Preliminary results of the phylogeography of the montane caddisfly *H. tenuis* and a comparison of its population structure with the one of *D. discolor*

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Abstract

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In this study we compared mitochondrial sequence data (COI) to infer the population structure of the two montane caddisflies *Hydropsyche tenuis* and *Drusus discolor*. The two species represent different models of montane aquatic insects: *D. discolor* is restricted to elevations above 600m, *Hydropsyche tenuis* is limited to the same mountain ranges in central Europe but inhabits much lower elevations. Thus the two represent different models for montane insects. To determine the basic population structure of both species conventional population genetics analyses were applied to mitochondrial sequence data. We collected and sampled 121 specimens of *H. tenuis* from 29 sites in 10 different regions of the central European highlands and 138 individuals of *D. discolor* from 40 sites in 11 different regions. Nine unique haplotypes were identified for *H. tenuis* and 34 haplotypes for *D. discolor*. We observed unexpected differences comparing the population structure of this two montane caddisflies.

Keywords: population structure, mitochondrial DNA, montane caddisflies, *Hydropsyche tenuis, Drusus discolor*

Introduction

In central Europe the legacy of the Pleistocene ice ages and their effects on the European biota have been subject to much examination over the last few decades. Many studies have focussed on examining population genetic structure using a variety of fingerprinting methods to infer the Pleistocene history of European species (see Hᴇᴡɪᴛᴛ 2004a, b, Sᴄʜᴍɪᴛᴛ 2007 for recent reviews). Although the patterns of species response to glacial cooling are currently only understood for individual species, there is accordance that most temperate species currently occurring in central Europe survived glaciations in one or more refugia in more favourable climates in southern Europe. During ice free periods like interstadials, interglacials or postglacial times, the recolonisation of central Europe started from these refugia (e.g. Hᴇᴡɪᴛᴛ 1996, 2000, 2004a, Zᴡɪᴄᴋ 1981, Bᴇʀɴᴀᴛᴄʜᴇᴢ & Wɪʟsᴏɴ 1998, Sᴄʜᴍɪᴛᴛ & Sᴇɪᴛᴢ 2001, Hᴀ̈ɴꜰʟɪɴɢ ᴇᴛ ᴀʟ. 2002, Bᴜɴᴊᴇ 2005, Pᴀᴜʟs ᴇᴛ ᴀʟ. 2006). In this study, we will compare the signals from mitochondrial sequence data to infer the population structure of aquatic insects using two montane caddisflies, *Hydropsyche tenuis* (Navas, 1932) (Trichoptera: Hydropsychidae, Hydropsychinae) and *Drusus discolor* (Rambur, 1842) (Trichoptera: Limnephilidae, Drusinae) as our models. *H. tenuis* is distributed across the central Euro-

pean mountain ranges and the Apennine Mountains whereas the distribution of *D. discolor* extends further East and West (Pɪᴛsᴄʜ 1993, Pᴀᴜʟs 2004, Fauna Europaea Web Service 2004). Both species are cold-resistent (Pɪᴛsᴄʜ 1993, Lᴀᴠᴀɴᴅɪᴇʀ 1992) and intolerant to pollution. This makes them excellent biological indicators of water quality (Pɪᴛsᴄʜ 1993, Hᴀᴀsᴇ 1999, Wᴀʀɪɴɢᴇʀ & Gʀᴀꜰ 1997). Both species are montane, being distributed across mountain ranges with elevations exceeding 800 m asl (Hᴀᴀsᴇ 1999), and therefore show an insular distribution pattern. They are character species of montane silicious cobble-bottom streams (Hᴀᴀsᴇ 1999). *Hydropsyche* larvae are netspinners and build silk retreats and filtering nets. *Drusus* larvae on the other hand show case-making behaviour (Pɪᴛsᴄʜ 1993, Wɪᴄʜᴀʀᴅ ᴇᴛ ᴀʟ. 1995, Wᴀʀɪɴɢᴇʀ & Gʀᴀꜰ 1997). *H. tenuis* shows a more varied feeding behavior (filtering: 50%; predatory: 30%; grazing: 20%) than *D. discolor* (grazeing: 10%; filtering predator: 90%) (Gʀᴀꜰ et al.1995).

One aim of this study is to examine whether the insular distribution pattern of *H. tenuis* results in detectable population differentiation as is the case in *D. discolor* (Pᴀᴜʟs et al. 2006). Both examined species exhibit differences but also commonalities with regards to their ecology and local distribution. These drive the second question our study aims

to answer: Do both species have a similar population structure due to their commonalities or is the population structure vastly different? To determine the basic population structure of *H. tenuis* and *D. discolor* conventional population genetics analyses were applied to mitochondrial sequence data for both species.

Materials and Methods

We collected and sampled 121 specimens of *H. tenuis* from 29 sites in 10 different regions of the central European highlands. Specimens were collected using light traps or hand nets and preserved in 70-96% EtOH until DNA-Extraction. We identified specimens to species rank using PITSCH (1993), WARINGER & GRAF (1997) and NEU & TOBIAS (2004) for larvae and MALICKY (2004) for adult males. To compare the population structure of *H. tenuis* with that of *D. discolor*, we analysed a subset of sequence data from approximately the same geographic range that we sampled for *H. tenuis* of previously published data of *D. discolor* (PAULS ET AL. 2006). We analysed the homologous 498bp long fragment of mtCOI sequence data for both species. The subset for *D. discolor* comprised 138 individuals from 40 sites in 11 different regions in central Europe.

We extracted total genomic DNA from legs, thorax or abdomen using DNEasy Blood & Tissue Kit (Qiagen) and the QIAmp Microkit (Qiagen) following the protocol for purification of total DNA from insects. A homologous fragment of mitochondrial DNA encoding for cytochrome oxidase I (mtCOI) was amplified by PCR. PCR primers were Dave and Inger (ZHANG & HEWITT 1996) for *H. tenuis*. PCR amplification was performed in 25μl reactions contained one Ready-To-Go PCR Bead (GE Healthcare) 0.8μM of each primer and 2μl to 8μl DNA. PCR cycling conditions were: 5min at 95°C; 36 cycles of 1min at 95°C, 1min at 50°C, 2min at 72°C; and a final extension of 7min at 72°C. Laboratory procedures for *D. discolor* are described in PAULS ET AL. (2006). Sequences were generated by two commercial sequencing companies, GATC Ltd. and Nano+BioCenter Kaiserslautern, Germany, using the PCR primers. ABI traces were aligned and manually checked and edited in Seqman 4.0 (Lasergene). The identity of sequences was verified using blast search (ALTSCHUL ET AL. 1997). Sequences were aligned using Clustal W (THOMPSON ET AL. 1994) as implemented in Bioedit 7.0.0 (HALL 1999). Alignment parameters were default.

We generated a haplotype matrix of 121 sequences of *H. tenuis* and 138 sequences of *D. discolor* using DNAsp 4.10.9 (ROZAS ET AL. 2003). For both species a median joining network was calculated in Network 4.2.0.1 using the default settings (Fluxus Technoloy 2004-2006).
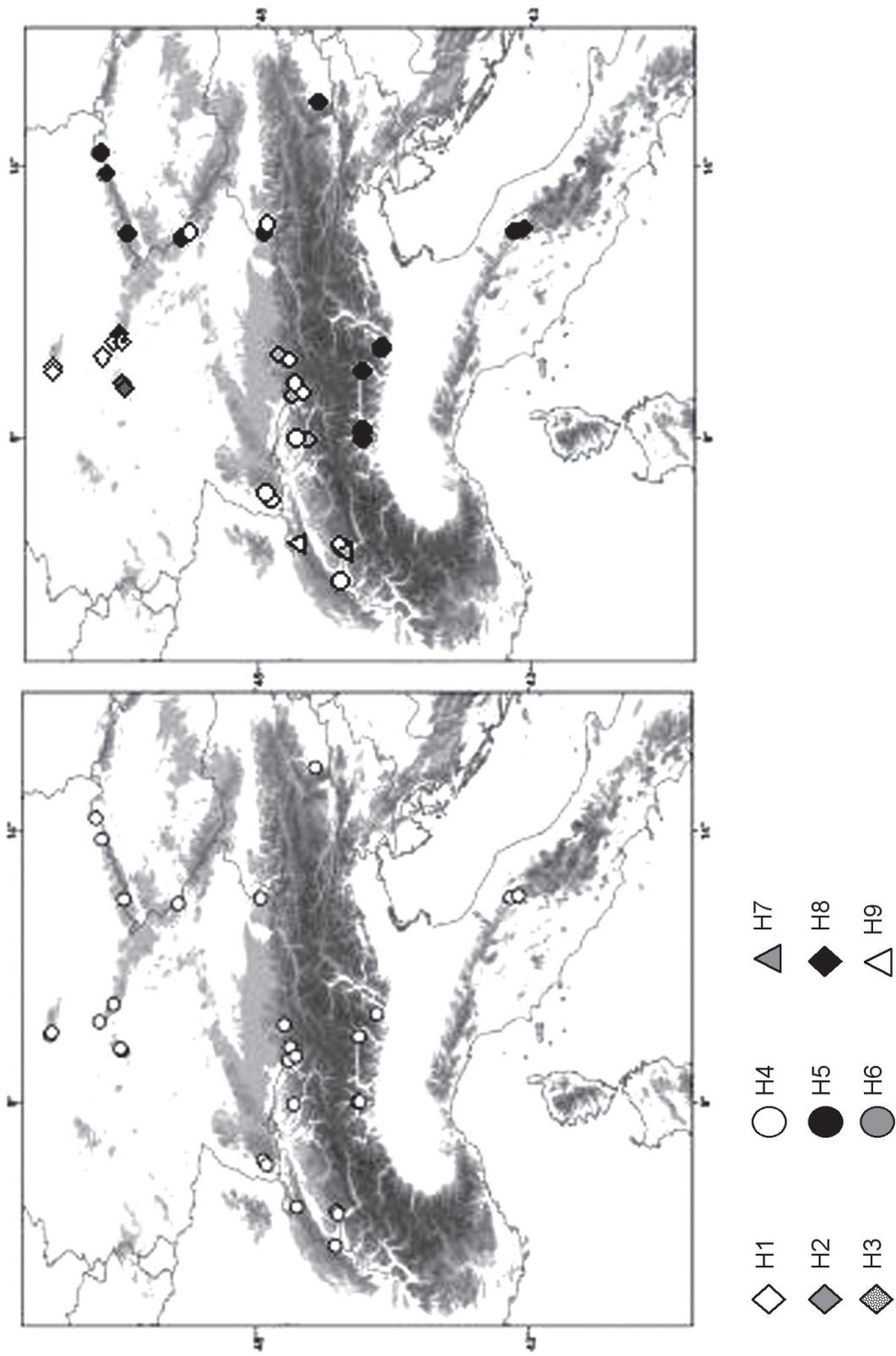
Results

We generated new mtCOI sequences for 121 *H. tenuis* specimens. These were aligned into a 498bp alignment. A homologous alignment was generated for 138 *D. discolor* specimens from a comparable geographic range. The alignments did not contain ambiguous sites or gaps. Eight variable positions were found for *H. tenuis*, 55 for *D. discolor*.

Nine unique haplotypes were identified for *H. tenuis*, 34 haplotypes for *D. discolor*. The maximum difference between all haplotypes is 0.8% (4 bp) for *H. tenuis* and 4.2% (21 bp) for *D. discolor*.

A median-joining network was calculated for each of the two species. For *H. tenuis* one mutational step between haplotypes is sufficient to combine all haplotypes, while for *D. discolor* up to twelve steps are necessary. We observed haplotype overlap between geographic regions for both species. The most common haplotype of *H. tenuis* H5 was found in every mountain range from the studied regions except for the Northern German Highlands and the Apennine Mountains (Fig. 1). The second most common haplotype of *H. tenuis* H4 was found in the Alps, Apennine Mountains and the Bohemian Massif. In the Northern populations (Harz, Rhoen and Thuringian Forest) there were four haplotypes H1, H2, H3 and H8 that show no overlap with southern regions. In the Rhoen there is one endemic haplotype of *H. tenuis*, H2. In the Thuringian Forest we observed two endemic haplotypes, H3 and H8 whereas in the Alps there were three endemic haplotypes, H6, H7 and H9.

We observed four haplotype groups (≥3 mutational steps divergence) in the *D. discolor* data set. Group 1 comprises 13 haplotypes and individuals from the Alps, Bohemian Massif, Erzgebirge and Fichtelgebirge. Group 2 represents a second, predominantly western Alpine lineage, which is quite diverged (≥11 mutational steps). Group 3 comprises 10 haplotypes and individuals from the Erzgebirge, Fichtelgebirge, Harz, Rhoen, Rothaargebirge and Thuringian Forest. Group 4 comprises 7 haplotypes from the Black Forest, Jura Mts and Vosges Mts. The number of haplotypes per region ranged from one in Jura Mts to 13 in the Alps. In total 30 *D. discolor* haplotypes (88%) and six *H. tenuis* haplotypes (66%) are endemic to a single region.

Fig.1. Collection sites of *H. tenuis* (left) and distribution of *H. tenuis* haplotypes (right).

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Discussion

Results of mtCOI sequence data show that the variability between haplotypes is very low in *H. tenuis* compared to that of *D. discolor*. However, population structure is detectable in both species. Clear patterns of genetic population structure due to limited gene flow are common in insects inhabiting high montane regions, or sky islands (KNOLL & ROWELL-RAHIER 1998; KNOWLES 2001; MARDULYN 2001).

All haplotypes differ from one another by one mutational step in *H. tenuis*. Hence the maximum difference between all haplotypes are four steps (0.8%). Compared to *D. discolor* (4.2%) and to another Hydropsychidae from New Zealand, *Orthopsyche fimbriata* (5.2%) the differences between haplotypes is very small in *H. tenuis*. In their study of *Orthopsyche fimbriata* SMITH ET AL. (2006) also investigated mtCOI, but a shorter section of the gene. In comparison to *D. discolor* and *O. fimbriata* one might have expected a higher level of divergence between haplotypes of *H. tenuis*. But another caddisfly with insular distribution pattern, *Rhyacophila pubescens* also shows strong population structure with small differences (0.84%) between haplotypes for almost the same fragment of mtCOI (pers. comm. Christine ENGELHARDT).

There are remarkable differences in the population structure of the two study species. There are different possible explanations why there are such big differences between the two montane species *H. tenuis* and *D. discolor*. These could e. g. be the result of different colonisation patterns in the past and/or different dispersal abilities. As with most caddisflies larval movement of *H. tenuis* and *D. discolor* is limited to upstream or downstream movement in the water course. Lateral dispersal between different streams or stream systems is presumably limited to a few kilometres at the winged adult stage as is the case for other caddisflies. Although our knowledge of dispersal capabilities of caddisflies is limited, most existing studies show that most adult aquatic insects tend to stay close to the stream from which they emerge (SODE & WIBERG-LARSEN 1993; KOVATS ET AL. 1996; COLLIER & SMITH 1998, PETERSEN ET AL. 2004). But MALICKY (1987) showed that the flight range of the montane *Hydropsyche saxonica* is a lot more than three kilometers.

A second reason for the differences in the patterns observed between *H. tenuis* and *D. discolor* could be varying Pleistocene survival strategies and postglacial recolonisation patterns. Haplotype distribution of *H. tenuis* shows a gradient from South to North. Such a clear gradient is not apparent in *D.* *discolor* haplotype distribution. There is one very common haplotype H5 for *H. tenuis* which is widespread over the central distribution and is only missing in the most northern (Rhoen, Thuringian Forest and Harz) and most southern (Apennine Mountains) populations. This could indicate that H5 is an ancestral haplotype and that the other eight haplotypes were derived from it. The limited divergence could result from a younger range expansion or a bottleneck. A population bottleneck is an evolutionary event in which a significant percentage of a population goes extinct or is otherwise lost from the gene pool (FREELAND 2005). A founder effect can occur if a small group of individuals, carrying only a small fraction of the original population's genetic variation becomes reproductively separated from the main population and acts as the main source for recolonising a large range (FREELAND 2005).

It is possible that *H. tenuis* was distributed in the recent past only in the central parts of its current distribution e.g. in the Central Alps and expanded from there. After the expansion into other montane regions, gene flow was limited between populations from different mountain ranges, allowing endemic haplotypes to evolve. With only single base pair changes and so few haplotypes, divergence time cannot be calculated with much credibility. However, it seems that the time since divergence is limited and insufficient for haplotypes to have diverged more than a single base pair from one another. In contrast to that scenario, PAULS et al. (2006) show that *D. discolor* did not follow a classic pattern of glacial persistence in only southern European refugia with postglacial re-colonisation of central Europe. Instead, *D. discolor* appears to have persisted in many different, isolated refugial populations over several glacial cycles throughout the Pleistocene (PAULS ET AL. 2006, Pauls ET AL. in press). The long-time persistence of isolated populations of *D. discolor* across much of its present-day range could be one reason for the different population structure of *D. discolor* and its larger differences between haplotypes. For *H. tenuis* it is also plausible that the todays distribution pattern is a result of expansion from one or few southern refugia potentially in the Apennine Mountains to the northern parts of its current distribution. As it is not possilbe to reliably calculate divergence times between lineages based on only one mutational step between haplotypes for *H. tenuis* we cannot test this hypothesis with the data at hand. For *D. discolor* the differentiation between the insular populations could be calculated and it seems to exist since well before the last glacial maximum (PAULS et al. 2006).

Another possibility for the very different population structure and the high differences between haplotypes of *D. discolor* is that individual populations of *D. discolor* may be diverged to such a degree that populations of *D. discolor* are no longer compatible and may represent subspecies or species.

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