Higher β- and γ-diversity at species and genetic levels in headwaters than in mid-order streams in *Hydropsyche* (Trichoptera)

CESC MÚRRIA*,†,‡, NÚRIA BONADA*, MIQUEL A. ARNEDO§, NARCÍS PRAT* AND ALFRIED
P. VOGLER†,‡

Grup de Recerca Freshwater Ecology and Management (FEM), Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Barcelona, Catalonia, Spain

†*Department of Entomology, Natural History Museum, London, U.K.*

‡*Department of Life Sciences, Imperial College London, Ascot, U.K.*

§*Institut de Recerca de la Biodiversitat & Departament de Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Barcelona, Catalonia, Spain*

SUMMARY

1. The influence of local habitat characteristics and landscape barriers on the distribution of species and genetic diversity remains poorly known. We tested for differences in turnover and total diversity in aquatic insect assemblages among headwater and mid-order reaches, two habitats presumably differing in their connectivity between catchments.

2. The genus *Hydropsyche* (Trichoptera) includes 17 species in the eastern Iberian Peninsula and northern Morocco. Cytochrome *c* oxidase subunit I (*cox1*) gene was sequenced for *Hydropsyche* assemblages at 38 headwater sites and 12 mid-order sites. Local species and intraspecific genetic diversity and differentiation among populations were measured.

3. A total of 231 *cox1* sequences resulted in 83 unique haplotypes that clustered into 19 genetic species entities. We find that α-diversity did not differ between headwater and mid-order habitats at species and genetic levels, but total species numbers and intraspecific genetic diversity (γ-diversity) were higher for headwater than for mid-order or lowland clades, due to greater turnover among sites (β-diversity).

4. The study revealed lineage-specific evolutionary responses to different river zones, presumably because of differences in the strength of landscape barriers. The multispecies community genetic approach provides a comprehensive picture of diversity patterns in *Hydropsyche*, as it links habitat heterogeneity and dispersal limitations to genetic structure and lineage diversification.

Keywords: community ecology, gene flow, habitat type, population genetics, stream ecology

Introduction

Understanding what determines spatial patterns of biodiversity is one of the most challenging issues in population and community ecology and evolution. The patterns of local richness (α-diversity) and their variation across geographical ranges (β-diversity) are primarily determined by a combination of local habitat features, landscape barriers and dispersal-related biological traits (Brown & Swam, 2010). These factors impose constraints at both the population (genetic diversity within species) and the community (species diversity) levels that, if effective over large temporal and spatial scales, drive intraspecific genetic divergence, lineage diversification and macroecological patterns (Vellend & Geber, 2005; Papadopoulou *et al.*, 2011; Baselga *et al.*, 2013). Hence, the links between ecology and evolution are important to characterise diversification processes and to understand spatial patterns at various scales, both of which provide essential information in the present-day global

biodiversity crisis. However, the relative influence of various factors on the patterns of genetic and species diversity remains poorly understood.

The spatial organisation of river ecosystems and their associated local habitat characteristics are critical to freshwater species diversity (Poff, 1997; Brown & Swam, 2010). Rivers have a dendritic structure, with a strong longitudinal gradient of chemical, physical and biotic variables from headwaters to river mouths. Discharge, temperature, salinity and sediments increase downstream, while mean flow velocity decreases. These factors change microhabitat conditions and hydraulic forces (Ward, 1998; Fausch *et al.*, 2002; Benda *et al.*, 2004). The marked heterogeneity of river habitats has prompted species diversification and resulted in highly differentiated communities along river zones (Heino, 2009). The genus *Hydropsyche* (Trichoptera), a common, widespread and diverse group of aquatic insects, provides a clear example of how river zonation drives evolutionary processes (Statzner & Dolédec, 2011; Múrria *et al.*, 2012). The larvae of *Hydropsyche* are filter feeders that process suspended particulate matter. Local habitat differences from headwaters to lowland reaches that affect particle size, oxygen concentration and near-bottom velocity coincide with species-specific mesh sizes

of filtering nets and longitudinal differentiation of communities (Hildrew & Edington, 1979). Longitudinal stream habitat preferences were found to be phylogenetically conserved, and *Hydropsyche* first diversified into these river zones, as suggested by recent phylogenetic analyses (Múrria *et al.*, 2012) that split the genus into four clades composed of headwater (clade B), mid-order (clades C and D) and lowland species (clade A) (Table 1). Subsequent species diversification within these clades can be explained by vicariance driven by the geological and climatic history of the western Mediterranean basin (Múrria *et al.*, 2012). Because closely related lineages of *Hydropsyche* are associated with different river zones, the genus provides an opportunity to test the effect of different habitat types on species and genetic diversification, in particular, as these habitats differ in spatial structure and levels of connectivity among catchments.

Rivers are confined by landscape barriers that limit the movement of organisms among catchments (Bilton, Freeland & Okamura, 2001; Bohonak & Jenkins, 2003). From headwaters to lowland reaches, rivers usually traverse landscapes that are characterised by greater relief and higher potential dispersal barriers at high altitudes but whose effects usually recede towards the river

Table 1 GMYC and *cox1* haplotype diversity among the sampled *Hydropsyche* species

Morph. sp.	Phyl.	GMYC	N (HW*,MO*)	H (HW*,MO*)	π	Segr. sites	Geo. reg.	HW sites	MO sites	LL sites
H. modesta	A	1	3	1	–	–	H	–	–	1
H. maroccana	A	2	6	1	–	–	R	–	–	–
H. exocellata	A	3	30 (6,19)	6 (4,3)	0.0043	7	H, T, B	6	8	2
H. teruela	A	4	6 (3,3)	2 (2,1)	0.0017	1	T	1	1	–
H. sp2	B	5	4 (4,0)	4 (4,0)	0.0068	8	B	1	–	–
H. siltalai	B	6	40 (34,6)	13 (12,4)	0.0099	23	H, T, B	11	3	–
H. infernalis	B	7	7 (7,0)	2 (2,0)	0.0017	1	B	4	–	–
H. fezana	B	8	6	2	0.0017	1	R	–	–	–
H. fontinalis	B	9	2 (2,0)	1 (1,0)	–	–	T	1	–	–
H. sp1	B	10	8 (8,0)	3 (3,0)	0.0147	3	H	3	–	–
*H. instabilis*1	B	11	7 (7,0)	1 (1,0)	–	–	H	3	–	–
*H. instabilis*2	B	12	8 (8,0)	5 (5,0)	0.0196	27	H, B	5	–	–
H. dinarica	–	17	12 (12,0)	8 (8,0)	0.0042	8	H	6	–	–
H. lobata	C	18	3	2	0.0203	12	R	–	–	–
H. brevis	C	19	20 (16,2)	15 (14,2)	0.0072	21	H, T, B	8	2	1
H. pellucidula	D	13	46 (28,12)	10 (10,4)	0.0066	16	H, T, B	16	4	2
*H. incognita*1	D	14	4 (4,0)	2 (2,0)	0.0118	7	T, B	2	–	–
*H. incognita*2	D	15	4 (1,3)	2 (1,2)	0.0051	3	H, T	2	1	–
H. iberomaroccana	D	16	15 (0,5)	3 (0,2)	0.018 (0.0051*)	16	B, R	–	1*	–
Total			231 (140, 50)	83 (69,18)						

GMYC, generalised mixed Yule coalescent.

For each morphological species, clade membership in Múrria *et al.* (2012) (in the 'Phyl.' columns); number of specimens sequenced (N) and total haplotypes (H) and their distribution at headwaters (HW) and mid-order (MO) reaches; genetic diversity measures average nucleotide diversity (π) and number of segregated sites (Segr. Sites); the biogeographical region (Geo. Reg.) of each species; and the number of sites where each species was found in headwaters (HW), mid-order reaches (MO) and lowlands (LL) are indicated.
*Only considering specimens located in the Iberian Peninsula.

mouth. These differences in landscape features would predict that dispersion between catchments and sub-catchments is limited in particular between headwater habitats, and therefore, high heterogeneity in species composition among individual streams is expected. This has been confirmed in studies that showed higher β-diversity in species composition among headwaters than among mid-order or lowland reaches, although these studies could not distinguish the effects of dispersal limitation and high habitat heterogeneity (Finn & Poff, 2005; Clarke *et al.*, 2008, 2010). Equally, population studies have shown higher intraspecific genetic structure among catchments in headwater than in mid-order or lowland species (Hughes, 2007; Hughes, Schmidt & Finn, 2009). These findings are consistent with the notion that dispersal and gene flow among reaches are more constrained across high mountains in headwaters than across the typically lower barriers affecting mid-order or lowland reaches. A first attempt to analyse species diversity and intraspecific genetic diversity together based on literature surveys revealed that the increased β-diversity in headwaters is correlated on both levels (Finn *et al.*, 2011). However, this and other population studies that aimed to test effects of dispersal barriers on genetic structure have focused either on a single species (Finn *et al.*, 2006; Múrria & Hughes, 2008) or on a few unrelated species with potentially contrasting dispersal abilities (Monaghan *et al.*, 2002; Lehrian, Pauls & Haase, 2009; Alp *et al.*, 2012). Hence, the spatial patterns described may differ simply because of contrasting vagility between taxonomic groups. Moreover, these approaches lack the evolutionary perspective that reveal the effect of landscape barriers across hierarchical levels from population structure to species diversification and, thus, will fail to link population and community levels. Intra- and interspecific patterns of diversity capture different portions of the diversification process and their combined study provide insights into the role of evolutionary and ecological factors at different temporal scales (Craft *et al.*, 2010; Papadopoulou *et al.*, 2011; Dexter, Terborgh & Cunningham, 2012).

Here, we use a community-level genetic approach to test the previous finding of increased β- and γ-diversity in headwater habitats (Finn *et al.*, 2011) and its presumed cause of greater landscape barriers upstream. Multispecies sequencing of mtDNA across communities provides information both at the population and at the species levels, whereby the sequence variation can usually be partitioned into discrete clusters of haplotypes broadly corresponding to species entities, to separate species and genetic levels (Pons *et al.*, 2006). Specifically,

we investigate the effects of landscape barriers associated with river zonation by comparing the four clades of *Hydropsyche* adapted to headwater, mid-order and lowland river sections (Múrria *et al.*, 2012). The use of phylogenetically close relatives, which revealed high dispersal in previous genetic studies indicated by high gene flow (Guinand & Tachet, 2000; Baker, Williams & Hughes, 2003; Lehrian *et al.*, 2009; Múrria *et al.*, 2010), presumably minimises potential differences in dispersal-related biological traits and instead reflects the effects of landscape and habitat features. In addition, community-level sequence data provide information simultaneously at species and genetic levels, which are expected to be correlated if the patterns are mainly explained by dispersal (Vellend, 2003; Vellend & Geber, 2005), while non-neutral processes determining species distribution would only affect the species level (assuming that habitat associations are due to filtering at species level) (Dexter *et al.*, 2012; Baselga *et al.*, 2013). These data therefore may provide a means of discriminating between the effects of habitat heterogeneity (high β-diversity only at species level) from those of dispersal limitation (high β-diversity at genetic level). Finally, if we detect correlated effects on population and species diversity, this will be extremely important for generalising results from previous studies (Hughes, 2007; Hughes *et al.*, 2009; Finn & Poff, 2011; Finn *et al.*, 2011). Demonstrating headwater species have higher genetic structuring and narrower geographical ranges than species distributed across lowland reaches is also of major conservation relevance.

Methods

Study area

The study was conducted on the eastern coastal region of the Iberian Peninsula and northern Morocco, hereafter referred as the western Mediterranean basin (Fig. 1). This region has high species richness and levels of endemism of aquatic insects compared with other Palaearctic areas (Graf *et al.*, 2008). Such species richness and endemism are likely the result of a dynamic geology and climatology. Tectonic events that occurred from the early Eocene (*c.* 30 My ago) to the Miocene (*c.* 5 My ago) divided the western Mediterranean basin into four main geological regions, namely (from north to south) the Iberian plate, the transitional zone, the Betic region and the Rif region (Fig. 1). In addition, the western Mediterranean basin endured several climatic shifts, including the quaternary glacial cycles (for more details about these regions and how they originated, see Bonada *et al.*,
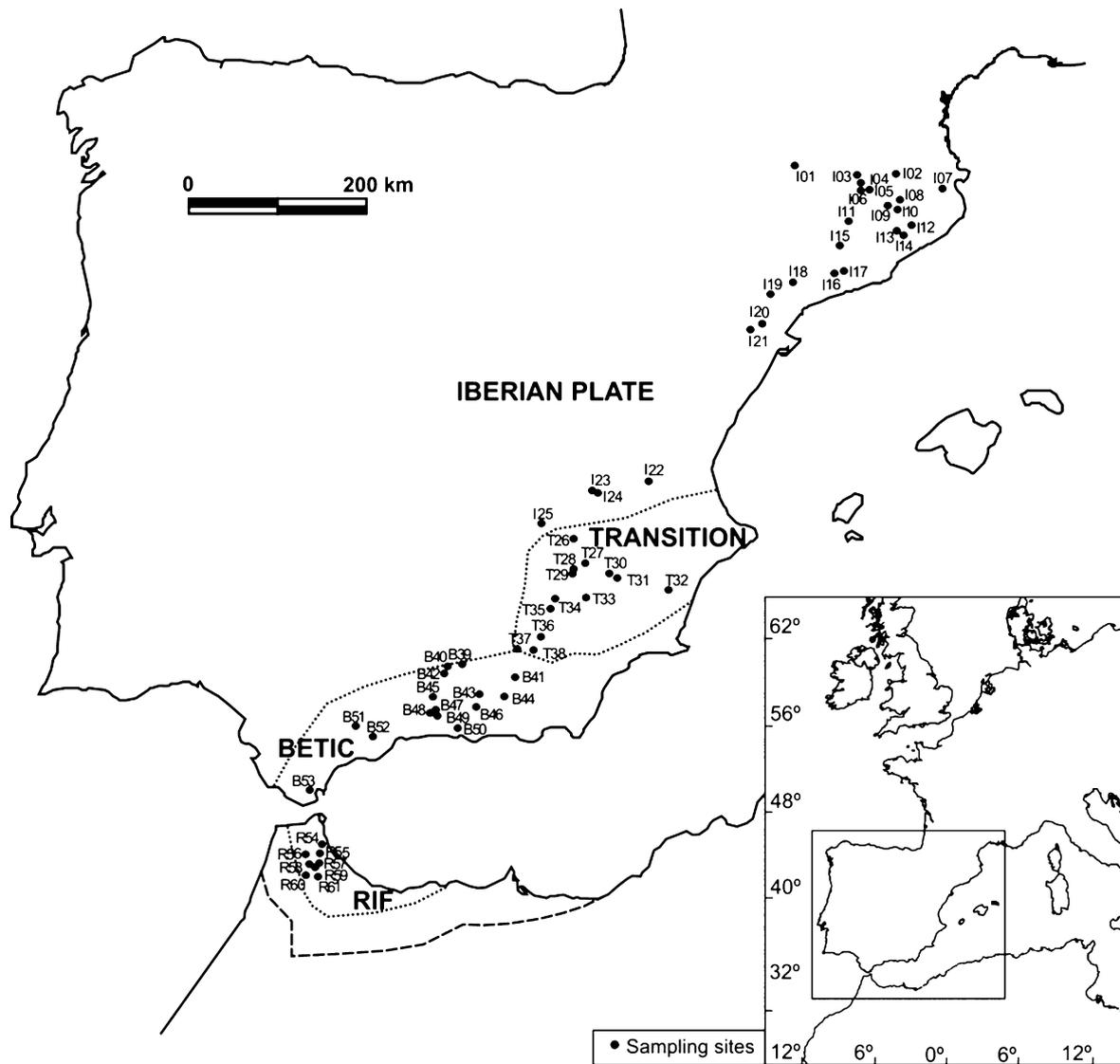
Fig. 1 Sampling sites in the western Mediterranean basin located in Iberian (I), transitional (T), Betic (B) and Rif (R) regions. Sites were numbered from north to south (for further details see, Bonada *et al.*, 2009).

2009). Present-day geographical distributions of species have the signature of these biogeographical processes (Múrria *et al.*, 2012; Table 1).

Hydropsyche data set and landscape measures

Using an existing database of non-polluted sites in western Mediterranean basin where *Hydropsyche* species are present (Múrria *et al.*, 2012), sampling sites were selected first for the highest number of co-occurring species, after which sites containing unsampled species were added to obtain all *Hydropsyche* species of the region. This resulted in 61 sites distributed in 22 catchments (see Table S1 in Supporting Information). These sites were plotted in a geographical information system

(Arcview GIS version 3.1, Environmental Systems Research Institute, Inc., Redlands, CA, U.S.A.) and assigned Strahler stream order on 1 : 24 000 maps, classifying each site as either 'headwaters' (first and second order), 'mid-order' (third and fourth order) or 'lowland' (higher than fifth order) reaches. The dendritic network structure of streams implies that around 75% of sites are located in headwaters (Benda *et al.*, 2004), so a disproportional higher number of headwater sites than mid-order or lowland sites was expected. Only three sites were located in lowland reaches (sites H19, H22 and T37), and therefore, only headwater and mid-order sites were included in the comparisons of habitat types. Moreover, the 8 sites in the Rif region that are separated from the Iberian Peninsula by the Alboran Sea were excluded from

statistical analyses because of their high degree of isolation unrelated to the landscape barriers that was the focus here. However, sequences of *cox1* found in this region were kept in the analysis of species-level boundaries.

Molecular analyses and estimates of molecular species entities

Previous studies of several *Hydropsyche* species showed low haplotype accumulation per site (commonly one or two haplotypes/site; Lehrian *et al.*, 2009; Múrria *et al.*, 2010) and low intraspecific genetic differentiation independently of river section and despite genetic structure at large geographical scales. For example, only nine unique haplotypes were identified for *Hydropsyche tenuis* for 121 specimens from 29 localities across Central Europe (Lehrian *et al.*, 2009), whereas seven different haplotypes based on six polymorphic sites were identified for 101 individuals of *Hydropsyche siltalai* in the north-east Iberian Peninsula (Múrria *et al.*, 2010). Therefore, when available, the *cox1* gene of three individuals per species for each site was sequenced for a total of 231 individuals across 61 sites. DNA was extracted from abdominal segments I–V using Promega WizardSV extraction plates. A total of 592 bp of *cox1* was sequenced following Múrria *et al.* (2010). GenBank accession numbers are shown in Appendix S2 in Supporting Information.

The generalised mixed Yule coalescent (GMYC) model (Pons *et al.*, 2006) was used to identify species-level boundaries based on sequence data. The GMYC method delimits genetic clusters that can be used as a surrogate for species entities in a statistical, repeatable framework. The GMYC method classifies the observed time intervals between branching events as either interspecific ('diversification') or intraspecific ('coalescent') processes, identifying a change in branching rates that correspond to the transition from species- to population-level relationships. The GMYC analysis was conducted on the *cox1* haplotypes after all sequences were collapsed to unique haplotypes with the 'single threshold' option. *Cheumatopsyche lepida* was used as outgroup (GenBank accession number JQ687925). The online version of the program Modeltest (version 3.7) (Posada, 2006) was used to select the substitution model that best fitted the data with the fewest parameters, as indicated by the Akaike information criterion (AIC) (Akaike, 1973). Bayesian inference (BI) was conducted with MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) and run remotely at the University of Oslo's bioportal computer resources (http://www.bioportal.uio.no). Two independent runs

with four simultaneous Markov Chain Monte Carlo (MCMC) chains (one cold and three heated), each with random starting trees, were carried out simultaneously, sampling 1000 generations until the standard deviation of the split frequencies of these two runs dropped below 0.01 (10 million generations). The program Tracer version 1.4 (Rambaut & Drummond, 2003) was used to ensure that the Markov chains had reached 'stationarity' by examining the effective sample size (ESS) values and to determine the number of generations to discard as burn-in. The majority rule consensus topology obtained from the BI was transformed to an ultrametric tree using the Langley–Fitch method as implemented in the computer program r8s (Sanderson, 2003). A preliminary cross-validation analysis (Sanderson, 2002) selected the molecular clock-like Langley–Fitch method over the NPRS. It should be noted, however, that all smoothing parameter values assayed for the penalised likelihood method returned failure, probably suggesting that the actual best smoothing value is either lower or higher (Sanderson, 2003). A likelihood ratio test further suggested that the assumption of a molecular clock was only marginally worse than not enforcing a molecular clock (LRT = 96.23, critical value = 101.8 for $P = 0.05$). The ultrametric tree was subsequently used to implement the GMYC method. The code implementing the model was kindly provided by T. Barraclough. A current version is available in 'Splits' from the R package (R Development Core Team, 2011) (http://r-forge.r-project.org/projects/splits/).

Analysis of diversity

To compare the diversity accumulated within clades and river zones, the number of GMYC entities and *cox1* haplotypes for each clade (defined in Múrria *et al.*, 2012) was recorded. In addition, the number of polymorphic nucleotide sites and nucleotide diversity π (i.e. the average number of nucleotide differences per site between two sequences, Nei, 1987) were measured for each GMYC entity. α-diversities for headwater and mid-order reaches were compared by calculating the number of GMYC entities per site and *cox1* haplotype richness and average nucleotide diversity per GMYC entity and per site as estimates of genetic diversity. β-diversity at the species and the genetic levels among sites within headwater or mid-order habitats was assessed by measuring Jaccard's similarity index of the presence/absence data for both the GMYC entities and the *cox1* haplotypes (Koleff, Gaston & Lennon, 2003; Anderson *et al.*, 2011). The number of sites in headwater and the number of sites in

mid-order streams are expected to differ given their disproportionate distribution in the dendritic network structure (Benda *et al.*, 2004). Therefore, the significance of differences in α- and β-diversity between headwater and mid-order reaches was tested with a nonparametric Mann–Whitney U-test. γ-diversity was estimated as the total number of GMYC entities and *cox1* haplotypes, irrespective of species membership, in headwater and in mid-order reaches. To test differences in γ-diversity among habitat types, using diversity composition data for the 38 headwater sites and 12 mid-order sites, 100 matrices of 11 sites each were randomly generated. For each simulated matrix, γ-diversity was calculated, and differences between headwater and mid-order reaches were tested using a Kruskal–Wallis test. This procedure was carried out to avoid the differences in sample size between headwater and mid-order reaches, an inherent difference resulting from the dendritic structure of river basins. Measures of genetic diversity were calculated with DnaSP version 5.10.01 (Librado & Rozas, 2009) and the R packages (R Development Core Team, 2011) *Vegan* (Oksanen *et al.*, 2011) and *Ape* (Paradis, Claude & Strimmer, 2004).

Results

The 231 *cox1* sequences resulted in 83 unique haplotypes and 19 GMYC entities (Table 1). GMYC entities were highly congruent with the morphologically defined species, with the exception of *Hydropsyche instabilis* and *H. incognita*, each of which split into two GMYC entities. Composition and number of GMYC entities and unique *cox1* haplotypes for each clade of *Hydropsyche* defined in Múrria *et al.* (2012) are included in Table 1. The highest species diversity was found in the headwater clade B, with a total 8 GMYC entities. In contrast, the mid-order clades D and C included 4 and 2 GMYC groups, respectively, and the lowland clade A included 4 GMYC groups. Similarly, the total number of unique *cox1* haplotypes was highest for the headwater clade (31 unique *cox1* haplotypes) followed by the mid-order clades (20 and 17 *cox1* haplotypes, respectively, for clades C and D) and the lowland clade (ten unique *cox1* haplotypes). For each GMYC entity with at least five individuals sequenced, the highest genetic diversity as estimated by the average nucleotide diversity (π) was found for *H. instabilis 2* and *H.* sp1 (clade B), whereas the lowest values were revealed in *H. infernalis* (clade B) and *H. teruela* (clade A) (Table 1). Average nucleotide diversity per GMYC entity within a clade was highest for clade B (π = 0.0115) followed by clades C (π = 0.0072), D (π = 0.0059) and A (π = 0.003).

Thirty-eight sites were located in headwater, and 12 sites, in mid-order (Table 1 and see Appendix S1 in Supporting Information). Differences in α-diversity based on the number of GMYC entities, *cox1* haplotypes and average nucleotide diversity per GMYC per site were non-significant between both habitat types (GMYC entities: 1.75 ± 1.00 and 1.66 ± 0.98, respectively, for headwater and mid-order reaches; $U = 238.5$, $P = 0.8$; *cox1* haplotypes: 2.81 ± 1.85 and 2.33 ± 1.37, respectively, $U = 256$, $P = 0.51$; average nucleotide diversity: 0.0035 ± 0.004 and 0.0014 ± 0.0017, respectively, $U = 108.5$, $P = 0.16$) (Fig. 2a,b). In contrast, β-diversity at the GMYC and *cox1* haplotype levels was significantly higher for headwater than for mid-order sites (GMYC entities: 0.14 ± 0.24 and
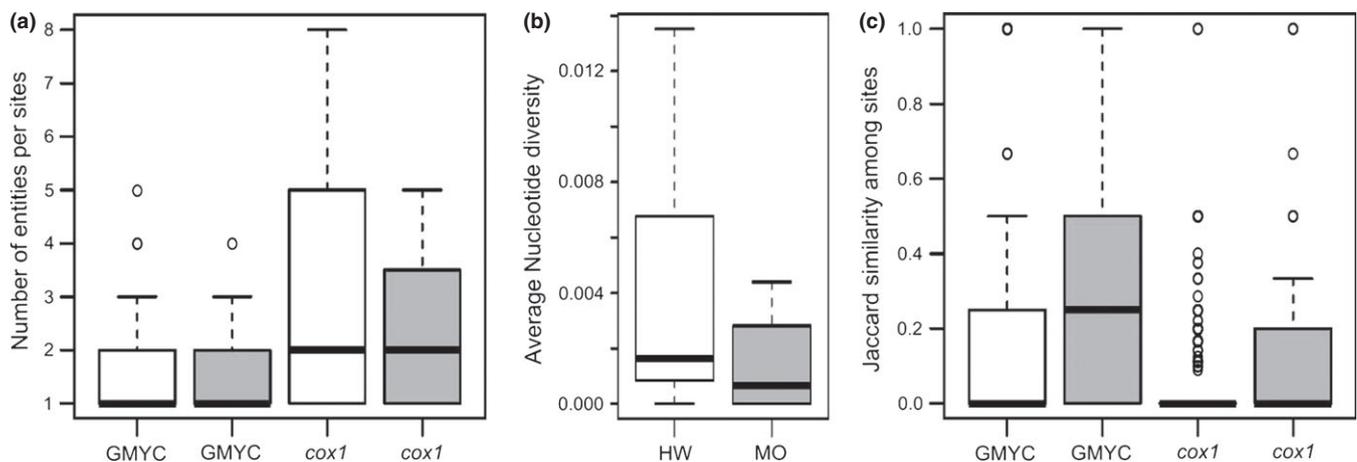

Fig. 2 Box plots of diversity distribution at generalised mixed Yule coalescent (GMYC) and *cox1* haplotype levels. a/α-diversity measured as the number of entities (either GMYC or *cox1* haplotypes) per site, b/α-diversity measured as the average number of nucleotide diversity, and c/β-diversity measured using the Jaccard's similarity index among sites. Headwaters are shown in the white box-plot, and mid-order reaches, in the grey box-plot. *Significant differences between headwater and mid-order reaches ($P < 0.05$).
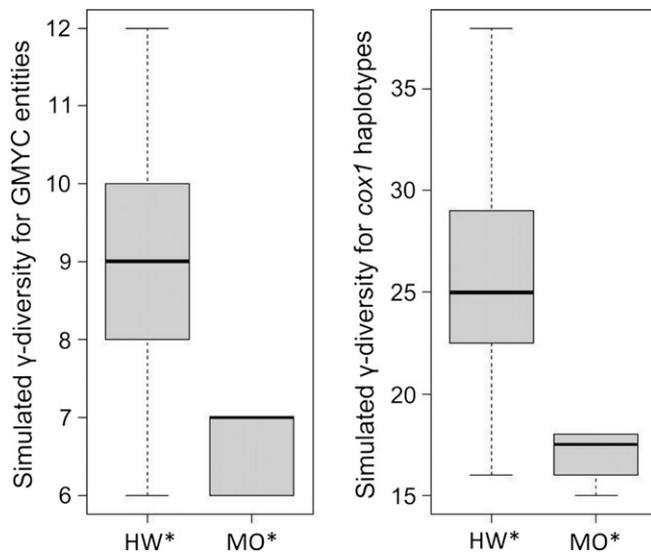
Fig. 3 Box plots of simulated γ-diversity at generalised mixed Yule coalescent and *cox1* haplotype levels for headwaters (HW) and mid-order reaches (MO). *Significant differences between headwater and mid-order reaches (*P* < 0.05).

0.26 ± 0.31, respectively, $U = 17897.5$, $P < 0.01$; *cox1* haplotypes: 0.03 ± 0.01 and 0.14 ± 0.25, respectively, $U = 17116$, $P < 0.01$) (Fig. 2c). Similarly, γ-diversity at the GMYC level reflected the population level: 14 GMYC entities were located in headwaters, whereas 7 were located in mid-order reaches; headwaters had 69 unique *cox1* haplotypes, whereas only 18 were found in mid-order reaches (Table 1). These differences were significant between headwaters and mid-order sites for GMYC entities ($\chi^2 = 136.29$, d.f. = 1, *P*-value < 2.2e-16) and *cox1* haplotypes ($\chi^2 = 131.64$, d.f. = 1, *P*-value < 2.2e-16) (Fig. 3).

Discussion

The dendritic nature of river ecosystems has an important effect on the evolutionary history of *Hydropsyche*. We found that levels of both genetic and species diversity were strongly affected by river zonation, consistent with the notion that dispersal limitations among catchments and subcatchments increase from lowlands to headwaters with an increasing influence of landscape barriers. This confirms previous studies that found increased genetic diversity in headwater habitats (Hughes *et al.*, 2009; Finn *et al.*, 2011). However, due to the focus on a whole lineage, our study demonstrates for the first time that these landscape effects simultaneously acted on intraspecific genetic divergence and species-level diversity, as suggested by higher β- and γ-diversity at both levels.

At both species and population levels, α-diversity was low and did not differ between headwater and mid-order sites. The low local species diversity may be explained by aggressive species interactions of *Hydropsyche* competing for the best sites to filter (Hildrew & Edington, 1979; Múrria *et al.*, 2012). However, species competition would not explain the low local intraspecific genetic variation in the presumed neutral mtDNA used here. Correlations between species and genetic diversity may result when the processes effective on one level can also constrain or enhance the other level (Vellend, 2003; Vellend & Geber, 2005). For instance, factors determining local species diversity may also affect genetic diversity, for example, if competition at the species level not only prevents immigration of other species but also selects against the establishment of certain genotypes, or if different genotypes vary in their efficiency of resource use or ability to cope with species competition (Vellend & Geber, 2005). Genetic diversity in competitive communities may also be reduced due to stronger drift effects resulting from bottlenecks and reduced establishment of immigrating individuals. Based on the genetic data generated here, these processes do not differ in headwater and mid-order stream habitats, and local community composition is unrelated to different levels of community isolation. Hence, local processes such as competition may override the effects of differences in levels of dispersion that would have predicted higher α-diversity in mid-order than in headwater habitats, due to the higher in-stream energy available and larger patch or area sizes at mid-order sustaining bigger populations and greater genetic diversity.

The explanation for finding the same pattern in species and genetic β-diversity is more straightforward. The river dendritic network structure predicts a decrease in β- and γ-diversity downstream because limited migration among headwater sites retains potentially unique species and alleles, while these barriers are less efficient among mid-order sites (Morrissey & De Kerckhove, 2009; Brown & Swam, 2010; Finn *et al.*, 2011). This prediction was confirmed in a study of diversity patterns along streams with high, medium and low glacial influence (as a proxy of isolation), which found a correlated higher β- and γ-diversity at species and genetic levels in the more isolated streams with greater glacial influence than in more connected mid- and low-glaciality streams (Finn, Khamis & Milner, 2013). However, in that study, the species diversity of a site was based on a measure of total taxonomic richness of whole macroinvertebrate communities, while genetic diversity was assessed based on a single species, the mayfly *Baetis alpinus* (Ephemeroptera). In this paper, we refined the analysis by assessing species diversity in a well-defined

phylogenetic lineage that exhibits species association with different river reaches (Múrria *et al.*, 2012) and by assessing intraspecific genetic diversity for the members of an entire genus, rather than for single species. Our results are consistent with the involvement of landscape barriers over an ecological and evolutionary time scale in the generation of the observed patterns by accumulating diversity from genetic to species levels. Moreover, the similarity of β-diversity at both species and genetic levels may argue against the alternative hypothesis of higher habitat heterogeneity in headwaters than in lower reaches (Clarke *et al.*, 2008, 2010), because habitat heterogeneity among reaches may affect only spatial turnover of species composition but not genetic diversity in mitochondrial (neutral) markers simultaneously. These findings add to an emerging macroecological pattern of greater turnover in upland compared with lowland ecosystems by limited dispersal. Thus, for example, three herbivorous guilds feeding on foliage (Lepidoptera), wood (Coleoptera) and fruit (Diptera) revealed low β-diversity in lowlands rainforest in Papua New Guinea (Novotny *et al.*, 2007). In contrast, upland forest communities showed high β-diversity, narrow geographical ranges and allopatric speciation in herbivorous moths in the genus *Eois* (Geometridae) in Costa Rica and Ecuador (Rodríguez-Castañeda *et al.*, 2010) and in scarabaeid assemblages (Dynastinae, Rutelinae, Melolonthinae) across mountain ranges in Costa Rica (García-López *et al.*, 2013).

Our community genetic approach supports explanations for patterns observed in existing single-species genetic studies in stream ecology (e.g. Hughes, 2007; Hughes *et al.*, 2009; Finn & Poff, 2011; Finn *et al.*, 2011). An earlier study of phylogenetic relationships of *Hydropsyche* at the species level in the western Mediterranean basin has already linked the basal diversification of the genus to river zonation (Múrria *et al.*, 2012). The current study now reveals lineage-specific differential responses of *Hydropsyche* to habitat type along the river continuum apparently due to different degrees of dispersal constraints. Landscape barriers effectively separate populations between catchments and initially reduce gene flow and increase genetic differentiation through drift, and continued limitations to gene flow through time eventually lead to speciation in allopatry. Therefore, habitat drives differences in *Hydropsyche* diversification, and this process is more relevant in headwater than in mid-order or lowland reaches. This is analogous to the result from similar community genetic approaches that have revealed habitat type and dispersal-related biological traits as the main drivers of genetic diversification and

evolution; in the Aegean archipelago, for example, tenebrionid beetles associated with stable habitats showed higher species diversity and deeper genetic structure compared with genetically homogenised lineages in ephemeral coastal sand dune habitats (Papadopoulou *et al.*, 2009). It thus seems that dispersal limitations resulting from both intrinsic (e.g. dispersal-related biological traits) and extrinsic (e.g. landscape barriers, habitat stability) factors not only constrain the pattern of community composition, but may also influence deeper levels of genetic structuring and lineage divergence within clades, and these patterns can be detected using a community genetic approach.

Our result that *Hydropsyche* diversity patterns differ among river zones has implications for the conservation of diversity in streams. Species in headwater habitats have been geographically isolated in each mountain range and have narrow geographical range and high species turnover (e.g. distinct headwater Trichoptera communities in the western Mediterranean basin are generally restricted to a single mountain range, Bonada *et al.*, 2009). In contrast, less constrained dispersal among mid-order and lowland reaches has favoured species homogenisation, large geographical ranges and low species turnover among reaches (and mid-order Trichoptera communities are widely distributed across different catchments in the western Mediterranean basin, Bonada *et al.*, 2009). Moreover, our finding of high genetic divergence among headwater reaches associated with high genetic species endemism suggests that more isolated highlands may harbour unidentified cryptic genetic and species diversity (e.g. Habel *et al.*, 2011; Finn *et al.*, 2013). Consequently, the contribution of headwater streams to global diversity may be greater than currently recognised (Finn *et al.*, 2011). Unfortunately, species and genetic diversity in headwaters may be highly vulnerable to predicted climatic changes, and conservation measures will be required to preserve this unique diversity (Parmesan, 2006; Bálint *et al.*, 2011). As we show here, a complete view of diversity patterns for conservation purposes will require studies that link habitat heterogeneity and intrinsic and extrinsic dispersal limitations to genetic structure and lineage diversification, both at the local and regional levels and across multiple spatiotemporal scales.

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Supporting Information

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

Table S1. Location of sampled reaches.

Table S2. GenBank accession numbers for DNA sequences used.

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