Intraspecific genetic and phenotypic diversity: parallel processes and correlated patterns?

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Introduction

In non-clonal species, all individuals are genetically and phenotypically unique, which constitutes the most elemental facet of biological diversity. Intraspecific biodiversity plays a key role in evolutionary and ecological dynamics (Bolnick et al. 2003, Odling-Smee et al. 2003). It is the raw material on which selection does act, potentially leading to adaptation to environmental changes, and improving population resilience to disturbances (Jung et al. 2013, Moran et al. 2015). Intraspecific diversity also affects the way populations modulate their biotic and abiotic environment, thus impacting community structure and ecosystem functioning (Hughes et al. 2008, Bolnick et al. 2011). Therefore, understanding patterns and underlying determinants of intraspecific diversity is of critical importance for ecological, evolutionary and conservation sciences (Chave 2013, Mimura et al. 2017).

As proposed for interspecific diversity, intraspecific diversity can be decomposed into two components: within-population (intraspecific Ŭ-diversity) and between-population intraspecific diversity (intraspecific ɓ-diversity) (Loreau 2000). Within-population intraspecific diversity corresponds to the diversity space covered by individuals composing a population, whereas between-population intraspecific diversity corresponds to the differentiation observed among populations pairs. Intraspecific diversity also comprises a genetic and a phenotypic facet, the former being inherited from the parents and the later being affected by both inherited and non-inherited (environmental) information. Intraspecific genetic diversity is here defined as the variability of neutral and non-neutral genetic sequences observed within and among populations (Holderegger et al. 2006), whereas phenotypic diversity encompasses the diversity of individuals' traits and includes behavioural, morphological and physiological traits (Violle et al. 2007).

Understanding how intraspecific diversity is maintained at the population level has long attracted ecologists and evolutionary biologists. For instance, the rise of molecular tools

in the last decades has generated many studies describing patterns of intraspecific neutral genetic diversity (e.g. through allelic richness and F_{ST}), so as to unravel the demographic and evolutionary history of populations, and hence to improve their conservation and management (Manel et al. 2003, Reed and Frankham 2003, Blanchet et al. 2017). From an adaptive point of view, the relative importance of divergent natural selection in shaping the distribution of phenotypic traits across landscapes -and hence phenotypic ß-diversity- has been the focus of studies combining quantitative genetics and experimental approaches (Kawecki and Ebert 2004, Leinonen et al. 2013, Blanquart et al. 2013). In parallel, ecologists have recently focused on the distribution of intraspecific phenotypic Ŭ-diversity across species and landscapes in order to better appraise its roles for community dynamics (Violle et al. 2012, Moran et al. 2015, Siefert et al. 2015). However, the study of intraspecific diversity still lacks an integrative framework in which patterns of genetic and phenotypic (Ŭ- and ß-) diversity, as well as their underlying determinants, would be investigated simultaneously and considered as two potentially covarying facets of biological diversity. Remarkably, a framework in which two facets of biodiversity (namely species diversity and intraspecific genetic diversity) are studied in an integrative way has been introduced by Vellend (2005) and has generated an increasing number of studies (reviewed in Vellend et al. 2014, Lamy et al. 2017). These studies on species-genetic diversity correlations (SGDCs) led to a better understanding of the relationships between species and genetic diversity, as well as the processes shaping these facets of biodiversity in similar or contrasting ways (Taberlet et al. 2012, Vellend et al. 2014).

Studying genetic-phenotypic intraspecific diversity correlations (GPIDCs) within a framework analogous to the SGDCs framework is attractive since genetic and phenotypic intraspecific diversity are intrinsically related and can be influenced by the same set of adaptive and neutral processes (Lowe et al. 2017). For instance, in the case of non-neutral genetic markers and adaptive traits, a positive GPIDC is expected when genetic and

63 phenotypic non-neutral diversity are directly affected by environmental conditions (through
64 selection and/or plasticity). In this case, both genetic and phenotypic Ŭ-diversity are expected
65 to be high in populations inhabiting highly heterogeneous environments, and both genetic and
66 phenotypic ɓ-diversity are expected to be high between populations experiencing contrasting
67 environmental conditions (Leimar 2005, Hedrick 2006, Wang and Bradburd 2014). Genetic
68 and phenotypic diversity are also expected to be positively correlated if they are driven by
69 neutral processes such as drift and dispersal (but see Edelaar et al. 2008, Lowe and McPeek
70 2014), which can notably be the case for neutral genetic markers and phenotypic traits that are
71 weakly affected by selection (Hartl and Clark 2007). In that case, genetic and phenotypic Ŭ-
72 diversity should be high in populations with large effective sizes and/or experiencing strong
73 immigration. At the ɓ-level, genetic and phenotypic diversity should be high between
74 populations of small population sizes (Prunier et al. 2017) and/or geographically isolated from
75 one another (Hutchison and Templeton 1999). Finally, a positive GPIDC can be explained by
76 a direct relationship between genetic and phenotypic diversity, notably when genetic diversity
77 directly codes for the considered traits, or appropriately describes the whole genomic diversity
78 (Hoffman et al. 2014). Conversely, when genetic and phenotypic diversity are driven by
79 (uncorrelated) divergent processes, GPIDCs are expected to be weak and non-significant.

80 Here, we aimed at testing spatial covariations in genetic and phenotypic intraspecific
81 diversity in two parapatric species inhabiting a spatially-structured landscape, and at
82 unravelling underlying determinants of each diversity facet at the landscape scale. More
83 specifically, we first quantified and described genetic and phenotypic intraspecific diversity in
84 two parapatric freshwater fish species (*Gobio occitaniae* and *Phoxinus phoxinus*) across an
85 entire river drainage. We then investigated both Ŭ- and ɓ-GPIDCs for these two species, and
86 we finally deciphered the parallel or independent determinants shaping Ŭ- and ɓ- genetic and
87 phenotypic diversity using causal analyses (Fourtune et al. 2018). To this end, we gathered

88 neutral genetic diversity and morphological diversity (a supposedly non-neutral type of trait)
89 in both *G. occitaniae* and *P. phoxinus* so as to test whether or not the relative importance of
90 main determinants of GPIDCs varied for species sharing a similar environment but with
91 different life-history traits. We predicted that GPIDCs should be weak for the two species
92 since neutral genetic diversity should mainly be driven by gene flow and/or drift, whereas
93 morphology should be determined by local environmental characteristics. Alternatively, in
94 river networks (Grant et al. 2007), factors affecting neutral processes (e.g. carrying capacity,
95 geographic isolation, etc.) and adaptive processes (e.g. physico-chemical conditions such as
96 water temperature, habitat heterogeneity, etc.) tend to covary along the network (e.g. upstream
97 areas are generally homogeneous habitats with small carrying capacities whereas downstream
98 areas are heterogeneous habitats with large carrying capacities) and these spatial covariation
99 in underlying processes might generate strong GPIDCs. Moreover, the specific structure of
100 river networks (treelike branching, constrained dispersal corridors, upstream-downstream
101 environmental gradient) has already been theoretically and empirically shown to affect
102 patterns of neutral and non-neutral diversity (Paz-Vinas and Blanchet 2015, Fronhofer and
103 Altermatt 2017). Testing GPIDCs in highly spatially-structured landscapes such as dendritic
104 riverine networks thus appears of particular interest.
105
106
107 **Materials and Methods**
108
109 *Collection of genetic and phenotypic data*
110 *Study species. Gobio occitaniae* (the Occitan gudgeon) and *Phoxinus phoxinus* (the
111 European minnow) belong to the Cyprinidae family. Both species are insectivorous but differ
112 in their foraging mode: *G. occitaniae* feeds predominantly on the bottom, whereas *P.*

113 *phoxinus* feeds in the water column. *Gobio occitaniae* mean body length (120-150mm) is
114 slightly larger than that of *P. phoxinus* (80-90mm). Moreover these parapatric species have
115 contrasting levels of habitat specialisation: *G. occitaniae* lives in many habitat types and is
116 ubiquitous in many river basins whereas *P. phoxinus* is more likely to occur in upstream
117 (cold) areas.

118 *Study area and sampling.* Fish were sampled across 48 sites evenly scattered across
119 the Garonne-Dordogne river drainage (South-Western France). This river drainage covers a
120 79 800km² area and sites were selected so as to cover the whole distribution of the two fish
121 species, and hence their entire realized environmental niches. Electrofishing sampling was
122 conducted during summers 2014 (42 sites) and 2015 (6 sites) and each site was visited once.
123 Sampled area was of ~500-1000 m2 to adequately represent the local habitat heterogeneity.
124 *Gobio occitaniae* and *P. phoxinus* individuals were found in 39 and 34 sites respectively, with
125 25 sites in which the species were found in sympatry (see Fig. 1). We sampled up to thirty
126 individuals per species and per sampling site (range, 21-30 and 24-30 for *G. occitaniae* and *P.*
127 *phoxinus r*espectively, see Table S1), leading to the sampling of 1119 gudgeons and 978
128 minnows. Sampled individuals were anaesthetised using oil of clove before being carefully
129 aligned on their right side on a white dashboard including a reference scale. The left side of
130 each individual was photographed using a digital camera (Canon G16©) mounted on a tripod.
131 Subsequently, we collected on each individual a small piece of pelvic fin which was preserved
132 in 70% ethanol for genetic analyses. Individuals were then released alive in their respective
133 sampling site.

134 *Genetic data.* Genetic DNA was extracted from all samples using a salt-extraction
135 protocol (Aljanabi and Martinez 1997). Genotyping was performed using 15 and 18
136 microsatellite loci in *G. occitaniae* and *P. phoxinus* respectively. Accession numbers and
137 conditions for polymerase chain reactions (PCR) are provided as supplementary material

(Appendix S1). Genotypes were analysed using GENEMAPPER 5.0 (Applied Biosystems©). The presence of null alleles was assessed at each locus using MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004). We also checked for gametic disequilibrium using GENEPOP 4.2.1 (Rousset 2008) after sequential Bonferroni correction to account for multiple tests. We discarded from further analyses any locus showing significant gametic disequilibrium and/or evidence of null alleles, leading to a total of 13 and 17 loci for *G. occitaniae* and *P. phoxinus* respectively (Appendix S1).

As a measure of genetic Ŭ-diversity, we computed -for each species- the allelic richness as the mean number of alleles across loci for a standardized sample size of 20 using ADZE 1.0 (Szpiech et al. 2008). As a measure of genetic ɓ-diversity, we used three common indices based on allelic frequencies: Roussetôs linearized F_{ST} ($F_{ST}/(1-F_{ST})$), hereafter denoted as F_{ST} (Rousset 1997), Neiôs version of Cavalli-Sforzaôs chord distance *Da* (Nei et al. 1983) and Jostôs D (Jost 2008). Whatever the dataset, these three indices were highly correlated (Mantel $r > 0.85$, $p < 0.001$): for the sake of simplicity, we thus only retained F_{ST} as a measure of genetic differentiation. This metric of genetic differentiation is indeed the most commonly used on population genetics and most theoretical works have been developed on this metric.

Phenotypic data. Individuals morphology was analysed using a landmark-based geometric morphometrics approach (Rohlf and Marcus 1993). Sixteen homologous landmarks were defined so as to capture the overall body shape of each individual (Fig. 2). Landmarks coordinates were obtained from digitized pictures using the Pointpicker plugin (http://bigwww.epfl.ch/thevenaz/pointpicker/) in the ImageJ software (Schneider et al. 2012). As the distance between the camera and the fish slightly varied between sites, we size-corrected landmarks coordinates using the reference scale. For each species, landmarks were aligned using Generalized Procrustes Analysis (Rohlf and Slice 1990) with the R package *geomorph* (Adams and Otárola-Castillo 2013) in order to remove the effects of rotation,

translation and scale on shape variation. Relative warps (n = 32) were computed for each individual by performing a Principal Component Analysis (PCA) on the aligned landmark coordinates of each species (Rohlf 1993). As the majority of the 32 relative warps explained a very small amount of variation, we only conserved for further analyses the first nine relative warps that together explained more than 85% of the variance in each data set (85.69% and 85.32% for *G. occitaniae* and *P. phoxinus* respectively). These relative warps were used as shape variables. Individual centroid size, which is the square root of the sum of squared distances from landmarks to their centroid, was used as a surrogate of overall body size of each individual (Bookstein 1991).

Since relative warps are PCA coordinates, they can be seen as coordinates in a nine dimensions shape space (i.e. the hyperspace in which each point represents a configuration of landmarks) of each individual. Morphological Ŭ-diversity was thus computed as the proportion of the total shape space (i.e. the shape space covered by all individuals from all populations for a given species) occupied by all individuals from a given population (see Fig. S2 for a graphical example), after accounting for differences in sampling sizes among populations using a random resampling approach. This index is equivalent to the functional richness index developed by Villéger et al. (2008) at the interspecific level. Morphological ɓ-diversity was computed -for each species- as the euclidean distance between the consensus (i.e. the average shape in a population) of each populations pair (see Fig. S2). Additionally, in order to further decompose this index of differentiation, we computed the functional dissimilarity index $F_ɓ$ initially developed by Villéger et al. (2011) for interspecific diversity and informing the proportion of the total shape space that is not shared between two populations from a given pair:

$$F_ɓ = 1 ï \text{ (Volume shared)/(Total volume)}$$

187 $F_ß$ ranges from 0 to 1, with 0 indicating a perfect overlap in shape space occupation and 1
188 indicating that no space is shared between two populations. Intermediate $F_ß$ can result from
189 two (non-exclusive) mechanisms: turnover (the two populations fill distinct parts of the shape
190 space with weak overlap) and nestedness (one population fills a small proportion of the shape
191 space filled by the other) (Baselga 2010, Villéger et al. 2013). Consequently, we further
192 computed F_{pturn}, the proportion of $F_ß$ that is due to trait turnover so as to tease apart the effect
193 of nestedness and turnover. Because of computing limitations, these two indices were
194 computed only from the first three relative warps coordinates. All indices were computed
195 using functions available at http://villeger.sebastien.free.fr/Rscripts.html.
196
197 *Collection of environmental data*
198 We gathered several variables related to environmental characteristics and river
199 topography. These variables are likely to impact intraspecific diversity through evolutive
200 and/or neutral processes. General predictions related to the effect of each variable on
201 intraspecific diversity are listed in Table 1.
202 *Environmental characteristics.* Substrate type covering the river bed (i.e. the habitat
203 for these fish species) was evaluated visually on each site following a predefined protocol:
204 substrate was classified into nine categories based on particle size, ranging from silt (<
205 0.05mm) to solid bedrock (see Table S3), and the percentage of each category composing the
206 river bed of each site was estimated visually within a predefined area of ~100 m^2 that was
207 representative of the sampling area. From these data, habitat heterogeneity was computed as
208 the Pielou's evenness index, with low values identifying sites in which one of the substrate
209 categories was dominant and hence relatively homogeneous habitats (large values indicated
210 heterogeneous habitats with various substrate types). Habitat dissimilarities between sites
211 were computed from percentages of substrate categories as Bray-Curtis distances, with a

212 value of 1 identifying two sites sharing no substrate categories (i.e. sites highly dissimilar in
213 substrate types). The other environmental variables were obtained for each site from the
214 database of the Water Information System of the Adour Garonne basin (SIEAG, ôSystème
215 dôInformation sur lôEau du Bassin Adour Garonneõ; http://adour-garonne.eaufrance.fr) that
216 gathers physico-chemical characteristics of surface water measured several times every year
217 at numerous sites in the river catchment. Only sites for which data were available for July (a
218 month in which the two species are highly active) of the years 2013, 2014 and 2015 were
219 selected from the SIEAG database. The mean of the three values was calculated to inform the
220 physico-chemical quality of the sites according to several parameters. We notably focused on
221 two parameters directly affecting fish populations, i.e. oxygen saturation (%) (Crispo and
222 Chapman 2008) and water temperature (°C) (Buisson et al. 2008). We gathered eleven
223 additional variables informing overall water quality: concentrations in ammonium, azote,
224 organic carbon, nitrate, nitrite, orthophosphate and phosphorus (mg/L), Biological Oxygen
225 Demand (mg/L), water conductivity (mS/cm), pH and suspended matter (mg/L). We
226 performed a PCA on these variables using R package ñade4ò (Dray and Dufour 2007), and
227 gathered the coordinates of each site on the first two axes (representing respectively 36.85%
228 and 19.73% of the total variance) to create two synthetic variables (hereafter named
229 *chemicals1* and *chemicals2*) informing water quality. High values of *chemicals1* correspond
230 to high concentrations in ammonium, azote, organic carbon, phosphorus and a high Biological
231 Oxygen Demand, whereas high values of *chemicals2* correspond to high concentrations in
232 nitrate and nitrite and high values of conductivity, pH and suspended matter (see Fig. S4 for
233 the graphical representation of the PCA).

234 *River topography*. River distances from the outlet and from the source for each site, as
235 well as river distance between each pair of sites, were computed using QuantumGIS software
236 (QGIS; Quantum GIS Development Team 2017). Elevation for each site was obtained from

237 the French Theoretical Hydrological Network (ôRéseau Hydrologique Théorique françaisõ;
238 Pella et al. 2012). A PCA was performed on elevation and distance from the outlet. The
239 coordinates of each site on the first axis, accounting for 92.99% of the variance, were used to
240 create a synthetic variable, hereafter named *isolation*, with high values corresponding to sites
241 of high altitude, located far from the outlet and that are expected to be highly isolated
242 geographically. Additionally, the cumulative altitude differences between each pair of sites
243 along the riverine network were computed using the MATLAB software-coding environment
244 (Mathworks, Inc., scripts available upon request). River width, used as a proxy for habitat
245 area and hence carrying capacity (Raeymaekers et al. 2008), was characterised by measuring
246 river bed width at two randomly selected locations for each sampling site, and subsequently
247 computing the mean of these two values. The betweenness centrality value of each site was
248 computed using ComplexNetGIS toolbox in ArcGIS (Caschili 2010). Betweenness centrality
249 is an index quantifying the connectivity and positional importance of a node within a network
250 (Freeman 1977, Estrada and Bodin 2008).
251
252 *Statistical analyses*
253 Intraspecific genetic and phenotypic Ŭ- and ɓ-diversities were compared between
254 species using Wilcoxon rank sum test. Spearman rank correlations and Mantel tests were then
255 used to assess and statistically test the significance of the correlations -for each species
256 separately- between genetic and phenotypic diversity, at the Ŭ- and ɓ-levels respectively.
257 The d-sep test (Shipley 2000, 2013) was used to unravel the relationships between
258 environmental characteristics, topographical variables and intraspecific phenotypic and
259 genetic diversity at the Ŭ- and ɓ-levels. The d-sep test is a type of path (causal) analysis
260 method computing the significance and likelihood of a causal model through the test of the
261 conditional independences (named d-separations; Pearl and Verma 1987) that should be true

if the model fits the data. A non-significant p-value associated with the null hypothesis that ñthe model fits the dataò indicates that the observed data are consistent with the tested model. This method is very flexible as the statistical method used to test the independences is selected according to the data, which allowed in our case modelling both point summary (Ŭ-diversity) and pairwise (ɓ-diversity) data types (Fourtune et al. 2018). Prior to analyses, environmental variables were log-transformed if needed to obtain a normal distribution, and all variables were centred to the mean and scaled.

At the Ŭ-level, we defined a causal model in which intraspecific genetic and phenotypic diversity were both linked one to the other and linked to oxygen saturation, water temperature, habitat heterogeneity, *chemicals1*, *chemicals2*, connectivity, *isolation*, and habitat area (see Table 1 for specific predictions). As some of the topological and environmental variables are expected to covary spatially, paths taking into account these covariations were included when needed. This model was tested using a d-sep test in which d-separations (i.e. path coefficients) were tested using linear regressions. This model was then simplified by removing paths one by one until reaching the model with the lowest Akaike Information Criteria (hereafter AIC; Burnham and Anderson 2002) so as to identify the main determinants underlying phenotypic and genetic Ŭ-diversity.

For genetic and phenotypic ɓ-diversity, four environmental variables (oxygen saturation, temperature, *chemicals1* and *chemicals2*) were converted into pairwise environmental differences between sites using euclidean distances. Differences in habitat area, used as a proxy for carrying capacity and hence for the effect of genetic drift, were computed as *di* (distance based on the inverse; Relethford 1991) as recommended in Prunier et al. (2017). Additionally, we considered the three variables already taking the form of pairwise matrices: topographic distances, differences in cumulative altitude and habitat dissimilarities between sites. We defined a model in which genetic and phenotypic ɓ-diversity

were both linked one to the other and linked to these eight explanatory variables. A full model was tested using a d-sep test procedure recently developed for handling pairwise matrices (Fourtune et al. 2018) and that uses permutations-based linear regressions. This model was simplified using the same procedure as above, until reaching the model with the lowest AIC score.

As a side objective aiming at better understanding the spatial distribution of phenotypic diversity in the two fish species, we investigated phenotype-environment relationships by assessing and testing relationships between the individual shape of fish and raw environmental variables. For the sake of clarity, only the first two relative warps (respectively encompassing 31.3% and 15.5% of the variance in *G. occitaniae* and 27% and 21.9% of the variance in *P. phoxinus*) were separately considered in this analysis combining model selection and model averaging. Global models (one per relative warp and per species) linking relative warps to the environmental variables and their associated quadratic terms were implemented using the *lme* function in R package ónlmeô (Pinheiro et al. 2016) with the population identity included as a random-intercept effect. We also added individual centroid size and its quadratic term as explanatory variables to take the effects of allometry into account (Outomuro and Johansson 2017). All possible models were generated from the global model and their AIC were computed using the *dredge* function in the R package ôMuMInô (BartoŒ2016). Full model averaging was then applied across the best models (ΔAIC < 4; Burnham and Anderson 2002) with the function *model.avg* in order to estimate the relative importance of each explanatory variable and weighted estimates associated to explanatory variables. All statistical analyses were performed with the R software (R Development Core Team 2017).

Results

Alpha- and β-intraspecific diversity

Both genetic and phenotypic Ŭ-diversity were higher for *P. phoxinus* than for *G. occitaniae* (Wilcoxon rank sum tests, W = 149, P < 0.001 for genetic Ŭ-diversity; W = 340, P < 0.001 for phenotypic Ŭ-diversity; Fig. 3a and 3b), indicating that minnow populations were on average more diverse genetically and phenotypically than gudgeon populations. However, within-species, we did not find significant correlations between genetic and phenotypic Ŭ-diversity (i.e. Ŭ-GPIDCs) for any of the two species (Spearman rank correlation tests, ɟ = 0.105, P = 0.521 in *G. occitaniae* and ɟ = 0.016, P = 0.927 in *P. phoxinus*, Fig. 4a and 4b).

Mean between-sites genetic ɓ-diversity was in average lower in *G. occitaniae* than in *P. phoxinus* (Wilcoxon rank sum test, W = 245, P < 0.001, Fig. 3c), whereas the reverse held true for mean phenotypic ɓ-diversity per site (Wilcoxon rank sum test, W = 1284, P < 0.001, Fig. 3d); gudgeon populations were -in average- less genetically differentiated than minnow populations but more phenotypically differentiated. The fact that *G. occitaniae* populations were more phenotypically differentiated was confirmed using $F_{ɓ}$ (Wilcoxon rank sum test, W = 1259, P < 0.001; Fig. S5). We further found that phenotypic turnover (measured as F_{pturn}) was also higher for *G. occitaniae* populations than for *P. phoxinus* populations (Wilcoxon rank sum test, W = 981, P < 0.001; Fig. S5). Remarkably, for 116 out of 741 populations pairs of gudgeon, $F_{ɓ}$ and F_{pturn} were equal to 1, indicating no overlap in the portions of the shape space occupied by populations, whereas in *P. phoxinus*, none of the populations pair had values of $F_{ɓ}$ and F_{pturn} equal to 1. The correlation between genetic and phenotypic ɓ-diversity was positive and significant in *G. occitaniae* (i.e. significant ɓ-GPIDC, Mantel test, r = 0.358, P = 0.001, Fig. 4c) but not in *P. phoxinus* (Mantel test, r = -0.011, P = 0.521, Fig. 4d).

337 *Determinants of* ***Ŭ*** *and* ***ɓ****-GPIDCs.*

338 ***Ŭ****-GPIDCs.* In *G. occitaniae* and *P. phoxinus*, the models with the lowest AIC scores
339 were well supported by the data, as indicated by non-significant p-values associated with the
340 tests of conditional independences (C = 75.485, d.f. = 74, P = 0.389 for *G. occitaniae* and C =
341 76.524, d.f. = 72, P = 0.335 for *P. phoxinus*, Table 2a). In *G. occitaniae*, we found a negative
342 effect of *isolation* on both genetic and phenotypic Ŭ-diversity, indicating that populations
343 were genetically and phenotypically impoverished in sites situated at high altitude and far
344 from the river mouth. Additionally, phenotypic Ŭ-diversity tended to be negatively related to
345 connectivity (Fig. 5a). In *P. phoxinus*, genetic Ŭ-diversity was also negatively related to
346 *isolation*, but not phenotypic Ŭ-diversity (Fig. 5b). Genetic Ŭ-diversity was also positively
347 related to habitat area. Phenotypic Ŭ-diversity was negatively correlated to oxygen saturation,
348 which was in turn positively associated with *isolation* and habitat area (Fig. 5b).

349 ***ɓ****-GPIDCs.* The models with the lowest AIC scores were well supported by the data in
350 both species (C = 109.167, 92 d.f., P = 0.130 in *G. occitaniae* and C = 97.699, 94 d.f., P =
351 0.575 in *P. phoxinus*, Table 2b). For *G. occitaniae*, genetic ɓ-diversity was positively related
352 to the cumulative difference in altitude, which was itself related to riverine distance (leading
353 to an indirect relationship between genetic ɓ-diversity and riverine distance, Fig. 5c).
354 Phenotypic ɓ-diversity was positively correlated to three environmental variables (difference
355 in oxygen concentration, difference in water temperature and habitat dissimilarity, Fig. 5d).
356 Additionally, we found a positive relationship between genetic and phenotypic ɓ-diversity
357 (Fig. 5d). Regarding *P. phoxinus*, genetic ɓ-diversity was positively related to riverine
358 distance both directly and indirectly through difference in altitude and difference in habitat
359 area. Genetic ɓ-diversity was also negatively related to difference in oxygen. Phenotypic ɓ-
360 diversity was directly related to difference in connectivity and indirectly related to pairwise
361 riverine distance through difference in altitude and habitat area (Fig. 5d).

362

363 *Phenotype-environment relationships.*

364 In *G. occitaniae*, the first relative warp had high values in individuals living in sites
365 with high concentration in oxygen (ɓ = 0.272, CI = [0.138; 0.406]) and low water temperature
366 (ɓ = -0.236, CI = [-0.367; -0.105]), and where the proportion of silt in the substrate was low
367 (ɓ = -0.268, CI = [-0.401; -0.136]) (Table 3). Additionally, the first relative warp was related
368 to individual centroid size (used as a proxy for individual size) and its quadratic term (ɓ =
369 0.513, CI = [0.469; 0.557] and ɓ = -0.106, CI = [-0.136; -0.076] respectively), suggesting
370 allometric relationships with this relative warp. None of the environmental variables we
371 considered were likely to be associated to the second relative warp. In *P. phoxinus*, the first
372 and second relative warps were significantly associated to the individual centroid size (ɓ =
373 0.455, CI = [0.392; 0.518] and ɓ = -0.342, CI = [-0.413; -0.271] respectively), which also
374 suggests allometric relationships. Surprisingly, we found no phenotype-environment
375 relationships in *P. phoxinus*, suggesting that, in this species, most of the phenotype variations
376 we detected were independent of environmental characteristics and topography.

377

378

379 **Discussion**

380

381 In this study, we tested for Genetic-Phenotypic Intraspecific Diversity correlations
382 (GPIDCs) in two parapatric freshwater fish species, and explored the processes shaping the
383 spatial distribution of their genetic and phenotypic characteristics. Our results revealed
384 disparities in the distribution of genetic and phenotypic diversity in the two species, as well as
385 common and contrasted processes shaping diversity at the Ŭ- and ɓ-levels.

386

387 In terms of genetic diversity, we found that, overall, *P. phoxinus* populations were
388 more locally diverse (higher neutral genetic Ŭ-diversity) and more differentiated (higher
389 neutral genetic ɓ-diversity) than *G. occitaniae* populations (Fig. 3a and 3c). The overall
390 higher genetic Ŭ-diversity in *P. phoxinus* may indicate different evolutionary histories
391 between the two species. For instance, this may indicate that ancient effective population sizes
392 were higher and more stable over time in *P. phoxinus* than in *G. occitaniae*, thus limiting the
393 impact of drift, and/or that multiple glacial refugia existed in *P. phoxinus*, hence favouring the
394 maintenance of a high neutral genetic diversity (Hewitt 1999). The overall high genetic Ŭ-
395 diversity found in *P. phoxinus* populations may also result from high levels of biological
396 connectivity in this species when compared to *G. occitaniae* (Frankham 1996) as suggested
397 by spatial patterns of isolation-by-distance (Appendix SX). We also found that local levels of
398 neutral genetic Ŭ-diversity in *P. phoxinus* were lower in sites of small habitat area (Fig. 5a),
399 indicating an increased effect of genetic drift in habitat with small carrying capacity. This
400 relationship between neutral genetic Ŭ-diversity and habitat area was not observed in *G.*
401 *occitaniae*, probably because of a sampling bias, as *G. occitaniae* was generally found at
402 lower altitudes and thus in stretches of higher carrying capacity than *P. phoxinus* (mean
403 habitat area: 29.9 m and 16.2 m in *G. occitaniae* and *P. phoxinus* respectively; anova on log-
404 transformed data: $F = 4.16$, $df = 71$, $p = 0.06$). Higher overall levels of neutral genetic ɓ-
405 diversity in *P. phoxinus* (Fig. 3c) may also be explained by this possible altitudinal sampling
406 bias, responsible for higher spatial heterogeneity in the influence of drift (Prunier et al. 2017).
407 Accordingly, we observed a positive impact of differences in habitat areas on genetic ɓ-
408 diversity in *P. phoxinus* (Fig. 5d), indicating that -as expected- populations experiencing
409 contrasted intensity of genetic drift were more genetically differentiated (Prunier et al. 2017).
410 Despite these differences, we found common processes driving genetic diversity in
411 both species. First, we found that neutral genetic Ŭ-diversity was strongly related to

412 geographic isolation in both species, with lower genetic diversity observed in highly isolated
413 sites, i.e. sites at high altitude and far from the river mouth (Fig. 5a-b). This decrease in
414 neutral genetic diversity in geographically isolated sites has already been reported, and has
415 actually been suggested to be a general pattern in riverine networks (Paz-Vinas et al. 2015).
416 Two non-exclusive hypotheses can explain this pattern. First, movements between
417 populations might be directionally-biased due to water flow (Morrissey and de Kerckhove
418 2009, Paz-Vinas et al. 2013). This asymmetric dispersal leads to an increase in gene flow
419 from upstream (isolated sites) to downstream, generating an upstream loss of genetic diversity
420 through emigration (Kawecki and Holt 2002). Alternatively, a decrease in genetic diversity in
421 upstream sites might reflect the species colonization history from downstream glacial refugia.
422 Second, genetic ϐ-diversity was driven by topographic features in both species (Fig. 5a-b); in
423 *G. occitaniae*, genetic differentiation was higher between sites isolated from each other by
424 high altitude drops along the network, whereas in *P. phoxinus*, genetic differentiation was
425 higher between sites separated by a high riverine distance. These two latter patterns confirm
426 the existence of a process of isolation-by-distance (Hutchison and Templeton 1999) in the two
427 species (supplementary material Appendix SX).
428
429 At the phenotypic level, our findings suggest that the regional pool in *G. occitaniae*
430 was composed of poorly diverse local populations (low phenotypic Ŭ-diversity; Fig. 3b) that
431 were highly dissimilar from one site to another (high phenotypic ϐ-diversity with high
432 turnover between populations, i.e. different populations display different phenotypes; Fig. 3d).
433 Conversely, in *P. phoxinus*, phenotypic Ŭ-diversity was higher and phenotypic ϐ-diversity was
434 lower than in *G. occitaniae*, which suggests that the regional pool of *P. phoxinus* was
435 composed of highly diverse local populations that were highly similar from one site to
436 another. The contrasted morphological patterns found in these two parapatric species may

result (i) from higher effective population sizes in *P. phoxinus* than in *G. occitaniae* (as suggested by measures of genetic Ŭ-diversity, see above), and/or (ii) from stronger effects of selection (or environmental effects in general) in *G. occitaniae* than in *P. phoxinus*. Indeed, a stronger effect of selection is expected to lead to environmental filtering and hence to less phenotypically diverse populations at the local scale (local adaptation) as well as to a high phenotypic ɓ-diversity between populations resulting from adaptive divergence (and/or strong plastic effects) (Blanquart et al. 2013). This later hypothesis was strengthened by the significant relationships found between the individual shapes in *G. occitaniae* and three environmental variables (see below), and by the limited scale of gene flow in *G. occitaniae* when compared to isolation-by-distance pattern in *P. phoxinus* (supplementary material Appendix SX). Despite the strong environmental heterogeneity measured among sites, *P. phoxinus* populations appeared highly similar suggesting a higher level of generalism in *P. phoxinus* populations than in possibly more specialist *G. occitaniae* populations, as well as a homogenizing influence of effective dispersal in *P. phoxinus.*

In line with this result, we found highly contrasted processes shaping phenotypic diversity in both species. In *G. occitaniae*, phenotypic Ŭ-diversity was lower in highly-connected sites, with a high centrality index (Fig. 5a). This result was unexpected since highly central sites are expected to receive more dispersers, hence enhancing phenotypic diversity and impeding local adaptation. However, the observed pattern could be explained by a higher efficiency of selection in central sites in which dispersal introduces additional phenotype variability necessary for adaptation (Lenormand 2002), potentially in combination with a habitat matching process, that would hinder the negative impact of gene flow on local adaptation (Edelaar et al. 2008). Alternatively -and not-exclusively- this negative relationship could arise from a statistical bias, for instance if an unmeasured collinear variable explained both centrality and phenotypic Ŭ-diversity. However, phenotypic Ŭ-diversity tended to be

462 lower in isolated sites (in which, according to our former hypothesis, populations are expected
463 to be less locally adapted and hence more diverse), which may suggest an effect of neutral
464 processes (ñphenotypicò drift) as observed in neutral genetic diversity, and/or stronger effects
465 of environmental filtering in isolated sites (high altitude and far from the river mouth) than in
466 less isolated sites. This latter hypothesis of strong environmental filtering is likely given that
467 upstream (isolated) sites are known to experience harsh environmental conditions (Vannote et
468 al. 1980). Furthermore, in *G. occitaniae*, phenotypic ß-diversity was primarily shaped by
469 environmental variables related to habitat and water features (namely, difference in oxygen
470 saturation, temperature and habitat dissimilarity) such that mean phenotype was different
471 between sites displaying contrasted abiotic conditions (Fig. 5c). This impact of environment
472 on phenotype was strengthened by the direct relationships found between individual
473 phenotype and oxygen saturation, temperature and proportion of silt in the habitat. These two
474 results confirm the hypothesis that selection (or environment in general) has strong effects on
475 phenotype in *G. occitaniae*, however it remains unclear whether these effects originate from
476 heritable differentiation or environmentally induced plasticity.

477 In *P. phoxinus*, phenotypic Ŭ-diversity was higher in sites with low oxygen
478 concentration, suggesting a positive influence of stressful conditions on phenotypic Ŭ-
479 diversity (Fig. 5b). This result was surprising as we expected that a low saturation in oxygen
480 would sustain small population sizes, hence reducing phenotypic diversity. Moreover,
481 stressful conditions were expected to strengthen selection pressure. However, stressful
482 conditions have already been proven to have a positive effect on intraspecific diversity,
483 notably (i) when they lead to an increase of mutation and recombination rates in non-neutral
484 parts of the genome (Badyaev 2005), and (ii) when they lead to an increase in phenotypic
485 plasticity (Ghalambor et al. 2007, Rey et al. 2016). Phenotypic ß-diversity was increased
486 between populations inhabiting sites of different area and different connectivity (Fig. 5d).

These relations may suggest an effect of neutral processes associated with population sizes and gene flow on phenotypic diversity, which is likely as local adaptation does not appear to be high in this species.

We found no GPIDCs at the Ŭ-level in either species, indicating that neutral genetic Ŭ-diversity and phenotypic Ŭ-diversity are driven by independent processes. Although consistent with our theoretical expectations, this result was surprising in *G. occitaniae* as we found a similar impact of isolation on genetic and phenotypic Ŭ-diversity. This absence of correlation suggests that the influence of other processes (related to connectivity) were strong enough to break spatial covariation between these two facets of diversity in this species.

At the ɓ-level, we found a significant and positive GPIDC in *G. occitaniae*, such that populations being genetically different were also phenotypically different. However, this correlation did not seem to originate from similar environmental processes shaping both facets of ɓ-diversity but appeared to be mainly caused by a direct effect of one facet of ɓ-diversity on the other. It was not possible to statistically determine the direction of this relation (genetic diversity to phenotypic diversity or phenotypic diversity to genetic diversity) due to methodological limitations. However, given that we focused on neutral genetic markers, a direct impact of genetic ɓ-diversity on phenotypic ɓ-diversity seems unlikely except if we assume (i) that the here chosen microsatellite markers properly reflect the genomic diversity in this species and (ii) that phenotypic diversity in this species is mainly driven by the genetic background of individuals. Alternatively, positive assortative mating (i.e. the propensity to mate with phenotypically similar individuals) has been shown to be particularly strong in fish (Jiang et al. 2013) and could explain this direct relation between phenotypic and genetic differentiation (Wang and Summers 2010). Yet, although our dataset encompasses the main environmental variables known to be involved in adaptive and neutral

512 processes in freshwater fish, we cannot rule out the influence of a possible unmeasured abiotic
513 or biotic factor impacting both facets of ϐ-diversity. In *P. phoxinus*, genetic and phenotypic ϐ-
514 diversity were not correlated despite of a similar impact of habitat area on both facets of
515 diversity. Other important processes involving riverine distance and connectivity could
516 impede spatial covariation between these two facets of diversity in this species.
517
518 The use of an integrative framework allowed us to unveil striking dissimilarities
519 between the patterns and drivers of genetic and phenotypic intraspecific diversity in two
520 parapatric freshwater fish species. First, we found indications of limited gene flow and of
521 local adaptation in *G. occitaniae* populations. Second, we observed that, in *P. phoxinus*,
522 populations were phenotypically more diverse and that gene flow occurred at a larger spatial
523 scale. This high phenotypic diversity could indicate a bet-hedging strategy (i.e. the
524 augmentation of phenotypic diversity to optimize fitness in varying environments), possibly
525 in response to inter-annual variation in local flow regimes (Lytle and Poff 2004). Studying
526 neutral genetic diversity and phenotypic diversity within an integrative framework hence
527 appeared as a valuable way of deciphering the complex and diverse impacts of neutral and
528 adaptive processes on intraspecific diversity.
529 While introducing the novel framework of Species-Genetic Diversity Correlation,
530 Vellend (2005) stated that treating interspecific and intraspecific diversity as independent
531 phenomena in community ecology and population genetics was irrelevant. Similarly, genetic
532 and phenotypic diversity are clearly interrelated but are mainly studied separately in
533 population genetics and functional ecology. We advocate for a greater integration across
534 disciplinary boundaries in future studies in order to advance our understanding of the
535 distribution of intraspecific diversity.
536

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775 **Table 1**: General predictions (and underlying processes) regarding the influence of environmental

776 variables on intraspecidific α-diversity (a) and on intraspecidific β-diversity.

(a) Environmental variables	**Expected influence on intraspecific α-diversity**
Habitat heterogeneity	Highly heterogeneous sites should harbour populations with higher phenotypic α-diversity. Neutral genetic α-diversity should not be affected.
Chemicals1 Chemicals2 Oxygen saturation Temperature	Stressful conditions (i.e. high concentrations of chemicals, low oxygen saturation, high temperature) should reduce phenotypic α-diversity by strengthening selective pressures. They should also reduce effective population size and hence both neutral genetic and phenotypic α-diversity through genetic drift.
Habitat area	Populations living in large habitats should harbour high population sizes and experience low genetic drift (Prunier et al. 2017), hence increasing both neutral genetic and phenotypic α-diversity.
Connectivity	Sites with high connectivity should receive a high proportion of migrants and hence harbour populations with higher genetic and phenotypic α-diversity.
Isolation	Highly isolated sites should suffer higher genetic drift relatively to gene flow (Fourtune et al. 2016), hence reducing both neutral genetic and phenotypic α-diversity.

(b) Environmental variables	**Expected influence on intraspecific β-diversity**
Habitat dissimilarity Difference in chemicals1 Difference in chemicals2 Difference in oxygen saturation	Sites with highly dissimilar abiotic conditions should display high phenotypic β-diversity due to divergent selection. Additionally, if gene flow between environmentally different sites is hindered by the maladaptation of immigrants (isolation-by-environment, Sexton et al. 2014), we also expect a high genetic β-diversity between environmentally dissimilar sites.
Difference in habitat area	Heterogeneity in the intensity of genetic drift between sites due to contrasting population sizes should increase both neutral genetic and phenotypic β-diversity (Prunier et al. 2017).
Difference in connectivity	Dissimilarities in the intensity of gene flow experienced by populations due to contrasting connectivities should increase both neutral genetic and phenotypic β-diversity (Prunier et al. 2017).
Riverine distance Cumulative altitude difference	Sites highly isolated one from each other should experience a decrease of the homogeneizing effect of gene flow and an increase of genetic drift between them (isolation-by-distance, Hutchinson and Templeton 1999), hence enhancing both genetic and phenotypic β-diversity.

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779 **Table 2**: D-sep test statistics used to disentangle the effects of environmental variables on genetic and
780 phenotypic Ŭ-diversity (a) and on genetic and phenotypic ɓ-diversity (b) in *Gobio occitaniae* and
781 *Phoxinus phoxinus*. For each species and diversity facet, we simplified a full model (i.e. a model
782 including all paths described in the main text) until reaching the models with the lowest AIC score
783 represented in Figure 5.

(a) Alpha-intraspecific diversity	C statistics	d.f.	p-value	AIC
Gobio occitaniae				
Complete model	32.569	30	0.342	128.569
Optimal model	75.485	74	0.430	116.791
Phoxinus phoxinus				
Complete model	51.971	30	0.008	147.971
Optimal model	76.524	72	0.335	122.524
(b) Bêta-intraspecific diversity	C statistics	d.f.	p-value	AIC
Gobio occitaniae				
Complete model	47.866	30	0.020	167.866
Optimal model	108.119	92	0.120	150.119
Phoxinus phoxinus				
Complete model	50.105	30	0.012	170.105
Optimal model	97.058	94	0.394	133.058

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786 **Table 3**: Coefficients estimates and significance obtained through full model averaging on the best
787 (qpAIC < 4) linear mixed-effects models (one per relative warp and per species) linking relative warps
788 to the environmental variables and their associated quadratic terms models.(*** : p-value < 0.001)

Environmental variables	Relative warp 1	Relative warp 2	Relative warp 1	Relative warp 2
	Gobio occitaniae		*Phoxinus phoxinus*	
Centroid size	0.513***		0.455***	-0.342***
Centroid size²	-0.106***			
Connectivity			-0.017	
Distance from the mouth				0.018
Distance from the source			-0.025	
Habitat area			-0.016	
Slope		0.009		0.134
Carbone	0.008	-0.048		-0.002
Carbone²		-0.063		0.007
Conductivity				-0.035
Biological Oxygen Demand			0.007	
Suspended matter				0.013
pH		0.010		
pH²		0.009		
Oxygen saturation	0.272***	0.089	0.001	0.005
Oxygen saturation²			0.006	
Temperature	-0.236***			
Silt	-0.268***	-0.016		
Cobble	-0.023			
Large cobble		0.021		
Boulder		0.007		
Large boulder		0.045		0.080

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792 **FIGURE 1**

793 Location of the 48 sites sampled during summers 2014 and 2015 colored according to the

794 species present.

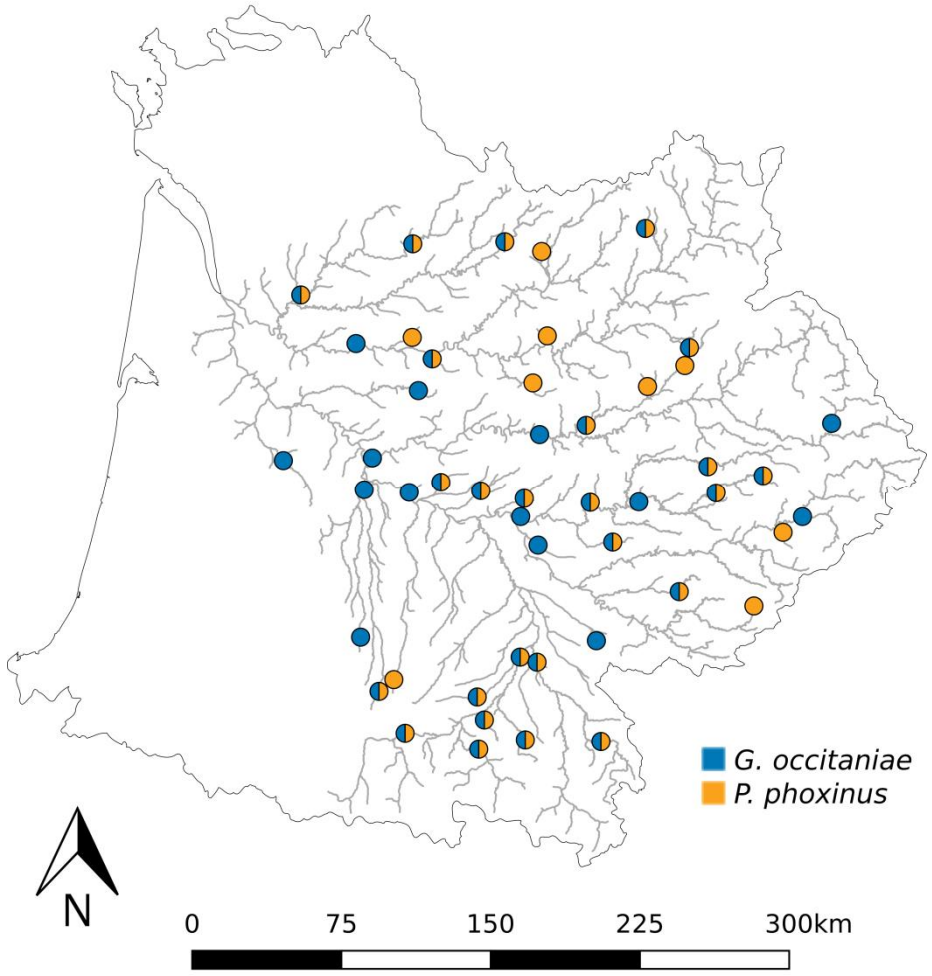

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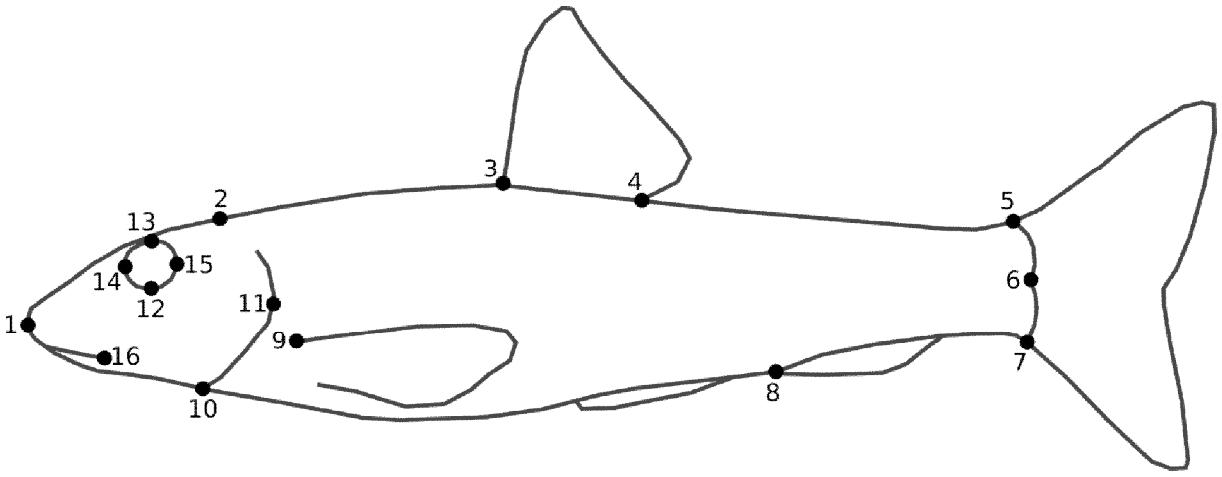
FIGURE 2

Location of 16 homologous landmarks used to assess phenotypic diversity in Gobio occitaniae and Phoxinus phoxinus. Landmarks refer to (1) tip of the snout, (2) beginning of scales coverage on the dorsal outline, (3) anterior and (4) posterior insertions of the dorsal fin, (5) dorsal insertion of the caudal fin, (6) posterior extremity of the body, (7) ventral insertion of the caudal fin, (8) anterior insertion of the anal fin, (9) superior insertion of the pectoral fin, (10) posterior border of the operculum, (11) posterior extremity of the operculum, (12) the inferior, (13) superior, (14) anterior and (15) posterior extremities of the orbital circumference, (16) posterior extremity of the premaxillar.



812 **FIGURE 3**

813 Boxplots summarizing the genetic Ŭ-diversity (allelic richness) (a), phenotypic Ŭ-diversity
814 (proportion of shape space occupied by each population) (b), genetic ɓ-diversity (F_{ST}) (c) and
815 phenotypic ɓ-diversity (euclidean distance between the consensus shapes of each pair of
816 populations) (d) in *Gobio occitaniae* and *Phoxinus phoxinus*. The solid line within each box
817 marks the median; the length of the box is the interquartile range (from the first to the third
818 quartile). The lower whisker extends to the first quartile minus 1.5 times the interquartile
819 range; the upper whisker extends to the third quartile plus 1.5 times the interquartile range.
820 Diamonds represent the data points which are beyond the whiskers.

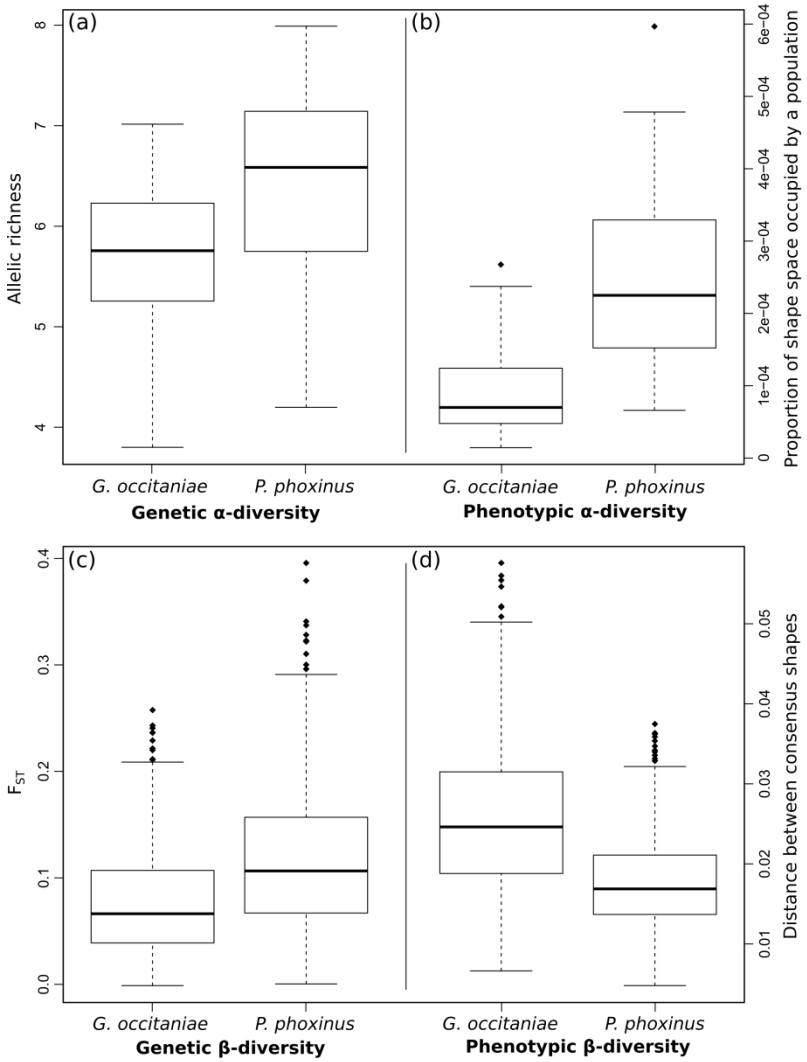


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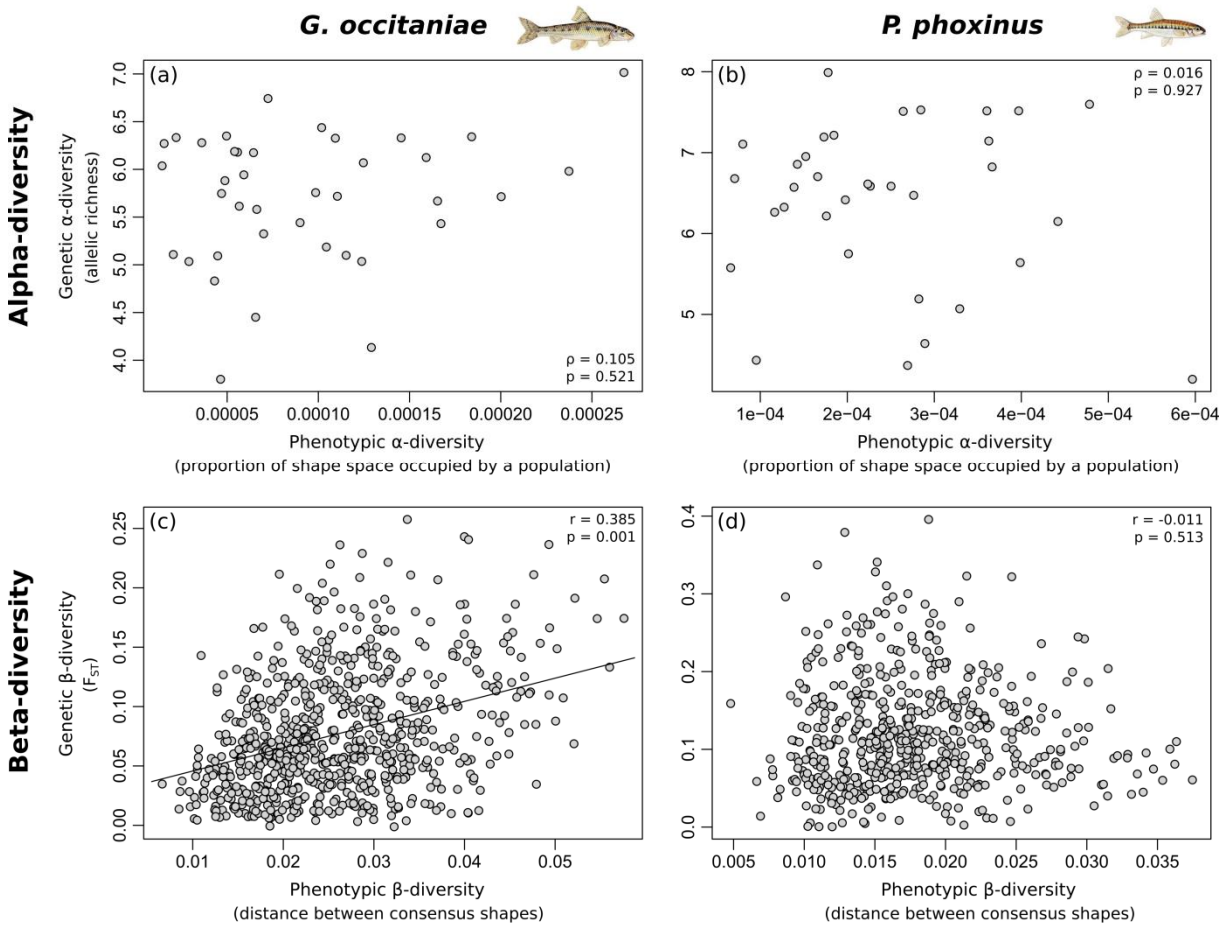
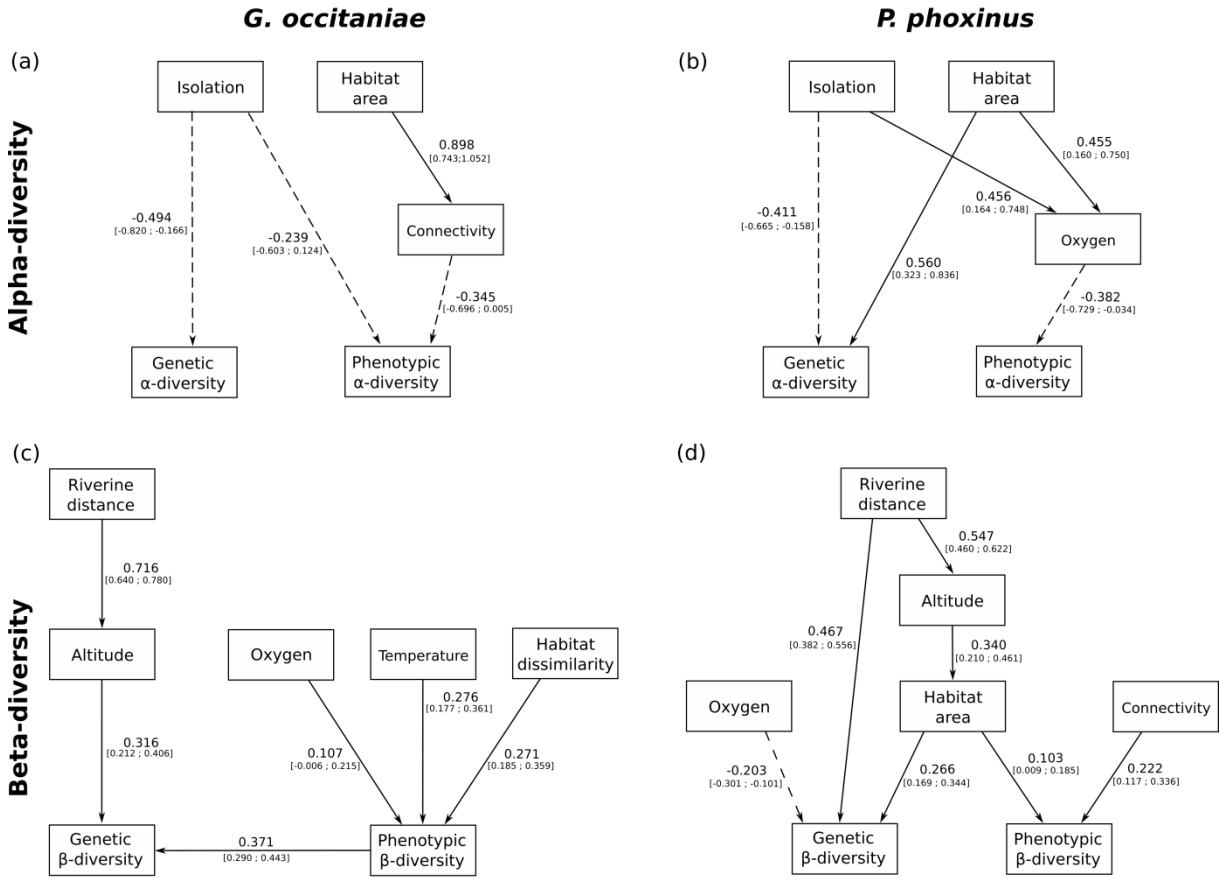
FIGURE 4

Genetic Ŭ-diversity (allelic richness) of Gobio occitaniae (a) and Phoxinus phoxinus (b) plotted against phenotypic Ŭ-diversity (proportion of shape space occupied by each population) with Spearmanôs rho and associated P-values; and genetic ɓ-diversity (FST) of Gobio occitaniae (c) and Phoxinus phoxinus (d) plotted against ɓ-diversity (euclidean distance between the consensus shapes of each pair of populations) with Mantelôs r and associated P-values.



832 **FIGURE 5**

833 Graphical representations of the models describing the causal relationships between
834 environmental variables and genetic and phenotypic Ŭ-diversity in *Gobio occitaniae* (a) and
835 *Phoxinus phoxinus* (b), and between environmental variables and genetic and phenotypic ɓ-
836 diversity in *Gobio occitaniae* (a) and *Phoxinus phoxinus* (b), obtained using the d-sep test.
837 Single-headed arrows indicate a causal path. Solid and dashed lines stand for positive and
838 negative values, respectively.



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