Contrasting patterns of population structure in the montane caddisflies *Hydropsyche tenuis* and *Drusus discolor* in the Central European highlands

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**SUMMARY**

1. In this study, we compared mitochondrial sequence data (cytochrome oxidase I) to infer the population structure of the two montane caddisflies *Hydropsyche tenuis* and *Drusus discolor*. The two species are contrasting examples of montane aquatic insects with insular distributions: *D. discolor* is restricted to altitudes above 600 m, *H. tenuis* is limited to the same mountain ranges in Central Europe but inhabits lower altitudes.

2. In particular, we ask whether these two species with similar regional distributions show similar patterns of population structure and haplotype diversity, and whether any differences can be attributed to population history and/or autecology.

3. To determine the population structure of both species, we applied conventional population genetics analyses 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.

4. Nine unique haplotypes were identified for *H. tenuis* and 34 for *D. discolor*. There were eight variable positions in *H. tenuis* and 41 in *D. discolor*. The maximum difference between haplotypes was 0.8% (4 bp) for *H. tenuis* and 4.2% (21 bp) for *D. discolor*. We observed haplotype overlap between geographic regions for both species. Analysis of molecular variance showed that two-thirds of the total variance in *H. tenuis* was found among regions while in *D. discolor*, a larger portion of variance was found within regions and populations due to a higher number of haplotypes observed within regions. Mantel test showed a significant relationship between genetic and geographic distance in *D. discolor*, but no significant relationship in *H. tenuis*.

5. Our analyses show that, despite their very similar overall distribution pattern in Europe, the two species exhibit distinct population structures, which may reflect differences in phylogeographic history, dispersal capabilities, habitat specificity or within-region geographic occurrence.

**Keywords**: cytochrome oxidase I, mitochondrial DNA, montane, population structure, Trichoptera
Introduction

Genetic population structuring results from limited gene flow between subpopulations at different temporal or spatial scales. In the northern hemisphere, especially in Central Europe, climatic shifts in the Pleistocene may have been responsible for structuring populations. The effects of climatic shifts on the European biota have been widely studied and much debated (e.g. Hewitt, 1999, 2000, 2004a). Pleistocene climate oscillations drove numerous range expansions and contractions, which had especially severe consequences in Europe where the Alps form a major barrier for populations moving south or north (Thienemann, 1950; Holdhaus, 1954; De Lattin, 1967; Taberlet et al., 1998; Hewitt, 2000). Retreat into stable isolated refugial populations can isolate populations that subsequently accumulate genetic differences. These genetic differences may be maintained or enforced if a species continues to have an insular or discontinuous distribution or has limited powers of dispersal (Bennett, Tzedakis & Willis, 1991; Taberlet et al., 1998; Hewitt, 1999; Conroy & Cook, 2000; Schmitt & Seitz, 2001; Whorley, Alvarez-Castaneda & Kenagy, 2004; Schmitt, 2007). Thus, genetic markers can be used to reconstruct fragmentation events and secondary contact and/or hybridization between lineages previously isolated in independent refugia (Taberlet et al., 1998; Hewitt, 2000; Barton, 2001; Pfenninger & Posada, 2002). Many studies have focussed on examining population genetic structure using a variety of fingerprinting methods to infer the Pleistocene history of European species (see Hewitt, 2004a,b; Schmitt, 2007 for recent reviews).

Although the patterns of species response to climatic cooling are currently only understood for some species, most workers agree that the majority of temperate species currently occurring in Central Europe survived glaciations in refugia with more favourable climates in Southern Europe. During ice free periods, such as interstadials, interglacials or in postglacial times, the recolonization of Central Europe started from these refugia (e.g. Zwick, 1981, 1982; Hewitt, 1996, 2000, 2004a; Bernatchez & Wilson, 1998; Schmitt & Seitz, 2001; Hänfling et al., 2002; Bunje, 2005).

To date, several studies have examined the genetic population structure of aquatic organisms in Europe (e.g. Kelly, Bilton & Rundle, 2001; Wilcock, Hildrew & Nichols, 2001; Kelly, Rundle & Bilton, 2002; Monaghan et al., 2002; Wilcock, Nichols & Hildrew, 2003; Wilcock et al., 2005, 2007; Pauls, Lumbsch & Haase, 2006). In Europe, aquatic and terrestrial species may have reacted differently to historic climate change due to the comparatively buffered nature of the thermal regime in aquatic ecosystems compared to that of terrestrial systems (Malicky, 1983; Pauls et al., 2006). Several studies of European aquatic insects have found some novel and unexpected patterns. For example, Monaghan et al. (2002) found differentiation within and among streams in the mayfly Baetis alpinus (Pictet, 1843), but not among major drainages of the Alps. Cryptic genetic diversity was found in sympatric populations of the mayfly Baetis rhodani (Pictet, 1843) (Williams, Ormerod & Bruford, 2006) and the flatworm Crenobia alpina (Dana, 1766) (Brändle et al., 2007). In contrast, Pauls et al. (2006) observed Central European Pleistocene persistence and high levels of geographically associated population divergence in the caddisfly Drusus discolor (Rambur, 1842). Together, these studies show that a variety of different patterns of population structure exist in the diverse European aquatic insect fauna.

Generally, variation in patterns of population structure depend on the life history and evolution of the taxa examined (Avise, 2000, 2007; Wilcock et al., 2007). We thus propose that closely related taxa and those occupying the same ecological niche or geographic distribution may show similarities in genetic population structure. While Wilcock et al. (2007) compared the genetic population structure of two caddisfly species in the U.K. in great detail, no studies have addressed this hypothesis in a range-wide, directly comparative framework. Here, we compare the population structure of two aquatic insects using homologous mitochondrial cytochrome oxidase I (mtCOI) sequence data. We examined the same 498 bp for both species to allow direct comparison between the two species and avoid potential issues of rate heterogeneity within mtCOI, as shown by Roe & Sperling (2007). Two montane caddisflies, Hydropsyche tenuis Navas, 1932 (Trichoptera: Hydropsychidae, Hydropsychinae) and Drusus discolor (Trichoptera: Limnephilidae, Drusinae) were assayed. Both species are montane, extending to altitudes above 800 m above sea level (asl; Haase, 1999). H. tenuis is distributed across the Central European mountain ranges and the Apennines of Italy; D. discolor occurs in the
same mountain ranges in Central Europe, but its range extends further east and west (Pitsch, 1993; Fauna Europaea Web Service, 2007; Pauls, 2004). Both species are cold-tolerant (Lavandier, 1992; Pitsch, 1993) and geographically limited to these montane regions. Thus they exhibit insular distribution patterns. However, within montane regions, *D. discolor* is restricted to altitudes above 600 m asl, whereas *H. tenuis* occupies a wider altitudinal range from 200 m asl to above 1200 m asl (Haase, 1999; Voigt et al., 2006).

Our comparative study had two main aims. First, we wanted to examine whether *H. tenuis* exhibits population structure and if this is comparable to that of *D. discolor*. Second, we wanted to elucidate how the contrasting ecology and local distribution of these two species affects their population structure. In particular, we asked if the regionally co-distributed species show similar patterns of population structure and haplotype diversity, and whether similarities and differences can be attributed to population history and/or autecology. To address these questions, we applied conventional population genetics analyses to mitochondrial sequence data for both species, as these approaches are ideally suited to elucidating subdivision among such isolated populations (Knowles, 2000; Finn & Adler, 2006).

**Methods**

**Study material**

We collected and sampled 121 specimens of *H. tenuis* (23 adults) from 29 sites in 10 different regions of the Central European highlands (Appendix S1, Fig. 1a). We defined a ‘population’ as all specimens collected from a single stream location. Specimens were collected using light traps or hand nets and preserved in 70–96% EtOH until DNA extraction. We identified specimens to species using the study of Pitsch (1993), Waringer & Graf (1997) and Neu & Tobias (2004) for larvae and Malicky (2004) for adults. Voucher specimens were deposited in the collections of the Senckenberg Natural History Museum, Frankfurt, Germany or in the private collection of Ralf Kütter (Appendix S1). To compare the population structure of *H. tenuis* with that of *D. discolor*, we included a subset of previously published data on *D. discolor* (Pauls et al., 2006). This subset was selected to cover approximately the same geographic range that we sampled for *H. tenuis*. The analysed subset for *D. discolor* comprised 138 individuals (14 adults) of *D. discolor* from 40 sites in 11 different regions in Central Europe (Appendix S2, Fig. 1b).

Laboratory procedures

We extracted total genomic DNA from the thorax, abdomen or legs, using DNEasy Blood & Tissue Kit or QIAMP Microkit (both QIAGEN, Hilden, Germany) following the protocol for purification of total DNA from insects or tissue samples. A homologous fragment of mitochondrial DNA encoding for COI (mtCOI) was amplified by PCR. PCR primers were Dave (5′-AGTTITAGCAGGACATTAT-3′, position 2059 at 3′ end relative to Drosophila yakuba Burla, 1954: Zhang & Hewitt, 1996) and Inger (5′-AAAAATGTGAGGGAAAAATGTTA-3′, position 2735 at 3′ end relative to D. yakuba: Zhang & Hewitt, 1996) for H. tenuis. PCR amplification was performed on a Biometra T-Gradient thermal cycler in each primer and 2 μl of 5 μl DNA template. PCR cycling conditions were: 5 min at 95 °C, 36 cycles of 1 min at 95 °C, 1 min at 50 °C, 2 min at 72 °C and a final extension of 7 min at 72 °C. Laboratory procedures for D. discolor were almost the same as for H. tenuis. PCR primers were Jerry (5′-CAACATT TATTGATTTG-3′, position 2183 at 3′ end relative to D. yakuba: Simon et al., 1994) and S20 (5′-GGGAAAAGGTAAAAATTACTCC-3′, position 2724 at 3′ end relative to D. yakuba: Pauls, Lumbsch & Haase, 2003) following the protocol published in Pauls et al. (2006).

Sequences were generated by two commercial sequencing companies, GATC Ltd., Konstanz, Germany, and Nano+BioCenter, Kaiserslautern, Germany, using the PCR primers. Sequences were aligned, manually checked and edited in Seqman 4.0 (DNASTar, Madison, WI, U.S.A.). Only unambiguous sequences without double peaks were included in the study. The identity of sequences was verified using a BLAST search (Altschul, Madden & Schäffer, 1997). Sequences were aligned using Clustal W (Thompson, Higgins & Gibson, 1994) as implemented in Bioedit 7.0.0 (Hall, 1999). Alignment parameters were default.

Population genetic analyses

To allow a direct comparison between the two species, we analysed the same homologous 498 bp long segment of mtCOI sequence data. We generated a haplotype matrix from 121 sequences of H. tenuis and 138 sequences of D. discolor using DNAsp 4.10.9 (Universitat de Barcelona, Spain) (Rozas et al., 2003). Median-joining (MJ) networks (Bandelt, Forster & Röhl, 1999) were calculated in Network 4.2.0.1 Fluxus Technology Ltd (2004–2007, Suffolk U.K.) using the default settings. We calculated haplotype frequencies and mean pairwise differences between geographical regions and each population as well as genetic differentiation with pairwise FST and exact test of population differentiation (ETPD) (Raymond & Rousset, 1995) using the default parameters in Arlequin 3.11 (Excoffier, Laval & Schneider, 2005). Analysis of molecular variance (AMOVA, Excoffier, Smouse & Quattro, 1992) was performed in Arlequin 3.11 to examine the distribution of total genetic variation at different geographic hierarchies (within populations, between populations within groups and among groups). The a priori grouping design was based on major mountain ranges and sampled populations were grouped accordingly. This approach is non-random, but reflects the natural insular distribution pattern of both species. This procedure resulted in 10 groups for H. tenuis and 11 groups for D. discolor. A Mantel test was applied to the matrices of pairwise FST and geographical distance between each mountain range to assess isolation-by-distance (Mantel, 1967). Geographic distances were based on the geographic centre of populations within each mountain range. Ten thousand permutations were run. For mountain ranges and the total data set, we calculated Tajima’s D (Tajima, 1989) and Fu’s F (Fu, 1997). Neutrality tests, gene diversity (Nei, 1987) and pairwise uncorrected distances within mountain ranges were calculated in Arlequin 3.11 (Excoffier et al., 2005) using default settings.

Results

We generated a 498-bp aligned mtCOI sequence fragment of 121 H. tenuis specimens. A homologous sequence fragment was generated for 138 D. discolor specimens from a comparable geographic range (Fig. 1) using previously published data. The data set comprised all specimens published by Pauls et al. (2006) from 11 regions: the Harz, Rothaargebirge, Thuringian Forest, Rhoen, Erzgebirge, Fichtelgebirge, Bohemian Massif, Black Forest, Vosges Mountains, Jura Mountains and Alps (Appendix S2). The alignments did not contain ambiguous sites or gaps. Eight variable
positions were found for *H. tenuis* and 41 variable positions for *D. discolor*. Nine unique haplotypes were identified for *H. tenuis* (Fig. 2; GenBank Accession numbers EU884268–EU884276). Across a comparable geographic range, 34 haplotypes were identified in the data set of *D. discolor* (Fig. 2; GenBank Accessions: AY954396, AY954397, DQ351157–DQ351162, DQ351164–DQ351166, DQ351168–DQ351170, DQ351171, DQ351173–DQ351175, DQ351181, DQ351183, DQ351184–DQ351188, DQ351190–DQ351194, DQ351196, DQ351206, DQ351211).

**Table 1** Summary of population differentiation values within populations and mountain ranges for both species

<table>
<thead>
<tr>
<th></th>
<th><em>H. tenuis</em></th>
<th><em>D. discolor</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Populations</td>
<td>18/62</td>
<td>37/92.5</td>
</tr>
<tr>
<td>Regions</td>
<td>9/90</td>
<td>11/100</td>
</tr>
<tr>
<td>ETPD results</td>
<td>22/75.9</td>
<td>37/92.5</td>
</tr>
</tbody>
</table>

ETPD, exact test of population differentiation. Detailed pairwise tests are shown in Appendix S3.

Population genetic analyses and haplotype distribution

A MJ network was calculated for each species (Fig. 2). For *H. tenuis* eight mutational steps were sufficient to combine all haplotypes, while for *D. discolor* 55 steps were necessary. We observed haplotype overlap between geographic regions for both species. The numbers of haplotypes per region ranged from one in the Rhoen to four in the Alps.

Fig. 2 Upper: median-joining network of *Hydropsyche tenuis*; lower: median-joining network of *Drusus discolor*. Haplotype diameter reflects the number of individuals carrying a given haplotype. Missing intermediates are added to the network and called median vectors (Posada & Crandall, 2001). Median vectors are illustrated with boxes in the network. Colour coding refers to geographic origin of specimens.

for \( H. \text{tenuis} \) and one to 13 in \( D. \text{discolor} \) (Jura Mountains and Alps).

In \( H. \text{tenuis} \), the most common haplotype (H5) was found in the Erzgebirge, Bohemian Massif, Elbsandsteinengebirge, Black Forest, the Alps and in the Jura Mountains (Fig. 2 upper). The second most common haplotype (H4) was found in the Alps, Apennine Mountains and the Bohemian Massif. In the northern populations (Harz, Rhoen and Thuringian Forest), four haplotypes (H1, H2, H3, H8) occurred that showed no overlap with the southern populations. Private haplotypes were found in four localities: one (H2) in the Rhoen, two (H3, H8) in Thuringian Forest, two (H6, H7) in the Alps and one (H9) in the Jura Mountains. In total, six haplotypes (67%) were private to a single mountain range, while the remaining haplotypes were either restricted to the northern or southern populations.

In \( D. \text{discolor} \), we found four haplotype groups (≥3 mutational steps divergence) (Fig. 2 lower). Group 1 (left) consisted of 13 haplotypes and individuals from the Alps, Bohemian Massif, Erzgebirge and Fichtelgebirge. Group 2 (top) represents a second Alps lineage, which was quite diverged (≥11 mutational steps). Group 3 (central) comprised ten haplotypes and individuals from the Erzgebirge, Fichtelgebirge, Harz, Rhoen, Rothaargebirge and Thuringian Forest. Group 4 (right) consisted of seven haplotypes from individuals from the Black Forest, Jura and Vosges Mountains. Haplotype overlap between geographic regions was observed in the most common and central haplotypes of groups 1 and 3 (H6 and H12 respectively) and within group 4. In total, 30 \( D. \text{discolor} \) haplotypes (88%) were private to a single region. Twelve private haplotypes occurred in the Alps; four in the Thuringian Forest; three in the Black Forest and Bohemian Massif; two each in the Erzgebirge, Rothaargebirge and Vosges Mountains; and one in both the Harz and Fichtelgebirge. Potentially the private haplotypes of \( H. \text{tenuis} \) and \( D. \text{discolor} \) are endemic. However, due to small sampling size in some regions with private haplotypes (i.e. two individuals in Jura Mountains for \( H. \text{tenuis} \) and four individuals in Rothaargebirge for \( D. \text{discolor} \)), their restricted occurrence could be a sampling effect for some of these haplotypes (H9 in \( H. \text{tenuis} \), and H05 and H07 in \( D. \text{discolor} \)).

Exact tests of population differentiation showed that 22 of 29 populations of \( H. \text{tenuis} \) and 37 of 40 populations of \( D. \text{discolor} \) were significantly differentiated. The ETPD revealed significant differentiation in nine of 10 regions for \( H. \text{tenuis} \) and all 11 regions for \( D. \text{discolor} \) (Table 1, Appendix S3). The region Elbsandsteinengebirge, which did not show significant difference in \( H. \text{tenuis} \), comprised only one individual with the common haplotype H5. \( F_{ST} \)-values supported the ETPD analyses. Significant differentiation occurred between 18 of 29 populations of \( H. \text{tenuis} \) and between 37 of 40 populations of \( D. \text{discolor} \). When regions were compared, nine of 10 regions of \( H. \text{tenuis} \) (all except Elbsandsteinengebirge) and all 11 regions of \( D. \text{discolor} \) were significantly differentiated based on \( F_{ST} \)-values (Table 1, Appendix S3).

The results of the AMOVA from both species are summarized in Table 2. The highest variance (58.15%) for \( H. \text{tenuis} \) was found among mountain ranges. Among populations within mountain ranges and within populations variation is about equal (c. 20%). For \( D. \text{discolor} \), similar levels of genetic variance were found among populations within mountain ranges (39%) and among mountain ranges (38%). In \( D. \text{discolor} \), within population variation explained 23% of the total variation.

Within mountain ranges, gene diversity ranged from 0.295 ± 0.156 to 1 ± 0.500 in \( H. \text{tenuis} \) and from 0.333 ± 0.215 to 0.833 ± 0.222 in \( D. \text{discolor} \) (Table 3). Pairwise uncorrected distances between haplotypes within mountain ranges ranged in \( H. \text{tenuis} \) from 0% to 1.0% and in \( D. \text{discolor} \) from 0% to 6.31%. Mantel test did not demonstrate a significant isolation by

### Table 2 Analysis of molecular variance for the groupings of Hydropsyche tenuis and Drusus discolor populations

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>% of variation</th>
<th>P-value</th>
<th>( \phi )-statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( Ht )</td>
<td>( Dd )</td>
<td>( Ht )</td>
</tr>
<tr>
<td>Among regions</td>
<td>58.15</td>
<td>37.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Among populations within regions</td>
<td>22.12</td>
<td>39.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within populations</td>
<td>19.73</td>
<td>23.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\( Ht \), Hydropsyche tenuis; \( Dd \), Drusus discolor.
distance effect in *H. tenuis* (*r* = 0.106, *P* = 0.31), while isolation by distance was inferred for *D. discolor* (*r* = 0.574, *P* = 0.00). Neutrality tests showed no evidence that either species experienced departures from selective neutrality when we examined the entire populations. This suggested that neither species has recently experienced recent demographic expansion as a whole. However, in particular regions, there was evidence for demographic expansion based on significantly negative Tajima’s *D* and Fu’s *F* (Table 4). This was the case in the Thuringian Forest populations for both species, although only Fu’s *F* was significantly negative (*−1.40*, *P* < 0.02) for *H. tenuis*. In *D. discolor*, there was also evidence for demographic expansion in the Eastern Alps.

**Discussion**

**Contrasting patterns of population structure**

Our ETPD, **AMOVA** and **F**<sub>ST</sub> analyses all indicated that both species have significant population structure. However, these structures are different. Both species carry numerous regionally private haplotypes (six in *H. tenuis*, 30 in *D. discolor*). Three of these (H9 in *H. tenuis*, and H05 and H07 in *D. discolor*) could result from small sample size within some regions (two individuals in Jura Mountains for *H. tenuis* and four individuals in Rothaargebirge for *D. discolor*). However, the consistent occurring of private haplotypes across several regions in both species (Rhoen, Thuringian Forest, Jura Mountains, Alps in *H. tenuis*; all regions except Jura Mountains and Rhoen in *D. discolor*) suggests that these could be regionally endemic, in particular those that are carried by two or more individuals. Most populations and mountain ranges are significantly differentiated from one another in both species.

In contrast, stark differences occur with respect to haplotype diversity and variability. *Drusus discolor* has more than three times as many haplotypes as *H. tenuis* across a similar geographic range and with comparable sample sizes. Also, *D. discolor* only has four haplotypes shared between neighbouring regions (H06, H12, H13, H36), whereas *H. tenuis* has two widespread haplotypes (H4, H5). Also gene diversity within *D. discolor* (*H* = 0.875) is higher than in *H. tenuis* (*H* = 0.708).

These results clearly show that *D. discolor* is genetically more diversified in the Central European highlands than *H. tenuis*. A major difference between *D. discolor* and *H. tenuis* is the within- and among-mountain range divergence between haplotypes and haplotype clades. Within *H. tenuis* all haplotypes can

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**Table 3** Gene diversity (Nei, 1987) and pairwise uncorrected distances within mountain ranges for *Hydropsyche tenuis* and *Drusus discolor*

<table>
<thead>
<tr>
<th>Mountain range</th>
<th><em>n</em></th>
<th>Gene diversity</th>
<th>Uncorrected pairwise distances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Ht</em></td>
<td><em>Dd</em></td>
</tr>
<tr>
<td>Harz</td>
<td>11</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>Rothaargebirge</td>
<td>n.d.</td>
<td>4</td>
<td>n.d.</td>
</tr>
<tr>
<td>Elbsandsteingebirge</td>
<td>1</td>
<td>n.d.</td>
<td>–</td>
</tr>
<tr>
<td>Erzgebirge</td>
<td>4</td>
<td>16</td>
<td>–</td>
</tr>
<tr>
<td>Thuringian Forest</td>
<td>13</td>
<td>16</td>
<td>0.295 ± 0.156</td>
</tr>
<tr>
<td>Rhoen</td>
<td>5</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Fichtelgebirge</td>
<td>n.d.</td>
<td>12</td>
<td>n.d.</td>
</tr>
<tr>
<td>Bohemian Massif</td>
<td>2</td>
<td>8</td>
<td>1.000 ± 0.500</td>
</tr>
<tr>
<td>Black Forest</td>
<td>9</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Vosges Mountains</td>
<td>n.d.</td>
<td>11</td>
<td>n.d.</td>
</tr>
<tr>
<td>Jura Mountains</td>
<td>2</td>
<td>5</td>
<td>1.000 ± 0.500</td>
</tr>
<tr>
<td>Alps</td>
<td>63</td>
<td>48</td>
<td>0.573 ± 0.044</td>
</tr>
<tr>
<td>Apennine Mountains</td>
<td>9</td>
<td>n.d.</td>
<td>–</td>
</tr>
<tr>
<td>all populations</td>
<td>121</td>
<td>138</td>
<td>0.708 ± 0.027</td>
</tr>
</tbody>
</table>

*Ht*, *Hydropsyche tenuis*; *Dd*, *Drusus discolor*.

’n’ refers to the number of specimens studied from each mountain range; ‘−’ indicates there was only one haplotype; ‘n.d.’ means there were no data available.

be connected by a single base pair change and the MJ network spans eight nucleotide changes. In *D. discolor*, the MJ network spans 55 nucleotide changes. The maximum difference between haplotypes in *H. tenuis* is four basepairs (0.8%) compared to 21 basepairs (4.2%) in *D. discolor*. The larger genetic differences between haplotypes show that isolation mechanisms between populations and mountain ranges seem to have been stronger and/or in place for a longer time in *D. discolor* than in *H. tenuis*.

Reasons for differences in divergence and diversification could lie in the species’ ecology and life-history traits (Avise, 2000, 2007; Wilcock et al., 2007). These species’ different altitude preferences may have affected their lateral dispersal among mountain ranges. *H. tenuis* tends to occupy lower altitudes (>200 m asl) than *D. discolor* (>600 m asl) within the same montane mountain ranges. Therefore, the average distance between suitable habitats among mountain ranges is shorter for *H. tenuis* than for *D. discolor*. The shorter distances could allow more frequent dispersal events between geographic isolates. As with most caddisflies, larval movement of *H. tenuis* and *D. discolor* is limited to the upstream or downstream movement in the water course. Therefore, lateral dispersal between different courses of running water is limited to the winged adult stage. Although our knowledge of dispersal capabilities of caddisflies is limited, most existing studies show that adult aquatic insects generally tend to stay close to the stream from which they emerge (Sode & Wiberg-Larsen, 1993; Kovats, Ciborowski & Corkum, 1996; Collier & Smith, 1998; Petersen et al., 2004). On the other hand, Hydropsychidae are considered relatively good fliers. Malicky (1987) showed that the flight range of *Hydropsyche saxonica* McLachlan, 1884 extends beyond 3 km. A combination of better flight dispersal and shorter distances between populations could favour gene flow between *H. tenuis* populations within and between mountain ranges. However, in a scenario of more frequent dispersal between populations, we would also expect less population differentiation in *H. tenuis*. Our *F*<sub>ST</sub>-values and ETPD indicate that most populations and regions in *H. tenuis* are significantly differentiated, thus inferring limited or no gene flow. Therefore, we find no evidence that differences in niche distribution and adult dispersal play an important role for the distinct present-day patterns of population differentiation.

If adult dispersal within regions was frequent, we would expect an isolation-by-distance pattern, which was rejected for *H. tenuis* by the Mantel test. In contrast, isolation-by-distance was observed in *D. discolor* in Central Europe. This result is somewhat
unexpected based on findings across the whole distribution range of *D. discolor*, where neighbouring mountain ranges in western Europe (Western Alps, Massif Central, Pyrenees) are among the most diverged (Pauls *et al.*, 2006). However, the clustering of only minimally diverged haplotypes into regional clades leads to a pattern of isolation-by-distance in the data analysed. The different genetic structure between northern and southern populations suggests that processes structuring the Central European and Southern European populations of *D. discolor* may be different.

While they do not seem to be the primary source for differences in population structure, the differences in life history of both species may have a pronounced and varying effect on their respective population histories. For *D. discolor*, a pattern of Pleistocene persistence in mountain ranges in Central Europe has been inferred based on the species distribution (Malicky, 1983), its extreme cold tolerance (Lavandier, 1992) and its strong genetic population structure (Pauls *et al.*, 2006; Pauls, Lumbsch & Haase, in press). Lower tolerance of extremely cold habitats and tolerance of much warmer streams may have driven *H. tenuis* into milder Pleistocene refugia in southern Europe. The most common haplotype in *H. tenuis*, (H5), is widespread across the central distribution area and is missing only in the most northern (Rhoen, Thuringian Forest and Harz) and most southern (Apennine Mountains) populations. Its geographic distribution and central position in the network indicate that H5 is an ancestral haplotype (Posada & Crandall, 2001) and that the other eight haplotypes were derived from it. Also, with only single basepair changes and so few haplotypes, time since divergence in *H. tenuis* appears limited compared to *D. discolor*. Although divergence times cannot be calculated with much credibility based on single basepair changes, the pattern observed in *H. tenuis* is consistent with isolation occurring after last glacial maximum (LGM) c. 12 000 years ago. In contrast, the pattern in *D. discolor* is dated to well before the LGM (Pauls *et al.*, 2006). Further analyses of more variable markers are needed to obtain more robust estimates of intraspecific divergence times for *H. tenuis*.

The population structure and intraspecific divergence and diversity of *H. tenuis* are not typical of species like *D. discolor* that show northern Pleistocene persistence. Rather, the pattern is consistent with a warm-tolerant aquatic species that postglacially expanded northward from a southern refugia. The data collected for *H. tenuis* has characteristics of two patterns that are consistent with such a recolonization event: reduced genetic variation (Hewitt, 1999; Muster & Berendonk, 2006; Pabijan & Babik, 2006), either as a consequence of bottlenecks in the refugial population, and/or founder effects during the recolonization (Freeland, 2005), and a haplotype gradient along the recolonization route(s). Very little haplotype diversity exists, and the northern populations (Harz, Thuringian Forest, Rhoen) only have regionally private, ‘northern’ haplotypes. These data support a scenario of postglacial recolonization of *H. tenuis* from one or a few refugia, presumably in Southern Europe. After expansion into other montane regions, gene flow could have become limited between populations from different mountain ranges, allowing endemic haplotypes to evolve. However, with only three *H. tenuis* samples from two localities (Bohemian Massif and Elbsandsteingebirge), our sampling is limited in the transitional area between the ‘northern’ and ‘southern’ clade. We also do not see an isolation-by-distance effect in *H. tenuis*, or significant results in neutrality tests, which could indicate recent demographic expansion of the transitional populations or the entire species (Tajima, 1989; Fedorov & Stenseth, 2002; Lopes, Miño & Del Lama, 2007). Thus, we cannot confidently resolve whether there is a north–south haplotype gradient with our data. More detailed sampling of the entire distribution range combined with detailed phylogeographic analyses of mtCOI and other independent nuclear markers (e.g. microsatellites) are needed to test our hypothesis of southern refugia, expansion and subsequent isolation in *H. tenuis*.

General patterns of population structure in caddisflies

Clear patterns of genetic population structure due to limited gene flow and high levels of microendemic diversity are common in insects inhabiting high montane regions, or ‘sky islands’ (Knoll & Rowell-Rahier, 1998; Knowles, 2001; Mardulyn, 2001; Finn *et al.*, 2006; Pauls *et al.*, 2008; W. Graf, J. Waringer & S. U. Pauls, unpubl. data) and several studies show that aquatic insects with insular distribution patterns in Europe exhibit population structure. Kelly *et al.* (2001, 2002) found that two caddisfly species endemic
to the Canary Islands show high levels of population structure, although this is not hierarchically structured. A similar pattern is presented for *D. discolor* across mountains in Europe (Pauls et al., 2006). However, in *D. discolor*, the degree of population differentiation is very high. Each region may maintain independent evolutionary entities (e.g. cryptic species) that have not accumulated sufficient morphological characters to allow easy distinction. Similarly, in their study of *Orthopsyche fimбриata* (McLachlan, 1862), a North Island endemic of New Zealand, Smith, McVeagh & Collier (2006) observed up to 5.2% mtCOI sequence divergence between different regional haplotype clades. The overall pattern of population structure showed population differentiation among more distant populations and between catchments and regions, while closer populations were not significantly differentiated. However, in contrast to *D. discolor*, *O. fimбриata* does not exhibit an insular distribution pattern. Another caddisfly with insular distribution pattern is *Rhyncophila pubescens* Pictet, 1834. This species is restricted to limestone tufa streams in mountain regions across Central Europe. The species exhibits strong population structure but only little differences (0.84%) between haplotypes across a shorter but homologous fragment of mtCOI (Engelhardt, Pauls & Haase, in press). Haplotype differences are almost the same in *H. tenuis* (0.80%) and *R. pubescens*.

Varying patterns of population differentiation have also been observed in species with less insular distribution patterns. Wilcock et al. (2003, 2007) found contrasting patterns of population genetic structure between two cofamilial caddisflies *Plectrocnemia conspersa* (Curtis, 1834) and *Polycentropus flavomaculatus* (Pictet, 1834), which have less pronounced insular distribution patterns than *H. tenuis* and *D. discolor*. In two regions in south-east and north-west England, *P. conspersa* showed varying patterns of population structure. While there was no relationship of increasing genetic differentiation and geographic distance below 100 km in the south-east, such a pattern was observed at different levels below 40 km in the north-west. *Polycentropus flavomaculatus* exhibited much stronger genetic structure in south-east England than *P. conspersa* in either region (Wilcock et al., 2007).

Although the number of studies on European caddisfly population genetics is limited to date, different patterns of gene flow and population structure exist across different geographic scales. Most of the studies observe different, and often unexpected and contrasting patterns between different species, even when they occupy a similar ecological niche and have comparable geographic distributions. Our study, for example, shows that *H. tenuis* and *D. discolor* may have similar geographic distribution patterns, but that their population structure is very different. It would appear that the differences in local niche occupancy and local spatial distribution are not responsible for these differences. Rather, the phylogeographic history of the two species, related to their different ecologies, may have played a major role in shaping contemporary patterns. The reported diversity in patterns of population genetic structure in caddisflies suggests that many more interesting and unique patterns will be found by studying the population genetics and phylogeography of caddisflies and other aquatic insects. These patterns can then serve as an important basis for addressing more general questions on their recruitment, dispersal, diversification and evolution.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Material of *Hydropsyche tenuis* used in this study.

**Appendix S2.** Material of *Drusus discolor* used in this study.

**Appendix S3.** Results of exact tests of regional differentiation (ETPD) and pairwise regional *F*<sub>ST</sub>-values for *Hydropsyche tenuis* (HT) and *Drusus discolor* (DD).

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