaq Ready-to-Go PCR Beads (GE Healthcare Lifesciences, Freiburg, Germany). PCR

cycling conditions followed Hebert et al. (2003). PCR products were purified using NucleoSpin Extract II kits (Macherey & Nagel, Düren, Germany). Purified PCR products were sequenced on a 16 capillary sequencer (ABI 3733). ABI traces of each individual were aligned in the Seqman software (DNASTAR, Lasergene, Madison, USA). No sequences contained ambiguous sites. We generated mtCOI sequences for 266 individuals from 32 sampling localities (populations) in 14 mountain ranges (regions) for *A. inopinatus* (Figure 1.1). A summary of all sampling localities and the number of sampled individuals per population is given in Table 1.1.

Statistical analyses

Genetic population structure

For the mtCOI sequence data we calculated Median Joining networks (Bandelt et al. 1999) using the default settings in Network 4.6 (Fluxus Technology 2009). We subsequently colour-coded the origin of each specimen carrying a given haplotype to illustrate haplotype distribution, identify number of haplotypes, and tally the number of endemic haplotypes. We further examined the relationship of haplotype in BEAST (see below). We examined population genetic differentiation using analysis of molecular variance (AMOVA, Excoffier et al. 1992) and exact tests of population differentiation (ETPD, Raymond & Rousset 1995) as implemented in ARLEQUIN 3.11 (Excoffier et al. 2005).

Demographic history

We inferred demographic history using Tajima's *D* (1989), Fu's *Fs* (1997), and mismatch distributions (Rogers & Harpending 1992) under the model of recent population expansion as implemented in ARLEQUIN 3.11 (Excoffier et al. 2005). We analyzed past population dynamics with Bayesian Skyline Plots (BSP; Drummond &

Rambaut 2007), implemented in BEAST 1.6.1 (Drummond et al. 2005) and Tracer 1.5 (Rambaut & Drummond 2005). We calculated the timing of divergence between the 42 haplotypes of *A. inopinatus* using BEAST (Drummond et al. 2005). Lacking fossil data to calibrate a species-specific molecular clock, we used a divergence rate of 2.3% for the gene COI (Brower 1994) under the relaxed uncorrelated lognormal model (Drummond et al. 2006). The substitution model was set to HKY+I as suggested by the Akaike Information Criterion implemented in jModelTest (Posada 2008). Four independent runs with 10 million generations each were performed on the dataset. Chains were sampled every 1000 states; the initial 10% of the samples were removed as burn-in. A UPGMA starting tree was constructed before each run. The results of the independent runs were combined in the program LogCombiner v1.5.2 (http://beast.bio.ed.ac.uk/LogCombiner) to check for convergence and mixing of independent chains. The 40.000 trees resulting from the four independent runs were summarized in a consensus tree with TreeAnnotator v1.5.2 (http://beast.bio.ed.ac.uk/TreeAnnotator). The ultrametric consensus tree was visualised in FigTree (http://beast.bio.ed.ac.uk/FigTree). The output plot was edited in Inkscape v0.47 (http://www.inkscape.org).

RESULTS

Genetic population structure

In the 620 bp alignment, 63 positions were variable. We found 42 unique mtCOI haplotypes (Genebank accession numbers will be provided upon acceptance of the manuscript). Haplotype diversity was 92.95%. Numbers of mtCOI haplotypes found in each population are listed in Table 1.1. For all but one mountain range (PI: Pirin Mountains) the total sample size exceeded 5 for mtCOI and 4 for microsatellites. The

two individuals from Pirin Mountains were only used in the analyses of haplotype relationships (network, BEAST).

The median joining (MJ) network with colour-coded mountain ranges is shown in Figure 1.2. The maximum number of base pair changes between haplotypes was 44 (7.1%). Thirty-eight haplotypes (95%) were endemic to one of the fourteen regions. Four haplotypes were shared among mountain ranges. The eastern European mountain ranges (High Tatra [HT], Sudety Mountains [SM], Beskides [BK]) exhibited high genetic variation (14 haplotypes = 35%), while the Erzgebirge (EG) populations showed moderate genetic variation (7 haplotypes = 17.5%). The only haplotype found in the Western Alps (WA) population clustered with haplotypes associated with populations in the eastern European Mountain ranges (HT and SM). The network comprises four main clades: 1) A Central European clade, consisting of the eastern Mountain range and the German highlands (Bohemian Massive [BM], Thuringian Forest [TF], EG), the Eastern Alps (EA), the British Peninnes (PE), and WA; 2) A Western European group, consisting of the Jura Mountains (JM), the Vosges Mountains (VM), and the Black Forest (BF); 3) The Pirin (PI) clade had only two haplotypes differing by two bp; 4) A highly divergent Fennoscandian (FS) clade with max. intra-clade divergence of 14 bp (2.3%). The Fennoscandian clade differed by 17 bp changes (2.7%) to the closest haplotype from Western Europe. One common haplotype (H06) occured in all FS populations. The strong division into four main clades was confirmed with the BEAST analysis (see below).

Taubmann et al. (2011) applied a spatial clustering analysis (GENELAND) to define thirteen groups of populations within our sampling, which also correspond to the thirteen sampled mountain ranges (without PI): PE, FS, WA, JM, VM, BF, TF, EG, BM, EA, HT, BK, and SM. The results of the AMOVA for these thirteen mountain ranges and three predefined hierarchical groups (Central Europe, Western Europe,

Table 1.1 Sampling localities for *Ameletus inopinatus* from 14 mountain ranges and 32 populations across Europe. Mountain ranges are presented from West to East. Given are the number sequenced individuals (N), occurring haplotypes, and haplotype frequency in brackets.

Mountain range	Locality	Pop code	Latitude [°N]	Longitude [°E]	Altitude [m]	Collector	N	mtCOI haplotypes
Pennines	(UK) Appleby, Milburn Beck	PE	54.670	-2.446	628	Theissinger, Taubmann	11	H02 (11)
Western Alps	(CH) Alt St. Johann, Thur	WA	47.193	9.288	840	Wagner	5	H39 (5)
Jura		JM					10	
	(F) Foncine le haut, spring Saine	JM_1	46.663	6.074	910	Wagner	5	H11 (5)
	(F) Doubs, Mouthe	JM_2	46.714	6.196	930	Wagner	5	H40 (5)
Vosges		VM					10	
	(F) La Bresse, Tourbiere de Marchais	VM_1	48.008	6.963	1012	Theissinger, Taubmann	5	H11 (5)
	(F) La Bresse, Rue de Crete	VM_2	48.030	7.001	1200	Theissinger, Taubmann	5	H11 (5)
Black Forest		BF					22	
	(D) Bühlertal, Schwarzenbach	BF_1	48.660	8.247	802	Theissinger, Taubmann	2	H11 (2)
	(D) Forbach	BF_2	48.471	8.318	862	Theissinger, Taubmann	5	H12 (4); H13 (1)
	(D) Kleiner Kinzig	BF_3	48.435	8.373	717	Theissinger, Taubmann	10	H11 (10)
	(D) Rechtmurg	BF_4	48.521	8.226	864	Theissinger, Taubmann	5	H12 (5)
Thuringian Forest	(D) Göritzgrund, Steinheid	TF	50.475	11.115	775	Brettfeld	8	H10 (8)
Erzgebirge		EG					20	
	(D) Pöhlbach	EG_1	50.414	12.955	915	Voigt	6	H32 (3); H33 (1); H34 (1); H35 (1)
	(D) Fünfenbach, Sehma	EG_2	50.455	12.973	800	Voigt	5	H32 (3); H36 (1); H37 (1)
	(D) Zschopau	EG_3	50.453	12.939	900	Voigt	9	H32 (6); H34 (2); H38 (1)

Table 1.1 continued

Mountain range	Locality	Pop code	Latitude [°N]	Longitude [°E]	Altitude [m]	Collector	N	mtCOI haplotypes
Bohemian Massiv		BM					26	
	(D) Frauenau, Hirschbach	BM_1	49.042	13.374	1105	Theissinger, Taubmann	5	H10 (5)
	(D) Frauenau, Hochschachten	BM_2	49.042	13.385	1079	Theissinger, Taubmann	9	H10 (7); H14 (1); H15 (1)
	(D) Schimmelbach	BM_3	48.759	13.772	752	Theissinger, Taubmann	7	H10 (4); H16 (1); H17 (2)
	(AUT) Zwettl, Höllbach	BM_4	48.421	15.085	883	Taubmann	5	H01 (5)
Eastern Alps	(AUT) Andertal Moor, St. Lorenzen	EA	46.865	13.931	1400	Theissinger, Pauls, Graf	17	H02 (17)
High Tatras		HT					36	
	(SK) Podbanske, Bela River	HT_1	49.158	19.923	1095	Taubmann	12	H18 (10); H19 (1); H20 (1)
	(SK) Zdiar, Tokarensky potok	HT_2	49.261	20.267	1052	Taubmann	13	H19 (2); H21 (6); H22 (2); H23 (3)
	(SK) Javorina	HT_3	49.256	20.149	1206	Taubmann	11	H19 (4); H22 (6); H24 (1)
Beskides	(PO) Nowy Targ, Jasiruowka	BK	49.554	19.547	919	Taubmann	10	H19 (2); H25 (1); H26 (5); H27 (1); H28(1)
Sudety Mts.	(CZ) Karlova Studanka, Bila opava	SM	50.077	17.297	864	Taubmann	12	H29 (2); H30 (9); H31 (1)
Pirin Mountains	(BG) Pirin Mts., Argirovo lake	PI	41.697	23.138	2356	Vidinova	2	H41 (1); H42 (1)
Fennoscandia		FS					80	
	(NO) Jotunheimen: Ovre Heimdalen	FS _1	61.449	8.841	1300	Brittain	20	H03 (14); H04 (2); H05 (1); H06 (6)
	(NO) Jotunheimen: Valdresflya	FS _2	61.385	8.867	1400	Theissinger, Theobald	4	H03 (1); H05 (3)
	(NO) Dovrefjell: Vinstradalen	FS _3	62.417	9.737	1272	Theissinger, Theobald	19	H06 (4)

Table 1.1 continued

Mountain range	Locality	Pop code	Latitude [°N]	Longitude [°E]	Altitude [m]	Collector	N	mtCOI haplotypes
	(SWE) Skorovatn: Skorovass gruver	FS _5	64.632	13.107	650	Theissinger, Theobald	5	H06 (5)
	(SWE) Burgfjellet: Gellvernokko Mt	FS _6	65.058	14.367	686	Theissinger, Theobald	15	H06 (14); H09 (1)
	(SWE) Abisko, lake Vassijaure	FS _7	68.427	18.185	473	Theissinger, Theobald	4	H06 (4)
Total							266	42

Table 1.2 Results of the analysis of molecular variance (AMOVA). Hierarchical substructuring into groups of populations is based on GENELAND results of the microsatellite data (Taubmann et al. 2011). The tripartite partitioning of the data set (Central Europe, Western Europe, and Fennoscandia, without Pirin) is based on MJ network results. Shown are the percentage of the total variance, fixation indices and their significance (*** = highly significant) based on 1000 random permutations.

Within populations	Among populations within groups	Among groups
9.29 %	6.82 %	83.90 %
$F_{ST} = 0.907^{***}$	$F_{SC} = 0.423^{***}$	$F_{CT} = 0.839^{***}$

Fennoscandia, without PI) based on MJ network results are shown in Table 1.2. F_{ST} values (F_{ST} = 0.907, p < 0.000) indicated strong population structure and high genetic diversity across the whole data set. Exact tests for population differentiation showed that 97.4% of all population pairs were significantly differentiated (Table 1.3).

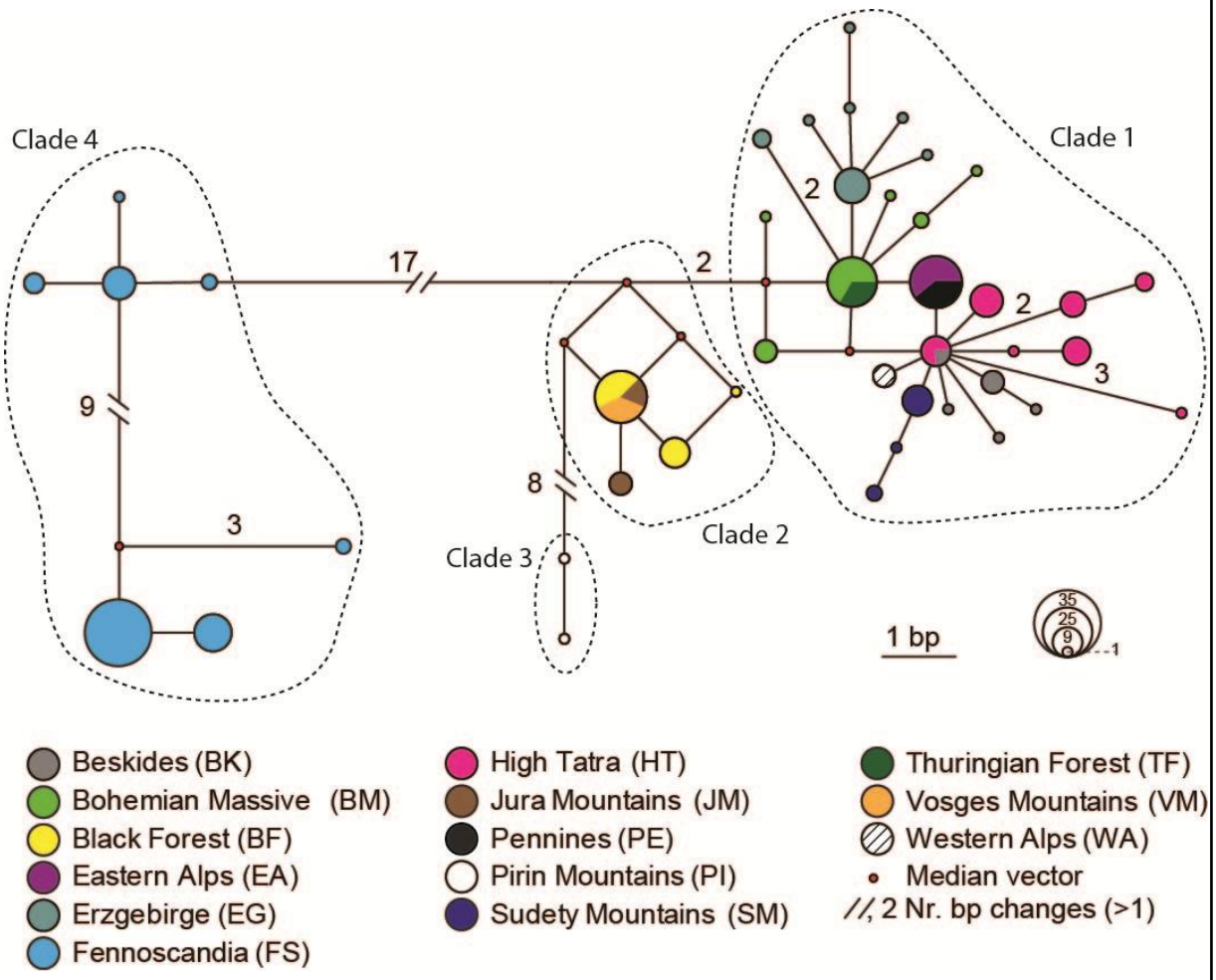


Figure 1.2 Median joining (MJ) haplotype network based on 620 bp mtCOI sequence data for *Ameletus inopinatus* across 14 mountain ranges in Europe (n=266). Haplotype circle size denotes for number of sampled individuals. Colours correspond to different mountain ranges. Given are number of bp changes (no number = 1 bp) and the numbers of haplotypes shared among mountain ranges. Abbreviations of mountain ranges refer to nomenclature in text. Dotted lines indicate the inferred four main clades.

Table 1.3 Exact tests of population differentiation (ETPD) based on mtCOI sequence data of the 13 mountain ranges (without PI).

	EA	BM	TF	HT	BK	SM	EG	PE	WA	BF	VM	JM
EA												
BM	+											
TF	+	-										
HT	+	+	+									
BK	+	+	+	+								
SM	+	+	+	+	+							
EG	+	+	+	+	+	+						
PE	-	+	+	+	+	+	+					
WA	+	+	+	+	+	+	+	+				
BF	+	+	+	+	+	+	+	+	+			
VM	+	+	+	+	+	+	+	+	+	+		
JM	+	+	+	+	+	+	+	+	+	+	+	
FS	+	+	+	+	+	+	+	+	+	+	+	+

Demographic history and timing of divergence

Tajima´s *D* and Fu´s *Fs* did not reveal significant departure from neutrality for any of the thirteen population groups derived from GENELAND (Taubmann et al. 2011). After grouping the populations into three main clusters (Fennoscandia, Central Europe, Western Europe, without PI) both measures returned significant negative departures from neutrality for Central Europe (D = -1.415, p = 0.05; Fs = -26.246, p < 0.0001). Mismatch distributions for the thirteen population groups revealed uni-modal patterns in four mountain ranges: BF, HT, SM, and JM, as well for the Central Europe and Western European clades. The uni-modal distributions suggest negative *D* and *Fs* are caused by recent demographic expansions (Rogers & Harpending 1992) rather than selection.

The BEAST haplotype tree based on the 42 haplotypes (Fig. 1.3) revealed the same main clades (Western Europe, Central Europe, Fennoscandia, and Pirin) as the MJ network (pp>0.95). Further substructure is supported within Fennoscandia

and the Central Europe clade. The lineage split between Fennoscandia and Central Europe was estimated to more than 1.5 my ago. The four supported haplotype groups could all have diverged as recent as 250.000 years ago. The BSP indicated a population expansion about 150.000 years ago.

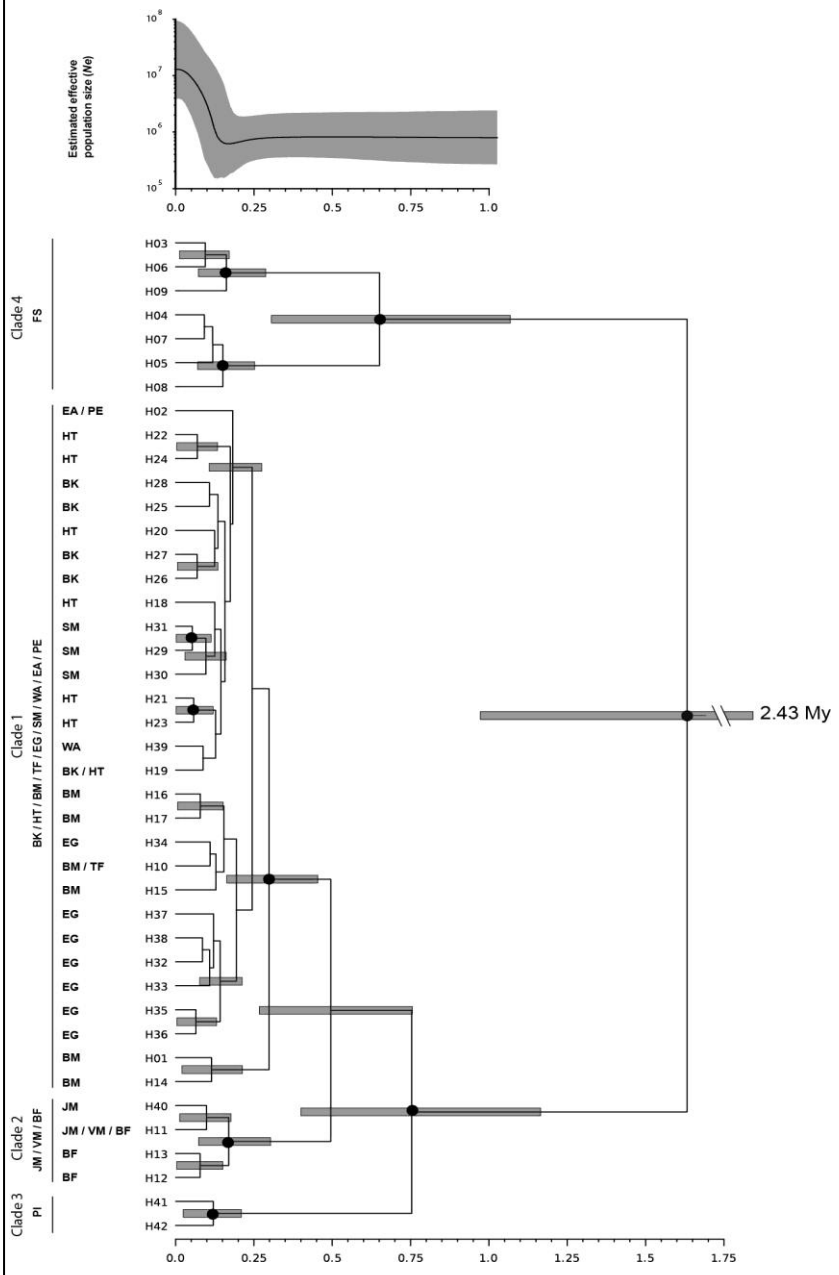


Figure 1.3 Ultrametric consensus tree of haplotypes as calculated in BEAST v1.5.2. Given are relationships of 42 unique haplotypes (H01 – H42). H03 – H09 belong to Fennoscandian populations and form a well supported clade versus the rest of Central Europe. Within Central Europe H11 – H13 and H40 belong to the Western European group and also form a supported clade. Black dots indicate 95 % posterior probability values. Grey lines are the highest and the lowest 95% probability densities of the node ages. Changes in effective population size over a comparable timescale are represented by a Bayesian Skyline Plot. The black line represents the median value of effective population sizes over time. Areas shaded grey show effective population sizes within 95 % lower and upper highest probability densities (HPD). Population size estimates are limited by the timing with the lower 95% HPD at the tree's root height.

DISCUSSION

The main aim of this study was to investigate the Pleistocene history of the rare mayfly *Ameletus inopinatus* in Europe. We applied SDM to develop two hypotheses about the population demography and migratory past of the species and tested those using two genetic data sets. This methodology was recently promoted by Richards et al. (2007) and is regarded as a key innovation in modern phylogeography (Hickerson et al. 2010). In the following we discuss the two hypotheses in light of our *a priori* expectations and the observed results.

Population history in Central Europe

Distinct mtCOI lineages and strong genetic differentiation among all mountain ranges support hypothesis 1 of a Pleistocene periglacial survival of *A. inopinatus* in refugia located in the Eastern European mountain ranges. Endemic haplotypes dominate the genetic structure of *A. inopinatus* (Fig. 1.2). This indicates that lineage sorting among regions and mountain ranges is almost complete. The only exception is the British (PE) population, which had one haplotype identical to the likewise monomorphic eastern Alps (EA) population (Fig. 1.2). The haplotype is situated within the Central European clade. This pattern contradicts the SDM which suggests independent, *in situ* refugia for PE and EA. Instead, the genetic data show that PE and EA populations did not persist in their current regions during LGM and were likely colonized from a common Central European source. The strong differentiation between most other mountain ranges indicates restricted gene flow among sky island populations and long-term isolation of European populations, sufficient to fix population-specific haplotypes. This is a common pattern in cold-adapted species in general (Schmitt 2009) and montane aquatic insects in particular (e.g. Pauls et al.

2006, 2009, Finn et al. 2007, Bálint 2008, Kubow et al. 2010, Lehrian et al. 2010). Small genetic distances among most Central European haplotypes indicate that in this region populations would have expanded from a shared Pleistocene refuge. Most arctic-alpine species were widespread during the glacial, and retreated to small refuges during the interglacials (Schmitt et al. 2006, Brändle et al. 2007). High genetic diversity and an internal haplotype suggest that the Pleistocene refuge may be associated with the High Tatra region. This region was part of the permafrost zone during the LGM. *In situ* interglacial/glacial Pleistocene persistence of cold-stenotherm, rheophile aquatic insects in the vicinity of mountain ranges within permafrost regions was first suggested within the Dinodal theory by Malicky (1983) and supported for selected caddisfly species by Pauls et al. (2006) and Lehrian et al. (2010). Based on its habitat plasticity *A. inopinatus* does not fit the strict model of a Dinodal species, i.e. a species restricted to cold, turbulently flowing mountain streams that flowed permanently through glacial periods providing comparatively warm, azonal environments. However, it is conceivable that a cold-water tolerant mayfly like *A. inopinatus* also persisted in suitable habitat pockets in permafrost regions, consistent with Jacob (1979) who suggested *A. inopinatus* has persisted in extra-Mediterranean refugia during the LGM.

Our demographic history inferences support this interpretation and indicate that *A. inopinatus* experienced population expansions particularly in Central Europe. The BEAST analysis suggests that the Central European populations were prevalent throughout the LGM, since the BSP (Fig. 1.3) show that the species experienced range expansions about 150 to 30 thousand years ago. The separation of northern and Central European lineages happened relatively early during the Pleistocene (Fig. 1.3), after which the two lineages experienced independent LGM histories. The

calibrated BEAST haplotype tree (Fig. 1.3) shows that almost all divergences within the four major haplogroups coincide with the second part of the Riss/Saalian glaciations, dated to 352 to 130 thousand years ago (Lisiecki & Raymo 2006).

Based on microsatellite data Taubmann et al. (2011) found high levels of genetic diversity and strong genetic differentiation across all Central European populations of *A. inopinatus*. Furthermore, they found that in Central Europe genetic diversity decreased along an East-West gradient and is highest in the Eastern European populations, indicating important Pleistocene refuge regions especially for the Eastern Alps and the northern Carpathians. Although the Eastern Alps population appear monomorph for mtCOI haplotype variation, microsatellites indicate an important refugial region due to exceptionally high number of private alleles. A similar pattern of higher genetic diversity in the East associated with lower genetic diversity along a gradient to the West was reported for many other taxa and might indicate better glacial survival conditions in the eastern parts of Europe (see Schmitt 2009 for review). The microsatellite data from Taubmann et al. (2011) thus confirm the geographical aspect of Haybach´s (2003) hypothesis, who proposed a recolonization of *A. inopinatus* into Central Europe from one or more eastern refugia, though this colonization presumably occurred well before the LGM as shown with the demography analyses of the sequence data.

Recolonization of Fennoscandia

The SDM suggests that the arctic range was not inhabited by *A. inopinatus* during the LGM. Our data show that we can reject a putative recolonization of Fennoscandia – a region completely covered by glaciers during the LGM – from a Central European source. The Fennoscandian lineage appears totally independent of the sampled

Western, Central, or Eastern European populations (Fig. 1.2 and 1.3). This is evident from the lack of shared haplotypes and the high degree of genetic differentiation among Fennoscandian and all other populations based on microsatellite data (Taubmann et al. 2011). The genetic divergence among Fennoscandian and Central European haplotypes suggests long-term separation of the lineages. Our analysis dates the split to the early Pleistocene (approx. 2 mya, Fig. 1.3). Microsatellite data support this strong separation between Fennoscandia and Central/Eastern Europe, as shown with an unrooted UPGMA tree (Taubmann et al. 2011). Unlike several other species inhabiting Fennoscandia (Taberlet et al. 1998, Haas & Brodin 2005) *A. inopinatus* does not show a pattern of secondary contact between Fennoscandian and Central European lineages.

We observed strong differentiation among Fennoscandian populations. The strong genetic divergence between two groups of haplotypes indicates that Fennoscandia was recolonized by more than one lineage (Fig. 1.2). Colonization from multiple, independent genetic lineages is a frequent pattern in the Scandinavian biota, as shown for bear (*Ursus arctos,* Taberlet et al. 1995), hedgehog (*Erinaceus europaeus,* Santucci et al. 1998), fishes (*Cottus gobio* Kontula & Väinölä 2001; *Gasterosteus aculeatus*, Mäkinen et al. 2006), trees (*Betula pendula*, Palmé et al. 2003); adder (*Vipera berus,* Carlsson et al. 2004), vole (*Microtus oeconomus*, Brunhoff et al. 2006), seaweed (*Fucus serratus*, Hoarau et al. 2007), and frog (*Rana arvalis*, Knopp & Merilä 2008). This pattern was also inferred for the recolonization of the North American fishfly *Nigronia serricornis* from multiple southern refugia, resulting in several areas of secondary contact in or near previously glaciated regions for that species (Heilveil & Berlocher 2006). However, the mtCOI structure in *A. inopinatus* within Fennoscandia is not apparent in the microsatellite data (Taubmann

et al. 2011). This incongruence of mitochondrial versus nuclear data is a common issue in phylogeographic studies (Zink & Barraclough 2008). Here, this could indicate a secondary contact zone of two maternal evolutionary lineages without sexual incompability, or potentially sex-biased dispersal, which has already been shown for other mayfly species (Flecker & Allan 1988, Caudhill 2003). Microsatellite data furthermore showed lower genetic diversity in Fennoscandian populations compared to Central Europe (Taubmann et al. 2011). This pattern is consistent with a founder effect in the northern populations caused by relatively few individuals dispersing over long distances to recolonize Fennoscandia. Interestingly, within Fennoscandia a decrease in genetic diversity from North to South was observed based on microsatellite data (Taubmann et al. 2011), whereas our mtCOI data exhibit higher genetic diversity in southern Fennoscandia compared to the northern Fennoscandian populations (Table 1.1). This inconsistency could be due to a secondary contact zone of two lineages with different levels of ancestral genetic diversity, a result of 1) different refugial regions, with one region being more diverse than the other; 2) different age of lineages; or 3) lineages experienced bottlenecks to different degrees. Other Fennoscandian taxa, e.g. the moor frog *Rana arvalis* (Babik et al. 2004), have been associated with glacial refugia in south-eastern Russia. A recolonization from a north-eastern refuge suggests an independent postglacial history of the Fennoscandian and Central European populations. Despite the small PI sample size, our data provide no evidence for a recolonization from the Balkan Peninsula via the Carpathian range. Fennoscandia was probably recolonized from more than one refugial source that were not accounted for in our sampling, e.g. the southern Ural, Siberia, or Mongolia (Haybach 2003), a common pattern in the Scandinavian biota (Taberlet et al. 1998). The observed pattern is also consistent with one or several

northern periglacial refugia along the southern fringe of the northern glaciation. This type of survival has been suggested for other aquatic invertebrates and fish in Europe (Thienemann 1950) and North America (e.g. Heilveil & Berlocher 2006, Faber et al. 2009). We currently cannot fully resolve the situation without additional samples from potential eastern or Manchurian refugial areas.

ACKNOWLEDGEMENTS

[redacted]

APPENDIX: SDM

For the present-day species distribution modelling of *Ameletus inopinatus* we used 88 data points of known occurrences across Eurasia, which arose from our own collections (Table 1), international databases such as GBIF and the literature (see Table 1.4 below). Climate information for SDMs came from the WorldClim database (http://www.worldclim.org), which contains sets of various global climate layers.

Bioclimatic variables are derived from monthly temperature and rainfall values in order to generate more biologically meaningful variables (Hijmans et al. 2005). Data were gridded in a spatial resolution of 2.5´ on an equal area grid. In many studies, the spatial resolution of modelled last glacial maximum (LGM) climates is typically on grid cells of 50 square km or greater (Waltari et al. 2007). We used a downscaled high-resolution estimate on 4 square km grids, providing a more detailed picture of LGM environments. Many studies use all 19 BIOCLIM variables (e.g. Waltari et al. 2007, Cordellier & Pfenninger 2008) and do not consider the potential problem of correlation between those variables. The use of significantly correlated climatic variables hinders the interpretation of the contribution of single variables to the occurrence probability of the species (Phillips et al. 2006). We used only those climatic variables that were uncorrelated and most likely to influence the occurrence of *A. inopinatus* (BIO1 = Annual Mean Temperature; BIO12 = Annual Precipitation; and BIO15 = Precipitation Seasonality). Past climate layers (LGM, 20 thousand ybp) were based on two general climate models, the Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC). These data are downscaled layers generated from the Paleoclimate Modelling Intercomparison Project Phase II (PMIP2; <http://pmip2.lsce.ipsl.fr/>http://pmip2.lsce.ipsl.fr/) (Braconnot et al. 2007). Downscaling used the projected change in a given weather variable, which was computed as the difference between the output of a given general climate model and WorldClim current climate (http://www.worldclim.org/downscaling). Distribution modelling was performed with Maxent 3.3.0 (Phillips et al. 2006), using default settings. Maxent utilizes a maximum entropy approach to make predictions from incomplete information. It estimates the most uniform distribution across the study

area given the constraint that the expected value of each environmental predictor variable under this estimated distribution matches its empirical average (Phillips et al. 2006). Maxent outputs a continuous probability value, ranging from 0 to 100, an indicator of relative suitability for the species, based on the principle of maximum entropy, as constrained by the input occurrence data. We evaluated the overall model fit using receiver operating curves (ROC), which are threshold independent and include both commission and omission error, and a tenfold cross-validation as implemented in Maxent 3.3.0.

The average test AUC for the replicate runs of the tenfold cross-validation was 0.925 ± 0.038 S.D. The occurrence probability maps of current distribution (A) and modelled distribution during the LGM (B) based on the MIROC model are shown in Figure 1. An LGM distribution using the CCSM model produced similar results (data not shown). The current SDM correctly projects suitable habitat in regions we could not sample (Eastern and Western Balkans, the Carpathians, and the Harz), but also overpredicts the range in the Eiffel, Westerwald, Denmark, the Massive Central, the Pyrenees, and the Italian highlands. There are no known records for these regions. Overpredictions leading to “false positives” are common in SDM, as these do not take biotic interactions or microhabitat conditions into account (e.g. Lobo et al. 2006). The LGM SDM projects suitable climate conditions on all three Mediterranean Peninsulas. However, as the species is presently not known from the Pyrenees and the Italian highlands we did not consider these regions as possible Pleistocene refugia. Suitable climate conditions during the LGM are also projected northwest of the Alps and in the EA, and to a lesser degree in the TM and the Eastern Balkan Peninsula.

Table 1.4 Reference data points (N = 57) used for species distribution modelling of *Ameletus inopinatus* in addition to the sampled populations (s. Table 1.1). Geographic coordinates are given in decimal degrees. Country codes: UK = United Kingdom; GER = Germany; ROU = Romania; SVK = Slovakia; SWE = Sweden; MGL = Mongolia.

Country	Latitude (ºN)	Longitude (ºE)	Altitude (m)	Source
UK	54.22	-2.33	300	RIVPACS (Wright et al. 2000)
UK	54.35	-1.24	227	RIVPACS (Wright et al. 2000)
UK	54.13	-2.15	303	RIVPACS (Wright et al. 2000)
UK	54.67	-2.45	590	RIVPACS (Wright et al. 2000)
UK	54.72	-2.38	518	RIVPACS (Wright et al. 2000)
UK	56.33	-4.53	135	RIVPACS (Wright et al. 2000)
UK	57.43	-3.36	135	RIVPACS (Wright et al. 2000)
UK	57.48	-5.33	45	RIVPACS (Wright et al. 2000)
UK	57.53	-5.15	170	RIVPACS (Wright et al. 2000)
UK	58.15	-4.98	70	RIVPACS (Wright et al. 2000)
UK	58.41	-3.88	70	RIVPACS (Wright et al. 2000)
UK	57.55	-5.13	170	RIVPACS (Wright et al. 2000)
UK	58.15	-4.24	246	RIVPACS (Wright et al. 2000)
UK	58.12	-4.13	120	RIVPACS (Wright et al. 2000)
UK	56.38	-4.64	176	RIVPACS (Wright et al. 2000)
UK	57.15	-4.43	327	RIVPACS (Wright et al. 2000)
UK	56.88	-5.52	45	RIVPACS (Wright et al. 2000)
UK	58.12	-4.96	149	RIVPACS (Wright et al. 2000)
GER	48.72	8.46	642	Böhmer *et al.* 2004
GER	48.10	8.16	703	Böhmer *et al.* 2004
GER	48.62	8.44	655	Böhmer *et al.* 2004
GER	49.01	13.40	684	Böhmer *et al.* 2004
GER	47.78	8.21	735	Böhmer *et al.* 2004
GER	48.98	13.40	705	Böhmer *et al.* 2004
GER	48.93	13.39	668	Böhmer *et al.* 2004
GER	49.77	12.42	541	Böhmer *et al.* 2004
GER	47.92	7.92	677	Böhmer *et al.* 2004
GER	50.65	10.76	839	Ralf Brettfeld, pers. comm.
ROU	46.00	25.00	669	European Environment Agency
SVK	48.00	18.00	200	European Environment Agency
SWE	62.87	17.38	221	GBIF-Sweden
SWE	63.88	20.07	50	GBIF-Sweden
SWE	64.18	17.27	283	GBIF-Sweden
SWE	64.42	16.30	355	GBIF-Sweden

Table 1.4 continued

Country	Latitude (ºN)	Longitude (ºE)	Altitude (m)	Source
SWE	65.61	19.08	363	GBIF-Sweden
SWE	66.479	17.66	564	GBIF-Sweden
SWE	67.68	17.62	586	GBIF-Sweden
SWE	67.86	19.44	400	GBIF-Sweden
SWE	67.61	23.47	218	GBIF-Sweden
SWE	68.38	18.12	396	GBIF-Sweden
SWE	64.34	16.45	355	GBIF-Sweden
SWE	65.22	17.05	459	GBIF-Sweden
SWE	63.68	19.22	154	GBIF-Sweden
SWE	64.00	19.06	298	GBIF-Sweden
MGL	50.69	100.25	1650	Soldán et al. 2009
MGL	50.96	100.75	1646	Soldán et al. 2009
MGL	51.63	100.53	1724	Soldán et al. 2009
MGL	51.57	100.48	1721	Soldán et al. 2009
MGL	50.60	100.48	1620	Soldán et al. 2009
MGL	51.00	100.71	1655	Soldán et al. 2009
MGL	50.95	100.75	1640	Soldán et al. 2009
MGL	50.92	100.25	1577	Soldán et al. 2009
MGL	50.99	100.54	1785	Soldán et al. 2009
MGL	51.61	100.60	1854	Soldán et al. 2009
MGL	51.65	100.53	2088	Soldán et al. 2009
MGL	50.96	100.54	1661	Soldán et al. 2009
MGL	50.76	100.23	1970	Soldán et al. 2009

CHAPTER 2

Postglacial disjunct distribution in a true arctic-alpine freshwater insect (*Arcynopteryx compacta*, Plecoptera, Perlodidae)

ABSTRACT

Arctic-alpine distributed species exhibit various complex patterns of population histories and are thus of growing interest among biogeographers, though genetic studies remain rare. Here, we investigate the European Pleistocene and Holocene history of the true arctic-alpine stonefly species *Arcynopteryx compacta* (Perlodidae) and compare our results with recent studies on other arctic-alpine invertebrates. We used species distribution models (SDM) to derive two hypotheses: *A. compacta* (1) is a representative of the postglacial disjunct distribution pattern with extra Mediterranean refugia, and (2) postglacially recolonized Fennoscandia from the closest, Central European distribution areas. We tested these hypotheses with two genetic data sets: mitochondrial sequence data (mtCOI) and microsatellite data. Eighty unique mtCOI haplotypes were detected, of which 77 were endemic to a single region, indicating almost complete lineage sorting among regions. The number of divergent lineages reflects the distribution of sampled sky islands. Shared haplotypes were only detected between the Black Forest and Fennoscandian populations. Both mtCOI and microsatellite data suggest strong population differentiation between mountain ranges (mtCOI: F_{ST} = 0.207, p < 0.0001; msat: F_{ST} = 0.455, p < 0.0001; R_{ST} = 0.491, p < 0.0001). Microsatellite data exhibit private alleles to different degrees in all but two mountain ranges (Fennoscandia, Ural), with the highest rate found in the Eastern Alps (38.2 %). The genetic data supports

hypothesis (1) that *A. compacta* survived glacial cycles in periglacial, extra Mediterranean refugia. MtCOI data exhibit signals of a postglacial disjunct distribution pattern for the Black Forest / Fennoscandian clade, providing evidence that *A. compacta* recolonized Fennoscandia from source populations in the Black Forest area, thus confirming hypothesis (2). Microsatellite data, however, do not support this connection between Black Forest and Fennoscandia. The discrepancies could be explained with ancestral polymorphism or female-biased dispersal. This study represents the first genetic evidence of a postglacial-disjunct distribution pattern in an arctic-alpine freshwater insect and provides a next step in filling the knowledge gap regarding molecular studies of the arctic-alpine invertebrate fauna.

INTRODUCTION

The distribution pattern of European arctic-alpine disjunct species is of growing interest among biogeographers due to the arising variety of inferred demographic histories. However, phylogeographic or genetic studies of species with arctic-alpine disjunct distribution are still relatively rare (Schmitt et al. 2010). Species with arctic-alpine distribution ranges are cold adapted, typically inhabiting arctic habitats as well as the alpine altitudes of more southern mountain ranges (DeLattin 1967). Current distribution patterns of species adapted to high altitudes and latitudes are often strongly disjunct (DeLattin 1967), with continuous distribution in the North and patchy “sky island” distribution at high elevations of southern mountain ranges. Schmitt (2007) proposes two different biogeographical arctic-alpine distribution types: 1) Interglacial disjunct distribution: disconnected populations during glacial phases, thus resulting in comparably larger genetic divergence among southern mountain ranges due to long-time lacking gene flow; 2) Postglacial disjunct distribution: widely connected populations throughout glacial periods, which only experienced interrupted

gene flow during recent climate warming, thus resulting in relatively low genetic differentiation compared to the interglacial disjunct distribution type. For the postglacial disjunct distribution type genetic evidence is still rather poor (Schmitt 2007).

Molecular models for understanding the climate-driven processes of postglacial colonization are mainly derived from studies on taxa from the temperate zone (Hewitt 2004, Schmitt et al. 2010). However, cold-adapted species, such as those found in arctic-alpine environments, should tolerate or even thrive under colder climate conditions. Consequently, they should exhibit widespread glacial distributions, as well as bottlenecks and range contractions during interglacials (DeLattin 1967, Hewitt 2004). This should result in signatures of population expansion during the last glacial (12,000 to 110,000 years ago), followed by a population decline during the Holocene due to climate warming, and range regression into cold refugial habitats (Schmitt 2007). Recent work on arctic and alpine species has revealed that this pattern is not true for cold-adapted species in general (Schmitt 2007). In fact, there is evidence that some arctic animal and plant species survived the LGM even at high latitudes or north of the extensive alpine ice shields (Hewitt 2004). Moreover, some arctic-alpine species did not necessarily respond to climate change through large-scale latitudinal shifts (i.e. long distance dispersal) but rather through elevation shifts, which often resulted in mountain system specific genetic lineages (Schmitt 2007).

Most molecular studies on the phylogeography of arctic-alpine species are from plants, exhibiting various genetic distribution patterns, i.e. connection of Northern and Central European populations, strong separation of Northern and Alpine populations, connection of Alpine and Scandinavian populations with Siberia and Ural (see Schmitt et al. 2010 for recent review). Similar arctic-alpine distribution patterns are

also observed in animal species (Holdhaus 1954), but genetic studies remain rare. Schmitt et al. (2010) compared mitochondrial sequence variation between three arctic-alpine invertebrates: the wolf spiders of the *Pardosa saltuaria* group (Muster & Berendonk 2006), the ground beetle *Nebria rufescens*, and the butterfly *Erebia pandrose*. Despite differences in sequence divergence, Schmitt et al. (2010) found some common genetic pattern among these co-distributed species, namely a highly distinct Balkan clade, and low divergence among Scandinavia, the Alps, and several lower Central European mountain ranges, which were grouped in a combined "northern clade". In sharp contrast, northern populations of the mayfly *Ameletus inopinatus* showed no relationship to potential Central European source populations (Theissinger et al. *submitted*). These fundamental differences in Pleistocene histories demonstrate the biogeographical peculiarity of European arctic-alpine invertebrate species.

Here, we aim to investigate the Pleistocene and Holocene history of a true arctic-alpine stonefly species, *Arcynopteryx compacta* (Perlodidae). Our model species exhibits the typical arctic-alpine disjunction sensu De Lattin (1967), with restriction to the highest elevations in the southern European mountains (sky island populations) and a continuous holarctic distribution (Illies 1955). It is a comparably large predator and adapted to springs and spring brooks in Central Europe (Graf et al. 1995). At high latitudes it is occasionally also found on stony lake shores (Lillehammer 1974). *A. compacta* must have recolonized these northern populations of Fennoscandia after the retreat of the ice sheet. The Central European populations of *A. compacta* appear to be glacial relicts that either continue to inhabit their pre-glacial distribution areas (interglacial disjunct) or represent small relict populations of the species wider glacial distribution (postglacially disjunct).

In this study we used species distribution models (SDM) to derive specific hypotheses on the glacial survival of *A. compacta* during the LGM. The LGM SDM predicts 1) suitable climatic conditions continuously along the glacial fringe in Central Europe, 2) a patchy sky island distribution of suitable climate conditions at high altitudes of the southern and eastern mountain ranges, and 3) unsuitable areas in the glaciated northern habitats (Figure 2.1C; for detailed SDM methods and results see Appendix). Thus, we hypothesize (1) that *A. compacta* is a representative of the postglacial disjunct distribution pattern (*sensu* Schmitt 2007), surviving glacial cycles in large populations of the periglacial belt in Central Europe and also in isolated periglacial populations associated with the southern and eastern European mountain ranges, and (2) that *A. compacta* postglacially recolonized Fennoscandia from the closest, Central European distribution areas, following the retreat of the ice sheet. Although other studies showed that the geographically closest area is not always equivalent to the refugial region, this recolonization pattern seems to be the most parsimonious approximation according to our SDM results. We expect I) young lineages in Central Europe consistent recent postglacial population fragmentation; II) mountain system specific lineages in isolated southern and eastern European sky island populations consistent with multiple extra-Mediterranean refugia; III) shared haplotypes and alleles between Fennoscandia and the potential source populations of the Central European mountains, which should all be the descendants of large, more or less continuous populations of the former periglacial belt; and IV) comparably lower genetic diversity in the recolonized populations when considering a colonization event from a single source population.

We tested our hypotheses by analysing mitochondrial sequence data (mtCOI) and nuclear fragment data (microsatellites) in a phylogeographic and population genetics framework. As many species, and particularly arctic-alpine ones, inhabit large and

complex ranges, it is essential for phylogeographical research to take into account the entire range to sufficiently clarify its evolutionary history (Bunje 2005). Here, we present a complete and consistent dataset across Europe, containing samples of all mountain ranges inhabited by *A. compacta*. Additionally, we included some extra European samples to gain information of the world-wide genetic divergence for this holarctic species.

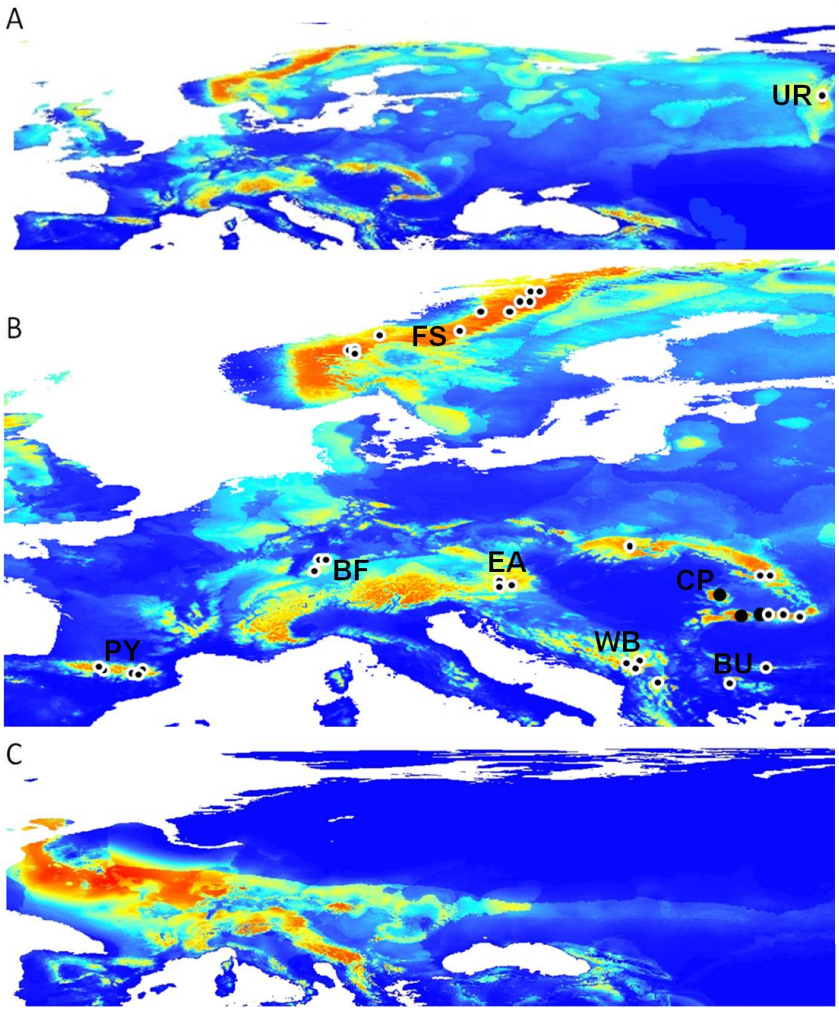


Figure 2.1
Current and past SDM for *A.compacta* and 46 sampling localities across eight mountain ranges in Europe. A: current SDM for Europe, showing the Ural (UR), population. B: current SDM with zoom to Central Europe, showing the Pyrenean (PY), Black Forest (BF), Eastern Alps (EA), Western Balkan (WB), Carpathian (CP), Bulgarian (BU), and Fennoscandian (FS) populations. Big black dots indicate multiple sites in close distance. C: LGM SDM based on MIROC algorithm.

MATERIAL AND METHODS

Field work

Larval and adult specimens of *A. compacta* were collected by using water nets and manually searching preferred microhabitats. Collected specimens were identified in

the field under a field microscope to ensure collection success. Specimens were collected in individual tubes in 96% EtOH and stored in cooler boxes until they were brought to the lab. In the lab specimens were stored individually in fresh 96% EtOH and kept in the refrigerator until DNA extraction.

Laboratory work

We used DNEasy tissue kits (QIAGEN, Hilden, Germany) to extract whole genomic DNA following the manufacturer's protocol. After removing the gut, DNA was extracted from the abdomen. Abdomens were lysed in ATL tissue lysis buffer and Proteinase K at 55°C. Cleared abdomens were placed in individual tubes together with the torso and head as vouchers. All vouchers were labelled and stored at 8°C in 96% EtOH in the Senckenberg Museum (SMF1967– SMF2104). We eluted final DNA extracts in ddH_2O and stored them at 8°C until polymerase chain reaction (PCR) was complete. DNA extracts were stored long term at –80°C.

We generated mitochondrial DNA sequence data from the "DNA barcode" region (Hebert et al. 2003) of the cytochrome oxidase I (mtCOI) gene. We used the primers LCO1490 (5´ GGT CAA CAA ATC ATA AAG ATA TTG G 3´) and HCO2198 (5´ TAA ACT TCA GGG TGA CCA AAA AAT CA 3´) (Folmer et al. 1994) to produce a 658 base pair fragment. PCR was performed using PuRe*Taq* Ready-to-Go PCR Beads (GE Healthcare Lifesciences, Freiburg, Germany). PCR cycling conditions followed Hebert et al. (2003). PCR products were purified using NucleoSpin Extract II kits (Macherey & Nagel, Düren, Germany). Purified PCR products were sequenced on a 16 capillary sequencer (ABI 3733). ABI traces of each individual were aligned in the Seqman software (DNASTAR, Lasergene, Madison, USA). The starting alignment was generated using Clustal W (Thompson et al. 1994) as implemented in

BioEdit 9.0 (Hall 1999). The final alignment was 620 unambiguous base pairs (bp) long.

Microsatellite genotyping was performed using six species-specific primer pairs (Arco_8, Arco_79, Arco_123, Arco_126, Arco_102, Arco_157; Theissinger et al. 2009). We used a high throughput automatic pipetting robot (MWG, Ebersberg Germany) to guarantee highly standardized amplifications. PCR amplification and thermal cycling parameters was performed following Theissinger et al. (2009). We repeated the PCR up to two times before rejecting a sample as non-amplifying. The PCR amplification product (1 µL) of each individual was applied to 11.7 µL HiDi-formamide, 0.3 µL ROX 500 standard (Applied Biosystems, Carlsbad USA) and scored on an ABI 3733 16 capillary sequencer. Loci were genotyped using the software GENEMAPPER 4.0 (Applied Biosystems) and were analyzed for possible null allele issues using MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004) for each separate sampling locality. We additionally estimated the genotyping error rate by randomly re-amplifying 32 individuals (10 %) across all loci (Bonin et al. 2004). The error rate was 0.5% and should not bias our results.

Statistical analyses

mtCOI sequence data

For the mtCOI sequence data, we calculated Median Joining (MJ) networks (Bandelt et al. 1999) with the default settings in Network 4.516 (Fluxus Technology 2009). We subsequently colour-coded the origin of each specimen carrying a given haplotype to illustrate haplotype distributions, identify number of haplotypes, and tally the number of endemic haplotypes. Final illustrations were generated using Network Publisher (Fluxus Technology 2009) and Adobe Illustrator CS5.

We conducted a spatial analysis of molecular variance (SAMOVA) to test whether these geographically defined groups correspond to the genetic variance within our sampling. We used the software SAMOVA 1.0 (Dupanloup et al. 2002) which, given an a priori number of groups (*K*), uses a simulated annealing procedure to define the group composition in which populations within a group are as genetically homogeneous as possible (Θ_{SC} minimized) and groups are maximally differentiated from each other (Θ_{CT} maximized). The analysis was run for $K = 2$ to $K = 20$ and the significance of fixation indices was tested with 1000 permutations. We then estimated the genetic differentiation among clusters with analysis of molecular variance (AMOVA, Excoffier et al. 1992) based on F_{ST}, and exact tests of population differentiation (ETPD, Raymond and Rousset 1995a) as implemented in Arlequin 3.5 (Excoffier et al. 2005).

We inferred demographic history using Tajima's *D* (1989), Fu's *Fs* (1997),, and mismatch distributions (Rogers & Harpending 1992) as implemented in Arlequin 3.5 (Excoffier et al. 2005). We estimated the timing of divergence of the 80 haplotypes of *A. compacta* in BEAST 1.5.2. (Drummond et al. 2005). Lacking fossil calibration points we did not enforce an *a priori* rate of molecular rate of evolution to date our ultrametric tree. The substitution model was set to GTR-I as suggested by the Akaike Information Criterion implemented in jModelTest (Posada 2008). Three independent runs (20 million generations each) were performed on the dataset. Chains were sampled every 2,000 states; the initial 10% of the samples were removed as burn-in. A UPGMA starting tree was constructed before each run. The results of the independent runs were combined in the program LogCombiner 1.5.2 (http://beast.bio.ed.ac.uk/LogCombiner) to check for convergence and mixing of independent chains. The 27 000 trees resulting from the three independent runs were summarized in a consensus tree using TreeAnnotator 1.5.2

(http://beast.bio.ed.ac.uk/TreeAnnotator). The ultrametric consensus tree was visualised in FigTree (http://beast.bio.ed.ac.uk/FigTree). The output plots were edited and combined in Inkscape 0.47 (http://www.inkscape.org). Past population dynamics were analyzed with Bayesian Skyline Plots (BSP; Drummond and Rambaut 2007).

Microsatellite data

Number of alleles (N_A) and number of private alleles (N_P) per population were calculated using the software CONVERT (Glaubitz 2004). Expected (H_E) and observed (H_O) heterozygosity as well as departure from Hardy-Weinberg equilibrium (HWE) were calculated for each locus, over all loci for each population, and across mountain ranges with default parameters in GENEPOP (Raymond & Rousset 1995b). Linkage disequilibrium between pairs of loci was tested in the web-based version of GENEPOP (Raymond & Rousset 1995b) using default parameters and applying a Bonferroni correction for multiple comparisons (Rice 1989). Five loci showed signs of null alleles in few populations across mountain ranges exclusively due to homozygote excess. We therefore used the program FreeNA (Chapuis and Estoup 2007) to calculate null allele frequencies and to estimate global and pairwise F_{ST} values *sensu* Weir (1996) with the *ENA*-method as described by Chapuis and Estoup (2007). This method was found to efficiently correct for the positive bias induced by the presence of null alleles on F_{ST} estimation and provide accurate estimation of F_{ST} in presence of null alleles (Chapuis and Estoup 2007). We conducted a Mantel test (Mantel 1967) between pairwise F_{ST} uncorrected for null alleles and F_{ST} corrected for null alleles with 10,000 random permutations to test whether they were significantly correlated. H_E was used as a measure of genetic variability within populations because it is robust to the presence of null alleles (Chapuis et al. 2009). This is because H_E is independent of

the mating system since it is based on expected frequencies in the population, not on individual heterozygosity.

We applied a Bayesian spatial modelling of genetic population structure using the program BAPS5 (Corander et al. 2008) to define population partitioning based on microsatellite data. BAPS uses Bayesian mixture modelling to describe variation within subpopulations using a separate joint probability distribution over multiple loci. We used the group analysis mode, treating individuals of each sampling locality as separate groups (N=46). We applied the spatial model that uses individual geo-referenced multilocus genotypes to assign a biologically relevant non-uniform prior distribution over space of clustering solutions, thereby increasing the power to detect correctly the underlying population structure (Corander et al. 2006). The program was initially run in fixed *K* modus with *K*=46. Thereafter, we repeated the analysis with the five best partitions, using the vector K= {26 26 26 26 26 27 27 27 27 27 28 28 28 28 28 29 29 29 29 29 30 30 30 30 30} for repeating the run five times for each *K*. For each *K* value, BAPS determines the optimal partitions, stores these internally, and, after all *K* values have been processed, it merges the stored results according to the log-likelihood values. We calculated admixture of individuals, excluding populations with less than three specimens, to estimate gene flow rates (*r*) between given clusters, i.e. the relative average amounts of ancestry in the source cluster among the individuals assigned to the target cluster (Corander et al. 2006). Settings for estimation of admixture coefficients for individuals was 100, number of reference individuals from each population was 200 with 100 iterations.

To estimate genetic differentiation among sampled mountain ranges we conducted analysis of molecular variance (AMOVA, Excoffier et al. 1992) using both the infinite allele model (Kimura & Crow 1964) implemented in F_{ST}, and the stepwise mutation model (Kimura & Ohta 1978) implemented in R_{ST}. We also calculated

population pairwise R_{ST} (Weir & Cockerham 1984) using Arlequin 3.5 (Excoffier et al. 2005). Significance for fixation indices was established with 1,000 permutations for AMOVA and default parameters

RESULTS

We sequenced 334 and genotyped 366 individuals for *A. compacta* from eight regions (mountain ranges) and 46 sampling sites (populations) across Europe, including the Ural (see Figure 2.1A and B). We also analysed sequences from two sites from Far East Siberia (FES) (N = 8) and one Alaskan (AL) specimen to gain information about the world wide genetic divergence of this Holarctic species. These sequences (N = 9) were only used in the analyses of haplotype relationships (MJ network, BEAST). A summary of all sampling localities and their abbreviations, the number of sequenced (mtCOI) and genotyped (msat) individuals, the number of mtCOI haplotypes per population and mountain range, the number of microsatellite alleles (N_A) and number of private alleles (N_P), as well as expected (H_E) and observed (H_O) heterozygosity, and departure from Hardy-Weinberg equilibrium (HWE) across all loci for each population and mountain range, is given in Table 2.1.

mtCOI sequence data

Eighty unique haplotypes were detected of which 77 were endemic to a single region, indicating almost complete lineage sorting among regions. The number of variable sites was 72. The maximum number of steps between haplotypes was 24. Overall haplotype diversity was 0.9507. The mtCOI MJ network (Fig. 2.2) was characterized by six branches, one to five bp in length, diverging from the network's central median vector: 1) The Carpathian (CP) range formed a highly diverse group, with a haplotype

Table 2.1 Sampling localities for *A. compacta* from eight mountain ranges and 46 populations across Europe. Additionally, two populations from eastern Siberia and one specimen from Alaska were analysed for holarctic comparison. Mountain ranges are presented from West to East. Given are number (N) of sequenced (COI) and genotyped (msat) individuals, number of mtCOI haplotypes found in each mountain range as well as mtCOI haplotypes per locality, with respective numbers of individuals carrying the haplotype as indicated in brackets, number of microsatellite alleles (N_A) and private alleles (N_P), expected heterozygosity (H_E), and conformance (+) to Hardy-Weinberg expectations (HWE) across all loci. Deviation from HWE (-) after sequential Bonferroni corrections; *P < 0.05, **P < 0.01, ***P < 0.001. na: not available information due to low sample size or single haplotypes.

Mountain range	Locality	Pop code	Latitude [°N]	Longitude [°E]	Altitude [m]	Collector	N mtCOI / msat	mtCOI haplotypes	Msats: N_A /N_P	H_E	HWE
Pyrenees		PY					32/67	4	44/1	0.598	- ***
	(F) Les Camporeilles, Formigueres	PY_1	42.6231	1.997	2300	Theissinger, Theobald	7/12	H01 (5); H02 (2)	25/0	0.562	- *
	(AND) Tristaina stream	PY_2	42.6421	1.7073	2400	Theissinger, Theobald	8/19	H01(7); H03 (1)	20/1	0.401	+
	(E) Posets Maladeta, lakeObaga	PY_3	42.5889	0.674	2300	Theissinger, Theobald	7/15	H04 (7)	12/0	0.224	+
	(E) Posets Maladeta, lakeLlauset	PY_4	42.5972	0.6824	2500	Theissinger, Theobald	8/19	H04 (8)	22/0	0.444	+
	(E) Valle d´Aran	PY_5	42.7225	0.8372	1800	Pauls	2/2	H01 (2)	11/0	0.389	+
Black Forest		BF					28/30	6	37/2	0.636	- ***
	(D) Stollenbach springs	BF_1	47.899	7.984	1100	Theissinger, Pauls	19/20	H05 (18); H06 (1)	17/2	0.505	+
	(D) Hundseck	BF_2	48.623	8.242	730	Bálint, Neu	7/7	H07 (5); H08 (1); H09(1)	16/0	0.552	+
	(D) Seebach, Mummelsee	BF_3	48.581	8.218	700	Bálint, Neu	2/3	H08 (1); H10 (1)	23/0	0.578	+
Eastern Alps		EA					20/20	8	55/21	0.783	- ***
	(AUT) Saualpe, Ladinger Hütte	EA_1	46.686	14.676	1730	Theissinger, Pauls, Graf	14/15	H11 (6); H12 (7); H13 (1)	42/16	0.720	- ***
	(AUT) Rettenbach, Brentehütte	EA_2	46.900	15.080	1400	Stradner	1/1	H14 (1)	10/1	na	na
	(AUT) Soboth, Krumbach Quellen	EA_3	46.700	15.110	1500	Stradner, Graf	5/4	H15 (1); H16 (1); H17 (2); H18 (1)	17/4	0.682	+
Carpathians		CP					125/126	39	137/30	0.826	- ***
	(ROU) Apuseni, Someşul Calde	CP_1	46.640	22.736	1150	Theissinger, Bálint	14/14	H19 (14)	22/0	0.630	- *
	(ROU) Apuseni, Albac	CP_2	46.626	22.993	1023	Bálint	6/6	H20 (6)	25/0	0.644	+
	(ROU) Apuseni, Someşul Gorge	CP_3	46.640	23.040	1900	Bálint	4/4	H20 (1); H21 (1); H22 (1); H23 (1)	23/0	0.726	+
	(ROU) Apuseni, Someşului Calde	CP_4	46.641	22.823	1020	Theissinger, Bálint	3/2	H19 (1); H22 (1), H23 (1)	18/1	0.917	+
	(ROU) Apuseni, Băişoara	CP_5	46.533	23.288	1500	Theissinger, Bálint	15/21	H19 (6); H24 (8); H25 (1)	46/8	0.750	- ***

Table 2.1 continued

Mountain range	Locality	Pop code	Latitude [°N]	Longitude [°E]	Altitude [m]	Collector	N mtCOI / msat	mtCOI haplotypes	Msats: N_A /N_P	H_E	HWE
	(ROU) Retezat, Câmpu Lui Neag	CP_6	45.320	22.914	1330	Theissinger, Bálint	6/5	H26 (1); H27 (1); H28 (2); H29 (1); H30 (1)	28/2	0.696	+
	(ROU) Retezat, Câmpu Lui Neag	CP_7	45.316	22.914	1320	Theissinger, Bálint	4/4	H30 (2); H31 (1); H32 (1)	25/1	0.744	+
	(ROU) Retezat, Gales lake	CP_8	45.38	22.9		Bálint	14/14	H30 (7); H32 (1); H33 (1); H34 (3); H35 (1); H36 (1)	37/2	0.880	- ***
	(ROU) Parâng, Lotru Valley	CP_9	45.415	23625	1370	Theissinger, Bálint	12/11	H30 (4); H37 (1); H38 (1); H39 (4); H40 (1); H41 (1)	37/2	0.662	+
	(ROU) Parâng, Jiet	CP_10	45.40	23.59	1570	Theissinger, Bálint	12/12	H30 (7); H32 (2); H38 (1); H39 (1); H42 (1);	49/4	0.747	- *
	(ROU) Parâng, Obârşia	CP_11	45.438	23.629	1578	Theissinger, Bálint	2/1	H39(1); H43 (1)	49/0	na	na
	(ROU) Sibiului, Păltiniş	CP_12	45.654	23.932	-	Theissinger, Bálint	4/4	H30 (1); H43 (1); H44 (2)	22/6	0.756	+
	(ROU) Făgăraş, Bâlea Valley	CP_13	45.610	24.618	1890	Theissinger, Bálint	16/17	H44 (11); H45 (1); H46 (1); H47 (1); H48(1); H49(1)	63/0	0.800	- **
	(ROU) Bucegi, IalomiţaValley	CP_14	45.346	25.414	1450	Theissinger, Bálint	6/5	H30 (1); H44 (1); H50(2); H51 (2);	30/3	0.691	- *
	(ROU) Maramureş, Borşa Valley	CP_15	47.682	24.880	1573	Murányi	3/2	H52 (1); H53 (1); H54 (1)	10/1	0.827	+
	(ROU) Rodnei Mts, Lala Valley	CP_16	47.551	24.937	1277	Bálint	1/1	H55 (1)	14/0	na	na
	(PO) Tatranska	CP_17	49.664	20.073	1900	Murányi	3/3	H56 (3)	9/0	0.344	+
Western Balkan		WB					40/40	10	66/3	0.768	- ***
	(ALB) Korab Mts, DibreCounty	WB_1	41.802	20.555	2300	Murányi	6/6	H57 (6)	21/1	0.663	+
	(MNE) Prokletije Mts	WB_2	42.550	19.825	935	Murányi	24/24	H58 (1); H59 (6); H60 (6); H61 (8); H62 (3);	18/0	0.688	+
	(MNE) Sinjajevina Mts, Bistrica spring	WB_3	42.872	19.481	1069	Murányi	4/4	H61 (2); H63 (2)	40/2	0.627	- ***
	(KOS) Hajta Mt, Novo Sole	WB_4	42.737	20.307	580	Murányi	6/6	H64 (4); H65 (1); H66 (1)	20/0	0.563	+
Bulgarian Highlands		BU					14/14	5	21/1	0.535	- ***
	(BUL) Centralna Stara Planina Mt	BU_1	42.602	23.257	1750	Kovács	2/2	H19 (1); H67 (1)	10/1	0.333	+
	(BUL) Pirin, Roalog, Bausho	BU_2	41.751	23.414	2060	Murányi	12/12	H68 (8); H69 (2); H70 (2)	14/0	0.411	+

Table 2.1 continued

Mountain range	Locality	Pop code	Latitude [°N]	Longitude [°E]	Altitude [m]	Collector	N mtCOI / msat	mtCOI haplotypes	Msats: N_A/N_P	H_E	HWE
Fennoscandia		FS					69/71	5	14/0	0.237	- ***
	(NO) Jotunheimen, Valdresflya (a)	FS_1	61.387	8.807	1400	Theissinger, Theobald	8/9	H06 (8)	7/0	0.061	+
	(NO) Jotunheimen, Valdresflya (b)	FS_2	61.390	8.892	1300	Theissinger, Theobald	13/13	H06 (12); H71 (1)	6/0	0.000	+
	(NO) Jotunheimen, Valdresflya (c)	FS_3	61.409	8.804	1300	Theissinger, Theobald	11/10	H06 (10); H72(1)	7/0	0.072	+
	(NO) Dovrefjell, Orkelsjöhytta	FS_4	62.517	9.836	1152	Theissinger, Theobald	1/1	H06 (1)	6/0	0.000	na
	(SWE) Burgfjället, Gellvernokko	FS_5	65.058	14.367	880	Theissinger, Theobald	2/2	H08 (1); H06 (1)	7/0	0.125	+
	(SWE) Abisko, Torneträsk lake	FS_6	68.318	19.183	345	Theissinger, Theobald	8/8	H73 (8)	9/0	0.176	+
	(NO) Finnmark, Stokkedalen lake	FS_7	70.154	23.716	372	Theissinger, Theobald	3/3	H06 (3)	10/0	0.328	+
	(NO) Finnmark, SilesjavviLake	FS_8	69.638	23.444	341	Theissinger, Theobald	16/16	H06 (16)	10/0	0.205	++
	(NO) Finnmark, Eibyelva stream	FS_9	69.823	23.201	92	Theissinger, Theobald	6/6	H08 (1); H06 (5)	9/0	0.100	+
	(NO) Finnmark, Stuaoravari lake	FS_10	69.043	22.911	373	Theissinger, Theobald	2/2	H06 (2)	8/0	0.194	+
	(NO) Finnmark, Polarcircle, E6	FS_11	66.552	15.317	654	Theissinger, Theobald	1/1	H06 (1)	7/0	na	na
Ural	(RU) Maly Patok River	UR	64.4316	59.3974	-	Loskutova	5/5	H74 (4); H75 (1)	16/0	0.560	+
total							334/366				
Far East Siberia		FES					9/7	4	49/1	0.821	- ***
	(RU) Amur, Selemdzha River	FES_1	52.310	130.520	-	Teslenko	3/3	H76 (2); H77 (1)	17/1	0.667	+
	(RU) Amur, Khabarovskiy Kray	FES_2	50.000	134.000	-	Teslenko	5/4	H76 (3); H78 (1); H79 (1)	32/0	0.556	+
Alaska	(USA) Bristol Bay Borough; AlagnakRiver	AL	59.250	-156.340	-	Beatty	1/0	H80 (1)	-	na	na

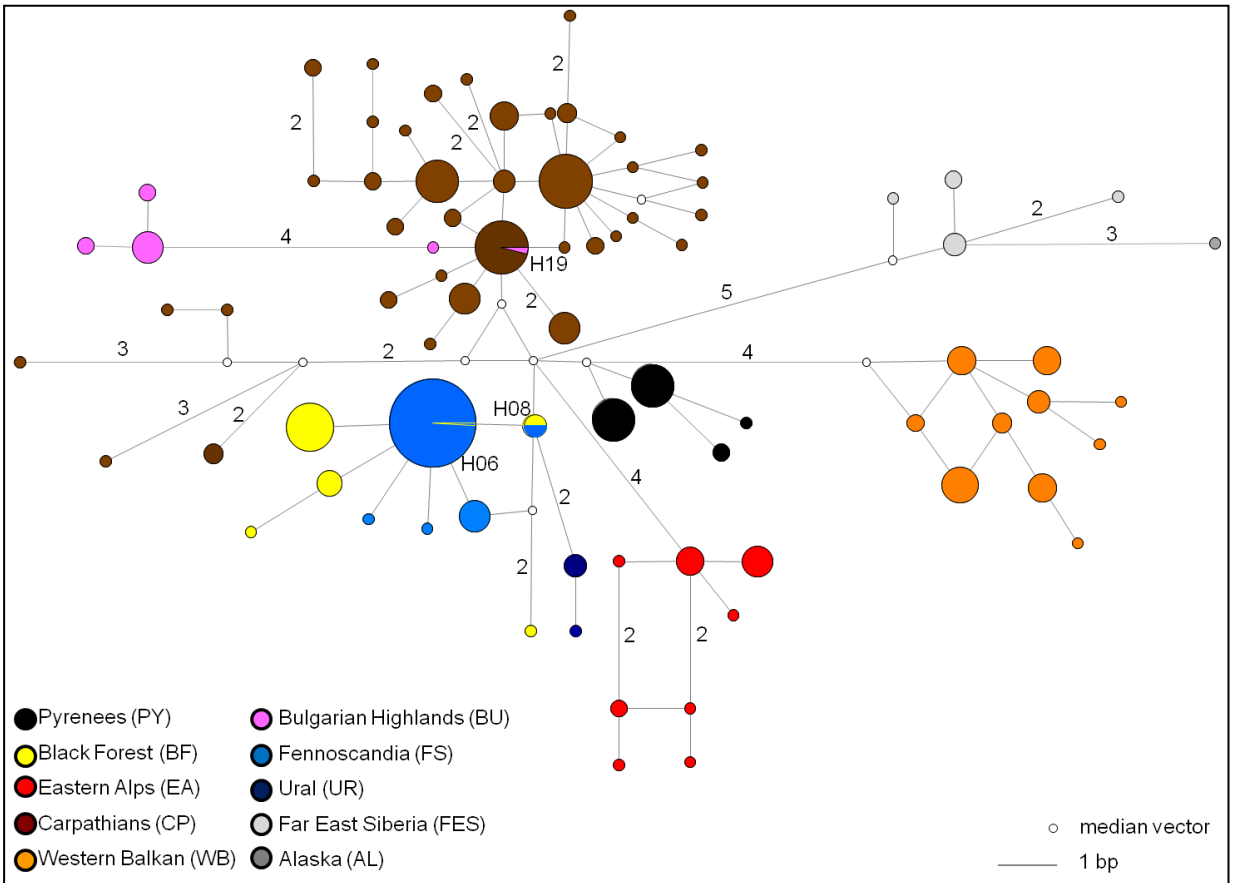


Figure 2.2 Median joining haplotype network based on 620 bp mtCOI sequence data for *A. compacta* across eight mountain ranges and 46 sampling localities in Europe (N = 334). Included are also specimens from Far East Siberia (N = 8) and one single specimen from Alaska for worldwide comparison of genetic variance. Haplotype circle size denotes for number of sampled individuals. Colours correspond to different mountain ranges. Given are number of bp changes (no number = 1 bp) and the number of shared haplotypes among mountain ranges.

diversity of (H_d) of 0.312. Two populations (CP_1, CP_14) shared a haplotype (H19) with one Bulgarian population (BU_1). The Pirin Mountains (BU_2) differed by 4 bp from BU_1; 2) The northern and eastern CP populations (CP_15 - CP_17) were separated from the southern and western CP populations by 6 bp; 3) The Black Forest (BF) haplotypes (H_d = 0.214) grouped together with the Fennoscandia (FS) populations (H_d = 0.072), exhibiting two shared haplotypes, H06 and H08, and were also close to the Ural (UR) haplotypes (H_d = 0.4); 4) The Eastern Alps (EA) were separated from the nearest clade by 5 bp and exhibited an H_d of 0.4; 5) The Pyrenean (PY) samples (H_d = 0.125) were connected to the remainder of the

network by the same median vectors as the Western Balkan (WB) haplotypes (H_d = 0.250). The PY and WB haplotypes were separated by ≥6 bp; 6) The Far East Siberian (FES) group (H_d = 0.444), including one Alaskan (AL) specimen, was separated from other clades by ≥6 bp.

Microsatellite data

All microsatellite loci were polymorphic, with a total of 165 alleles over six loci (min.: 10 alleles for Arco_79; max.: 47 alleles for Arco_157, Table 2.2). For each locus, the number of alleles (*A*), mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), proportion of populations with significant departure from Hardy–Weinberg equilibrium (HWE; $p < 0.05$), and mean null allele frequencies (*NA*) are given in Table 2. No linkage disequilibrium was observed between any loci.

In this study, all samples were collected from discrete stream sites which were initially treated as individual populations. Within streams nine populations showed significant deviations from HWE (Table 2.1). However, when sites from the same mountain range were pooled into regional groups strong deviations from HWE were noticeable for all mountain ranges (Table 2.1). Furthermore, MICROCHECKER indicated possible presence of null alleles at all loci except one (Arco_157) but only one locus (Arco_126) exhibited null allele frequencies > 0.2 (FS: *NA* = 0.236; PY: *NA* = 0.235). Simulations show that the bias induced by null alleles for population structure estimates is negligible at frequencies below 0.2 (Dakin & Avise 2004). To ensure that potential null alleles had no effect on our data, we also compared global F_{ST} values with and without using the *ENA* correction method as calculated in FreeNA. The two runs showed similar results (0.30 and 0.31, respectively). Furthermore, pairwise F_{ST} values corrected for null alleles by the *ENA* were strongly correlated with the uncorrected F_{ST} values in the Mantel test (R^2 = 0.99, $p < 0.0001$).

This suggests that all populations were similarly affected by null alleles and thus should not bias results (Wielgoss et al. 2008, Chun et al. 2010, Vandewoetijne & van Dyck 2010). Consequently, we used the original data set for subsequent analyses.

Table 2.2 Basic information on microsatellite loci used in this study. Given are the number of alleles (*A*), mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), proportion of populations with significant departure from Hardy–Weinberg equilibrium (HWE; $p< 0.05$), and mean proportion of null alleles (*NA*) estimated in FreeNA.

Locus	*A*	H_E	H_0	*HWE*	*NA*
Arco_8	19	0.473	0.424	0.032	0.086
Arco_79	10	0.423	0.362	0.065	0.076
Arco_123	29	0.495	0.408	0.129	0.104
Arco_126	26	0.475	0.419	0.097	0.117
Arco_102	25	0.346	0.308	0.065	0.071
Arco_157	47	0.599	0.585	0.065	0.059

Cluster analyses

Based on microsatellite data the spatial structuring program BAPS5 grouped the 46 sampling sites into 28 clusters (p = 0.96). Only six of these clusters contained multiple sampling sites (Figure 3A): BF_2 and BF_3 (northern BF); CP_2, CP_3, and CP_4 (western CP); CP_6, CP_7, CP_9, CP_10, CP_12 (south-western CP); CP_13, CP_14, CP_15 (southern and eastern CP); FS_1 to FS_4 (southern FS), FS_5 to FS_11 (northern FS). Running BAPS with the same settings but without spatial references of sampling localities produced similar clustering results (p = 0.99). Some of the clusters probably resulted from small sample sizes as indicated by narrow bars in Figure 2.3A (compare Table 2.1). This result indicated that most populations, even when they are geographically close, had a different genetic make-up. Accordingly, gene flow analysis in BAPS based on admixture analysis resulted in very low gene flow rates (r) between all cluster pairs ($0.05 > r > 0.0002$), except between southern and northern FS (r = 0.1).

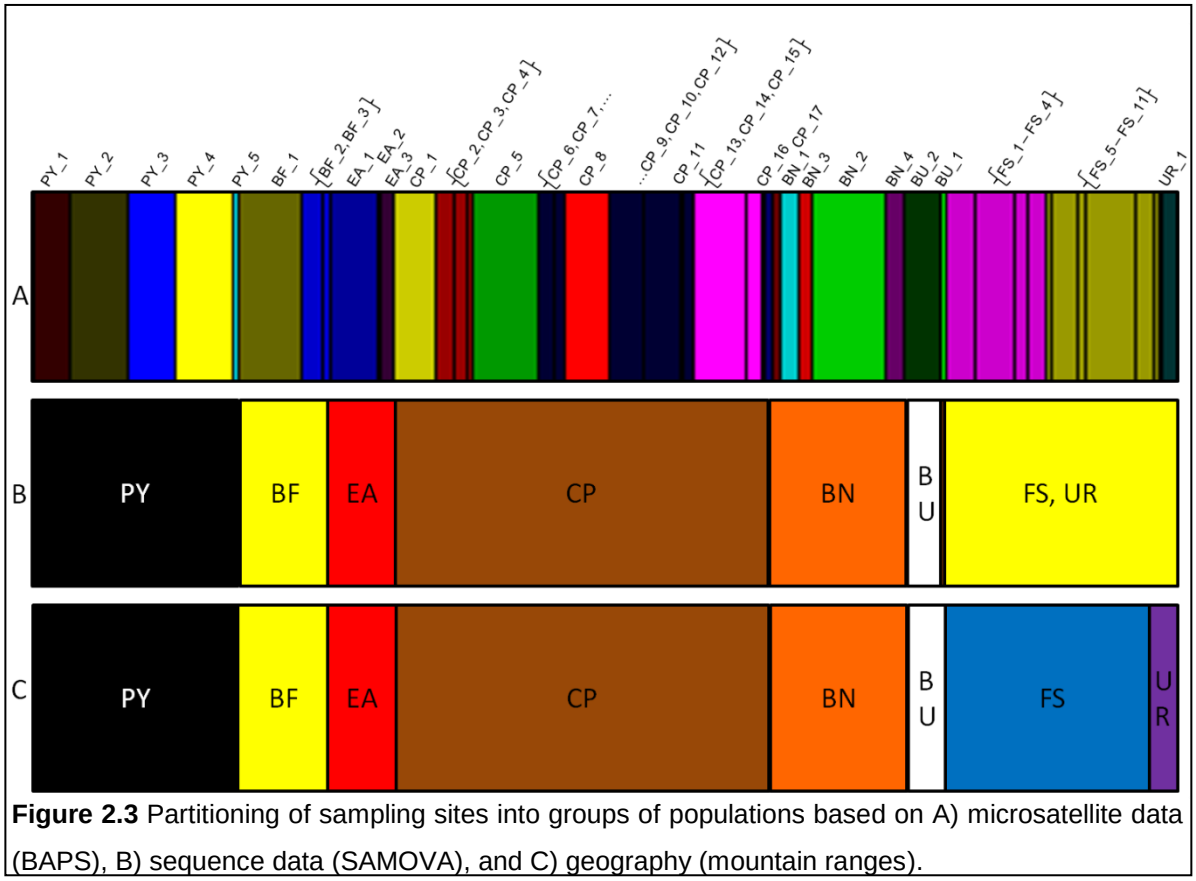


Figure 2.3 Partitioning of sampling sites into groups of populations based on A) microsatellite data (BAPS), B) sequence data (SAMOVA), and C) geography (mountain ranges).

The SAMOVA based on mtCOI sequence data grouped the 46 populations into only six clusters, which represented the sampled mountain ranges (Fig. 2.3B), with two exceptions due to shared or closely related haplotypes: 1) BU_1 was included in the CP group, BU_2 formed a separate group; 2) BF, FS, and UR were grouped in the same cluster.

Both markers showed more or less identical geographical groupings at the regional scale (Figure 2.3A and B).However, the microsatellite data had finer resolution and exhibited geographic substructuring within , but also clear separation among the sampled mountain ranges (based on BAPS). We therefore proposed eight geographical groups, corresponding to the eight sampled European mountain ranges: PY, BF, EA, CP, WB, BU, FS, and UR (Figure 2.3C). This hierarchical structuring was used for all subsequent population differentiation analyses.

Population differentiation

The results of the analysis of molecular variance (AMOVA) for the eight predefined geographical groups of populations, calculated for both sequence and microsatellite data, are given in Table 2.3. These results suggest strong differentiation between mountain ranges (mtCOI: F_{ST} = 0.207, p < 0.0001; msat: F_{ST} = 0.455, p < 0.0001; R_{ST} = 0.491, p < 0.0001). Pairwise F_{ST} and R_{ST} values for the eight geographical groups of populations are given in Table 4. For mtCOI sequence data, values ranged from 0.436 to 0.924 (Table 2.4A), with a median of 0.783 (lower quartile: 0.673, upper quartile: 0.828) and 100% significance. Pairwise R_{ST} values based on microsatellite data are presented in Table 2.4B. Values ranged from 0 to 0.780, with a median of 0.425 (lower quartile: 0.128, upper quartile: 0.579) and 86% significance. Microsatellite pairwise F_{ST} values (Table 2.4C) ranged from 0.109 – 0.665 (with ENA; median: 0.286, lower quartile: 0.200, upper quartile: 0.370) and 0.116 – 0.700 (without ENA; median: 0.314, lower quartile: 0.180, upper quartile: 0.194).

Table 2.3 Results of the analysis of molecular variance (AMOVA) for the eight predefined geographical groups of populations for *A. compacta*, calculated for A) mtCOI sequence data and B) microsatellite fragment data, using both F_{ST} and R_{ST}. Shown are the percentage of the total variance, fixation indices and their significance based on 1000 random permutations ($^{***}P$< 0.0001).

	Within populations	Among populations within groups	Among groups
A	41.48%	40.35%	18.17%
	F_{ST} = 0.207***	F_{SC}=0.202***	F_{CT} = 0.091***
B	54.50 %	18.80 %	26.60 %
	F_{ST} = 0.455***	F_{SC} = 0.257***	F_{CT} = 0.266***
	50.90%	14.50%	34.60 %
	R_{ST} = 0.491***	R_{SC} = 0.221***	R_{CT} = 0.346***

Table 2.4 Pairwise F_{ST} and R_{ST} values for the eight European mountain ranges of *A. compacta.* A) Pairwise F_{ST} values based on mtCOI sequence data as implemented in Arlequin 3.5. Significant values after Bonferroni corrections are indicated with*. B) Pairwise R_{ST} values based on microsatellite data as implemented in Arlequin 3.1.1. Significant values after Bonferroni corrections are indicated with*. C) Pairwise F_{ST} based on microsatellite data calculated with (below diagonal) and without ENA method (above diagonal) as implemented in the FreeNA software, applying 10.000 bootstraps.

A	PY	BF	EA	CP	WB	BU	FS	UR
PY	0							
BF	0.673*	0						
EA	0.782*	0.775*	0					
CP	0.562*	0.600*	0.684*	0				
WB	0.723*	0.803*	0.827*	0.712*	0			
BU	0.783*	0.795*	0.840*	0.578*	0.828*	0		
FS	0.851*	0.436*	0.903*	0.671*	0.891*	0.924*	0	
UR	0.773*	0.679*	0.813*	0.621*	0.818*	0.860*	0.917*	0
B	PY	BF	EA	CP	WB	BU	FS	UR
PY	0							
BF	0.722*	0						
EA	0.744*	0.624*	0					
CP	0.410*	0.030*	0.604*	0				
WB	0.451*	0.236*	0.579*	0.129*	0			
BU	0.458*	0.247*	0.584*	0.126*	0.177*	0		
FS	0.340*	0.777*	0.780*	0.384*	0.440*	0.533*	0	
UR	0.526*	0.135*	0.049	0.066	-0.067	-0.016	0.750*	0
C	PY	BF	EA	CP	WB	BU	FS	UR
PY	0	0.335	0.265	0.228	0.259	0.416	0.469	0.234
BF	0.304	0	0.301	0.162	0.216	0.396	0.638	0.394
EA	0.258	0.264	0	0.155	0.183	0.340	0.595	0.261
CP	0.215	0.137	0.143	0	0.116	0.174	0.390	0.180
WB	0.252	0.190	0.173	0.109	0	0.326	0.503	0.218
BU	0.412	0.364	0.331	0.182	0.318	0	0.700	0.454
FS	0.440	0.593	0.559	0.375	0.474	0.665	0	0.347
UR	0.239	0.370	0.268	0.200	0.227	0.455	0.309	0

For microsatellite data, H_E per population ranged from zero (FS_2, FS_4: homozygote at every locus) to 0.917 (CP_4); for mountain ranges H_E ranged from 0.237 (FS) to 0.826 (CP) (Table 2.1). Rates of allele endemism for mountain ranges, based on the number of private alleles (N_P, Table 2.1), ranged from zero (FS, UR) to 38.2 % (EA). Of the 14 alleles found in FS (Table 2.1), 8 alleles were shared with PY,

4 with BF, 3 with EA, 13 with CP, 10 with WB, 6 with BU, and 5 with UR (data not shown). Departures from HWE were observed in nine populations due to homozygote excess and/or population substructuring, and in all mountain ranges when populations were pooled due to genetic substructure within mountain ranges (Table 2.1).

Demographic history

Mismatch distributions (mtDNA) in the eight mountain ranges showed a multimodal pattern in PY, BF, EA, CP, and BU. Unimodal patterns were observed in WB, FS, and UR (Table 2.5), indicating recent demographic expansions in these regions (Rogers and Harpending 1992). Neutrality tests showed significant negative values for both Tajima´s *D* and Fu´s *Fs* in the CP populations. The FS range exhibited a significant negative *Fs* value (Table 2.5).

The BEAST haplotype tree is shown in Figure 2.4. The BSP showed that there was a recent population growth in Central European populations of *A. compacta*. With the exception of the BF, CP, and FS, all regional haplogroups were monophyletic, but only EA, UR, and WB represent highly supported clades (pp >0.95). Furthermore the connections among 1) BF, FS, and UR, as well as 2) FES and AL are significantly supported.

Table 2.5 Results for demographic expansions for the eight sampled European mountain ranges, calculated with Arlequin 3.5. Presented are patterns of mismatch distributions (MD: uni = unimodal distribution; multi = multimodal distribution) and Harpending´s raggedness index (R) as well as results from neutrality tests (Tajima´s *D* and Fu´s *Fs*) with levels of significance (*p<0.05, **p<0.005, ***p<0.0005).

	PY	BF	EA	CP	WB	BU	FS	UR
MD	multi	multi	multi	multi	uni	multi	uni	uni
R	0.683***	0.212	0.060	0.043	0.070	0.124	0.300	0.200
D	-0.171	-0.596	-0.113	-1.748*	0.049	-0.798	-1.106	-0.817
Fs	1.361	-0.261	-2.371	-26.718***	-2.615	-0.289	-2.097*	0.090

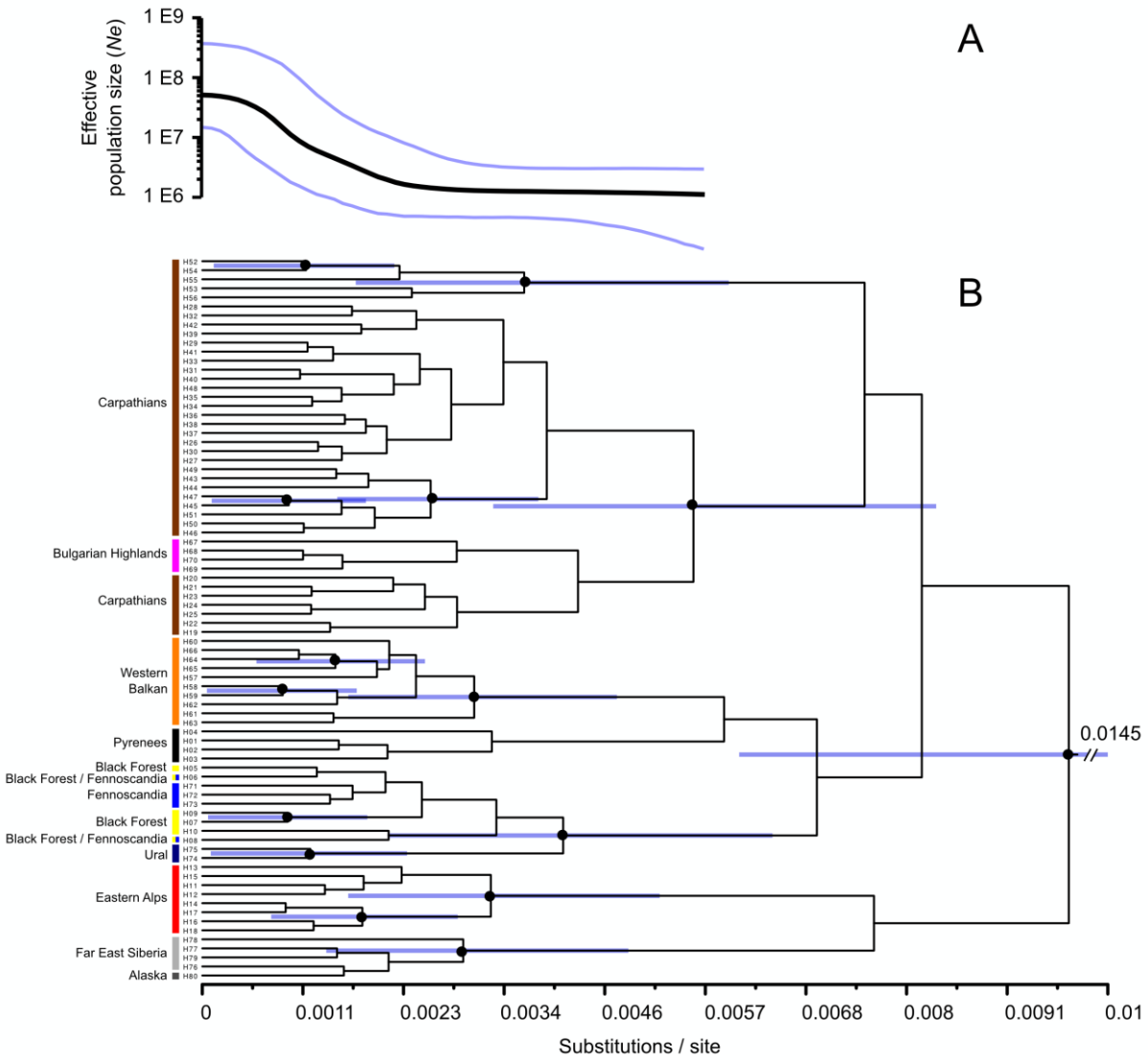


Figure 2.4 BEAST haplotype tree. A) Demographic changes of *A. compacta* populations are presented by a Bayesian Skyline Plot (BSP). Mean (black line) and 95% confidence intervals (grey) indicate effective sample size (N_e) estimates. The demographic changes reflected by the graphs are estimated back in time to the mean timing of the coalescent event. B) Bayesian phylogenetic tree of the eighty identified *A. compacta* haplotypes. Posterior probabilities ≥0.95 are marked by black circles. Grey bars represent 95% highest probability densities of the inferred mean divergence dates. Colours of the main clades correspond to the mountain range colour code in Figure 2.2.

DISCUSSION

Our study supports both of the hypotheses we derived from the SDMs: *A. compacta* (1) appears to be a representative of the postglacial disjunct distribution pattern and (2) recolonized the Fennoscandian range from a Central European periglacial refuge between the arctic and alpine glaciers. In the following we discuss the underlying

population genetic structure and the inferred population history of *A. compacta* in the light of our study hypotheses and compare our results with the respective literature.

Population genetic structure

Microsatellite data found high genetic structure among and even within mountain ranges. The underlying reasons for this subpopulation structure could be geographic barriers to gene flow due to low dispersal capabilities of *A. compacta*: all adult males and females of some populations are brachypterous, and stoneflies in general are poor fliers (Malmqvist 2000). It is thus not surprising that microsatellites show no gene flow among and within any mountain regions in Central Europe. Moreover, all sky island populations exhibit private microsatellite alleles. This is congruent with the high rate of endemic haplotypes within mountain ranges and regional differentiation in the mtDNA, which also indicates limited gene flow among populations. This pattern of strong genetic differentiation is common among European sky island distributed headwater stream insects (Finn et al. 2007). In aquatic insects, low gene flow rates are often explained by limited adult lateral dispersal capabilities or philopatry that restrict movement between sky island habitats at larger geographic scales (Pauls et al. 2006, 2009, Bálint 2008, Engelhardt et al. 2008, Kubow et al. 2010, Lehrian et al. 2010, Taubmann et al. 2011). At smaller spatial scales, however, Finn et al (2007) showed that for headwater stream insects lateral dispersal among reaches of same elevation is an important process for maintaining gene flow among catchments, and outweighs other processes like longitudinal within-stream movement. Benke et al. (2009) illustrated that low dispersal ability in cold-adapted spring snails and the isolation and fragmentation of spring habitats may account for lineage divergence between mountain ranges in Central Europe. For the putative glacial relict freshwater flatworm *Crenobia alpina* populations were effectively isolated even across small

spatial scales as shown with allozyme data (Brändle et al. 2007), again reflecting the species' very limited dispersal capacity. Lehrian et al. (2009) present a contrasting example of two montane Trichoptera species exhibiting similar insular distributions in Central Europe, with one species restricted to altitudes above 600 m, the other limited to the same mountain ranges but found at lower altitudes. The two species exhibited distinct population structures, with the high altitude species showing significantly higher haplotype diversity and isolation of mountain range lineages, while the species found at lower altitudes exhibited greater haplotype overlap between mountain ranges (Lehrian et al. 2009). This example indicates that there may be a link between cold-adaptation in sky island species and restricted gene flow. Suitable habitats for cold-adapted species in temperate regions are often restricted to isolated regions of higher elevation, thus limiting the availability of cool habitats and as well the connectivity among these habitats. For cold-stenotherm species with limited dispersal abilities, this should lead to relatively greater local genetic population structure. If this is the case, we would expect increasing isolation and genetic differentiation among sky island species in the face of future climate warming, where cold-adapted species of temperate regions will need to compensate increasing temperatures by local migrations to higher elevations (Hering et al. 2009, Taubmann et al. 2011).

Population history

Analyses of haplotype relationships (Figures 2.2 and 2.4) demonstrate that despite the great geographic distance and high geographic complexity the Far Eastern Siberian group and the Alaskan specimen are only minimally diverged from the closest Central European clade. This indicates that the world-wide genetic divergence of *A. compacta* is considerably smaller compared to other holarctic taxa

(see Hewitt 2004 for review), and demonstrates that our sampling is sufficient to understand the evolutionary history of this wide-spread species.

Our genetic data are generally consistent with a postglacial disjunct distribution pattern, confirming hypothesis 1. According to our initial expectations, we discovered I) relatively young genetic and mountain system specific lineages with very shallow divergence (Figures 2.2 and 2.4); II) low genetic divergence between sampled regions (Figure 2.2); III) shared haplotypes between Fennoscandia and the potential source populations of the Central European mountains (Figure 2.2); and IV) comparably lower genetic diversity in the recolonized populations (Table 2.1). The species was presumably widely distributed throughout the glacial steppes between the northern ice shield and the southern glaciers, so that gene flow between northern and southern populations was only interrupted by the postglacial disjunction among high mountain systems and the arctic (Schmitt et al. 2007). Populations of *A. compacta* also survived glaciations in isolated extra-Mediterranean refugia, such as the Eastern Alps and the northern and south-western Carpathians, but also in the Pyrenees and “classical” Mediterranean southern refuge areas of the Balkan Peninsula (Figure 2.5). This is genetically supported by monophyletic lineages in all mountain ranges (Figure 2.2), thus reflecting the elevation shift model as alternative to latitudinal migration (Schmitt et al. 2007, Galbreath et al. 2009). The relationship among several haplogroups is not resolved (Figure 2.4). This is not surprising when comparing the star-like shape of the MJ network (Figure 2.2). Here, we discovered complete lineage sorting among sampled sky islands, except for the Black Forest and Fennoscandia, which exhibit shared haplotypes (Figure 2) and comparably low pairwise F_{ST} values (Table 2.3A). This suggests that *A. compacta* recolonized Fennoscandia and the Black Forest from a common founder population, potentially located in Central Europe, confirming hypothesis 2.

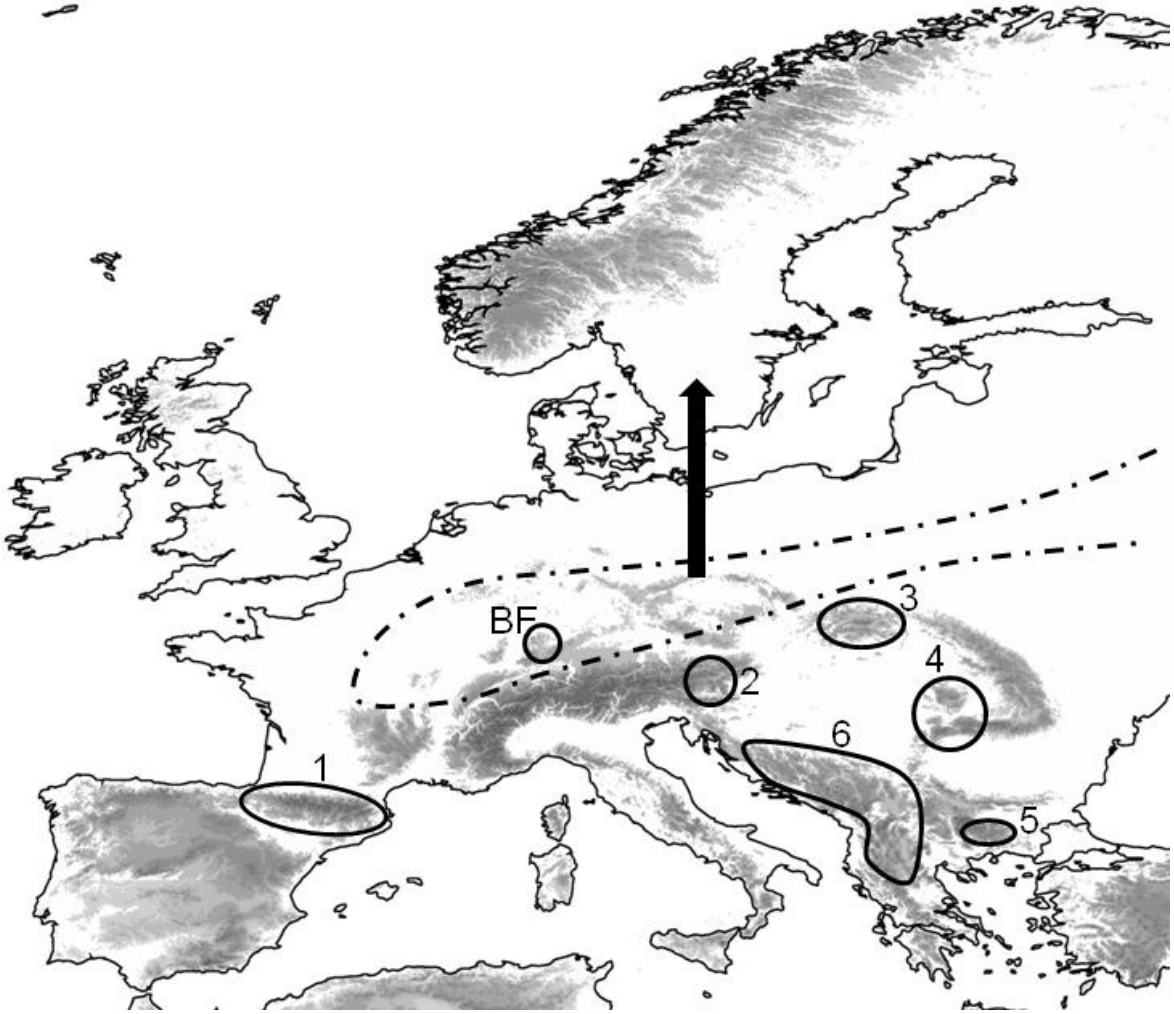


Figure 2.5 Inferred Central European refugial regions of *Arcynopteryx compacta*, based on genetic data and SDM results (compare Figure 2.1C and Appendix). 1) Pyrenees; 2) Eastern Alps; 3) Northern Carpathians; 4) South-Eastern Carpathians; 5) Bulgarian Highlands; 6) Western Balkan. The dotted line indicates the presumed periglacial belt population (including the Black Forest [BF] population) along the glacial fringe, from where the recolonization of Fennoscandia originated (arrow).

The genetic split seems relatively young compared to the other splits (shallow differences and shared mtCOI haplotypes), suggesting a postglacial disjunction of populations in these ranges. Thus, it seems plausible that the Black Forest and the Fennoscandian lineages both descended from large populations, which persisted in the periglacial belt during the last glaciations (Figure 2.5), or at least that substantial levels of late glacial gene flow among mountain ranges occurred (Schmitt et al. 2010). Moreover, the geographically distant Ural specimens group closely with

Fennoscandian and Black Forest haplotypes. This suggests a relatively recent common ancestry of these populations, which can be best explained by isolation by distance or recent isolation following a continuous distribution in the periglacial belt. The very low genetic diversity within Fennoscandia as revealed by mtCOI and microsatellite data (Table 2.1) indicates a strong founder effect. This also reflects the poor dispersal ability of *A. compacta*. The Black Forest populations, on the other hand, exhibit higher levels of genetic diversity, indicating that founder effects are not as prominent in this population. Whether the Black Forest population remained in place or retreated to higher elevations as a postglacial cold adapted relict nearby glacial refuge is unclear. Other studies of cold tolerant aquatic insects indicate that *in situ* persistence in Black Forest streams was possible (Pauls et al. 2006, Malicky 1983, 2006). However, those species are generally more range restricted than *A. compacta* and presently not found in Fennoscandia. This also suggests that *A. compacta* had a much wider periglacial range which may have included the Black Forest, and that the species was able to slowly expand its range over large distances during the postglacial northward recolonization.

However, the microsatellite data do not support the connection of Black Forest and Fennoscandia, as shown with the BAPS clustering (Figure 2.3). Moreover, only four alleles are shared between the Black Forest and Fennoscandia, ranging at the lower bound compared to the number of shared alleles of Fennoscandia with the other mountain ranges (data not shown). This incongruence of mitochondrial versus nuclear microsatellite data is a common issue in phylogeographic studies using both markers (Zink & Barraclough 2008) and emphasize the need to use various marker types with different modes of inheritance to sufficiently understand a species history (Godinho et al. 2008). In our case the discrepancies could be explained with 1) ancestral polymorphism, which is remnant in the mtCOI data while the microsatellite

data have diverged more quickly due to random mutations under strong genetic drift, or 2) female-biased dispersal, since brachypterous males of *A. compacta* could be disadvantaged in their dispersal capabilities. Unfortunately, there is insufficient data to examine the possibility of sex-biased dispersal. However, considering the large scale recolonization of *A. compacta* into Fennoscandia, the species holarctic distribution, and the limited divergence among mtCOI sequences from different regions and continents, it is plausible that long distance dispersal is primarily maintained by winged females, rather than brachypterous males.

A close relationship between Central and Northern European populations has often been suggested by classical biogeographers (e.g. De Lattin 1967). Schmitt et al. (2010) summarized several examples for this connection between Central and Northern Europe in plant species, which all colonized the North Atlantic region from source populations in the Alps. Most often in arctic-alpine plant species separations between the northern populations and the high mountain systems in Central and Southern Europe are postglacial phenomena (Schmitt et al. 2010), as found in this study between the Black Forest and the Fennoscandian populations. However, comparable studies on arctic-alpine invertebrates exist to date only for four species: 1) the wolf spiders of the *Pardosa saltuaria* group (Muster & Berendonk 2006), 2) the ground beetle *Nebria rufescens* (Schmitt et al. 2010), 3) the butterfly *Erebia pandrose* (Schmitt et al. 2010), and 4) the mayfly *Ameletus inopinatus* (Theissinger et al. *submitted*). *N. rufescens* and *A. inopinatus* represent the more boreo-montane disjunct distribution type as described by De Lattin (1967), whereas the *P. saltuaria* group and *E. pandrose* exhibit a classical arctic-alpine distribution pattern, similar to such of *A. compacta*. The first European-wide phylogeographical study of an arctic–alpine distribution in invertebrates focused on the *P. saltuaria* group (Muster & Berendonk 2006). In this study sequence data exhibited three clades of deep

mitochondrial divergence, a Pyrenean clade, a Balkan clade, and a “Northern clade” including Fennoscandia, the Alps, the Carpathians, the Giant Mountains, and the Bohemian Forest. Schmitt et al. (2010) compared these findings with preliminary sequence results of *N. rufescens* and *E. pandrose* and found similar genetic patterns among these species, i.e. a highly distinct Balkan clade, as well as low divergence among Scandinavia, the Alps, and several lower Central European mountain ranges. This also suggests a late glacial connection of Fennoscandian and Central European populations, as found in this study. In sharp contrast, Theissinger et al. (*submitted*) show that in *Ameletus inopinatus* Fennoscandia was a highly diverged and structured group and was not recolonized from Central Europe. Instead, Theissinger et al. (*submitted*) proposed the species extended its northern range during the early Pleistocene from multiple lineages, probably of north-eastern Siberian or Central Asian origin.

The observed differences between arctic-alpine distributed invertebrates concerning the recolonization pattern of Fennoscandia highlight the fact that species with similar present distribution patterns do not necessarily share the same evolutionary history. With this study we provide a next step in filling the knowledge gap regarding molecular studies of the arctic-alpine invertebrate fauna. Clearly, there is continued need to examine the phenomenon of arctic-alpine disjunctions and the multitude of arising patterns to better understand the processes and mechanisms of range expansion, regression, and lineage diversification in Europe’s high latitude and high altitude biota.

ACKNOWLEDGEMENTS

[redacted]

[redacted]

APPENDIX: SDM

For the present-day distribution modelling of *Arcynopteryx compacta* we used 171 data points of known occurrences across Eurasia, which arose from our own collections (N = 46; Table 2.1) and international databases (N = 125; see Table 2.6 below). Climate information for SDMs came from the WorldClim database (http://www.worldclim.org), which contains sets of various global climate layers. Bioclimatic variables are derived from monthly temperature and rainfall values in order to generate more biologically meaningful variables (Hijmans et al. 2005). Data were gridded in a spatial resolution of 2.5´ on an equal area grid. In many studies, the spatial resolution of modelled last glacial maximum (LGM) climates is typically on grid cells of 50 square km or greater (Waltari et al. 2007). We used a downscaled high-resolution estimate on 4 square km grids, providing a more detailed picture of LGM environments. We used only those climatic variables that were uncorrelated and most likely to influence the occurrence of *A. compacta* (BIO1 = Annual Mean Temperature; BIO12 = Annual Precipitation; and BIO15 = Precipitation Seasonality).

Past climate layers (LGM, 20 thousand ybp) were based on two general climate models, the Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC). These data are downscaled layers generated from the Paleoclimate Modelling Intercomparison Project Phase II (PMIP2; <http://pmip2.lsce.ipsl.fr/>http://pmip2.lsce.ipsl.fr/) (Braconnot et al. 2007). Downscaling used the projected change in a given weather variable, which was computed as the difference between the output of a given general climate model and WorldClim current climate (http://www.worldclim.org/downscaling). Distribution modelling was performed with Maxent 3.3.1 (Phillips et al. 2006), using default settings. Maxent utilizes a maximum entropy approach to make predictions from incomplete information. It estimates the most uniform distribution across the study area given the constraint that the expected value of each environmental predictor variable under this estimated distribution matches its empirical average (Phillips et al. 2006). Maxent outputs a continuous probability value, ranging from 0 to 100, an indicator of relative suitability for the species, based on the principle of maximum entropy, as constrained by the input occurrence data. We evaluated the overall model fit using receiver operating curves (ROC), which are threshold independent and include both commission and omission error, and a tenfold cross-validation as implemented in Maxent 3.3.1.

The average test AUC for the replicate runs of the tenfold cross-validation was 0.96 ± 0.015 S.D. The occurrence probability maps of current distribution (A, B) and modelled distribution during the LGM (C) based on the MIROC model are shown in Figure 2.1. An LGM distribution using the CCSM model produced similar results (data not shown). The current SDM overpredicts the distribution range mainly for the British Highlands, Massive Central, Jura, Vosges, Harz, Erzgebirge, Bohemian Forest, and

the Central Alps. There are no known records for these regions. Overpredictions leading to “false positives” are common in SDM, as these do not take biotic interactions or microhabitat conditions into account (e.g. Lobo et al. 2006). The LGM SDM predicts no occurrence within glaciated northern habitats, continuous appropriate climatic conditions along the glacial fringe in Central and Western Europe, and a patchy sky island distribution at high altitudes of more southern mountain ranges, i.e. Pyrenees, Massive Central, Western, Central, and Eastern Alps, Black Forest and Schwäbische Alb, Bohemian Massiv, High Tatras, and Western Balkan. Small patches of suitable habitat are also predicted for the South-western Carpathians (Apuseni, Retezat) and the Bulgarian Highlands.

Table 2.6 Reference data points (N = 125) used for species distribution modelling of Arcynopteryx compacta in addition to the sampled populations (s. Table 1). Geographic coordinates are given in decimal degrees. Country codes: AUT = Austria; BGR = Bulgaria; CH = Switzerland; ROU = Romania; RUS = Russia; SVK = Slovakia; SWE = Sweden.

Country	Latitude (ºN)	Longitude (ºE)	Source
AUT	46,733	15,010	Zobodat
AUT	46,940	15,150	Zobodat
AUT	46,917	15,117	Zobodat
AUT	46,860	14,670	Zobodat
BGR	42,883	24,717	Zobodat
BGR	42,117	23,667	Zobodat
BGR	41,833	23,483	Zobodat
BGR	42,300	23,700	Zobodat
BGR	41,733	23,150	Zobodat
BGR	41,733	23,150	GBIF
BGR	41,833	23,483	GBIF
BGR	42,117	23,667	GBIF
BGR	42,300	23,700	GBIF
BGR	42,883	24,720	GBIF
CH	46,600	6,500	GBIF
ROU	46,400	27,517	Zobodat

Table 2.6 continued

Country	Latitude (ºN)	Longitude (ºE)	Source
ROU	46,983	25,933	Zobodat
ROU	47,433	25,583	Zobodat
ROU	45,450	25,433	Zobodat
ROU	47,500	24,967	Zobodat
ROU	47,583	24,667	Zobodat
ROU	45,500	23,867	Zobodat
ROU	45,333	23,667	Zobodat
ROU	46,750	23,383	Zobodat
ROU	46,450	22,683	Zobodat
ROU	45,283	22,467	Zobodat
ROU	45,083	22,083	Zobodat
ROU	46,517	25,550	Zobodat
ROU	47,117	25,050	Zobodat
ROU	47,833	24,750	Zobodat
ROU	45,583	24,750	Zobodat
ROU	45,333	24,000	Zobodat
ROU	45,600	23,900	Zobodat
ROU	45,383	22,850	Zobodat
ROU	45,683	22,500	Zobodat
RUS	68,883	33,033	Zobodat
RUS	68,883	33,033	GBIF
RUS	66,686	34,489	GBIF
RUS	66,984	35,695	GBIF
RUS	66,838	35,959	GBIF
RUS	66,703	36,006	GBIF
RUS	66,533	36,209	GBIF
RUS	66,414	36,562	GBIF
RUS	64,356	58,279	GBIF
RUS	64,218	57,959	GBIF
RUS	65,508	59,796	GBIF
RUS	68,235	60,034	GBIF
RUS	66,399	62,827	GBIF
SVK	48,950	19,433	Zobodat
SVK	48,950	19,433	GBIF
SWE	66,667	19,733	Zobodat
SWE	68,267	18,967	Zobodat
SWE	68,417	18,167	Zobodat
SWE	67,900	17,933	Zobodat
SWE	66,950	17,783	Zobodat
SWE	67,667	17,267	Zobodat
SWE	67,300	16,683	Zobodat
SWE	67,300	16,683	Zobodat
SWE	67,217	16,583	Zobodat
SWE	67,200	16,417	Zobodat
SWE	65,150	16,033	Zobodat
SWE	65,850	15,433	Zobodat
SWE	65,850	15,433	Zobodat

Table 2.6 continued

Country	Latitude (ºN)	Longitude (ºE)	Source
SWE	66,050	14,883	Zobodat
SWE	65,517	14,867	Zobodat
SWE	65,483	14,633	Zobodat
SWE	62,333	13,067	Zobodat
SWE	62,333	13,067	Zobodat
SWE	62,767	12,633	Zobodat
SWE	62,933	12,617	Zobodat
SWE	62,967	12,567	Zobodat
SWE	62,867	12,530	Zobodat
SWE	62,983	12,367	Zobodat
SWE	63,033	12,217	Zobodat
SWE	68,417	19,350	Zobodat
SWE	68,367	19,100	Zobodat
SWE	68,370	19,010	Zobodat
SWE	66,000	19,000	Zobodat
SWE	68,450	18,950	Zobodat
SWE	68,333	18,850	Zobodat
SWE	68,330	18,850	Zobodat
SWE	68,350	18,780	Zobodat
SWE	66,330	18,380	Zobodat
SWE	68,367	18,200	Zobodat
SWE	67,300	16,750	Zobodat
SWE	66,950	16,500	Zobodat
SWE	62,933	12,550	Zobodat
SWE	62,933	12,550	Zobodat
SWE	62,917	12,450	Zobodat
SWE	63,283	12,350	Zobodat
SWE	63,217	12,350	Zobodat
SWE	62,030	13,078	GBIF
SWE	62,333	13,067	GBIF
SWE	62,767	12,633	GBIF
SWE	62,921	12,932	GBIF
SWE	62,867	12,533	GBIF
SWE	63,033	12,217	GBIF
SWE	62,898	12,156	GBIF
SWE	63,019	12,322	GBIF
SWE	64,022	13,589	GBIF
SWE	64,163	14,247	GBIF
SWE	64,304	14,137	GBIF
SWE	64,679	14,592	GBIF
SWE	64,863	14,719	GBIF
SWE	64,912	14,892	GBIF
SWE	65,067	14,667	GBIF
SWE	65,110	14,468	GBIF
SWE	64,985	14,208	GBIF
SWE	65,483	14,633	GBIF
SWE	65,517	14,867	GBIF

Table 2.6 continued

Country	Latitude (ºN)	Longitude (ºE)	Source
SWE	65,150	16,033	GBIF
SWE	65,758	15,271	GBIF
SWE	66,061	16,271	GBIF
SWE	66,479	15,574	GBIF
SWE	66,551	16,006	GBIF
SWE	66,950	16,500	GBIF
SWE	67,798	17,717	GBIF
SWE	67,876	19,202	GBIF
SWE	68,608	20,435	GBIF
SWE	68,626	20,919	GBIF
SWE	67,424	16,285	GBIF
SWE	67,200	16,417	GBIF
SWE	67,217	16,583	GBIF
SWE	68,417	19,350	GBIF

CHAPTER 3

Modelling range shifts and assessing genetic diversity distribution of the montane aquatic mayfly *Ameletus inopinatus* in Europe under climate change scenarios

ABSTRACT

Genetic diversity is one of the most important criteria to identify unique populations for conservation purposes. In this study we analyze the genetic population structure of the endangered montane mayfly *Ameletus inopinatus* in its European range. The species is restricted to unpolluted cold-water streams, and exhibits an insular distribution across highlands of Central Europe and a more continuous distribution across Fennoscandia and Northern Euro-Siberia. We genotyped 389 individuals from 31 populations for eight highly polymorphic microsatellite loci to investigate genetic diversity and population structure within and among European mountain ranges. Genetic diversity of *A. inopinatus* decreases along an east-west gradient in Central Europe and along a north-south gradient in Fennoscandia, respectively. Centres of exceptionally high genetic diversity are located in the Eastern Alps (Andertal Moor, Austria), the High Tatra, the Beskides, the Sudety Mountains and the Eastern German Highlands. Species distribution modelling for 2080 projects major regional habitat loss, particularly in Central Europe mountain ranges. By relating these range shifts to our population genetic results, we identify conservation units primarily in Eastern Europe, that if preserved would maintain high levels of the present-day genetic diversity and continue to provide long-term suitable habitat under future climate warming scenarios.

INTRODUCTION

Ecosystem diversity, species richness and genetic diversity represent the three fundamental levels of biodiversity (e.g. OTA 1987, Fiedler & Jain 1992, Hughes et al. 2008). The importance of preserving these levels of biodiversity has been widely acknowledged, and it has recently become technically feasible to consider intraspecific genetic diversity in conservation (e.g. McNeely et al. 1990, Taberlet 1998, Frankham et al. 2002). Preserving genetic variability is important to the overall health of populations, because decreased genetic variability may reduce fitness and limit the ability to adapt genetically to changing environments caused by e.g. recent global warming (Reed & Frankham 2003, Gienapp et al. 2008, Hoffmann & Willi 2008). Population genetic analyses can be used to identify management units based on ecological and genetic variation (Crandall et al. 2000) and to trace threatened populations in need of conservation priority (Frankham et al. 2002).

Concern over the implications of climate change for biodiversity has led to the use of bioclimatic models to forecast the range shifts of species under future climate conditions (Araújo & New 2006). Climatic data combined with species distribution models (SDM) provide an important tool to conservation biologists, since they can assist in determining high quality sites under present and future climate conditions, thus guiding additional surveys, and informing selection and management of protected areas (Graham et al. 2004). Apart from modelling terrestrial species, SDM can also be used for freshwater species with aquatic habitats (e.g. Cordellier & Pfenninger 2008). Freshwater invertebrates are surrogates of biodiversity in aquatic ecosystems, and represent most of the genetic variation at a site (Duelli 1997). Consequently, they play a key role in biomonitoring of aquatic ecosystems, not least because they are ubiquitous, diverse, and the sampling is more or less cost-efficient (Rosenberg & Resh 1993). Montane sky-island populations of benthic invertebrates

are especially threatened through global warming as such cold-adapted species may lose a large part of their range (Moussalli et al. 2009) and also a high amount of their present-day genetic diversity.

In this study, we predict the impact of climate change induced range shifts and habitat loss on the overall genetic diversity of the endangered mayfly *Ameletus inopinatus* Eaton 1887 (Malzacher et al. 1998). Like many montane aquatic insects, *A. inopinatus* is an indicator of unpolluted freshwater systems (Hendrey & Wright 1976). It is restricted to streams in mountain ranges of higher elevation (> 600 m asl) on the British Isles and in Central Europe; in Northern Eurasia it is known from Scandinavia to Western Siberia, where it occurs also at lower altitudes, and occasionally in lakes. It is considered a representative of the Eurasian, boreo-montane biome-type (Haybach 2003). We use species specific microsatellite markers to examine the fine-scale population structure and to identify genetic hotspots for *A. inopinatus* across its European range. We then incorporate our population genetic results and the SDM to estimate the future range of *A. inopinatus* to analyze how these genetic hotspots should be considered for conservation. In particular, we focus on i) how is the current genetic diversity distributed across Europe and ii) which populations may become extinct due to future climate change and iii) what consequences these extinctions may have for the overall genetic diversity and conservation of this rare mayfly species.

MATERIALS AND METHODS

Sample collection

We collected 389 specimens of *Ameletus inopinatus* from 31 localities within its European distribution range (Haybach 2003) from May to July in 2007 and 2008 (Figure 3.1; Table 3.1) using hand nets. The larvae were found in marsh lands, slow

running creeks, grassy lake shores and also in slow running branches of torrential rivers. We identified specimens under a field microscope to ensure correct identification. There is also evidence that this species occurs in the Pirin Mountains (Bulgaria; Russev & Vidinova 1994), the Apuseni Mountains (Romania; Biró I, pers. comm.) and the Harz (Germany; Hohmann M, pers. comm.), but unfortunately we were unable to collect individuals from these areas. We preserved specimens in 70–96 % EtOH, and kept them cool (4º C) until DNA extraction. All specimens are vouchered and deposited in the collections of the Senckenberg Natural History Museum, Frankfurt (SMF-No. 128–180).

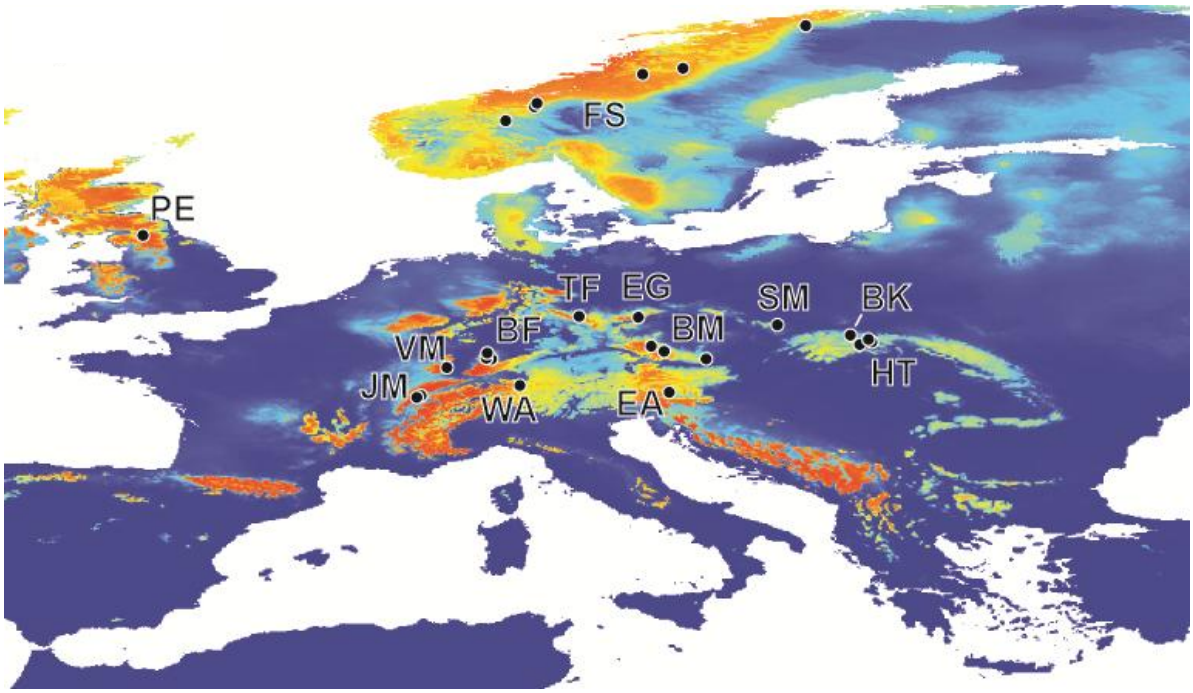


Figure 3.1 Map of sample sites and current species distribution model for *Ameletus inopinatus* in Europe. Labels indicate sampled mountain ranges (west to east): PE, Pennines; JM, Jura Mountains; VM, Vosges Mountains; BF, Black Forest; FS, Fennoscandia; WA, West Alps; TF, Thuringian Forest; EG, Erzgebirge; BM, Bohemian Massif; EA, East Alps; SM, Sudety Mountains; BK, Beskides; HT, High Tatra. Warmer colours show better predicted conditions. Best suitable conditions (from west to east) are predicted for the British Isles (Scottish Highlands and Northern England), the Pyrenees, Massive Central, VM, JM, WA, Central Alps, EA, German Highlands (BF, TF, EG, Harz, Rhön, BM), the Balkan Peninsula and FS. Moderate conditions (yellow-green) are predicted for the SM, BK, HT and the Carpathians.

Table 3.1 Material of *Ameletus inopinatus* used for population genetics (N = 389) and presence localities used for species distribution modelling (N = 31). Abbreviations of mountain ranges refer to codes used in Fig. 1a. Population codes refer to sampled localities. *N* = number of sampled individuals. Geographic coordinates are given in decimal degrees. Proximity to European Natura 2000 conservation areas are estimated based on data provided by Environment Directorate-General of the EU Commission (2010).

Mountain range	Pop.-code	*N*	Locality	Latitude (ºN)	Longitude (ºE)	Altitude (m)	Collector	Proximity to Natura 2000 conservation areas
Fennoscandia (FS)	FS_1	20	NO, Jotunheimen, Ovre Heimdalen	61.45	8.84	1300	Brittain	
	FS_2	3	NO, Jotunheimen, Valdresflya Moor	61.38	8.87	1400	Theissinger & Theobald	
	FS_3	20	NO, Dovrefjell, Vinstradalen	62.42	9.74	1272	Theissinger & Theobald	
	FS_4	12	NO, Dovrefjell, Orkelsjöhytta, river Almdalen	62.54	9.79	1199	Theissinger & Theobald	
	FS_5	6	SE, Skorovatn, Skorovass gruver	64.63	13.11	650	Theissinger & Theobald	
	FS_6	19	SE, Burgfjellet, Gellvernokko Mt.	65.06	14.37	868	Theissinger & Theobald	
	FS_7	4	SE, Abisko, Vassijaure	68.43	18.19	473	Theissinger & Theobald	
Pennines (PE)	PE	11	GB, Pennines Mountains, Appleby in Westmoreland	54.67	-2.45	628	Taubmann & Theissinger	within
Erzgebirge (EG)	EG_1	6	DE, Pöhlbach, Pöhlbach	50.41	12.96	915	Voigt	within
	EG_2	5	DE, Sehma	50.46	12.97	800	Voigt	< 5 km
	EG_3	6	DE, Zschopau, Zschopau	50.45	12.94	900	Voigt	< 5 km
Thuringian Forest (TF)	TF	20	DE, NSG Göritzgrund, Steinheid, Göritz	50.47	11.12	775	Taubmann & Theissinger	within
Black Forest (BF)	BF_1	3	DE, Bühlertal, Sandsee /Schwarzenbach	48.66	8.25	802	Taubmann & Theissinger	< 1 km
	BF_2	5	DE, Freudenstadt, Kniebis, Forbach	48.47	8.32	862	Taubmann & Theissinger	< 1 km
	BF_3	19	DE, Bulbach, Schliffkopf, Rechtmurg	48.52	8.23	864	Taubmann & Theissinger	within
	BF_4	5	DE, Kinzigtalstausee, Freudenstadt, Kleine Kinzig	48.43	8.37	717	Taubmann & Theissinger	< 1 km
Vosges Mountains(VM)	VM_1	5	FR, La Bresse, Route des Americains	48.01	6.96	1012	Taubmann & Theissinger	within
	VM_2	20	FR, La Bresse, Rue de Crete	48.03	7.00	1200	Taubmann & Theissinger	within
Jura Mountains (JM)	JM_1	20	FR, Foncine le haut, Source de la Saine	46.66	6.07	910	Wagner	< 5 km

Table 3.1 continued

Mountain range	Pop.-code	*N*	Locality	Latitude (ºN)	Longitude (ºE)	Altitude (m)	Collector	Proximity to Natura 2000 conservation areas
	JM_2	11	FR, Doubs, Mouthe, magasin ATAC	46.71	6.20	930	Wagner	< 1 km
West Alps (WA)	WA	4	CH, Schweiz, Alt St, Johann, Thur	47.19	9.29	890	Wagner	
Bohemian Massif (BM)	BM_1	7	DE, Buchenau/Frauenau, Hirschbach	49.04	13.37	1105	Taubmann & Theissinger	within
	BM_2	20	DE, Buchenau/Frauenau, outlet Latschensee	49.04	13.38	1079	Taubmann & Theissinger	within
	BM_3	10	DE, Schimmelbach	48.76	13.77	752	Taubmann & Theissinger	< 5 km
	BM_4	8	AT, Weinsberger Wald, Weidenegg, Höllbach	48.42	15.09	883	Taubmann	< 5 km
East Alps (EA)	EA	20	AT, Andertal Moor, St. Lorenzen	46.86	13.93	1550	Theissinger , Pauls & Graf	witin
High Tatra (HT)	HT_1	20	SK, Podbanske, Bela River	49.16	19.92	1095	Taubmann	within
	HT_2	20	SK, Zdiar, Tokarensky potok	49.26	20.27	1052	Taubmann	within
	HT_3	20	SK, Javorina, Javorinka River	49.26	20.15	1206	Taubmann	within
Beskides (BK)	BK	20	PL, Nowy Targ, Jasiruowka	49.55	19.55	919	Taubmann	within
Sudety Mountains (SM)	SM	20	CZ, Bruntal, Karlova Studanka, Bila opava	50.08	17.30	864	Taubmann	within
total	31	389						

Laboratory procedures and allele scoring

We extracted genomic DNA from the abdomen using DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturers' protocol with minor modifications: prior to final elution, spin columns were incubated for five minutes at 56 ºC to remove residual ethanol; final elution volume was 100 µL H_2O; spin columns were incubated for five minutes at 56 ºC prior to the final spin. We determined DNA concentration using the NanoDrop® (PEQLAB, Erlangen, Germany), and prepared working solutions (50 ng/µL).

We used nine species-specific primer pairs (Theissinger et al. 2008) to genotype all sampled individuals. We used a high throughput automatic pipetting robot (MWG, Ebersberg Germany) to guarantee highly standardized amplification procedure with minimized potential human error. PCR amplification was performed using PuRe*Taq* Ready-To-Go PCR beads (GE Healthcare, Chalfont St. Giles, Great Britain) using a reaction volume of 8 µL. We repeated samples that did not amplify directly up to two times. Thermal cycling parameters followed Theissinger et al. (2008). Samples were scored on an ABI 3130 capillary sequencer using 11.7 µL HiDi-formamide, 0.3 µL ROX 500 standard (Applied Biosystems, Carlsbad USA) and 1 µL of the PCR product. Loci were genotyped using GENEMAPPER 4.0 (Applied Biosystems). All loci were analyzed for possible null allele issues using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). We additionally estimated the genotyping error rate by re-amplifying 40 individuals (10 %) across all loci (Bonin et al. (2004). The error rate was 0.6%, and should not bias our results for localizing diversity centres.

Genetic diversity

Allele frequencies and private alleles were calculated using CONVERT (Glaubitz 2004). Correlation between sample size and number of alleles was examined in JMP 8.0 (SAS 2007). The number of alleles (N_A) was calculated with HP-RARE 1.0 (Kalinowski 2004). To produce an unbiased estimate of allele diversity, the allelic richness (A) and private allelic richness (A_p) was calculated with the same program. This approach uses the frequency of alleles at a locus to estimate the number of alleles which would occur in smaller samples of individuals (Petit et al. 1998, Leberg 2002). Allelic richness and allelic richness of private alleles was estimated over all loci per population (Table 4), based on the minimal sample size of three diploid individuals. A *t*-test was performed in JMP to test for significant differences in mean number of alleles and in levels of allelic richness between populations. Expected (H_E) and observed (H_O) heterozygosity and departure from Hardy-Weinberg equilibrium (HWE) were calculated at each locus and over all loci for each population using default parameters in GENEPOP 3.1 (Raymond & Rousset 1995). Linkage disequilibrium between pairs of loci was tested in the web-based version of GENEPOP using default parameters and applying a Bonferroni correction for multiple comparisons (Rice 1989).

Population differentiation

Fisher's exact tests of population differentiation were run in GENEPOP applying Bonferroni correction to determine the heterogeneity in allele and genotype distributions between pairs of populations. The relationship between all populations based on microsatellite variation was estimated by creating a dendrogram of relationships in the program PHYLIP 3.68 (Felsenstein 2008), using the UPGMA method (unweighted pair group method with arithmetic mean) because of no existing

outgroup. This method uses the Nei's D_A genetic distance (Nei *et al.* 1983), which is more appropriate for estimating evolutionary events than other genetic distances because of a higher P_C value (probability of correct topology), even with microsatellite DNA (Takezaki & Nei 1996). To estimate branch support, bootstrap values were computed by resampling loci over 1000 replications.

We used the GENELAND2.0.0 software (Guillot et al. 2005) implemented in R 2.4.1 (Ihaka & Gentleman 1996) to test for hierarchical structure within our sampling. This Bayesian clustering program uses both geographic and genetic information from the data and takes into account that genetically differentiated populations tend to be structured in spatially distinct areas. As recommended by Guillot et al. (2005) we first inferred the number of clusters with varying *K*, and in a second step we ran the analyses again with *K* fixed to the previously inferred value in order to estimate the assignment of individuals to the inferred populations. To verify the consistency of results we repeated each run five times, with $K_{min} = 1$ and $K_{max} = 20$, 100000 MCMC iterations and saved every 100^{th} iteration. The uncertainty of coordinates was fixed to 1 km. This allowed samples with the same coordinates to be assigned to different populations, which can be useful to detect migrants (Guillot et al. 2005). We then inferred the modal *K* of these five runs, and ran the MCMC ten times with *K* fixed to this number, with parameter settings same as above. We calculated the mean logarithm of the posterior probability for each of the ten runs and the posterior probability of population membership for each individual was then computed for the run with the highest value.

An analysis of molecular variance (AMOVA, Excoffier et al. 1992) was performed in the program ARLEQUIN 3.11 (Excoffier et al. 2005) to estimate the percentage of genetic variation on different hierarchical geographic levels: among the 13 sampled mountain ranges, among populations within mountain ranges and within

populations (N = 31). 1000 permutations were run to test for statistically significant fixation indices. The level of genetic differentiation among populations was estimated in ARLEQUIN 3.11 using pairwise R_{ST} (Slatkin 1995). The significance of multiple-pairwise comparisons was determined using default parameters in the permutation tests.

SDM

To explore how climate change may affect the range of *A. inopinatus*, we used species distribution modelling including 88 data points of known occurrences in Eurasia (Tables 3.1 and 3.2) to analyze both current and possible future distributions under two climate scenarios. Climatic data for models were taken from WorldClim (http://www.worldclim.org/), which is a database with sets of various global climate layers. Bioclimatic variables are derived from the monthly temperature and rainfall values in order to generate biologically meaningful variables (Hijmans et al. 2005). We used only those climatic three variables that were uncorrelated and most likely to influence the occurrence of *A. inopinatus*. (BIO1 = Annual Mean Temperature; BIO12 = Annual Precipitation; and BIO15 = Precipitation Seasonality).

Table 3.2 Reference data points (N = 57) used for species distribution modelling of *Ameletus inopinauts* in addition to the sampled populations (s. Table 3.1). Geographic coordinates are given in decimal degrees.

Country	Latitude (ºN)	Longitude (ºE)	Altitude (m)	Source
GB	54.22	-2.33	300	RIVPACS (Wright et al. 2000)
GB	54.35	-1.24	227	RIVPACS (Wright et al. 2000)
GB	54.13	-2.15	303	RIVPACS (Wright et al. 2000)
GB	54.67	-2.45	590	RIVPACS (Wright et al. 2000)
GB	54.72	-2.38	518	RIVPACS (Wright et al. 2000)
GB	56.33	-4.53	135	RIVPACS (Wright et al. 2000)
GB	57.43	-3.36	135	RIVPACS (Wright et al. 2000)
GB	57.48	-5.33	45	RIVPACS (Wright et al. 2000)
GB	57.53	-5.15	170	RIVPACS (Wright et al. 2000)
GB	58.15	-4.98	70	RIVPACS (Wright et al. 2000)

Table 3.2 continued

Country	Latitude (ºN)	Longitude (ºE)	Altitude (m)	Source
GB	58.41	-3.88	70	RIVPACS (Wright et al. 2000)
GB	57.55	-5.13	170	RIVPACS (Wright et al. 2000)
GB	58.15	-4.24	246	RIVPACS (Wright et al. 2000)
GB	58.12	-4.13	120	RIVPACS (Wright et al. 2000)
GB	56.38	-4.64	176	RIVPACS (Wright et al. 2000)
GB	57.15	-4.43	327	RIVPACS (Wright et al. 2000)
GB	56.88	-5.52	45	RIVPACS (Wright et al. 2000)
GB	58.12	-4.96	149	RIVPACS (Wright et al. 2000)
DE	48.72	8.46	642	Böhmer et al. 2004
DE	48.10	8.16	703	Böhmer et al. 2004
DE	48.62	8.44	655	Böhmer et al. 2004
DE	49.01	13.40	684	Böhmer et al. 2004
DE	47.78	8.21	735	Böhmer et al. 2004
DE	48.98	13.40	705	Böhmer et al. 2004
DE	48.93	13.39	668	Böhmer et al. 2004
DE	49.77	12.42	541	Böhmer et al. 2004
DE	47.92	7.92	677	Böhmer et al. 2004
DE	50.65	10.76	839	Brettfeld
RO	46.00	25.00	669	European Environment Agency
SK	48.00	18.00	200	European Environment Agency
SE	62.87	17.38	221	GBIF-Sweden
SE	63.88	20.07	50	GBIF-Sweden
SE	64.18	17.27	283	GBIF-Sweden
SE	64.42	16.30	355	GBIF-Sweden
SE	65.61	19.08	363	GBIF-Sweden
SE	66.479	17.66	564	GBIF-Sweden
SE	67.68	17.62	586	GBIF-Sweden
SE	67.86	19.44	400	GBIF-Sweden
SE	67.61	23.47	218	GBIF-Sweden
SE	68.38	18.12	396	GBIF-Sweden
SE	64.34	16.45	355	GBIF-Sweden
SE	65.22	17.05	459	GBIF-Sweden
SE	63.68	19.22	154	GBIF-Sweden
SE	64.00	19.06	298	GBIF-Sweden
MN	50.69	100.25	1650	Soldán et al. 2009
MN	50.96	100.75	1646	Soldán et al. 2009
MN	51.63	100.53	1724	Soldán et al. 2009
MN	51.57	100.48	1721	Soldán et al. 2009
MN	50.60	100.48	1620	Soldán et al. 2009
MN	51.00	100.71	1655	Soldán et al. 2009
MN	50.95	100.75	1640	Soldán et al. 2009
MN	50.92	100.25	1577	Soldán et al. 2009
MN	50.99	100.54	1785	Soldán et al. 2009
MN	51.61	100.60	1854	Soldán et al. 2009
MN	51.65	100.53	2088	Soldán et al. 2009
MN	50.96	100.54	1661	Soldán et al. 2009
MN	50.76	100.23	1970	Soldán et al. 2009

For our future modelling predictions we used the WorldClim data from two different emission scenarios, A2a and B2a (SRES – Special Report of Emission Scenarios; Alcamo et al. 1995). The A2 scenario describes a world with unreduced CO_2 emission and rapid global warming, henceforth the “business as usual” scenario. The B2 scenario describes a world with lower CO_2 emission and presumably slower climate change, henceforth the “reduced CO_2 emission” scenario. We used data from the CCCMA (Canadian Centre for Climate Modelling and Analysis) climate model of the A2a and B2a scenarios for the year 2080. Distribution models were generated in MAXENT 3.3.0 (Phillips et al. 2006), using default settings, to create present and future distribution maps. To assess model quality we performed a tenfold cross-validation and report the average test AUC (Area Under the receiver operating characteristic Curve) for the replicate runs.

RESULTS

We genotyped 389 specimens from 31 sample sites and 13 European mountain regions for nine microsatellite loci (Figure 3.1, Table 3.1). All loci were highly variable across all populations (N = 31), exhibiting 248 unique alleles across all loci. No sign of linkage disequilibrium was found in any of the populations ($P > 0.1$). Locus Ami_191 displayed frequent missing data (N = 30 of 389), homozygote excess in some populations (N = 9 of 31), and moderate to high null allele frequencies ($p = 0.33$) and was excluded from further analyses. Locus Ami_62 displayed an equally high null allele frequency ($p = 0.47$; Table 3.3, but only few missing data over all populations (N = 5 of 389) and departure from HWE in a single population (EA) only. We therefore did not exclude locus Ami_62.

Table 3.3 Summary table of microsatellite loci for *Ameletus inopinatus*. Given are number of alleles per locus (N_A), allele size range in bp (R), and null allele frequencies (p) across the entire data set (N = 389). ***** null allele sign due to one population (EA).

Locus	N_A	R	P
Ami_32	34	109 – 231	0.021
Ami_62	49	165 – 263	0.468*
Ami_188	30	193 – 257	0
Ami_67	24	86 – 182	0.066
Ami_109	24	116 – 170	0
Ami_95	40	235 – 321	0
Ami_185	24	183 – 265	0.065
Ami_202	23	165 – 234	0.050
Mean	31	-	-

Genetic diversity and HWE

Significant correlations between sample size and number of alleles were found across all populations (R^2 = 0.507, P < 0.0001). When excluding populations with less than eight individuals, the correlation was marginally significant (R^2 = 0.040, P = 0.06). Allele numbers (N_A) ranged from 23 to 49 with an average of 31 per locus (Table 3). The highest number of alleles over all loci was observed in populations of the HT, SM and BK (N_A = 85-99), in the BM (N_A = 54-98) and in the EA population (N_A = 91) (Table 4). Mean number of alleles across all loci was significantly lower for FS populations than for the remaining Central European populations (*t*-test: P < 0.001). Private alleles were observed in 21 of 31 populations (Table 3.4). The highest numbers of private alleles (N_P) across all loci were recorded for the EA population (N_P = 10) and for the BM_2 population (N_P = 4). Allelic richness and allelic richness of private alleles was the highest in the populations BM_1 (A = 2.58) and EA (A_p = 0.74), respectively. Allelic richness was significantly lower for FS populations than for all Central European populations (*t*-test: P < 0.01). The correlation between allelic richness and longitude was significant for Central European populations (R^2 = 0.472,

$P < 0.01$); the correlation between allelic richness and latitude was significant for FS populations ($R^2 = 0.875$, $P < 0.05$).

Expected (H_E) and observed (H_O) heterozygosity in the 31 populations ranged from 0.553 to 0.845 and from 0.531 to 0.850, respectively (Table 3.4). The FS_1 population had the lowest observed heterozygosity ($H_E = 0.553$) and BM_1 had the highest value ($H_E = 0.845$). Five of 31 populations showed significant deviation from HWE due to individual loci (Table 4). Also five of 13 mountain ranges showed deviations from HWE (Table 4).

Table 3.4 Summary statistics for 31 populations (Pops) and 13 hierarchical groups (Mountain ranges) of *Ameletus inopinatus*. Given are populations sample size (N), number of alleles across all loci (N_A), private alleles across all loci (N_P), allelic richness across all loci (A; based on minimal sample size of three individuals), private allelic richness (A_p; based on three individuals), expected (H_E) and observed (H_O) heterozygosities, and conformance to Hardy-Weinberg expectations ("+" means P > 0.05). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, deviation from HWE after sequential Bonferroni corrections. Mountain ranges*: deviations from HWE of single mountain ranges due to local subpopulation differentiation.

Mountain ranges	Pops	*N*	N_A	N_P	*A*	A_p	H_E	H_O	HWE
FS*		84					0.653	0.601	- ***
	FS_1	20	51	1	1.97	0.09	0.553	0.544	+
	FS_2	3	27	1	2.06	0.11	0.608	0.538	+
	FS_3	20	64	1	2.15	0.09	0.652	0.656	+
	FS_4	12	49	1	2.05	0.11	0.610	0.531	- *
	FS_5	6	41	-	2.22	0.21	0.676	0.667	+
	FS_6	19	69	3	2.17	0.23	0.655	0.599	- *
	FS_7	4	35	-	2.29	0.26	0.668	0.750	+
PE	PE	11	57	1	2.28	0.20	0.720	0.693	+
EG		17					0.817	0.772	+
	EG_1	6	51	1	2.52	0.20	0.822	0.771	+
	EG_2	5	40	-	2.43	0.20	0.789	0.750	+
	EG_3	6	49	1	2.51	0.21	0.820	0.792	+
TF	TF	20	74	2	2.43	0.26	0.784	0.744	+
BF*		32					0.711	0.641	- ***
	BF_1	3	23	-	2.15	0.02	0.658	0.626	+
	BF_2	5	32	-	2.29	0.22	0.703	0.700	+

Table 3.4 continued

Mountain ranges	Pops	*N*	N_A	N_P	*A*	A_p	H_E	H_O	HWE
	BF_3	19	69	1	2.27	0.10	0.694	0.632	+
	BF_4	5	36	-	2.29	0.08	0.708	0.633	- *
VM		25					0.661	0.655	+
	VM_1	5	32	-	2.15	0.14	0.653	0.725	+
	VM_2	20	64	1	2.18	0.12	0.662	0.638	+
JM		31					0.735	0.707	+
	JM_1	20	71	2	2.32	0.24	0.727	0.710	+
	JM_2	11	58	3	2.35	0.25	0.743	0.703	+
WA	WA	4	40	-	2.52	0.43	0.826	0.813	+
BM*		45					0.840	0.792	- ***
	BM_1	7	61	3	2.58	0.31	0.845	0.803	+
	BM_2	20	98	4	2.49	0.24	0.808	0.781	+
	BM_3	10	69	2	2.53	0.38	0.825	0.850	+
	BM_4	8	54	-	2.45	0.18	0.796	0.734	+
EA	EA	20	91	10	2.53	0.74	0.827	0.816	- ***
HT*		60					0.792	0.732	- ***
	HT_1	20	92	2	2.45	0.28	0.793	0.706	- ***
	HT_2	20	92	3	2.47	0.28	0.791	0.741	+
	HT_3	20	99	1	2.41	0.27	0.769	0.750	+
BK	BK	20	85	1	2.5	0.24	0.810	0.744	+
SM	SM	20	86	-	2.51	0.24	0.812	0.769	+

Various factors can cause deviations from HWE, such as inbreeding, the presence of null alleles or the Wahlund effect (Chakraborty et al. 1992, Freeland 2005). Inbreeding should affect all loci equally, and consequently lead to consistent heterozygote deficits over all loci (Daking & Avise 2004), while in this study only individual loci in few populations were affected. Deviations from the HWE due to null alleles could be estimated in one particular locus (Ami_191), which was excluded from further analyses. High levels of population differentiation within a sample or group of samples may result in a Wahlund effect. In aquatic insects population differentiation can be high both among and within reaches of the same stream, e.g. if larval drift and movement is restricted (Bunn & Hughes 1997). In this study all

samples were collected from discrete stream sites and each site was treated as an individual population. However, our results suggest that some of the 31 populations exhibit local subpopulation structure causing a Wahlund effect. This is especially obvious when sites were pooled, and HWE was calculated for mountain ranges (Table 3.4). In this species, local structuring could be the result of asynchronous generations within sites.

Population differentiation

The null hypothesis of equal allelic distribution between population pairs (N = 31) was rejected in 444 of 465 possible tests, and in 443 of 465 tests for equal genotypic distribution (Fisher's exact tests, $P < 0.05$). The UPGMA tree based on microsatellites described a distinct separation of FS and Central European populations (bs = 91.3 and 90.1, Figure 3.2). The PE population clustered within the Central European clade, which remained largely unresolved.

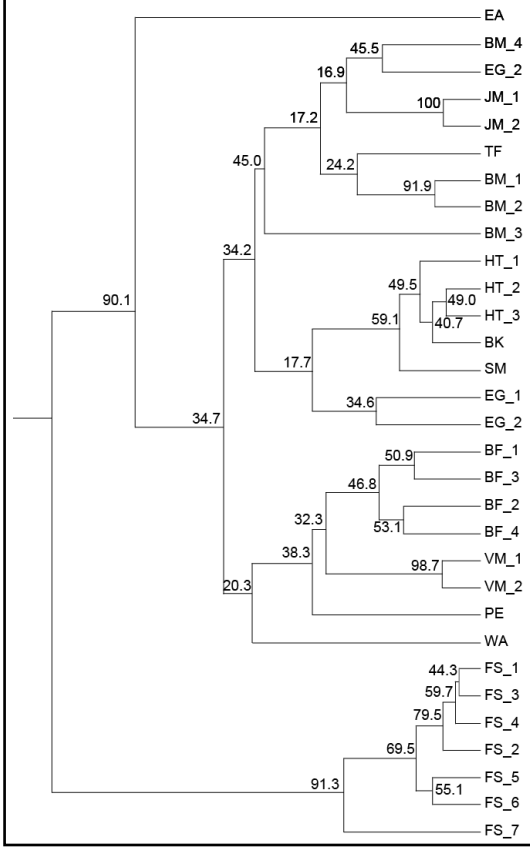


Figure 3.2
UPGMA (unweighted pair group method with arithmetic mean) dendrogram of the genetic relationships among 31 *Ameletus inopinatus* populations in Europe inferred from a matrix based on the Nei *et al.* (1983) *D*A distance. The dendrogram describes a distinct separation of FS and Central European populations (bs = 91.3 and 90.1). Bootstrap values were computed by resampling eight microsatellite loci over 1000 replications. For abbreviations of populations and their location see Fig. 1 and Table 3.1, respectively.

GENELAND grouping of individuals revealed a modal *K* of 13 for the first five runs. The number of inferred clusters was the same in all ten runs performed with *K* fixed to 13. These 13 groups of populations within our sampling also correspond to the sampled mountain ranges and we therefore defined 13 hierarchical geographic groups: the High Tatra (HT), the Sudety Mountains (SM), the Beskides (BK) the Bohemian Massif (BM), all Fennoscandian populations (FS), the Eastern Alps (EA), the Thuringian Forest (TF), the Black Forest (BF), the Vosges Mountains (VM), the Erzgebirge (EG), the British Pennines (PE), the Western Alps (WA) and the Jura Mountains (JM) (Figure 3.1, Table 3.1).

The results of the analysis of molecular variance (AMOVA) revealed that most genetic variances was observed within 31 populations (F_{ST}: 83.87, R_{ST}: 82.94), followed by variance among 13 mountain ranges (F_{ST}: 13.61, R_{ST}: 13.13), and finally variance among populations within mountain ranges (F_{ST}: 2.53, R_{ST}: 3.93). Genetic differentiation was significant among mountain ranges, among populations within mountain ranges and among populations for both F_{ST} and R_{ST} estimates (Table 3.5). Multilocus estimates of R_{ST} revealed significant differentiation between 136 of 156 pairs of mountain ranges (N = 13). R_{ST} estimates ranged from 0 to 0.47 with an average of 0.15 ± 0.1 (data not shown). The EA population showed high differentiation from all other populations; R_{ST} ranged from 0.20 (HT and EG, $P < 0.0001$) to 0.47 (FS, $P < 0.0001$) with an average of 0.27 ± 0.08. Noticeable was also the significant differentiation between the BF and the VM populations ($R_{ST} = 0.07$, $P < 0.0001$) with a mean geographical distance of 110 km. By comparison, the R_{ST} between the HT and the SM populations is not significant ($R_{ST} = 0.01$, $P = 0.16$) despite a mean geographical distance of 220 km.

Table 3.5 Genetic differentiation estimated with analysis of molecular variance (AMOVA) for *Ameletus inopinatus*. Shown are fixation indices and significance (*** $P < 0.0001$) based on 1000 random permutations.

Level of Differentiation	*F* Statistics	*R* Statistics
mountain ranges	$F_{CT} = 0.136^{***}$	$R_{CT} = 0.131^{***}$
populations within mountain ranges	$F_{SC} = 0.029^{***}$	$R_{SC} = 0.045^{***}$
populations (31)	$F_{ST} = 0.161^{***}$	$R_{ST} = 0.171^{***}$

SDM

The average test AUC for the replicate runs of the tenfold cross-validation was 0.925 ± 0.038. The maps in Fig. 1 show the MAXENT 3.3.0 predictions of current (Figure 3.1) and future (year 2080; Figure 3.3) climate niche distribution of *A. inopinatus*. The current distribution model (Figure 3.1) shows the most suitable conditions in the British Isles (Scottish Highlands and Northern England), the Pyrenees, Massif Central, VM, JM, WA, EA, German Highlands (BF, TF, EG, Harz, Rhön, BM), the Balkan Peninsula and FS. Moderate climatic suitability is attributed to the SM, BK, HT and the Carpathians. The Pyrenees, Massif Central and Rhön are identified as "false positive", as they show highly suitable conditions in the model, nevertheless the species is not known in these mountain ranges. In the future distribution model with the "business as usual" scenario (A2a; Figure 3.3) the best-suited climatic conditions are restricted to the Alps, parts of the Scottish Highlands and FS. However, highly reduced suitable microhabitats with varying magnitude remain for the Central and Eastern European mountain ranges: VM, JM, German Highlands (BF, TF, EG, Harz, BM), SM, BK, HT, Balkan Peninsula and the Carpathians. The future distribution model with the "reduced CO2 emission" scenario (B2a) predicted almost identical habitat loss as the A2a model.

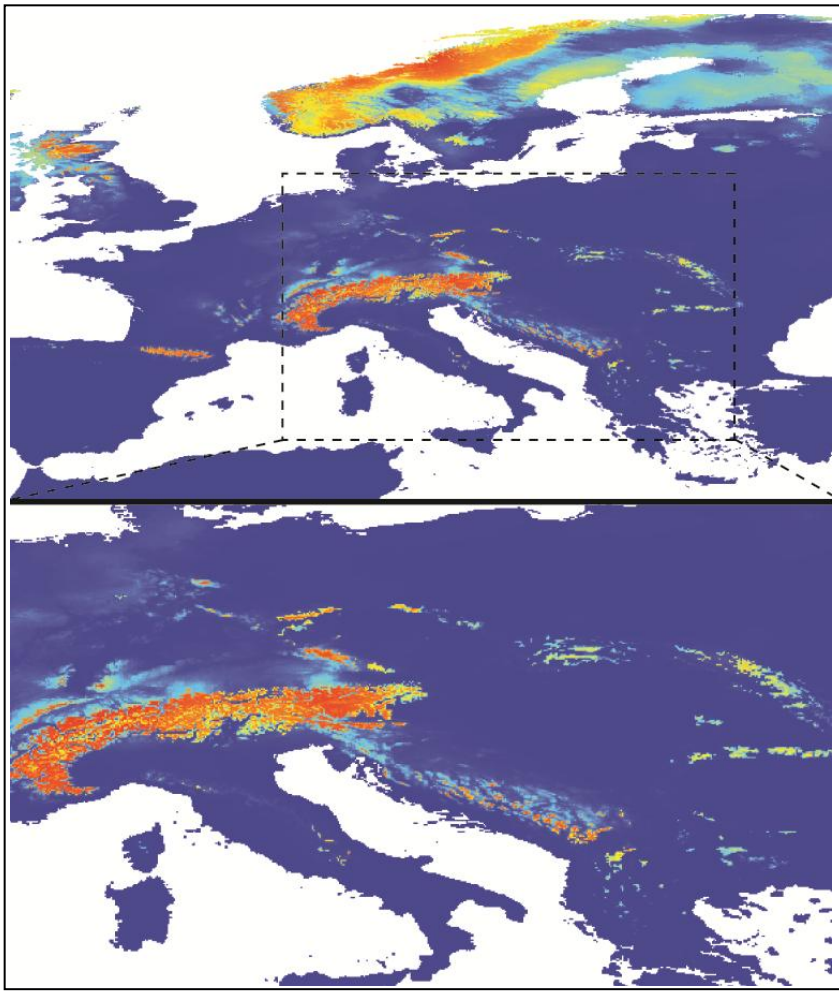


Figure 3.3
Future distribution model (scenario A2a; year 2080) for *Ameletus inopinatus* in Europe. Predicted main suitable habitats are the Alps, the Pyrenees, parts of the Scottish Highlands and FS. Reduced suitable microhabitats with varying magnitude remain for the Central European mountain ranges VM, JM, German Highlands (BF, TF, EG, Harz, BM) Balkan Peninsula, SM, BK, HT, Balkan Peninsula and the Carpathians. c) Zoom to Central Europe for the future distribution model of *A. inopinatus* (A2a; 2080)

DISCUSSION

Genetic distribution and genetic hotspots

Compared to other genetic surveys of aquatic insects (Beebee 2007, Wilcock et al. 2007) our study revealed high levels of genetic diversity across all Central European populations of the mayfly *A. inopinatus*. The general loss of alleles in FS compared to Central European populations may indicate a founder effect of the more northerly populations (Hewitt 1996, Freeland 2005). However, the FS populations belong to a separate phylogeographic lineage, which may also explain the general lower genetic diversity. In Central Europe genetic diversity decreases along an east-west gradient and is highest in the EA, BM, EG, SM, BK and HT populations. Private alleles were observed in 61 % of all European populations. In contrast, allelic richness of private

alleles revealed 96 % of all European populations have private alleles, after correcting for the sample size with the rarefaction method. The high degree of differentiation between EA and other populations is due to the preponderance of private alleles at the EA site. This could indicate that the population has been isolated for a long time and dispersal is restricted. In addition, allelic richness and expected heterozygosity were high compared to the other populations, suggesting a genetic hotspot and a large effective population size at EA. In this study we use the term ´genetic hotspot` to describe regions harbouring high genetic diversity and/or a specific allelic composition. We identified such genetic hotspots across the mayfly's Eastern European range (EA, BM, EG, SM, BK and HT) as well as in its Fennoscandian range.

Strong genetic differentiation among mountain ranges was prevalent in our study. This pattern in montane insects is common due to low gene flow rates between populations even in species capable of active flight (Jackson & Resh 1992, Pauls et al. 2006). Besides dispersal capabilities, geographical structures can act as dispersal barriers and lead to high genetic differentiation. *A. inopinatus* shows significant genetic differentiation between the BF and the VM across a moderate distance (110 km). The flood plain of the Rhine River is located between these populations and provides an unsuitable landscape for *A. inopinatus.* Hence, any gene flow between the concerned populations is based on rare long-distance dispersal events. The flood plain of the Rhine River seems to present a geographic dispersal barrier for *A. inopinatus*. In contrast, genetic differentiation between the SM and HT is not significant across a greater distance (220 km). In contrast to BF and VM, SM and HT are linked by a more or less continuous highland region in the Northwestern Carpathian Ranges. Lacking genetic differentiation across a great distance within highland areas suggests that short-distance (and maybe even long-

distance) dispersal is more likely to occur along mountain ranges, where suitable habitat is more widely available than in low gradient, low elevation areas. This is in line with current knowledge of the species habitat preferences (Buffagni et al. 2009). Due to limited gene flow among the inhabiting mountain ranges, we treated all mountain ranges as separate units for conservation purposes.

SDM and conservation implications

Recent and past climate changes have produced shifts in the distribution of many species (Parmesan & Yohe 2003). The anthropogenic influence on Earth's climate raises the possibility of rapid species extinction due to habitat loss in the near future (Houghton 2001, Thomas et al. 2004). Montane sky-island species like *A. inopinatus* are affected by climate change in particular, as their habitats are extremely isolated and are therefore more prone to local extinction under warmer and/or drier climates (Hering et al. 2009, Moussalli et al. 2009). By combining SDM and genetic data, we were able to identify regions and populations that should receive priority when developing conservation strategies for *A. inopinatus* in light of projected climate change. We refrain from explicitly determining management units sensu Crandall et al. (2000) for this species, because we lack specific data on correlations between genetic diversity and ecological plasticity or habitat specialisation. Instead, we identify conservation units, i.e. regions that harbour genetic hotspots and will provide suitable climate and habitat conditions under both climate change scenarios based on our SDM.

SDM for this mayfly predicts a loss of habitat due to climate change under both the "business as usual" (A2a; Figure 3.3) and "reduced CO2 emission" (B2a) scenarios primarily in the Central European mountain ranges and the Balkan Peninsula. Hereby the present populations would undergo a loss of genetic diversity

in general. SDM under both scenarios predicts future distribution areas with wide-range connectivity primarily in the Alps, Fennoscandia and the Scottish Highlands. It is reasonable to conserve mountain ranges with wide-range connectivity like Fennoscandia, which will maintain suitable and more or less stable climate conditions for *A. inopinatus* in the long run, particularly as these regions have been found to provide suitable conditions for other aquatic insects under climate change, e.g. for European caddisflies in Fennoscandia (Hering et al. 2009). Even if the FS populations do not provide as high genetic diversity as the Central European populations, they deserve consideration as a conservation unit because they harbour a diverse, viable, and independent genetic lineage.

Besides large, stable patches of areas predicted to be suitable, genetic diversity in itself is an important factor in identifying conservation units. This is because particularly diverse populations, i.e. EA, harbour greater adaptive potential, even if the suitable projected areas are relatively small (Harte et al. 2004). Alsos et al. (2009) explored the extent of climate change induced loss of genetic diversity in an arctic-alpine plant species (*Salix herbacea*) and found equally high genetic diversity in Northern and Central European populations, due to a broad fronted recolonization from Central Europe after the last ice age. Accordingly, the potential climate change induced habitat loss in Central Europe for the year 2080 will cause relatively low loss of overall genetic diversity for this plant species, as the remaining Northern populations will provide a similar level of genetic diversity compared to the past. In contrast, results in our study suggest that climate change induced regional extinction in Central Europe may cause a more severe loss of genetic diversity, and thus a potential reduction in adaptive potential. To conserve overall genetic diversity of *A. inopinatus*, it is thus most important to concentrate on the areas associated with the genetic hotspots and a stable climate in the future.

All of the predicted future Central European suitable microhabitats are currently within or very close to European nature reserves (EU Commission (2010), Table 3.1). In consideration of continuous climate warming and varying altitudes of the regions named above, particular attention should be paid to areas where the species has the chance to disperse to higher altitudes. Eight of ten populations that were closer than 20 km of each other were not significantly differentiated suggesting that gene flow across this distance is generally possible. We assume that this distance is at the low end of the intraregional dispersal capacity of *A. inopinatus*, as the species also showed no significant differentiation in 71% of the populations that were up to 100 km apart. We thus defined a 20 km radius around the sample sites as areas that can be reached during dispersal events. Higher altitudes within these 20 km ranges (300-1550 m above our sampling sites) provide potential immigration areas under climate change conditions. Such areas, which are particularly suitable under future climate change, are found in the Northern BM, Northern BF, EG, SM, VM, JM, Southern BM, Southern BF, BK and the HT (sorted by increasing potential for altitude shifts). When considering both genetic diversity and future climate warming, greatest importance for identifying conservation units within this selection should be given to HT and BK. SDM predicts smaller future habitats in HT and BK (Figure 3.3), but the current source populations are diverse and the region offers greatest altitude variation. Compared to Central Europe, HT currently has a more continental climate, which is characterized by very cold winters, hot summers, and lower annual precipitation. Based on its current distribution, *A. inopinatus* seems to inhabit areas under varying climatic conditions within its total climatic niche, with the continental climate zone in Eastern Europe compared to the more humid temperate zone in Central Europe. Additionally, we found the species inhabiting different stream structures in both zones, suggesting high ecological plasticity. In the continental

climate zone, the larvae were found within dead organic material in stony and slow running branches of big torrential rivers. But in the temperate zone, we found the species in marsh lands, slow running creeks, and grassy lake shores. Detailed habitat analyses would be needed to confirm the existence of suitable habitats in these proposed conservation units/priority areas.

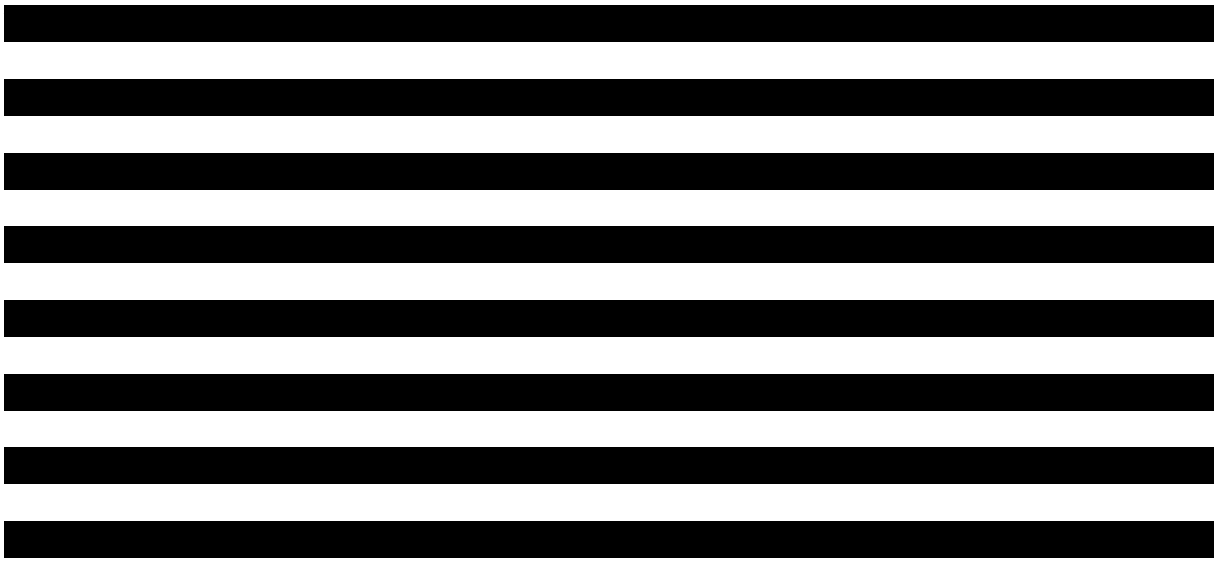
The EA population (Andertal Moor, Austria) currently represents a highly isolated population. Previous zoological and botanical investigations of the Andertal Moor showed that the area was temporarily ice-free during the Pleistocene (Hoelzel 1967). Thus, persistence in this refugium during the last glacial period may explain the high number of private alleles, genetic diversity and effective population size of the EA population. Concerning future climate change the EA population with its high genetic diversity has a great chance to adapt to climate change. In addition, the EA population may become a major colonization source for habitat becoming available in the Central Alps if the species is able to migrate on a long distance in the future. Long-distance dispersal seems more likely to occur along mountain ridges, e.g. across the North-western Carpathian Ranges, than across large lowland valleys, e.g. the Rhine valley between the BF and VM. Hence, the EA population could possibly serve as a colonization source and genetic stock for the Central Alps and maybe even for adjacent mountain ranges like the Balkan Peninsula or Bohemian Massif. Moreover, the EA population is the first known locality of *A. inopinatus* in the inner Alpine region (Schultz et al. 2004) and if the population is stable, the SDM predicts the region of the Andertal Moor will maintain suitable climate conditions for the species while climate change continues. *A. inopinatus* is listed as “endangered” in the German Red List (Malzacher et al. 1998), due to its rarity and loss of suitable, unpolluted habitats. Its rarity has not yet led to inclusion in the local Carinthian Red List in Austria (Graf et al. 2004). For conservation of the genetic resources and in

consideration of stable climatic predictions in the future, we recommend including the species in the Carinthian Red List of Austria and defining the Andertal Moor as a conservation unit.

CONCLUSIONS

Climate change has and will continue to influence species distributions. But the anthropogenic rapid climate warming requires conservation management practices that take future climate change conditions into account. Consequently, we must acknowledge that cold-water adapted aquatic invertebrates will be very sensitive to global warming, and may be among the most threatened species under a warming climate (Hering et al. 2009). Our study provides genetic, climatic and ecological data that can be used to prioritize conservation efforts for *A. inopinatus*, and possibly other montane aquatic insects. A specific genetic composition and stable habitat conditions are the keys for identifying suitable conservation units in the mayfly's European range. In this case, these areas are found in Eastern Europe (the Beskides, the High Tatra and the Eastern Alps) and in Fennoscandia.

ACKNOWLEDGMENTS



[redacted]

[redacted]

CHAPTER 4

Isolation and characterization of 10 highly polymorphic di- and trinucleotide microsatellite markers in the mayfly *Ameletus inopinatus* (Ephemeroptera: Siphlonuridae)

ABSTRACT

We describe the isolation of ten polymorphic microsatellite loci from the mayfly *Ameletus inopinatus*. Loci had di- or trinucleotide repeat motifs and were highly variable with three to 17 alleles (mean = 7.15). Observed heterozygosity ranged from 0.143 to 0.905. One locus (Ami_202) showed significant deviation from Hardy-Weinberg equilibrium in one population, but no evidence for null-alleles. One locus (Ami_73) was significantly linked with three other loci. The remaining nine loci should prove highly informative for population genetic studies.

INTRODUCTION

The mayfly *Ameletus inopinatus* inhabits mountain ranges of high elevation on the British Isles and in central Europe. In northern Eurasia, it is known from Scandinavia to Western Siberia, where it also occurs at lower altitudes. It is considered a representative of the Eurasic, boreo-montane biome-type (Haybach 2003). Its absence in the Alps and the Pyrenees indicates that *A. inopinatus* was not distributed in Europe during the last ice age (Haybach 2003), and is a recent colonizer of central Europe with postglacial retreat to higher elevations (Malicky 1988, Haybach 2003). *A. inopinatus* can be found in slow running water systems as well as little puddles or big lakes, exhibiting a high plasticity in niche occupancy. Here we present the

development of polymorphic microsatellite markers which will be used to examine the fine-scale population genetic structure of *A. inopinatus*.

LABORATORY PROCEDURES

Microsatellite markers were developed using an enrichment protocol developed by Glenn and Schable (2005). We extracted genomic DNA (gDNA) from one individual of *A. inopinatus* from the Andertal Moor, St. Lorenzen, Austria, using the DNEasy tissue kit (Qiagen). Approximately 4µg gDNA was digested with BstUI and XmnI (New England Biolabs, Ipswich, Massachusetts), and SuperSNX24 linkers were ligated onto the gDNA fragments. Linkers serve as priming sites for subsequent polymerase chain reactions (PCR) throughout the protocol. Different combinations of biotinylated trinucleotide and tetranucleotide probes were hybridised to gDNA. The biotinylated probe-gDNA complex was added to streptavidin-coated magnetic beads (Dynabeads® M-280, Invitrogen). This mixture was washed twice with 2xSSC, 0.1% SDS and four times with 1 x SSC 0.1% SDS at 53 °C. Between washes, a magnetic particle-collecting unit was used to capture the magnetic beads. After the last wash, enriched fragments were removed from the biotinylated probe by denaturing at 95 °C and precipitated with 95% ethanol and 3 M sodium acetate. To increase the amount of enriched fragments, a “recovery” PCR was performed in a 25 µl reaction containing 1X PCR buffer (Roche), 1.5 mM $MgCl_2$, 1X BSA, 0.16 mM of each dNTP, 0.52 µM SuperSNX24 forward primer, 1 U Taq DNA polymerase, and approximately 25 ng enriched gDNA fragments. Thermal cycling was performed in an MJ Research DYAD as follows: 95 °C for 2 min followed by 25 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 90 s, and a final elongation step of 72 °C for 30 min. Subsequent PCR fragments were cloned using the TOPO-TA Cloning® kit (Invitrogen) following the manufacturer’s protocol. White colonies (N=260) were amplified via PCR in 25 µl

reactions containing 1 X PCR buffer (Roche), 1.5mM $MgCl_2$, 1 X BSA, 0.12 mM of each dNTP, 0.25 μM M13 primers, and 1 U Taq DNA polymerase. Cycling conditions were: 7 min 95 °C, 35 cycles of 95 °C for 20 s, 50 °C for 20 s, 72 °C for 90 s, and a final elongation of 72 °C for 10 min. PCR products were cleaned using MultiScreen-PCR Filter Plates following the manufacturer's protocol (Millipore). All 260 colonies were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and run on an ABI 3730 DNA Analyzer. Sequences were assembled and edited in SEQUENCHER (GeneCodes) and visually checked for microsatellites. 41 clones had unique loci with repeat numbers greater than six. Primers flanking core microsatellite repeats were developed for 15 loci using PRIMER 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Primer pairs were developed for 15 loci. For fluorescent labelling we used the cost-efficient one tube single reaction nested PCR method as described by Schuelke (2000) and successfully applied by Pauls et al. (2007). An 18 basepair M13 primer was added to the 5´end of each forward primer and a fluorescent labelled M13 primer was added to the PCR. We genotyped 46 specimens of *A. inopinatus* from two populations in Southern Norway (Jotunheimen, Øvre Heimdalen: N = 21, and Dovrefjell, Vinstredalen: N = 25). PCR amplification was performed using PuReTaq Ready-To-Go PCR beads (GE Healthcare) following PCR instructions as described in Schuelke (2000). Cycling conditions were: 5 min at 94 °C, 30 cycles of 30 s at 94 °C, 45 s at 61 °C, 45 s at 72 °C, followed by 8 cycles of 30 s at 94 °C, 45 s at 53 °C, 45 s at 72 °C, and a final extension of 15 min at 72 °C. Samples were scored on an ABI 3130 using 11.7 µL HiDi formamide, 0.3 µL ROX 500 standard (Applied Biosystems) and 1 µL of the PCR product. Loci were genotyped using the GENEMAPPER 4.0 software (Applied Biosystems).

RESULTS

We established PCR protocols for ten polymorphic microsatellite loci that produce reliable and consistent results. The other five primer pairs are not listed because they either did not amplify reliably, or had evidence for null-alleles. Eight loci were dinucleotides, two were trinucleotide repeats (Table 4.1). Allele numbers ranged from three to 21 with average of 7.15 (Table 4.1). In the two populations allele numbers ranged from two to 14 and three to 17 per locus, with an average of 6.7 and 7.2, respectively. We calculated the observed and expected heterozygosity, and exact Hardy-Weinberg probability, using default parameters in the web-based version of GENEPOP (Raymond & Rousset 2004). Observed and expected heterozygosity in the two populations ranged from 0.143 – 0.905 and 0.215 – 0.848, respectively (Table 4.1). The global Hardy-Weinberg test across all loci for both populations showed significant deviation from Hardy-Weinberg equilibrium for one locus in one population (Table 4.1). We tested for null-alleles using the Micro-Checker version 2.2.3 (van Oosterhout *et al*. 2004) and found that no locus had evidence for the presence of null-alleles at the 99 % confidence level. The deviation from Hardy-Weinberg could result from a founder effect in postglacial recolonisation of Scandinavian populations. Tests for linkage disequilibrium applying a Bonferroni correction for multiple comparisons (Rice 1989), revealed that one locus (Ami_73) was significantly linked with three other loci.

CONCLUSIONS

The microsatellite loci that we developed primers for in this study are sufficiently variable to help detect population differentiation. Together with mitochondrial sequence data we will analyse microsatellite loci to detect patterns of population

structure and use coalescent estimates of migration rates to examine the phylogeography of the rare mayfly *A. inopinatus* across its European range.

ACKNOWLEDGEMENTS

[redacted]

Table 4.1 Characteristics and summary statistics for microsatellite loci from *Ameletus inopinatus*, genotyped on 21 and 25 individuals of two populations in southern Norway (Jotunheimen and Dovrefjell). Given are repeat motif, primer sequence, number of alleles, allele size ranges, and observed and expected heterozygosities (H_o, H_e) and *p*-values for deviation of Hardy-Weinberg equilibrium for each locus in both populations. "M" indicates M13 primer; "*" denotes significant deviation of Hardy-Weinberg equilibrium.

Locus ***GenBank Accession No.***	**Repeat motif**	**Primer sequences (5´-3´)***	**No. of alleles**	**Allele size range (bp)**	**Jotunheimen**		**Dovrefjell**	
					H_o	**H_e**	**H_o**	**H_e**
Ami_32	$(TC)_{32}$	F: M-TGCTGTTTCCGGCAGTTATC	16	109-157	0.905	0.771	0.680	0.753
EU729104		R: AGCTAGGAAGGCCAAGAAGG						
Ami_62	$(TC)_{32}$	F: M-ACACTGCGTACGAGGAGAGG	21	171-267	0.476	0.455	0.720	0.703
EU729105		R: CGTGATATTGGCGAGGAG						
Ami_67	$(TGC)_{15}$	F: M-CATGCATGACCCAGATTTTC	3	92-98	0.333	0.400	0.560	0.489
EU729106		R: TTCCTCCTACCCTTCTTTCTCC						
Ami_73	$(AG)_{19}$	F: M-CCTCGGGATTGAGAACTTGA	4	162-170	0.524	0.431	0.520	0.543
EU729107		R: AGAGAAGTCGAGTGGCCAGA						
Ami_95	$(AG)_{11}$	F: M-CGGGAACGTCATCGTCATAG	13	235-291	0.714	0.768	0.760	0.781
EU729108		R: GTGCGACTGGAGGAAAAGAG						
Ami_109	$(TC)_{12}$	F: M-TGTGCTCTAACCAACCGACA	8	116-160	0.714	0.670	0.680	0.740
EU729109		R: AAGATGCGGTGTTTTTACCG						
Ami_185	$(TC)_{12}$	F: M- CCTATGACGCAATGAACGTG	7	213-223	0.143	0.139	0.600	0.567
EU729110		R: CGAACGGTAGATAGAGAACGAGA						
Ami_188	$(CT)_{10}$	F: M-GGCAACTCTTCCTTCCATGT	16	193-257	0.810	0.875	0.840	0.848
EU729111		R: CCTACCCCACTTCGTCATGT						
Ami_191	$(AG)_{19}$	F: M-GAGTCGCTCTTGGGTTTCTG	13	159-195	0.667	0.750	0.760	0.793
EU729112		R: CCTCGCTTCTCTTGTGACAT						
Ami_202	$(AGC)_9$	F: M-GTGGTGTGTGCGAGAGAGAA	3	165-171	0.143	0.215	0.200*	0.313
EU729113		R: GATCCAGTGACGGTGTGTGT						

CHAPTER 5

Isolation and characterization of 11 polymorphic trinucleotide microsatellite markers in the stonefly *Arcynopteryx compacta* (Plecoptera: Perlodidae)

ABSTRACT

We describe the isolation of 11 polymorphic trinucleotide microsatellite loci from the stonefly *Arcynopteryx compacta*. Loci were highly variable with three to 14 alleles (mean = 6.45). Observed heterozygosity ranged from 0.000 to 0.867. Seven loci showed significant deviation from Hardy-Weinberg equilibrium across both populations. There was no evidence for null-alleles, so Hardy-Weinberg departures could result from genetic structure between populations or subpopulations. No linkage between loci was found. The 11 loci should prove highly informative for population genetic studies.

INTRODUCTION

Arcynopteryx compacta MACLACHLAN 1872 inhabits high altitude springs of isolated mountain ranges in western, central and eastern Europe and has a continuous holarctic distribution at high latitudes (Lillehammer 1988). It is a typical boreo-alpine-montane species, which putatively survived Pleistocene glaciations in the central European periglacial region and postglacially retreated to higher altitudes and latitudes (Illies 1955). Studying the intraspecific genetic variation of *A. compacta* will provide valuable additions to our understanding of how historic climate change impacted population regression, where refugia were localised and how isolated refugial populations diverged genetically (Hewitt 1999, Schmitt & Seitz 2001). To

examine *A. compacta's* population structure and phylogeography we developed 11 polymorphic microsatellite loci.

LABORATORY PROCEDURES

Microsatellite markers were developed using an enrichment protocol developed by Glenn and Schable (2005). We extracted genomic DNA (gDNA) from one individual of *A. compacta* from the Black Forest, Stollenbach, in Germany, using the DNEasy tissue kit (Qiagen). Approximately 4 µg gDNA was digested with RsaI and XmnI (New England Biolabs). SuperSNX24 linkers were ligated onto the gDNA fragments. Linkers serve as priming sites for subsequent polymerase chain reactions (PCR) throughout the protocol. Biotinylated trinucleotide probes [$(ACT)_8$ $(ACG)_6$ $(AAG)_8$ $(ATC)_8$ $(AAC)_6$ $(AAT)_{12}$] were hybridised to gDNA. The biotinylated probe-gDNA complex was added to streptavidin-coated magnetic beads (Dynabeads® M-280, Invitrogen). This mixture was washed twice with 2 x SSC, 0.1 % SDS and four times with 1xSSC, 0.1 % SDS at 53 °C. Between washes, a magnetic particle-collecting unit was used to capture the magnetic beads. After the last wash, enriched fragments were removed from the biotinylated probe by denaturing at 95 °C and precipitated with 95 % ethanol and 3 M sodium acetate. To increase the amount of enriched fragments, a "recovery" PCR was performed in a 25 µl reaction containing 1 X PCR buffer (Roche), 1.5 mM $MgCl_2$, 10 X BSA, 0.16 mM of each dNTP, 0.52 µM SuperSNX24 forward primer, 1 U Taq DNA polymerase, and approximately 25ng enriched gDNA fragments. Thermal cycling was performed in an MJ Research DYAD as follows: 95 °C for 2 min followed by 25 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 90 s, and a final elongation step of 72 °C for 30 min. Subsequent PCR fragments were cloned using the TOPO-TA Cloning® kit (Invitrogen) following the manufacturer's protocol. White colonies (N=188) were amplified via PCR in 25 µl

reactions containing 1 X PCR buffer (Roche), 1.5 mM $MgCl_2$, 10 X BSA, 0.12 mM of each dNTP, 0.25 μM M13 primers, and 1 U Taq DNA polymerase. Cycling conditions were: 7 min 95 °C, 35 cycles of 95 °C for 20 s, 50 °C for 20 s, 72 °C for 90 s, and a final elongation of 72 °C for 10 min. PCR products were cleaned using MultiScreen-PCR Filter Plates following the manufacturer's protocol (Millipore). All 188 colonies were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and run on an ABI 3730 DNA Analyzer. Sequences were assembled and edited in SEQUENCHER (GeneCodes) and visually checked for microsatellites.

Primer pairs were developed for 27 loci using PRIMER 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). We used the cost-efficient one tube single reaction nested PCR method described by Schuelke (2000) and applied by Pauls et al. (2007) to fluorescently label the loci. We genotyped 32 specimens of *A. compacta* from two proximate populations in the Apuseni Mountains (Somesul Calde: N = 14, and Baişoara: N = 18, Table 5.1) in Romania. PCR amplification was performed using PuReTaq Ready-To-Go PCR beads (GE Healthcare) following Schuelke (2000) and Theissinger et al. (2008). Samples were scored on an ABI 3733 using 11.7 μL HiDi formamide, 0.3 μL ROX 500 standard (Applied Biosystems) and 1 μL of the PCR product. Loci were genotyped using the GENEMAPPER4.0 software (Applied Biosystems).

RESULTS

Eleven polymorphic loci reliably amplified in all tested specimens and produced consistent results (Table 1). The other 16 primer pairs are not listed as they were either monomorphic or did not amplify reliably. Allele numbers ranged from three to 14, with an average of 6.45 (Table 5.1). Allele numbers per locus ranged from 2 to 6 (mean = 3.45) and 1 to 9 (mean = 4.46) in the two populations. We calculated

observed and expected heterozygosity, and exact Hardy-Weinberg probability, using default parameters in the web-based version of GENEPOP (Raymond & Rousset 2004). Observed and expected heterozygosity ranged from 0.000 to 0.833 and 0.000 to 0.867, respectively. One locus (Arco_46) was monomorphic in one population (Table 5.1). Tests for linkage disequilibrium applying a Bonferroni correction for multiple comparisons (Rice 1989), revealed no linkage between loci. The global Hardy-Weinberg test across all loci for both populations showed significant deviation from Hardy-Weinberg equilibrium for seven loci across both populations (Table 5.1). Using MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) we found no evidence for the presence of null alleles in any locus. Deviations from Hardy-Weinberg equilibrium could result from population substructuring (Wahlund Effect) and genetic structure between the two populations.

These loci are the first microsatellite markers developed for a stonefly species and will serve to examine the phylogeography of the rare stonefly *A. compacta* across its European range.

Acknowledgements

[redacted]

Table 5.1 Characteristics and summary statistics for microsatellite loci from *Arcynopteryx compacta*, based on genotyping 14 and 18 individuals of two proximate populations in the Apuseni Mts, Romania (Somesul Calde and Baişoara). Given are repeat motifs, primer sequences, number of alleles, allele size ranges, and observed and expected heterozygosities (H_o, H_e). "*" denotes for significant deviation of Hardy-Weinberg equilibrium. "M" indicates M13 sequence was added to the 5' end of the primer.

Locus ***GenBank Accession No.***	**Repeat motif**	**Primer sequences (5´-3´)**	**Tm (° C)**	**No. of alleles[1] (SC/B/total)**	**Allele size range (bp)**	**Somesul Calde**		**Baişoara**	
						H_o	**H_e**	**H_o**	**H_e**
Arco_8 *FJ007630*	$(TAG)_9$	F: M-GTCATCGCCACCTTGTT R: CCTAACAAACAATCCCCACAG	63	3/5/5	179 - 203	0.071*	0.262	0.278*	0.465
Arco_46 *FJ007631*	$(TTG)_5CTG(TTG)_{10}$	F: M-TCCAACAGACACATCGGGTA R: TACCCGATGTGTCTGTTGGA	61	3/1/3	149 - 165	0.500	0.429	0.000[2]	0.000[2]
Arco_53 *FJ007632*	$(GAT)_{13}$	F: M-CGAAACCACATGAATCATCAA R: TTGATGATTCATGTGGTTTCG	57	3/7/8	147 – 201	0.500	0.429	0.667	0.802
Arco_79 *FJ007633*	$(AAC)_9$	F: M-CCCCAAAGACGACAAGATTC R: TTCGGATACCCTATGGATGG	61	3/7/7	112 - 241	0.571	0.624	0.529*	0.827
Arco_102 *FJ007634*	$(TTG)_6TTA(TTG)_5$	F: M-CCGGAGTCTCACTTCTTGATG R: TCGTCTTGAATGGCATTTTG	63	3/4/6	186 - 219	0.308	0.283	0.294*	0.733
Arco_123 *FJ007635*	$(TAC)_{14}$	F: M-GCGGTATCTCCACAATATTACACA R: TGTGTAATATTGTGGAGATACCGC	63	4/9/11	247 - 325	0.643	0.690	0.833*	0.867
Arco_126 *FJ007636*	$(ATC)_{11}$	F: M-TCATTCCCTTGATTGAACTATTGA R: TCAATAGTTCAATCAAGGGAATGA	61	3/4/4	219 - 234	0.429*	0.601	0.722	0.694
Arco_138 *FJ007637*	$(AAC)_{12}$	F: M-TGACCCGATGTGTCTGTGTT R: AACACAGACACATCGGGTCA	61	2/3/3	142 - 151	0.357	0.389	0.444	0.481
Arco_144 *FJ007638*	$(TCC)_9(TAC)_4$	F: M-TTAGGGCGAACGCTGTTACT R: GAACTATTTGCAGGAGCAGGA	63	4/6/7	162 – 180	0.231*	0.674	0.412*	0.749
Arco_152 *FJ007639*	$(ATC)_9$	F: M-CCCCTCATCGTCTCGAATAG R: AGAGGGAATGCATCGTATGG	61	4/3/5	203 - 269	0.615	0.495	0.444	0.489
Arco_157 *FJ007640*	$(TTC)_{25}$	F: M-GATCGCTCGAGGTTTAACGA R: TCGTTAAACCTCGAGCGATC	61	6/9/14	141 - 357	0.714	0.646	0.667*	0.849

[1]number of alleles in Somesul Calde (SC), Baişoara (B) and overall (total)

[2]only one allele found in this population

SUMMARY OF RESULTS

The genetic data gathered in this thesis show strong genetic differentiation between mountain range populations for both *A. inopinatus* **(1, 3)** and *A. compacta* **(2)**. Most mountain range populations host genetically isolated lineages, emphasized by the presence of endemic haplotypes and private alleles. For both species the main centres of genetic diversity, and thus putative Pleistocene refugia, are localized in Eastern Europe **(1, 2)**. Additionally, both species exhibited comparably high genetic diversity in the Eastern Alps populations **(2, 3)**, suggesting an important refugial region. The results of this thesis confirm the SDM derived hypothesis that both species could have survived glacial cycles in periglacial, extra Mediterranean refugia, such as the Sudety Mountains, Beskides, and the High Tatra for *A. inopinatus* **(1, 3),** and the Carpathian bow and the Balkan for *A. compacta* **(2)**. However, regarding the recolonization of previously glaciated northern habitats I found contrasting patterns among the study species. *A. inopinatus* extended its northern range during the early Pleistocene from multiple lineages, probably of north-eastern Siberian or Central Asian origin **(1)**. In contrast, *A. compacta* recolonized the Fennoscandian range from a Central European periglacial refuge between the Arctic and Alpine glaciers **(2)**.

SDM projects major regional habitat loss for *A. inopinatus*, particularly in Central European mountain ranges under future climate change **(3)**. By relating these range shifts to our population genetic results, I identified conservation units primarily in Eastern Europe and the Eastern Alps, that if preserved would maintain high levels of present-day genetic diversity and continue to provide long-term suitable habitat under future climate change **(3)**. In the following I discuss the used methods and the main results of the research articles in a comparative framework. Figure 3 summarizes the

main study questions and corresponding answers provided in the articles constituting this thesis.

(1)	Did populations of *Ameletus inopinatus* survive glacial cycles in periglacial, extra-Mediterranean refugia during the LGM?	Yes – putative Central European refugia are localized in the Eastern Alps and the High Tatra.
	Did *A. inopinatus* recolonize Fennoscandia from Central Europe?	No – Fennoscandia was probably recolonized from multiple extra-European lineages.
(2)	Did populations of *Arcynopteryx compacta* survive glacial cycles in periglacial, extra-Mediterranean refugia during the LGM?	Yes – putative refugial areas are localized in the Pyrenees, the Eastern Alps, the Carpathians, and the Balkan.
	Did *A. compacta* recolonize Fennoscandia from Central Europe?	Yes – Fennoscandia was recolonized from a Central European periglacial refuge, probably close to the Black Forest.
(3)	How is the current genetic diversity distributed across Europe among populations of *Ameletus inopinatus*?	The main Centres of genetic diversity are located in the Eastern European mountain ranges and the Eastern Alps.
	Which populations of *A. inopinatus* may become extinct due to future climate change?	The most severe regional habitat loss will regard the Central European sky island populations.
	Which consequences will these extinctions have for the overall genetic diversity and conservation of *A. inopinatus*?	Local extinctions would significantly decrease the overall genetic diversity and thus the adaptive potential. Conservation units in Eastern Europe and the Eastern Alps should be protected to provide long-term suitable habitat under future climate warming scenarios.

Figure 3 The main study questions and corresponding results of the articles 1, 2, and 3 included in this thesis.

DISCUSSION

SAME PLACE, DIFFERENT STORY – VARYING RECOLONIZATION PATTERNS

Phylogeographic comparisons across co-distributed taxa can be informative about changes in the community structure of biogeographical regions over time (Richards et al. 2007). Co-distributed organisms may differ in dispersal capabilities and ecological plasticity, but do not necessarily produce incongruent phylogeographic patterns (Carstens & Richards 2007) and potentially occupied similar Pleistocene refugias (Waltari et al. 2007). However, species found in similar habitats, such as montane, cold adapted species found in crenal and epirhithral regions, could have responded vastly different to past climate change (Lehrian et al. 2009). SDM is particularly suited for comparative phylogeography of co-distributed species (Carstens & Richards 2007): the combined use of paleodistribution modelling and genetic techniques provide a well suited framework to determine whether differences in the genetic variation pattern among co-distributed species reflect varying responses to common historical events or incongruence among the species past distributions.

In this thesis, the genetic data sets suggest different phylogeographic histories of the study species despite the similarities of present day distribution patterns. The most striking difference relates to the postglacial recolonization of Fennoscandia. For *Ameletus inopinatus*, I detected a high degree of genetic separation among the Fennoscandian and Central European populations based on mtDNA (**1**, Figure 4), which was also confirmed by microsatellite data **(3)**. Microsatellite data furthermore exhibited lower genetic diversity in Fennoscandian populations compared to Central Europe **(3)**. This pattern is consistent with a founder effect in the northern populations caused by relatively few individuals dispersing over long distances to recolonize

Fennoscandia. Within northern populations I detected high genetic structure based on mtDNA haplotypes for *A. inopinatus* (Figure 4), indicating that *A. inopinatus* must have recolonized Fennoscandia from multiple refugia, representing distinct genetic lineages **(1)**. However, this structure was not apparent in the microsatellite data **(3)**. This could indicate a secondary contact zone of two maternal evolutionary lineages without sexual incompability, or potentially sex-biased dispersal. Interestingly, within Fennoscandia I observed a decrease in genetic diversity from North to South based on microsatellite data **(3)**, whereas the mtCOI data exhibit higher genetic diversity in southern Fennoscandia compared to the northern Fennoscandian populations. This inconsistency could be due to a secondary contact zone of two lineages with different levels of ancestral genetic diversity, a result of 1) different refugial regions, with one region being more diverse than the other; 2) different age of lineages; or 3) lineages experienced bottlenecks to different degrees. Although I could only analyse two samples from the Bulgarian Highlands, the genetic data provide no indication for a recolonization from the Balkan Peninsula via the Carpathian range. *A. inopinatus* might have recolonized Fennoscandia from different refugia not accounted for in my sampling.

In sharp contrast, Fennoscandian populations of *A. compacta* exhibited shared haplotypes with the Black Forest populations (Figure 4). This suggests that *A. compacta* presumably recolonized Fennoscandia and the Black Forest from a common refuge population, potentially located in Central Europe (Figure 5). Due to the low divergence between Fennoscandian and Black Forest haplotypes I assume that the separation of both lineages occurred recently, i.e. after the LGM. *A. compacta* thus represents an example for a postglacial disjunct distribution pattern as described by Schmitt (2007) **(2)**. The low genetic diversity within Fennoscandia based on microsatellite data indicates a strong founder effect, causing a severe loss

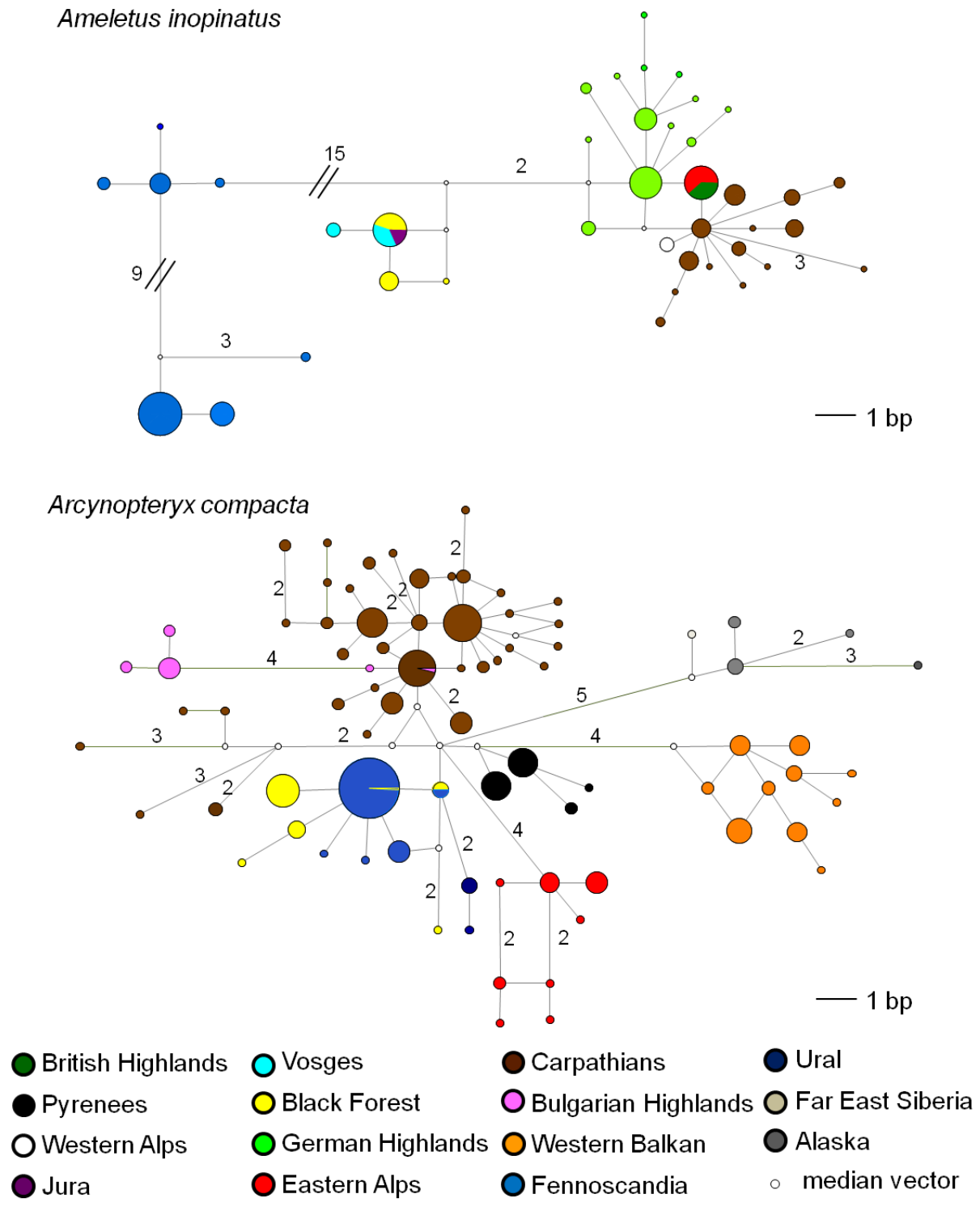


Figure 4 Median Joining networks for *Ameletus inopinatus* and *Arcynopteryx compacta* based on mtCOI haplotype variation.

of genetic diversity **(2)**. It seems possible that the Black Forest and Fennoscandia are descendants of large populations that persisted in the periglacial tundra belt during the last glaciations **(2)**. However, the connection of the Black Forest and Fenno-

scandia could not be confirmed with the microsatellite data due to the low number of shared alleles **(2)**. One possible explanation for this discrepancy could be female-biased dispersal, since brachypterous males of *A. compacta* could be disadvantaged in their dispersal capabilities. In this case the biparentally inherited microsatellite markers could indicate limited gene flow among populations, while the maternally inherited mitochondrial DNA exhibits a close relationship of both populations.

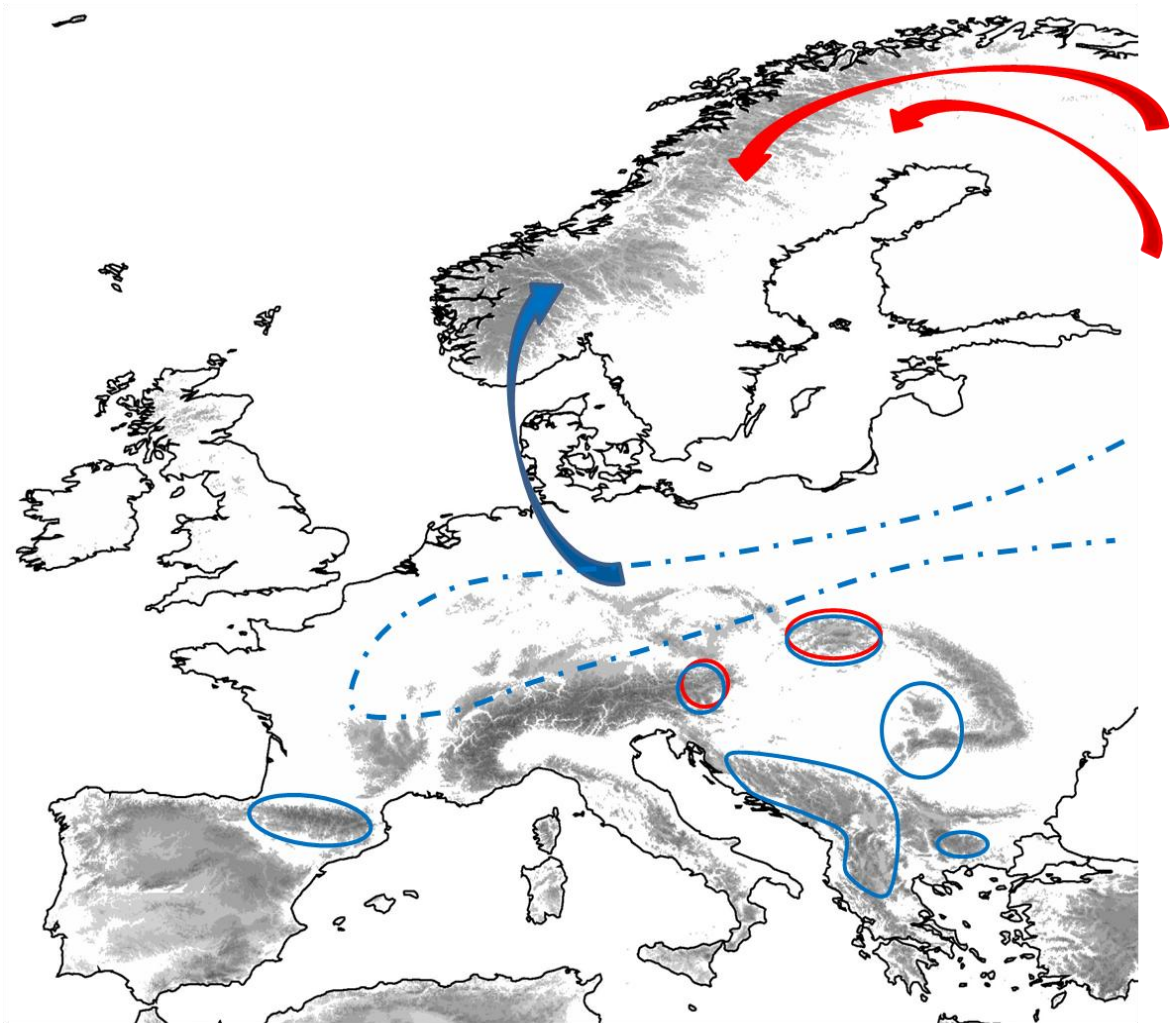


Figure 5 Proposed Pleistocene and Holocene history of the study species. Presented are the inferred Central European refugia and recolonization directions of Fennoscandia for both *Ameletus inopinatus* and *Arcynopteryx compacta*, based on genetic data and SDM results**.** The dotted line indicates the presumed periglacial belt popu-lation along the glacial fringe, from where the recolonization of Fennoscandia originated for *Arcynopteryx compacta*.

MOLECULAR MARKERS – CONSISTENCIES AND DISCREPANCIES

Based on microsatellite data I observed significant deviations from Hardy-Weinberg equilibrium (HWE) due to homozygote excess in some populations **(2, 3)**. Apart from potential null alleles, various other factors can cause deviations from HWE, such as inbreeding or subpopulation structure. Inbreeding should affect all loci equally, and consequently lead to consistent heterozygote deficits over all loci (Daking & Avise 2004). However, in both species only individual loci were affected, and hence inbreeding does not seem to be an issue. When populations from the same mountain range were pooled into regional groups highly significant deviations from HWE were noticeable in both species **(2, 3)**, revealing strong subpopulation structure. High levels of population differentiation within a sample or group of samples may result in a Wahlund effect. In aquatic insects population differentiation can be high both among and within reaches of the same stream, e.g. if larval drift and movement is restricted (Bunn & Hughes 1997). In *A. inopinatus* local structuring could be the result of asynchronous generations within sites **(3)**. The underlying causes of subpopulation structure in *A. compacta* could be geographic barriers to gene flow due to low dispersal capabilities, since adult males and females of some populations are brachypterous, and stoneflies in general are poor fliers (Malmqvist 2000). It is thus not surprising that microsatellites demonstrate that there is no gene flow between and within any mountain region in Central Europe **(2)**. Moreover, all sky island populations exhibit private microsatellite alleles in both species **(2, 3)**. This is congruent with the high rate of endemic haplotypes within mountain ranges and regional differentiation in the mtDNA, which also indicate high degree of local population differentiation and limited gene flow among populations **(1, 2)**.

Despite these congruent results between mtCOI and microsatellite data I also found discrepancies between the data sets in both species. For *A. inopinatus* I

observed a high degree of genetic structure within Fennoscandia based on mtCOI data **(1)**. However, this structure was not apparent in the microsatellite data **(3)**. For *A. compacta* mtCOI data suggested a connection of the Black Forest and the Fennoscandian populations due to shared haplotypes, which was not supported with microsatellite data **(2)**. This incongruence of mitochondrial versus nuclear data is a common issue in phylogeographic studies using both marker types (Zink and Barraclough 2008). In my studies these discrepancies could be due to 1) a secondary contact zone of two maternal evolutionary lineages without sexual incompability **(1)**; 2) ancestral polymorphism, which is remnant in the mtCOI data whereas the microsatellite data have diverged more quickly due to random mutations under strong genetic drift **(2)**; or 3) female-biased dispersal **(1, 2)**. The discrepancies between mtCOI data and microsatellite data found in this thesis emphasize the importance to use more than one marker type with different inheritance modes to sufficiently clarify the evolutionary history of a species.

SDM – OUTCOMES AND PITFALLS

Applying SDM approaches to freshwater species may appear challenging, as they are bound to the water column and may thus seem more influenced by local environmental and microhabitat conditions than by macroecological parameters, such as climate variables. SDMs are thus still rare in studies of aquatic insect phylogeography, and restricted to headwater mountain species (e.g. Lehrian et al. 2010). As for most lotic systems the environmental conditions of headwater mountain streams are largely determined by geology, slope, air temperature, and precipitation, but also by riparian vegetation. While some species may be adapted or restricted to certain geological features (e.g. Engelhardt et al. 2008), many headwater species occur in a variety of geological settings, and their presence is mostly determined by

water temperature and oxygen supply, parameters that are amongst others associated with air temperature and precipitation (Danks 2007). Hence, climate variables such as those used in this thesis can offer a reasonable, indirect way to predict the distribution of mountain dwelling aquatic species.

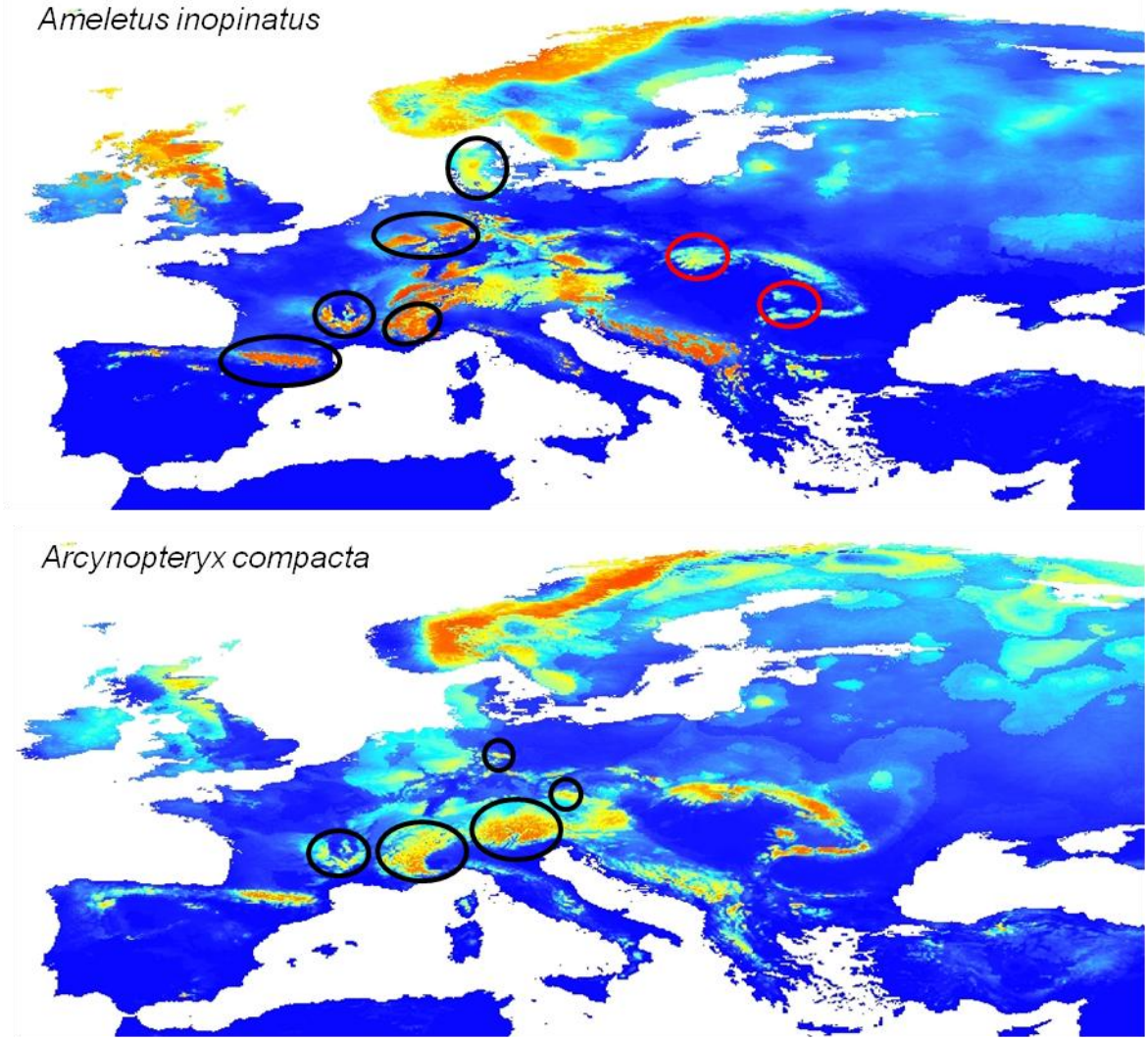


Figure 6 Current SDM for the two study species. Warmer colors indicate better climatic conditions for the occurrence of the species. Black circles denote for over-predictions (no current species records) and red circles for under-predictions (species recorded for the area) of the SDM.

However, SDM solely based on climate variables often tend to overestimate (over-predictions) or underestimate (under-predictions) range sizes. This is due to the fact that in addition to climate the distribution of a species is also shaped by biotic interactions (Duncan et al. 2009). Moreover, the SDM can only project a species range based on its current climatic preferences, which could have adapted over time.

In this thesis, the current SDM over-predicts the distribution ranges for both *A. inopinatus* **(1)** and *A. compacta* **(2)** in several mountain ranges (Figure 6). Noticeable is the over-prediction in the Western Alps (*A. inopinatus, A. compacta*) and the Central Alps (*A. compacta*), while correctly projecting suitable habitat in the proximity of the Eastern Alps. It is interesting that both species were able to postglacially extend their ranges from Central European refuges over thousands of kilometres northwards **(1, 2)**, but were not able to colonize the suitable microhabitats of the western and central alpine range. This issue was not only inferred by SDM but also observed by myself and colleagues. The reason could be either 1) dispersal barriers or 2) competitive exclusion. Population genetic analyses of *A. inopinatus* **(3)** show that besides dispersal capabilities, geographical structures, such as low river basins, can act as dispersal barriers and lead to high genetic differentiation.This suggests that dispersal is more likely to occur along mountain ranges, where suitable habitat is more widely available than in low gradient, low elevation areas. Possibility 2 of competitive exclusion might thus be more plausible. *A. compacta* is the top predator in springs and is as such unable to co-exist with e.g. the similarly carnivorous, cold-adapted stonefly *Dictyogenus alpines* or its sister species *D. fontinals*. Both species are frequently found in central alpine crenal or epirithral habitats (W. Graf, pers. com.). It seems likely that *A. compacta* is outcompeted by these species due to their similar ecological preferences. Also for *A.inopinatus* there are indications for competitive exclusion in the Central Alps. Various species of the genus *Baetis* and *Siphlonurus* are widely distributed across the Alps, exhibiting similar feeding attributes and habitat preferences as *A. inopinatus* (Brettfeld 1990). Accordingly, interspecific competition might have led to its restriction to the Eastern Alps. Pfenninger et al. (2006) present a similar case of competitive exclusion for the freshwater snail *Radix balthica*. The species currently does not occur in Eastern

Europe, but substantial areas were predicted as suitable on the Balkans in the LGM SDM. However, a closely related species of *R. balthica* occurs in the eastern predicted range. This demonstrates that suitable area for a species can be occupied by species with similar ecological requirements, thus leading to over-predictions in the SDM.

Over- and under-predictions such as those found in this thesis (Figure 6) are quite common in SDM (e.g. Lobo et al. 2006). The pitfall with such projections of fitted models to different times and ranges is that it assumes climate niche conservatism and species being at equilibrium with their climatic conditions. For several groups, including also freshwater invertebrates, these assumptions have been denied (Peterson & Vieglais 2001, Martínez-Meyer et al. 2004, Kozak & Wiens 2006, Martínez-Meyer & Peterson 2006, Pfenninger et al. 2007; Cordellier & Pfenninger 2008), and it is questionable if the assumption of niche conservatism fully applies to my study species. In this thesis I found indications for niche plasticity in both *A. inopinatus* and *A. compacta*. In Central Europe *A. compacta* occurs solely in crenal or epirithral regions of high altitudes, but at high latitudes it can also occasionally be found on rocky lake shores **(2)**. In northern America, *A. compacta* has also been shown to occur in alpine or arctic lakes, where the combination of gravelly substrates and cold, waveswept shallows simulate flowing water conditions (Stewart & Ricker 1997). These different habitats provide also varying levels of physiological parameters, such as oxygen supply and water temperature, due to different currents and snow coverage in winter (Danks 2007). This may thus reflect some degree of adaptive potential for *A. compacta*. For *A. inopinatus* there was evidence for adaptive potential in the High Tatra **(3)**, which is reflected by the under-prediction in the SDM (Figure 6). Based on its current distribution, *A. inopinatus* seems to inhabit areas under varying climatic conditions within its total climatic niche:

a continental climate zone in Eastern Europe and a more humid temperate zone in Central Europe. Additionally, I found the species inhabits different stream structures in both zones **(3)**. These examples suggest ecological plasticity to some degree for both species.

Cordellier & Pfenninger (2009) advise testing the assumption of niche conservatism before reasonably predicting the distribution of a particular species. Corresponding results between phylogeographically inferred refugial areas and the potential range of the species at the LGM can justify the use of SDM for future range predictions (Cordellier & Pfenninger 2009). For *A. inopinatus* the LGM SDM projects suitable climate conditions *inter alia* in the Eastern Alps and in the High Tatra, regions that showed highest genetic diversity and are thus regarded as potential Pleistocene refugia for this species **(1, 3)**. However, suitable climatic conditions during the LGM are also projected on all three Mediterranean Peninsulas **(1)**. The species is presently not known from the Pyrenees or the Italian highlands, although the present SDM predicts suitable climate conditions for these mountain ranges. Therefore I did not consider these regions as possible Pleistocene refugia. The mismatch between SDM and phylogeographical inferences could result from not considering biotic interactions and dispersal capabilities in the SDM (Waltari et al. 2007). A possible alternative explanation is that the species' climatic niche has evolved, as has been suggested for the land snail *Candidula* (Pfenninger et al. 2007). To clarify this issue more detailed analyses on habitat preferences of *A. inopinatus* and *A. compacta* would be needed.

DISTRIBUTION – A QUESTION OF DISPERSAL CAPABILITY?

The observed differences between recolonization patterns of Fennoscandia for *A. inopinatus* and *A. compacta* highlight the fact that species with similar present

distribution patterns do not necessarily share the same evolutionary history. The underlying causes can be found in the biology of the species. The most obvious parameter affecting distribution is the species dispersal capability. Malmqvist (2000) analysed the impact of wing length on the distribution patterns of stoneflies and mayflies. He found that in both orders wing lengths were positively correlated with range sizes. Short wings can be regarded as a potential for reduced dispersal capacity. Stoneflies in general have often evolved brachyptery, whereas in mayflies brachyptery is uncommon (Brittain 1990). Dispersal between habitats can be a challenging demand especially for freshwater invertebrates, as they occur at sites surrounded by unsuitable terrestrial environments (Bilton et al. 2001). Most population genetic studies on freshwater insects have found moderate to high levels of genetic differentiation in more distant streams even in species capable of flight (Jackson & Resh 1992, Hughes et al. 1999). Various studies thus suggest limited lateral dispersal in stream-dwelling insects (Kovats et al. 1996, Collier & Smith 1998, Griffith et al. 1998). Nevertheless, since my study organisms currently inhabit broad distributional scales (arctic-alpine), they must have been able to postglacially disperse over large distances to recolonize the arctic range. Many freshwater invertebrates use animal vectors, wind, or water flow for passive long-distance dispersal, but this involves a high probability of terrestrial deposition (Bilton et al. 2001, Kelly et al. 2001). Mayflies are capable of short-distance dispersal with active flight across hostile regions (Kelly et al. 2001), as they mate in flight, often in considerable distance from the aquatic habitat (Brittain 1990). In stoneflies, on the other hand, mating occurs on the ground or on the water surface besides the aquatic habitat (Brittain 1990), and stoneflies can generally be regarded as bad dispersers (Malmqvist 2000). The poorer dispersal ability of stoneflies compared to mayflies is also reflected in their higher numbers of endemic species (Brittain 1990).

I found indications for sex-biased dispersal in both of my study species **(1, 2)**. There exist data for sex-biased dispersal in mayflies due to female dispersal subsequent to mating, whereas males exhibited a more philopatric behaviour (Flecker & Allan 1988, Caudhill 2003). This supports the possibility of female-biased dispersal also for *A. inopinatus*. Conversely, population genetic analyses on the stonefly *Peltoperla tarteri* suggested that adult dispersal was limited and larval movement was the primary dispersal mechanism for this species (Schultheis et al. 2002), a result often found in Plecoptera species (Bittain 1990). Unfortunately, there was insufficient ecological data to examine the possibility of sex-biased dispersal for *A. compacta.* However, considering the large scale recolonization into Fennoscandia, a passive larval water transport seems unlikely to be the driving force for migration in this species. Moreover, the species holarctic distribution and the limited divergence among mtCOI sequences from different regions and continents make it plausible that long distance dispersal is primarily maintained by winged females, rather than brachypterous males. Such sexual dimorphism is a common trait-off between reproductive success and dispersal ability in Plecopterans (Brittain 1990). Since stoneflies mostly mate on the ground nearby their preferred habitat, the capability of flight is of inferior importance. This applies especially to arctic-alpine species where low air temperatures restrict flight activity.

Within the limits set by dispersal, distribution patterns are the result of life history traits. Temperature is probably the most important factor influencing the life cycle and occurrence of freshwater insects (Danks 2007). Brittain (1990) compared the general patterns in the life cycles of stoneflies and mayflies. He showed that Plecopterans are generally better adapted to cold environments than Ephemeropterans. Compared to mayflies, stoneflies were K-strategists, with lowered fecundity for the advantage of more robust egg shells. In frozen streams egg clutches

of *A. compacta* are able to overwinter at temperatures down to –29°C (Gehrken and Sømme 1987). However, in the arctic-alpine environment there was no difference in fecundity between stoneflies and mayflies (Brittain 1990). By comparing life history strategies of different species it is possible to evaluate present distributions with regards to the restrictions set by evolutionary processes and present selective pressures.

CONSERVATION IMPLICATIONS

Short wings, and hence a potential for reduced dispersal capability, can be one of several reasons for the rarity of many freshwater insects (Malmqvist 2000). When evaluating the role of dispersal in the maintenance of genetic diversity in stream invertebrates, interspecific differences in the ability to colonise or recolonize habitats should be considered (Elliot 2003). Since recolonisation by poor flyers can be very uncertain and slow after local extinction it is important for conservation to identify poor dispersers and describe their habitats. Therefore, localities with rare, poorly-dispersing species should be protected. Freshwater invertebrates are the indicators of biodiversity in aquatic ecosystems, and represent most of the genetic variation at a site (Duelli 1997). Consequently, they play a key role in biomonitoring of aquatic ecosystems. Like many montane aquatic insects, *A. inopinatus* and *A. compacta* are indicator species of unpolluted freshwater systems (Hendrey and Wright 1976). Their remote habitats of isolated mountain ranges make them less prone to direct anthropogenic disturbance. However, both species are listed as ‘‘endangered’’ in the German Red List due to their rarity and loss of suitable, unpolluted habitats (Binot et al. 1998). Furthermore, the human influence on Earth’s climate raises the possibility of rapid species extinction due to habitat loss in the near future (Houghton 2001; Thomas et al. 2004). Montane sky-island populations of freshwater invertebrates,

such as my study species, are especially threatened through global warming as such cold-adapted species may lose a large part of their range (Moussalli et al. 2009) and also a high amount of their present-day genetic diversity. Preserving genetic diversity, as one crucial sublevel of biodiversity, is essential to the overall fitness of a species, because decreased genetic variability may reduce the ability of single populations to adapt to changing environments triggered by current global warming (Reed & Frankham 2003; Gienapp et al. 2008; Hoffmann & Willi 2008) and may thus result in local extinctions.

Concern over the effect of climate change on biodiversity has led to the use of SDM to predict the range shifts of species under future climate change (Araújo & New 2006). SDM thus provide an important tool to conservation biologists, since they can assist in determining high quality sites under present and future climate conditions (Graham et al. 2004). Moreover, population genetic analyses can be employed to identify management units requiring conservation priority (Crandall et al. 2000, Frankham et al. 2002). By combining SDM and genetic data, I was able to identify regions (German highlands, High Tatra) and single populations (Eastern Alps: Andertal Moor) that should receive high attention when developing conservation strategies for *A. inopinatus* in light of predicted climate change **(3)**. Future SDM revealed serious habitat loss and thus severe reduction of genetic diversity especially in Central Europe. This highlights the importance to focus on today's genetic hotspots to effectively preserve the overall genetic diversity of a species. These genetic hotspots are most often associated with formerly Pleistocene refugia (Hewitt 1996). Phylogeography, especially when coupled with SDM, can thus guide the development of suitable conservation strategies for preserving the adaptive potential of a species, offering a great potential to infer the fate of species by revealing their evolutionary past.

CONCLUSIONS

The observed differences between recolonization patterns of Fennoscandia for *A. inopinatus* and *A. compacta* highlight the fact that species with similar present distribution patterns do not necessarily share the same evolutionary history. The underlying causes can be found in the biology of the species. By comparing life history strategies of different species it is possible to evaluate present distributions with regards to the restrictions set by evolutionary processes and present selective pressures. Climate change always has and will continue to impact species distributions. However, the anthropogenic influence causes such rapid climate warming in most parts of the world that many species might not be able to cope. Preserving the adaptive potential of a species is thus of primary importance to prevent local extinctions. Cold-water adapted aquatic invertebrates, such as the study species of this thesis, will be especially sensitive to global warming, and may be among the most threatened species (Hering et al. 2009). Conservation management practices have to take these future climate change conditions into account. In this thesis I present genetic, climatic, and ecological data that can be used to prioritize conservation efforts for cold-adapted freshwater insects. Furthermore, I provide a next step in filling the knowledge gap regarding molecular studies of the arctic-alpine invertebrate fauna. However, there is continued need to explore the phenomenon of arctic-alpine disjunctions to help understand the processes of range expansion, regression, and lineage diversification in Europe's high latitude and high altitude biota.

AUTHOR CONTRIBUTIONS

Chapter	1	2	3	4	5
Original idea	SUP, PH	SUP, PH	KT, SUP		
Laboratory work	KT	KT	JT, KT	KT, KAF	KT, KAF
Genetic data analyses	KT, MB	KT, MB	JT, KT	KT	KT
SDM	IL, KT	IL, KT	IL, KT		
Manuscript preparation	KT, SUP, JJ	KT, SUP, JJ	JT, KT, SUP, JJ	KT, SUP	KT, SUP

Kathrin Theissinger (KT), [redacted] (SUP), [redacted] (PH), [redacted] (JT), [redacted] (KAF), [redacted] (MB), [redacted] (IL), and [redacted].

I am very grateful for the contribution of all colleagues to the development of the articles constituting this thesis.

ACKNOWLEDGEMENTS

It is never easy to find the right words. There are so many people, who significantly influenced the development of this thesis during the last years - supportive colleagues by providing new ideas and critical feedback, and good friends by offering emotional support. Best of all: many of these people belong to both categories!

For a start, I would like to thank my two supervisors, [redacted] and [redacted], who guided the progress to arrange all my thoughts and results in an appropriate form, suitable for submitting the manuscripts that lastly constitute this thesis on hand. Jes, I always appreciated your in depth critical comments, that repeatedly made me reconsider my train of thoughts. And Evi, thanks for your competent advices and for always taking the time. I really enjoyed our talks at the coffee breaks!

[redacted], I think you are the person who taught me the most during my PhD, even though our communication was mainly restricted to email or skype. Thanks for your immense support during the past five years, and for your initial encouragement to carry out this thesis.

[redacted], catching flies across Europe we got to know each other quite well. Thanks for your physical effort, your mental input, and your personality, which enriched my project in various ways.

[redacted], I really enjoyed your company in Gelnhausen, on field trips, or at conferences. Thanks for the good teamwork, your great enthusiasm, and your always positive attitude.

[redacted], thanks for your lovely patience to give me an initial understanding of GIS, R and the wide field of ecological modeling. Now I know: R can do anything – if you know how ;)

Thanks to [redacted] and the team of the Senckenberg Research Institute for Limnology and Conservation, Gelnhausen, for giving me the chance to gain work experience in various projects, that in the end helped me to efficiently conduct this thesis, and for providing expertise and financial support for my research.

Also thanks to [redacted] from the Field Museum in Chicago for the cooperation and guidance in developing the microsatellite marker sets.

Numerous people helped me to complete my data sets by providing specimens, locality information, or helping me in the field. Thanks to [redacted].

To the great workgroup of the Department of Ecology at the Johannes Gutenberg University in Mainz: Thank you all so much for these wonderful years, the good atmosphere and the laughter on the corridors, the hidden biscuit supply behind the

lid, the joined BBQs and volleyball sessions, the comfy Advent seasons and funny Christmas parties, the many interesting and constructive talks, and simply for making it an everyday pleasure to go to uni! [redacted], it was so nice sharing the office with you – thank you for offering me a “home” in an exhaustive time. Special thanks also to [redacted] and [redacted], who took care of me and Lumpi by providing sweets and vitamins – you rescued me several times from complete exhaustion.

I happily enjoyed the ideational and financial support of a PhD fellowship from the Studienstiftung des deutschen Volkes. Thanks for the always unbureaucratic policy and for the fourth year monetary extension, signaling the general encouragement of having both career AND children!

Finally, I would like to thank my parents, my brother and my sister, who in the end built the basis for my walk of life. Without your ongoing encouragement that I can do anything I would not have been brave enough to go this whole way. I feel very blessed that I grew up in such a loving and supportive environment.

And then…Theo, my loving husband, my loyal partner, my best friend for over fifteen years – no words can sufficiently express my gratefulness… But I am sure you know without saying! Thank you for making it possible for me to combine both science and family, and for being such a wonderful father to our son! Matthis, my little sunshine – of all things I achieved so far, you are the best! Every day your smile makes me realize the only true meaning of life.

This thesis is dedicated to the memory of Professor Dr. [redacted], who passed away in January 2010.

REFERENCES

Alcamo J, Bouwman A, Edmonds J, Grübler A, Morita T, Sugandhy A (1995) An Evaluation of the IPCC IS92 Emission Scenarios. In: Climate Change 1994, Radiative Forcing of Climate Change and An Evaluation of the IPCC IS92 Emission Scenarios, Cambridge University Press, Cambridge, UK, pp 233–304.

Alsos IG, Alm T, Normand S et al. (2009) Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modelling. *Global Ecology and Biogeography*, 18, 223–239.

Araújo MB, New M (2006) Ensemble forecasting of species distributions. *Trends in Ecology and Evolution*, 22, 42–47.

Ashley MV, Dow B (1994) The use of microsatellite analysis in population biology: Background, methods and potential applications. In Schierwater B, Strait B, Wagner GP, Desalle R (Eds) *Molecular Ecology & Evolution: Approaches and Applications* (pp. 185-210). Basel: Birkhauser Verlag.

Avise JC (2004) Molecular Markers, Natural History, and Evolution. Sunderland: Sinauer Associates.

Avise JC (2000) Phylogeography. The history and formation of species. Harvard University Press, Cambridge, Massachusetts, USA, London, UK.

Babik W, Branicki W, Sandera M, Litvinchuk S, Borkin J, Irwin T, Rafinski J (2004) Mitochondial phylogeography of the moor frog, *Rana arvalis*. *Molecular Ecology*, 13, 1469-1480.

Bandelt, HJ, Forster P, Röhl A (1999) Median-Joining Networks for Inferring Intraspecific Phylogenies. *Molecular Biology and Evolution*, 16, 37-48.

Bálint M, Domisch S, Engelhardt CHM, Haase P, Lehrian S, Sauer J, Theissinger K, Pauls SU, Nowack C (*submitted*) Climate change may lead to massive loss of genetic and cryptic biodiversity.

Bálint M (2008) Pleistocene and Holocene history of *Rhyacophila aquitanica* (Insecta: Trichoptera) in the Carpathian Mountains, potential speciation centers. PhD thesis, Babes-Bolyai University, Faculty of Biology and Geology, Cluj, Romania.

Bandelt HJ, Forster P, Röhl A (1999) Median-Joining Networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16, 37-48.

Beebee TJC (2007) Population structure and its implications for conservation of the great silver beetle *Hydrophilus piceus* in Britain. *Freshwater Biology*, 52, 2101–2111.

Beebee TJC, Griffiths RA (2005) The amphibian decline crisis: a watershed for conservation biology? *Biological Conservation*, 125, 271-285.

Benke M, Brändle M, Albrecht C, Wilke T (2009) Pleistocene phylogeography and phylogenetic concordance in cold-adapted spring snails (*Bythinella* spp.). *Molecular Ecology*, 18, 890–903.

Beheregaray LB (2008) Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology*, 17, 3754–3774.

Bilton DT, Mirol PM, Mascheretti S, Fredga K, Zima J, Searle JB (1998) Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonisation. *Proceedings of the Royal Society of London B*, 265, 1219–1226.

Binot M, Bless R, Boye P, Gruttke H, Pretscher P (1998) Rote Liste gefährdeter Tiere Deutschlands, Bundesamt für Naturschutz (BfN). Bonn-Bad Godesberg, Germany, pp 264–267.

Böhmer J, Rawer-Jost C, Zenker A (2004) Multimetric assessment of data provided by water managers from Germany: assessment of several different types of stressors with macrozoobenthos communities. *Hydrobiologia*, 516, 215–228.

Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetic studies. *Molecular Ecology*, 13, 3261–3273.

Brändle M, Heuser R, Marten A, Brandl R (2007) Population structure of the freshwater flatworm *Crenobia alpina* (Dana): old lineages and low gene flow. *Journal of Biogeography,* 34, 1183–1192.

Brettfeld R (1990) Beiträge zur aquatischen Fauna des Schleuseeinzugsgebietes in Südthüringen. Teil 2: Verbreitung und Larvenökologie von *Ameletus inopinatus* EATON (Insecta, Ephemeroptera: Siphlonuridae). *Veröffentlichungen des Naturhistorischen Musseums Schleusingen*, 5, 58–62.

Brittain JE (1990) Life history strategies in Ephemeroptera and Plecoptera. *In*: Campbell I.C. (ed) Mayflies and Stoneflies, 1-12.

Braconnot P, Otto-Bliesner B, Harrison S, Joussaume S, Peterchmitt J-Y, Abe-Ouchi A, *et al*. (2007) Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum – Part 1: experiments and large-scale features, *Climatic Past*, **3**, 261–277.

Brochmann C, Gabrielsen TM, Nordal I, Landvik JY, Elven R (2003) Glacial survival or *tabula rasa*? The history of North Atlantic biota revisited. *Taxon*, 52, 417–450.

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from pattern of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences of the USA,* 91, 6491-6495.

Brunhoff C, Yoccoz NG, Ims RA, Jaarola M (2006) Glacial survival or late glacial colonization? Phylogeography of the root vole (*Microtus oeconomus*) in north-west Norway. *Journal of Biogeography*, 33, 2136–2144.

Buckley TR, Marske K, Attanayake D (2010) Phylogeography and ecological niche modelling of the New Zealand stick insect *Clitarchus hookeri* (White) support survival in multiple coastal refugia. *Journal of Biogeography*, 37, 682–695.

Buffagni A, Cazzola M, López-Rodríguez MJ, Alba-Tercedor J, Armanini DG (2009) Distribution and Ecological Preferences of European Freshwater Organisms. Volume 3 - Ephemeroptera. In Schmidt-Kloiber A, Hering D (ed.) Pensoft Publishers, Sofia-Moscow, pp 1-254.

Bunje PME (2005) Pan-European phylogeography of the aquatic snail *Theodoxus fluviatilis* (Gastropoda: Neritidae). *Molecular Ecology*, 14, 4323-4340.

Bunn SE, Hughes JM (1997) Dispersal and recruitment in streams: evidence from genetic studies. *Journal of the North American Benthological Society*, 16, 338–346.

Carlsson M, Söderberg L, Tegelström H (2004) The genetic structure of adders (*Vipera berus*) in Fennoscandia: congruence between different kinds of genetic markers. *Molecular Ecology*, 13, 3147-3152.

Carstens BC, Richards CL (2007): Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution,* 61, 1439–1454.

Caudhill CC (2003) Measuring dispersal in a metapopulation using stable isotope enrichment: high rates of sex-biased dispersal between patches in a mayfly metapopulation. *Oikos*, 101, 624–630.

Chakraborty R, De Andrade M, Daiger SP, Budowle B (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics*, 56, 45–57.

Chapuis M-P, Loiseau A, Michalakis Y, Lecoq M, Franc A, Estoup A (2009) Outbreaks, gene flow and effective population size in the migratory locust, *Locusta migratoria*: a regional-scale comparative survey. *Molecular Ecology*, 18, 792-800.

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. *Molecular and Biological Evolution*, 24, 621–631.

Chun YJ, Fumanal B, Laitung B, Bretagnolle F (2010) Gene flow and population admixture as the primary post-invasion processes in common ragweed

(Ambrosia artemisiifolia) populations in France. *New Phytologist*, 185, 1100-1107.

Corander J, Sirén J, Arjas E. (2008) Bayesian Spatial Modelling of Genetic Population Structure. *Computational Statistics,* 23, 111-129.

Corander J, Marttinen P (2006) Bayesian 530 identification of admixture events using multilocus molecular markers. *Molecular Ecology*, 15, 2833-2843.

Cordellier M, Pfenninger M (2009) Inferring the past to predict the future: climate modeling predictions and phylogeography for the freshwater gastropod *Radix balthica* (Pulmonata, Basommatophora). *Molecular Ecology*, 18, 534–544

Cordellier M, Pfenninger M (2008) Climate driven range dynamics of the freshwater limpet, Ancylus fluviatilis (Pulmonata, Basommatophora). *Journal of Biogeography*, 35, 1580–1592.

Collier KJ, Smith BJ. 1998. Dispersal of adult caddisflies (Trichoptera) into forests alongside three New Zealand streams. *Hydrobiologia*, 361, 53–65.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, 15, 290–295.

Danks H.V. (2007): How aquatic insects live in cold climates. *Canadian Entomology,* 139, 443–471.

Daking EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. *Heredity,* 93, 504–509.

Davis LA, Roalson EH, Cornell KL, McClanahan KD, Webster MS (2006) Genetic divergence and migration patterns in a North American passerine bird: implications for evolution and conservation. *Molecular Ecology*, 15, 2141-2152.

Deffontaine V, Libois R, Kotlik P (2005) Beyond the Mediterranean peninsulas: evidence of Central European glacial refugia for a temperate forest mammal

species, the bank vole (*Clethrionomys glareolus*). *Molecular Ecology*, 14, 1727–1739.

DeLattin G (1967) Grundriss der Zoogeographie. Fischer, Stuttgart.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7, 214.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology,* 4, 88.

Drummond AJ, Rambaut A, Shapiro B, Pybuss OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution,* 22, 1185-1192.

Duelli P (1997) Biodiversity evaluation in agricultural landscapes: an approach at two different scales. *Agriculture, Ecosystystems & Environment*, 62, 81–91.

Duncan RP, Cassey P, Blackburn TM (2009) Do climate envelope models transfer? A manipulative test using dung beetle introductions. *Proceedings of the Royal Society of London B*, 276, 1449-1457.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology,* 11, 2571-2581.

Engelhardt CHM, Pauls SU, Haase P (2008) Population genetic structure of the caddisfly *Rhyacophila pubescens*, Pictet 1834, north of the Alps. *Fundamental and Applied Limnology — Archiv für Hydrobiologie*, 173, 165-176.

Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE, Yates CJ (2011) A statistical explanation of MAxEnt for ecologists. *Diversity and Distributions*, 17, 43–57.

Elith J, Leathwick JR (2009) Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution and Systematics*, 40, 677–697.

Elliot JM (2003) A comparative study of the dispersal of 10 species of stream invertebrates. *Freshwater Biology*, 1652–1668.

Engelhardt C, Pauls SU, Haase P (2008) Population genetic structure of the caddisfly *Rhyacophila pubescens*, Pictet 1834, north of the Alps. *Fundamental and Applied Limnology — Archiv für Hydrobiologie*, 173: 165-176.

Estoup A, Taillez C, Cornuet JM, Solignac M (1995)Size homoplasy and mutational processes of interrupted microsatellites in two bee species, Apis mellifera and Bombus terrestris (Apidae). *Molecular Biology and Evolution*, 12, 1074-1084.

EU Commission (2010) http://ec.europa.eu/environment/nature/index_en.htm. Accessed 18 March 2010

European Environment Agency (2010) http://www.eea.europa.eu/de. Accessed 18 March 2010

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1, 47-50.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial-DNA restriction data. *Genetics*, 131, 479–491.

Faber JE, Rybka J, White MM (2009) Intraspecific phylogeography of the stonecat madtom, *Noturus flavus*. *Copeia*, 3, 563-571.

Felsenstein J (2008) PHYLIP (Phylogeny inference package) version 3.68. Available at http://evolution.genetics.washington.edu/phylip/software.html. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.Fiedler PL, Jain SK (1992) Conservation Biology: The Theory and Practice of Nature Conservation, Preservation and Management. Chapman and Hall, New York.

Finn, D, Blouin, MS, Lytle, DA (2007) Population genetic structure reveals terrestrial affinities for a headwater stream insect. *Freshwater Biology*, 52, 1881–1897.

Flecker AS, Allan JD (1988) Flight direction in some Rocky Mountain mayflies (Ephemeroptera), with observations of parasitism. *Aquatic Insects*, 10, 33– 42.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* 3, 294-299.

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge.

Freeland JR (2005) Molecular Ecology. John Wiley & Sons, Hoboken, USA.

Fu Y (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147, 915-925.

Galbreath KE, Hafner DJ, Zamudlo KR (2009) When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (american pika, *ochotona princeps*). *Evolution,* 63, 2848–2863

GBIF-Sweden (2010) GBIF Data Portal. http://data.gbif.org. Accessed 18 March 2010.

Gehrken U, Sømme L 1987. Increased cold hardiness in eggs of *Arcynopteryx compacta* (Plecoptera) by dehydration. *Journal of Insect Physiology*, 33, 987–991.

Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology,* 17, 167–178.

Glaubitz JC (2004) Convert: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes,* 4, 309-310.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, 395, 202–222.

Godinho R, Crespo EG, Ferrand N (2008) The limits of mtDNA phylogeography: complex patterns of population history in a highly structured Iberian lizard are

only revealed by the use of nuclear markers. *Molecular Ecology*, 17, 4670–4683.

Graf W, Lorenz A, de Figueroa JMT, Lücke S, López-Rodríguez MJ, Davies CE (2009) Plecoptera. *In*: Schmidt-Kloiber A, Hering D (eds.) Distribution and Ecological Preferences of European Freshwater Organisms. *Pensoft Publishers, Sofia-Moscow 2009*, 2, 262.

Graf W, Murphy J, Dahl J, Zamora-Muñoz C, López-Rodrguez MJ (2008) Trichoptera. *In*: Schmidt-Kloiber A (ed.) Distribution and Ecological Preferences of European Freshwater Organisms. *Pensoft Publishers, Sofia-Moscow 2008*, 1, 388.

Graf W, Schultz H, Janececk B (2004) Ökofaunistische Erhebungen und Bewertung im Natura 2000-Gebiet St. Lorenzener Hochmoor, Makrozoobenthos Endbericht.

Graf W, Grasser U, Weinzierl A (1995): Plecoptera. *In*: Moog O. (Ed.): Fauna Aquatica Austriaca, Lieferung Mai/95, Wasserwirtschaftskataster, Bundesministerium für Land- und Forstwirtschaft, Wien.

Graham CH, Ferrier S, Huettman F, Moritz C, Townsend Peterson A (2004) New developments in museum-based informatics and applications in biodiversity analysis. *Trends in Ecology and Evolution*, 19, 497–503.

Griffith MB, Barrows EM, Perry SA (1998) Lateral dispersal of adult insects (Plecoptera, Trichoptera) following emergence from headwater streams in forested Appalachian catchments. *Annals of the Entomological Society of America,* 91,195–201.

Guillot G, Mortier F & Estoup A (2005) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, 5, 712–715.

Gyllensten U (1985) Temporal allele frequency changes in density fluctuating populations of willow grouse (*Lagopus lagopus* L.). *Evolution*, 39, 115-121.

Haas F, Brodin A. (2005) The crow *Corvus corone* hybrid zone in southern Denmark and northern Germany. *Ibis*, 147, 649–656.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Harte J, Ostling A, Green JL, Kinzig A (2004) Climate change and extinction risk. *Nature* doi:10.1038/nature02718.

Haybach A (2003) Zoogeographische Aspekte der Eintagsfliegenbesiedlung Deutschlands (Insecta, Ephemeroptera). Verhandlungen der westdeutschen Entomologentagung Düsseldorf, 187–209.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B*, 270, 313–322.

Heilveil JS, Berlocher SH (2006) Phylogeography of postglacial range expansion in *Nigronia serricornis* Say (Megaloptera: Corydalidae). *Molecular Ecology,* 15, 1627–1641.

Hendrey GR, Wright RF (1976) Acid percipitation in Norway: Effects on aquatic fauna. J*ournal of Great Lakes Research,* 2, 192–207.

Hering D, Schmidt-Kloiber A, Murphy J, Lücke S, Zamora-Muñoz C, López Rodríguez MJ, Huber T, Graf W (2009) Potential impact of climate change on aquatic insects: a sensitivity analysis for European caddisflies (Trichoptera) based on distribution patterns and ecological preferences. *Aquatic Sciences*, 71, 3-14.

Hewitt GM (2004) The structure of biodiversity – insights from molecular phylogeography. *Frontiers in Zoology*, 1, 4.

Hewitt G.M. (2000) The genetic legacy of the Quarternary ice ages. *Nature*, 405, 907–913.

Hewitt GM (1999) Post-glacial re-colonization of European Biota. *Biological Journal of the Linnean Society*, 68, 87–112.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, 58, 247–276.

Hickerson MJ, Carstens BC, Cavender-Bares J, Crandall KA, Graham CH, Johnson JB, et al. (2010) Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution*, 54, 291–301.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal on Climatology, 25, 1965–1978.

Hoarau G, Coyer JA, Veldsink JH, Stam WT, Olsen JL (2007) Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. *Molecular Ecology,* 16, 3606–3616.

Hoelzel E (1967) Die Fauna des Hochmoores von St. Lorenzen in den Gurker Alpen. Carinthia 195–211.

Hoffmann AA, Willi Y (2008) Detecting genetic responses to environmental change. *Nature Reviews Genetics*, 9, 421–432.

Hoffman JI, Amos W (2005) Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Molecular Ecology*, 14, 599-612.

Holdhaus K (1954) Die Spuren der Eiszeit in der Tierwelt Europas. Abh zool-bot Ges Wien, 18,1–493.

Hopkins DM, Matthews Jr JV, Schweger CE, Young SB (1982.) Paleoecology of Beringia. Academic Press, New York.

Houghton J (2001) The science of global warming. *Interdisciplinary Science Review*, 26, 247–257.

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. *Ecology Letters*, 11, 609–623.

Ihaka R, Gentleman R (1996) R: A Language for Data Analysis and Graphics. J Comp Graph *Stat,* 5, 299-314.

Illies J (1955) Steinfliegen oder Plecoptera. In: Dahl F (Ed.): Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise. Jena.

Jackson JK, Resh VH (1992) Variation in genetic structure among populations of the caddisfly Helicopsyche borealis from three streams in northern California, U.S.A. *Freshwater Biology*, 27, 29–42.

Jakob SS, Martinez-Meyer E, Blattner FR (2009) Phylogeographic analyses and paleodistribution modeling indicate Pleistocene *in situ* survival of *Hordeum* species (Poaceae) in southern Patagonia without genetic or spatial restriction. *Molecular Biology and Evolution*, 26, 907-923.

Jacob U (1979) Die Ephemeropterenfauna aus zoogeographischer Sicht. In: Pasternak K, Sowa R (eds) *Proceedings of the Second International Conference on Ephemeroptera*, 21–26.

Kalinowski ST (2004) Counting alleles with rarefaction: private alleles and hierarchical sampling designs. *Conservation Genetics*, 5, 539–543.

Kelly LC, Bilton DT, Rundle SD (2001) Genetic differentiation and dispersal in the Canary Island caddisfly *Mesophylax aspersus* (Trichoptera: Limnephilidae). *Heredity*, 86, 370–77.

Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population, *PNAS*, 75, 2868-2872.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics*, 49, 725-738.

Knopp T, Merilä J (2008) The postglacial recolonization of Northern Europe by Rana arvalis as revealed by microsatellite and mitochondrial DNA analyses. *Heredity*, 102, 174–181.

Knowles LL, Carstens BC, Keat ML (2007) Coupling Genetic and Ecological-Niche Models to Examine How Past Population Distributions Contribute to Divergence. *Current Biology,* 17, 940–946.

Kontula T, Väinölä R (2001) Postglacial colonization of Northern Europe by distinct phylogeographic lineages of the bullhead, *Cottos gobio*. *Molecular Ecology*, 10, 1983-2002.

Kovats ZE, Ciborowski JJH, Corkum LD. (1996) Inland dispersal of adult aquatic insects. *Freshwater Biology*, 36, 265–76.

Kozak KH, Wiens JJ (2006) Does niche conservatism promote speciation? a case study in north american salamanders. *Evolution*, 60, 2604–2621.

Kubow KB, Robinson CT, Shama LNS, Jokela J (2010) Spatial scaling in the phylogeography of an alpine caddisfly, *Allogamus uncatus*, within the central European Alps. *Evolutionary Approaches in Benthic Ecology*, 29, 1089-1099.

Leathwick JR, Elith J, Chadderton WL, Rowe D, Hastie T (2008) Dispersal, disturbance and the contrasting biogeographies of New Zealand's diadromous and non-diadromous fish species. *Journal of Biogeography*, 35, 1481–1497.

Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology*, 11, 2445–2449.

Lehrian S, Bálint M, Haase P, Pauls SU (2010) Genetic population structure of an autumn emerging caddisfly with inherently low dispersal capacity and insights into its phylogeography. *Journal of the North American Benthological Society*, 29, 1100-1118.

Lehrian S, Pauls SU, Haase P (2009) Contrasting patterns of population structure in the montane caddisflies *Hydropsyche tenuis* and *Drusus discolor* in central European highlands. *Freshwater Biology,* 54, 283-295.

Lillehammer A (1988) Stoneflies (Plecoptera) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavica*, 21, Leiden.

Lillehammer A (1974): Norwegian Stoneflies. II. Distribution and relationship to the environment. *Norsk entomological Tidsskr*, 21, 195-250.

Lisiecki LE, Raymo ME (2006) Plio–Pleistocene climate evolution: trends and transitions in glacial cycle dynamics. *Quaternary Science Reviews*, 26, 56-69.

Lisiecki LE, Raymo ME (2005) A Pliocene-Pleistocene stack of 57 globally distributed benthic d18O records. *Paleoceanography,* 20, PA1003.

Lobo JM, Verdú JR, Numa C (2006) Environmental and geographical factors affecting the Iberian distribution of flightless *Jekelius* species (Coleoptera: Geotrupidae). *Diversity and Distributions*, 12, 179–188.

Lundqvist J, Mejdahl V (1995) Luminescence dating of the deglaciation in northern Sweden. *Quaternary international: the journal of the International Union for Quaternary Research*, 28, 193-197.

Mäkinen HS, Cano MS, Merilä J (2006) Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. *Molecular Ecology*,15, 1519-1534.

Malicky H (2006) Mitteleuropäische (extra-mediterrane) Arealkerne des Dinodal am Beispiel von Köcherfliegen (Trichoptera). *Beiträge zur Entomologie*, 56, 347–359.

Malicky H (2000) Arealdynamik und Biomgrundtypen am Beispiel der Köcherfliegen (Trichoptera). *Entomologica Basiliensia*, 22, 235-259.

Malicky H (1988) Spuren der Eiszeit in der Trichopterenfauna Europas. *Rivista di Idrobiologia*, 27, 247-297

Malicky H. (1983) Chorological patterns and biom types of European Trichoptera and other freshwater insects. *Archiv für Hydrobiologie,* 96, 223–244.

Malmqvist B (2000) How does wing length relate to distribution patterns of stoneflies (Plecoptera) and mayflies (Ephemeroptera)? *Biological conservation*, 93, 271-276.

Malzacher P, Jacob U, Haybach A, Reusch H (1998) Rote Liste der Eintagsfliegen (Ephemeroptera). In: Binot M, Bless R, Boye P, Gruttke H, Pretscher P (ed) Rote Liste gefährdeter Tiere Deutschlands, Bundesamt für Naturschutz (BfN), Bonn-Bad Godesberg, Germany, pp 264–267.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27, 209-220.

Martinez-Meyer E, Peterson AT (2006) Conservatism of ecological niche characteristics in North American plant species over the Pleistocene-to-Recent transition. *Journal of Biogeography*, 33, 1779–1789.

Martinez-Meyer E, Peterson AT, Hargrove WW (2004) Ecological niches as stable distributional constraints on mammal species, with implications for Pleistocene extinctions and climate change projections for biodiversity. *Global Ecology and Biogeography*, 13, 305–314.

McNeely JA, Miller KR, Reid WV, Mittermeier RA, Werner TB (1990) Conserving the world's biological diversity. World Conservation Union, World Resources Institute, Conservation International, World Wildlife Fund–US, and the World Bank, Washington, DC.

Mikko S, Andersson L (1995) Low major histocompatibility complex class II diversity in European and North American moose. *Proceedings of the National Academy of Sciences USA*, 92, 4259-4263.

Moussalli A, Moritz C, Williams SE, Carnaval AC (2009) Variable responses of skinks to a common history of rainforest fluctuation: concordance between phylogeography and palaeo-distribution models. *Molecular Ecology*, 18, 483–499.

Muster C, Berendonk TU (2006) Divergence and diversity: lessons from an arctic-alpine distribution (*Pardosa saltuaria* group, Lycosidae). *Molecular Ecology*, 15, 2921–2933.

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution*, 19, 153–170.

Nesbø CL. Fossheim T, Vollestad LA, Jakobsen KS (1999) Genetic divergence and phylogeographic relationships among european perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. *Molecular Ecology*, 8, 1387-1404.

OTA (US Congress Office of Technology Assessment) (1987) Technologies to Maintain Biological Diversity. US Government Printing Office, Washington, DC

Palmé AE, Su Q, Rautenberg A, Manni F, Lascoux M (2003) Postglacial recolonization and cpDNA variation of silver birch, *Betula pendula*. *Molecular Ecology*, 12, 201-212.

Palumbi SR (1996) Nucleic Acids II: The polymerase chain reaction. *In:* Molecular Systematics, Sinauer Associates: MA, 205-247.

Pamilo P, Nei M (1988) Relationship between gene trees and species trees. *Molecular Biology and Evolution*, 5, 568-583.

Pamilo P, Savolainen O (1999) Postglacial colonization, drift, local selection and conservation value of populations: a northern perspective. *Hereditas*, 130, 229-238.

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature,* 421, 37–42.

Pauls SU, Theissinger K, Ujvarosi L, Bálint M, Haase P (2009): Patterns of population structure in two closely related, partially sympatric caddisflies in Eastern Europe: historic introgression, limited dispersal, and cryptic diversity. *Journal of North American Benthological Society*, 28, 517–536.

Pauls SU, Feldheim KA, Haase P (2007) Isolation and characterization of microsatellite markers in the caddisfly *Drusus discolor* (Trichoptera: Limnephilidae). *Molecular Ecology Notes*, 7, 150-152.

Pauls SU, Lumbsch T, Haase P (2006) Phylogeography of the montane caddisfly Drusus discolor: evidence for multiple refugia and periglacial survival. *Molecular Ecology*, 15, 2153–2169.

Peterson AT, Vieglais DA (2001) Predicting Species Invasions Using Ecological Niche Modeling: New Approaches from Bioinformatics Attacka Pressing Problem. *BioScience,* 51, 363-371.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, 12, 844–855.

Pfenninger M, Cordellier M, Streit B (2006) Comparing the efficacy of morphologic and DNA-based taxonomy in the freshwater gastropod genus Radix (Basommatophora, Pulmonata*). BMC Evolutionary Biology*, 6, 100.

Pfenninger M, Nowak C, Magnin F (2007) Intraspecific range dynamics and niche evolution in Candidula land snail species. *Biological Journal of the Linnean Society*, 90, 303–317.

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropymodeling of species geographic distributions. *Ecological Modeling*, 190, 231–259.

Phillips SJ, Dudík M, Elith J, Graham CH, Lehmann A, Leathwick J, Ferrier S (2009) Sample selection bias and presence-only distribution models: implications for background and pseudo-absence data. *Ecological Applications*, 19,181–197.

Pinceel J, Jordaens K, Pfenninger M, Backeljau T (2005) Rangewide phylogeography of a terrestrial slug in Europe: evidence for Alpine refugia and rapid colonization after the Pleistocene glaciations. *Molecular Ecology*, 14, 1133–1150.

Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, 25, 1253-1256.

Rambaut A, Drummond AJ (2007) Tracer v1.5, Available from http://beast.bio.ed.ac.uk/Tracer, accessed on 10.11.2010

Raymond M, Rousset F (2004) GENEPOP on the web. http://wbiomed. curtin.edu.au/genepop/index.html.

Raymond M, Rousset F (1995a) An exact test for population differentiation. *Evolution*, 49, 1280-1283.

Raymond M, Rousset F (1995b) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248-249.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology*, 17, 230–237.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, 43, 223–225.

Richards CL, Carstens BC, Knowles LL (2007): Distribution modelling and statistical phylogeography: an integrative framework for generating and testing alternative biogeographical hypotheses. *Journal of Biogeography,* 34, 1833–1845.

Roderick GK (1996) Geographic structure of insect populations: Gene flow, phylogeography, and their uses. *Annual Review of Entomolgy*, 41, 325-352.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, 9, 552–569.

Rosenberg DM, Resh VH (1993) Freshwater biomonitoring and benthic invertebrates. Chapman & Hall, New York.

Rowe KC, Heske EJ, Brown PW, Paige KN (2004) Surviving the ice: Northern refugia and postglacial colonization. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 10355-10359.

Russev B, Vidinova Y (1994) Distribution and ecology of the representatives of some families of order Ephemeroptera (Insecta) in Bulgaria. *Lauterbornia,* 19, 107–113.

Santucci, F., Emerson, B.C., Hewitt, G.M. (1998) Mitochondrial DNA phylogeography of European hedgehogs. *Molecular Ecology*, 7, 1163-1172.

SAS Institute Inc. (2007) *JMP User Guide.* Cary, NC: SAS Institute Inc.

Schmitt T, Muster C, Schönswetter P (2010) Are disjunct alpine and arctic-alpine animal and plant species in the western Palearctic really “relics of a cold past”? In: Habel JC and Assmann T (eds.) *Relict Species: Phylogeography and Conservation Biology*. 239-252. Springer-Verlag Berlin.

Schmitt T (2009) Biogeographical and evolutionary importance of the European high mountain systems. *Frontiers in Zoology*, 6, 9.

Schmitt T (2007) Molecular biogeography of Europe: pleistocene cycles and postglacial trends. *Frontiers in Zoology*, 4, 11.

Schmitt T, Hewitt GM, Müller P (2006) Disjunct distributions during glacial and interglacial periods in mountain butterflies: *Erebia epiphron* as an example. *Journal of Evolutionary Biology,* 19, 108–113.

Schmitt T, Seitz A (2001) Allozyme variation in *Polyommatus coridon* (Lepidoptera: Lycaenidae): identification of ice-age refugia and reconstruction of post-glacial expansion. *Journal of Biogeography*, 28, 1129-1136.

Schönswetter P, Stehlik I, Holderegger R, Tribsch A (2005) Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molecular Ecology*, 14, 3547-3555.

Schuelke M (2000) An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology*, 18, 233–234.

Schultheis AS, Weigt LA, Hendricks AC (2002) Gene flow, dispersal, and nested clade analysis among populations of the stonefly *Peltoperla tarteri* in the southern Appalachians. *Molecular Ecology*, 11, 317-327.

Schultz H, Janecek B, Hess M, Reusch H, Graf W (2004) Das Makrozoobenthos des Natura 2000 Gebietes St. Lorenzener Hochmoor (Andertal, Kernten) unter

besonderer Berücksichtigung der Libellenfauna (Insecta: Odonata). *Carinthia II*, 196, 343–358.

Scott KD, Lawrence N, Lange CL, Scott LJ, Wilkinson KS, Merritt MA, Miles M, Murray D, Graham GC (2005) Assessing moth migration and population structuring in *Helicoverpa annigera* (Lepidoptera : Noctuidae) at the regional scale: Example from the Darling Downs, Australia. *Journal of Economic Entomology*, 98, 2210-2219.

Shepard DB, Burbrink FT (2008) Lineage diversification and historical demography of a sky island salamander, *Plethodon ouachitae*, from the Interior Highlands. *Molecular Ecology,* 17, 5315–5335.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, 139, 457–462.

Soldán T, Enktaivan S, Godunko RJ (2009) Commented checklist of mayflies (Ephemeroptera) of Mongolia. *Aquatic Insects*, 31, 653 – 670.

Stewart JR, Lister AM (2001) Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology & Evolution*, 16, 608-613.

Stewart KW, Ricker WE (1997) Stoneflies of the Yukon. *In*: Danks HV, Downes JA (eds) Insects of the Yukon. Biological Survey of Canada (Terrestrial Arthropods), Ottawa, 201 – 222.

Taberlet P (1998a) Biodiversity at the intraspecific level: The comparative phylogeographic approach. *Journal of Biotechnology*, 64, 91–100.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF (1998b) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, 7, 453–464.

Taberlet P, Swenson JE, Sandegren F, Bjärvall A (1995) Localization of a contact zone between two highly divergent mitochondrial DNA lineages of the brown bear (*Ursus arctos*) in Scandinavia. *Conservation Biology*, 9, 1255-1261.

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123, 585-595.

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics,* 144, 389–399.

Taubmann J, Theissinger K, Feldheim KA, Laube I, Graf W, Haase P, Johannesen J, Pauls SU (2011*)* Modelling range shifts and assessing genetic diversity distribution of the montane aquatic mayfly *Ameletus inopinatus* in Europe under climate change scenarios. *Conservation Genetics,*12, 503-515.

Theissinger K, Taubmann J, Feldheim KA, Laube I, Bálint M, Johannesen J, Haase P, Pauls SU (*submitted*) Species distribution models and molecular data reveal the Pleistocene history of the cold-adapted mayfly *Ameletus inopinatus* (Ephemeroptera: Siphlonuridae) in Europe. *Freshwater Biology*.

Theissinger K, Feldheim KA, Taubmann J, Seitz A, Pauls SU (2008) Isolation and characterization of 10 higly polymorphic di- and trinucleotide microsatellite markers in the mayfly *Ameletus inopinatus* (Ephemeroptera: Siphlonuridae). Mol Ecol Resour 8:1285–1287

Theissinger K, Feldheim KA, Seitz A, Pauls SU (2009) Isolation and characterization of 11 polymorphic trinucleotide microsatellite markers in the stonefly *Arcynopteryx compacta* (Plecoptera: Perlodidae). *Molecular Ecology Resources*, 9, 357–359.

Thienemann A (1950) Verbreitungsgeschichte der Süßwassertierwelt Europas. *Die Binnengewässer*, 18, 1–541.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC et al (2004) Extinction risk from climate change. *Nature*, 427, 145–147.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Research*, 22, 4673-4680.

Vandewoetijne S, van Dyck H (2010) Population Genetic Differences along a Latitudinal Cline between Original and Recently Colonized Habitat in a Butterfly. *PlosONE*, 5, e13810.

Van Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4, 535–538.

Varga ZS, Schmitt T (2008) Types of oreal and oreotundral disjunctions in the Western Palearctic. *Biological Journal of the Linnean Society*, 93, 415–430.

Waltari, E., Hijmans, R.J., Peterson, A.T., Nyári, A.S., Perkins, S.L., Guralnick, R.P. (2007): Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *PLoS ONE*, 7, 563.

Weir BS (1996) *Genetic Data Analysis II*. Sinauer Associates, Sunderland, Massachusetts.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, 38, 1358–1370.

Wielgoss S, Taraschewski H, Mexer A, Wirth T (2008) Population structure of the parasitic nematode *Anguillicola crassus*, an invader of declining North Atlantic eel stocks. *Molecular Ecology*, 17, 3478–3495.

Wilcock HR, Bruford MW, Nichols RA, Hildrew AG (2007) Landscape, habitat characteristics and the genetic population structure of two caddisflies. Freshwater Biology, 52, 1907–1929.

Wright JF, Sutcliffe DW, Furse MT (editors) (2000) Assessing the biological quality of fresh waters: RIVPACS and other techniques. Freshwater Biological Association, Ambleside, Cumbria, UK.

Zink RM, Barrowclough GF (2008) Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, 17, 2107-2121.

CURRICULUM VITAE

Kathrin Theißinger-Theobald

Institute for Zoology
Department of Ecology
Johannes Gutenberg-University Mainz
J.-J.-Becherweg 13
55128 Mainz
Germany

Email: theissinger@uni-landau.de
Tel.: 0049 6341 28031329
Fax: 0049 6341 28031326

since 04/11	Research Assistant at the Institute for Environmental Science, University Koblenz-Landau
07/07 – 03/11	PhD at the Johannes Gutenberg-University, Mainz, Germany, in cooperation with the Senckenberg Research Institute, Department of Limnology and Conservation, Germany
09/06-06/07	Researcher at the Senckenberg Research Institute, Department of Limnology and Conservation, Germany
08/06	Diploma in biology, Johannes Gutenberg-University of Mainz, Germany
11/04 – 10/05	Master Thesis at the University of Canberra, Australia
10/00 – 10/04	Biological studies at the Johannes Gutenberg-University, Mainz, Germany