Influence of isolation by environment and landscape heterogeneity on genetic structure of wild rice *Zizania latifolia* along a latitudinal gradient

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Abstract

Global aquatic habitats are undergoing rapid degradation and fragmentation as a result of land-use change and climate change. Understanding the genetic variability and adaptive potential of aquatic plant species is thus important for conservation purposes. In this study, we investigated the role of environment, landscape heterogeneity and geographical distance in shaping the genetic structure of 28 natural populations of *Zizania latifolia* (Griseb.) Turcz. Ex Stapf in China based on 25 microsatellite markers. Genetic structure was investigated by analysis of molecular variance (AMOVA), estimation of F_{ST}, Bayesian clustering and Thermodynamic Integration (TI) methods. Isolation by environment (IBE), isolation by resistance (IBR) and isolation by distance (IBD) hypotheses were compared using a reciprocal causal model (RCM). Further, generalized linear models and spatially explicit mixed models, by using geographic, landscape and genetic variables, were developed to elucidate the role of environment in driving *Z. latifolia* genetic diversity. The genetic differentiation across all populations was high: $F_{ST} = 0.579$; $Ø_{pt} = 0.578$. RCM exclusively supported IBE in shaping genetic structuring, only partial support for IBR, but not for IBD. Maximum temperature of the warmest month and precipitation seasonality were the plausible parameters responsible for genetic diversity. After controlling for spatial effect and landscape complexity, precipitation seasonality was significantly associated with genetic diversity. Based on these findings, genetic structure of *Z. latifolia* across China seem to be as a result of local adaptation. Environmental gradient and topographical barriers, rather than geographical isolation, influence genetic differentiation of aquatic species across China resulting in instances of local adaptation.

1 INTRODUCTION

Species adaptation to the changing environment mainly depend on the level of standing genetic variation in the populations (Lande and Shannon, 1996; Bell and Collins, 2008). Gene flow patterns, one of the factors influencing genetic variability, are affected by the movement of gametes, individuals and groups of individuals under the influence of landscape features (Sork et al, 1999). This further impacts the spatiotemporal patterns of genetic structure and evolution of natural populations (Slatkin, 1985). Climate and land-use changes are the major drivers of environmental alterations. For instance, global warming has led to temperature and precipitation fluctuations with instances of extreme episodes (Grant et al., 2017; Marrot et al., 2017), which result in plant populations' selective pressure (Hoffmann and Sgrò, 2011; Cao et al., 2015). On the other hand, increase in human population has resulted in rapid conversion of natural lands to farmlands, urban and industrial areas leading to habitat loss and fragmentation (Zhao et al., 2013; Davidson, 2014). The overall effect is decline in population size of native species, which is the initial stage of species local disappearance and eventual extinction (An et al., 2007; Qin, 2008; Halley et al., 2016; Pykälä, 2019). Habitat degradation has profound effects particularly on aquatic plants that live in fragmented islands within the terrestrial landscapes. Additionally, most aquatic plants persist as meta-populations, the long-term survival of which depends on continuous gene flow among populations (Barrett et al., 1993; Santamaría, 2002). Therefore, clear understanding of genetic variability of aquatic plant species occurring across a large spatial scale is important for estimation of the current vigor under the different abiotic and biotic conditions and hence the potential to adapt to the changing environment (Catullo et al., 2019). Knowledge about the major drivers of gene flow is important for the conservation and management of aquatic plants.

The rapid emergence and multidisciplinary nature of landscape genetics has introduced several hypotheses that assess the relationship between spatially explicit landscape variables and genetic measures (Manel and Holderegger, 2013; Hall and Beissinger, 2014). This is in an effort to understand whether dynamic landscape complexities limit gene flow thus resulting in discrete population structure (isolation by barrier, IBR), or clinal population structure is as a result of isolation by distance (IBD) and/or isolation by environment (IBE) (Meirmans, 2012; Landguth and Schwartz, 2014). Clustering of individuals in natural populations could be due to IBD, IBE, IBR or a combination of either (Cushman et al. 2006; Andrews, 2010; Wang and Bradburd, 2014; van Strien et al., 2015). IBD pattern results from geographical isolation, which limits gene flow and promotes genetic drift (Wright, 1943). This model assumes that genetic structure is driven by the linear relationship between geographic distance and genetic distance. It disregards the effects of nonlinear complex landscapes, environmental variables and historical processes that influence gene flow (Jenkins et al., 2010). IBR model considers landscape features and historical processes in explaining the impediment to gene flow (McRae, 2006). Both IBD and IBR are concerned with the limitations to the dispersal of gametes, but fails to accommodate the effects of environmental variables. Moreover, discrete population structure can be misinterpreted as an IBD or IBR pattern, and this may confound identification of environmental contributions resulting in overestimation of population clustering (Tucker et al., 2014). In contrast, IBE considers the contribution of environmental heterogeneity in shaping the distributions of spatial genetic variation independent of geographical isolation (Wang and Bradburd, 2014). Most empirical research on population genetics has focused on gene flow and genetic drift as drivers of genetic structure ignoring environmental-driven natural selection (Orsini et al., 2013). Understanding the environmental variables shaping genetic variation in natural populations is, therefore, important in evolutional studies and thus environmental gradients should be quantified and ecological approach included in population genetics studies (Lee and Mitchell-Olds,

2011). This information could be crucial in defining conservation and management units for the ecologically and economically important species (Segelbacher et al., 2010; Luque et al., 2012).

The wild rice *Zizania latifolia* is a perennial aquatic grass, and an important ecological and genetic resource in China. To date, the genetic differentiation of the wild rice across China has been attributed to IBD (Chen et al., 2017; Zhao et al., 2018; 2019). This is despite the fact that the distribution of wetlands in China, which represents the extant natural habitat for the species, has more unique features besides being expansive and patchy. The wetland ecosystem has a regional (Eastern) pattern with swamps distributed in the Northeast region and lakes in the middle-lower reaches of the Yangtze River and the Qinghai Tibet Plateau (Wang et al., 2012). Mangrove wetlands are distributed in the southeast coastal area. Topographically, the Eastern region, where most of the wetlands are found, is characterized by three plains; North, Northeast and Middle-Lower Yangtze plains amongst which foothills and hills are interspersed. This is the most economically important region characterized by a dense population, agriculture, and industries (An et al., 2007). China natural wetlands characteristics and distribution of *Z. latifolia*, therefore, presents an opportunity to study the landscape genetics of widespread species that have for long been neglected by conservation genetics community (Morente-López et al., 2018). This would aid in delineating the impact of island-like aquatic species distribution as well as human- and climate-change-induced habitat degradation (An et al., 2007) on the genetic structure of riparian plants at different spatial and environmental gradients. Further, the environmental gradient provides a perfect model for space-for-time substitution in assessing the long term aquatic ecosystem response to the changing environment (De Frenne et al., 2011).

In this study, we aimed to study the genetic diversity and population structure of 28 natural population of *Z. latifolia* based on 25 simple sequence repeat (SSR) markers and determine the influence of IBD, IBR and IBE in shaping the genetic structure. Based on the patchy nature of the wild rice habitat and the extensive latitudinal geographic range of the studied populations, it was predicted that *Z. latifolia* would show strong spatial genetic structure driven by diverse biotic and abiotic factors. It was expected that population decline and discontinuity, due to habitat degradation and fragmentation (An et al., 2007), could have considerable influence on wild rice reproduction system and gene flow, resulting in low genetic diversity. Further, we hypothesized that *Z. latifolia* genetic structure, in the expansive range and heterogeneous landscape, could be explained by combined effects of geographic isolation (IBD), gene-flow-limiting landscape variables (IBR) and differences in environmental variables between populations (IBE).

2 MATERIALS AND METHODS

2.1 Focal species, sampling and SSR data

The wild rice *Zizania latifolia*, commonly known as the Chinese wild rice, belongs to the family Poaceae, tribe Oryzeae. It shares the genus *Zizania* L. with other three species distributed in North America *(Z. aquatic Z. palustris* and *Z. texana*) (Xu et al., 2010). *Z. latifolia* is a perennial species distributed in Eastern Asia and well differentiated from the North American species (Brown, 1950; Dore, 1969; Chen et al., 1990; Kennard et al., 2000). It grows along the margins of lakes and rivers, ponds and marshes, and reproduces sexually by seeds or asexually by rhizomes (Xu and Zhou, 2017). In China, the wild rice is an important ecological species that is exploited for wastewater purification due to its high nutrient uptake capacity (Liu et al., 2007; Zhou et al., 2007; Peng et al., 2013) and high clonal reproduction (Chen et al., 2017). It also carries important genetic traits that include disease and pest resistance, elite grain, and tolerance to cold and flooding (Liu et al., 1999; Yu et al., 2006; Shen et al., 2011; Wang et al., 2013). Natural populations of *Z. latifolia* are

distributed along the East of China along a wide stretch of latitudinal zones (21°N -50°N) (Wagutu et al., 2020). This region has five major eco-geographic regions that vary in biotic and abiotic factors that influence gene flow and local adaptation (Wu et al., 2003).

Twenty-eight populations of *Z. latifolia* were collected across China from Heilongjiang province to Guangdong province (Table 1) in a cluster sampling approach (Li et al., 2017). This was based on the size of the population, which should be large enough to allow a random selection of at least 20-23 samples at intervals of 10 meters. Samples were collected in the natural wetlands; rivers, lake shores and marshes during early autumn as the wild rice approaches maturity. GPS coordinates were recorded at the center of each collection transect and mapped using ArcGIS (Figure 1). The two uppermost leaves from each selected individuals were harvested, individually dried in silica gel and stored in the laboratory for DNA extraction. Total genomic DNA was extracted from 0.5g dried leaves using a modified CTAB protocol (Doyle and Doyle, 1987). Twenty five SSR markers were used in this study, including 20 markers developed for *Z. latifolia* (Quan et al., 2008; Wagutu et al., 2020), three SSR markers for *Oryza sativa* (Wang et al., 2015) and two markers developed for *Z. texana* (Richards et al., 2007) (Supplementary Table S1). PCR amplification was performed following the protocol by Quan et al. (2008) and PCR products separated on a 6% denaturing polyacrylamide gel. Fragments were visualized by silver staining and alleles scored in reference to a 25bp DNA ladder (Promega, Madison, WI, USA).

GenoDive 2.0 (Meirmans et al., 2004) was used to identify clonal structure. At threshold zero, samples were assigned to their respective clones. The number of genotypes (G) was calculated and repeating genotypes excluded from further analysis. Micro-Checker 2.2.3 (Van Oosterhout et al., 2004) was used to test for null alleles and genotyping errors. Genetic diversity across all loci for every population and for each locus was estimated in terms of observed allele number (N_a), the effective number of alleles (N_e), and expected and observed heterozygosity (H_E and H_O) using GenAlEx software (Peakall and Smouse, 2012). FSTAT (Goudet, 2001) was used to calculate the inbreeding coefficient (F_{IS}), deviation from Hardy-Weinberg equilibrium and linkage disequilibrium.

2.2 Genetic structure among populations

FSTAT software was used to measure population genetic divergence by estimation of F_{ST} using 999 permutations for their significance. Based on F_{ST} values, an average level of gene flow (Nm) was estimated using the formula: [$Nm = (1- F_{ST})/4F_{ST}$] (Slatkin and Barton, 1989). ARLEQUIN software (Schneider et al., 2000) was used to perform analysis of molecular variance (AMOVA) to determine genetic variation among and within populations. STRUCTURE program (Pritchard et al., 2000) was used to perform Bayesian clustering of the samples. Ten independent runs for each number of K clusters from one to ten was performed. A 20,000 iteration burn-in period followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations were assumed for each run with correlated allele frequencies and admixture origin assumptions. To determine the value of K, the output was interpreted with Structure Harvester (Evanno et al., 2005; Earl and vonHoldt, 2012). However, Evanno's *delta K* method has been reported to suffer philosophical and statistical errors (Verity and Nichols, 2016). Therefore, it was supplemented with Thermodynamic Integration (TI) method (Verity and Nichols, 2016). Here, *rmaverick* R package was used to estimate the true value of K by running 20 rungs for $K = 1$ to 10 with a burn-in period of 10,000 iterations followed by 50,000 MCMC iterations under the admixture model. The value of K was estimated as described by Verity and Nichols (2016). Since both software did not differ in the estimation of K, Structure Harvester output was visualized by DISTRUCT v. 1.1 (Rosenberg, 2004). Identified clusters were analyzed for

molecular variations (AMOVA) using ARLEQUIN software. Geographical distribution of the different clusters identified was mapped using ArcGIS 10.0 (Esri, Redlands, CA, USA).

2.3 Geographic and environmental influence on the genetic structure

To determine the drivers of the observed genetic structure in *Z. latifolia*, we tested for isolation by distance (IBD), isolation by resistance (IBR) and isolation by environment (IBE) hypotheses. Four matrices were obtained in order to explore the three assumptions. Genetic distance was calculated as pairwise F_{ST} using FSTAT software, while the geographic distance matrix was based on Euclidean distance between populations calculated using GenAlEx. To linearize genetic and geographic distances relationship, both matrices were transformed: [F_{ST}/(1- F_{ST})] and log(Euclidean distance), respectively (Rousset, 1997). Nineteen environmental variables were extracted for the studied sites from BioClim's 30s resolution dataset (Busby, 1991) with GIS details using ArcGIS software (Supplementary Table S2). PAST ver. 3.24 (Hammer et al., 2001) was used to reduce climatic variables by principal component analysis (PCA) based on 20 variables (19 bioclimatic variables and elevation). The first principle components (PC) was used as it represented 99.32% of the variation in the data. Environmental distance matrix was calculated using the first principal component using *vegan* package in R (Oksanen et al., 2018). Lastly, resistance distance was calculated as the conductance matrix on a 30-arc seconds resolution digital elevation model (DEM) (Danielson and Gesch, 2011) of the study site using *gdistance* package implemented in R (van Etten, 2017). The resistance distance was obtained by calculating the effective resistance between populations when the DEM grid is conceived as an electrical circuit (McRae et al., 2008). Since wind dispersal is considered a major factor in *Zizania latifolia* gene flow, DEM was slope-modeled in ArcGIS 10.0. The grid cells with high values (high slope) contribute high resistance to gene flow and those with low value contribute the least resistance. Movement from any cell within the grid followed the standard eight directions (McRae et al., 2008).

To compete the three hypotheses, we used reciprocal causal modeling (RCM) (Cushman et al., 2006; 2013b) that eliminates the simple Mantel test's (Mantel, 1967) limitations of spurious correlations and type I error (Cushman and Landguth, 2010). The RCM method directly compares the different hypotheses and identifies whether any of them is relatively supported independently of the other alternatives. The approach is based on a pair of partial Mantel tests derived from two models at a time. First, partial Mantel was performed between genetic distance (Gen) and IBD, partialling out IBR (Gen~IBD|IBR). The second partial Mantel was performed between the genetic distance (Gen) and IBR, partialling out the IBD (Gen~IBR|IBD). The difference between the two partial Mantel tests (IBD|IBR- IBR|IBD) was the relative support for IBD relative to IBR (Cushman et al., 2013a). In that case, the difference between the partial mantel should be positive if IBD is correct, and zero or negative if the IBR is correct. The full matrix of the partial Mantel tests differences between pairs of alternative hypotheses was computed. A hypotheses was regarded as fully supported, independent of all alternatives, if all the values in its column were positive and the values in the row were negative (Cushman et al., 2013a, b). Correlation values and significance values for the Mantel model combinations were calculated through 9999 corrected permutations using *vegan* R package.

We further evaluated the relationship between genetic diversity estimators and geographic and environmental variables through generalized linear models (GLMs) using PAST ver. 3.24 (Hammer et al., 2001) and spatially explicit mixed modeling (Morente-López et al., 2018) using *spaMM* R package (Rousset and Ferdy, 2014). Firstly, generalized linear models were used to explore the contribution of each environmental variable to genetic diversity estimators (H_O, H_E and N_a). Secondly, spatially explicit generalized linear mixed models (spatial GLMMs) were developed using genetic, geographic (coordinates and elevation) and environmental data. Here, we used genetic

diversity estimators (H_O, H_E and N_a) as response variables, each of the 19 environmental variables as fixed effects and geographical coordinates and elevation as random effects. We transformed environmental variables as required, including using their squared values to account for non-linearity. Full models (e.g. [F_{IS} ~ bio_1 + (1|lat+long/Elevation)]) and null models (e.g. [F_{IS} ~ 1 + (1|lat+long/Elevation)]) were tested for associated likelihood ratio to obtain the *P*-value.

3 RESULTS

From 578 individuals, 570 multilocus genotypes were identified, and no genotype was shared among populations. In 19 populations, every individual was of unique genotype (Table 1). We did not find evidence of null alleles or genotyping error using Micro-Checker. At the population level, the range of observed heterozygosity (H_O) and expected heterozygosity (H_E) were 0.113 to 0.488 and 0.111 to 0.484, respectively (Table 1). Fixation index ranged from F_{IS} = -0.334 to F_{IS} = 0.104 with a mean of -0.036. Deviation from HWE was detected in 257 out of the 700 locus-population combinations (36.7%) at P = 0.05, which is expected for natural population at a broad geographical scale (Garnier-Géré and Chikhi, 2013). Significant genotypic disequilibrium was detected in 86 out of 300 locus pairs (P = 0.001), but after Bonferroni correction, none of the locus pairs was in significant genotypic disequilibrium. This indicated that loci were unlinked and statistically independent of each other.

3.1 Genetic structure and differentiation among populations

The genetic differentiation across all populations based on F_{ST} was 0.579 (Table 2). Pairwise comparisons of F_{ST} were significant for genetic differentiation between populations (P = 0.01). Moreover, the levels of differentiation were high; F_{ST} = 0.103 to 0.823 (Supplementary Table S3). AMOVA showed high population differentiation at $Ø_{pt}$ = 0.578 (P=0.000). Variation among populations was 58%, while 42% of the variation was within population, both statistically significant (P = 0.001) (Table 3). Bayesian clustering using structure identified optimum K=5 using the Evanno's delta K as well as TI method (Pritchard et al., 2000; Verity and Nichols, 2016). Populations from the five regions clustered according to their respective collection sites (Figure 2). Membership proportion of predefined populations in each of the 5 clusters ranged from 0.857 to 0.997. AMOVA showed that 36% of variation was among clusters (Table 3). The five identified clusters were mapped using ArcGIS (Figure 1).

3.2 Geographic and environmental influence on the genetic structure

IBE model was fully supported based on the relative support values of the reciprocal causal model. The column had positive values and the row had negative values (Table 4) indicating that it explained the genetic structure independent of the alternative hypotheses. Moreover, the partial Mantel tests between genetic distance and environmental distance controlling for both resistance and geopgraphy showed significant positive correlations (Table 4). IBR model had one positive value in the column when competed with IBD and partial Mantel tests were all significant. IBD model had all negative value in the column and thus had the least relative support. However, partial Mantel tests when controlling for IBR showed a significant positive correlation. In simple Mantel, environmental distance had the highest significant positive correlation with genetic distance followed by resistance distance and then geographical distance (Table 4).

Generalized linear models showed that seven of the 19 bioclimatic variables had significant contribution to genetic diversity of *Z. latifolia* (Table 5). However, spatially explicit mixed models

with coordinates as random effect showed no significant influence of environmental variables to genetic diversity. When elevation was used as the random effect in the mixed models, bio_15 showed significant influence ($P = 0.038$) on number of alleles (N_a). We tested the seven environmental variables showing significant contribution to genetic diversity in GLM for collinearity using variance inflation factor (VIF) analysis (Helsen et al., 2017). Two variables; bio_5 and bio_15 had VIF value below 2 and were therefore identified as the best environmental variables responsible for the genetic diversity of *Z. latifolia*.

4 DISCUSSION

In this study, *Zizania latifolia* showed relatively low genetic diversity ($H_E = 0.292$). A similar level of genetic diversity was reported in its natural populations from the Northeast wetland ($H_E = 0.328$; Chen et al., 2017) and the lower-middle Yangtze wetland ($H_E = 0.271$; Wang et al., 2015) using SSR markers. Our results are consistent with the expected average genetic variability for the family Poaceae ($H_E = 0.201$), which is estimated based on characteristics that influence gene flow (Hamrick and Godt, 1996). These results also correspond to Northern American wild rice *Z. palustris* genetic diversity ($H_E = 0.236$; Lu et al., 2005). Further, *Z. latifolia* shows lower genetic diversity compared to *Z. texana* ($H_E = 0.662$; Richards et al., 2007). However, the two studies employed different sampling approaches, with Richards et al. (2007) considering a single hydrologically connected river system, while we sampled different populations across large geographical and environmental gradient (Figure 1). A higher than average genetic diversity in populations in region III, along the Yangtze River ($H_E = 0.424$) and region IV, along the Yellow River ($H_E = 0.426$) was observed. Similar results were reported by Chen et al. (2012) and Zhao et al. (2018) who showed that *Z. latifolia* wild populations in the middle and lower reaches of the Yangtze basin exhibit high genetic diversity. This could be explained that, the central and southeast China wetlands are characterized by episodic floods that create temporary hydrological connectivity (Wang et al., 2008) resulting in gamete exchange between population. Genetic diversity is then maintained by the high clonal capacity of the species.

4.1 Genetic structure and differentiation among populations

High level of genetic differentiation among populations was detected ($F_{ST} = 0.579$ $D_{est} = 0.598$). Our results are comparable to *Z. latifolia* genetic differentiation reported recently ($F_{ST} = 0.481$, Xu et al., 2008; $F_{ST} = 0.405$, Chen et al., 2017). AMOVA analysis also showed high population differentiation at $Ø_{pt} = 0.578$ (P=0.001), with 58% variation attributed to between populations and 42% to within population ($P = 0.001$) variation. This was also comparable to *Z. latifolia* population differentiation ($Ø_{pt} = 0.598$) reported by Xu et al. (2015). Moreover, high population differentiation was also reported in the North American relatives of *Z. latifolia* (*Z. aquatica*: $F_{ST} = 0.607$; *Z. palustris*: $F_{ST} = 0.468$; Xu et al., 2015). The observed high genetic divergence is attributable to decreased gene flow between populations ($Nm = 0.061$). According to Wright (1969), when Nm is perceived as the exchange of migrants between demes per generation, a value > 1 result in little divergence, while value < 1 results in increased divergence. In our case, conventional fragmentation of wetlands into islands within the expansive terrestrial habitat and induced fragmentation could explain the negligible gene flow and the observed high genetic differentiation (Wagutu et al., 2020). Moreover, inbreeding system detected in some populations could have contributed to the high genetic differentiation. Bayesian clustering using STRUCTURE software and *rmaverick* (R package) identified five clusters (Figure 2). All the five clusters corresponded to the wetland from which the samples were collected. The clustering pattern can be attributed to the *Z. latifolia* habitat, which is discrete and patchy within an expansive geographical

range (Zhao et al., 2018). Further, dispersal of its gametes and somatic propagules depend on hydrological connectivity and landscape features. In such spatially isolated populations under a heterogenous landscape, wind and water dispersal is most effective locally creating locally identical genotypes. Environmental difference between the geographically isolated populations promote phenotypic plasticity and genetic changes.

4.2 Environmental, landscape and geographical influence on genetic structure and genetic diversity

For the first time, this study tested for IBE and IBR patterns, besides the commonly tested IBD pattern in the natural populations of *Z. latifolia*. Interestingly, we found exclusive relative support by RCM for IBE pattern, partial support for IBR, but not IBD, in shaping genetic structuring of the species. Similarly, Šurinová et al. (2019) reported that environmental variables, but not geographical distance, explained genetic relatedness among a perennial grass species (*Festuca rubra*) populations. Wu et al. (2019), when comparing IBD and IBE in aquatic species *Ranunculus subrigidus* on the Qinghai-Tibetan plateau, also found exclusive support for environmental isolation. Further, IBE has been reported as a more common phenomenon compared to IBD in a wide range of taxa (Shafer et al., 2013; Sexton et al., 2014), although it has for long been neglected in population genetic studies. IBE can be generated by various ecological processes that include; natural and sexual selection against maladapted immigrants, poor hybrid fitness and biased dispersal (Wang and Bradburd, 2014). In natural selection, populations evolve traits suitable for the environment and thus acquire higher fitness compared to immigrants from different environments. Sexual selection and dispersal bias may occur as a result of phenotypic plasticity. Here, environmental conditions influence reproductive physiology affecting synchronization of mating and dispersal processes such flowering and gamete release. Moreover, if immigrates survive long enough and are able to mate locally, hybrids would undergo selection against immigrant alleles (Sexton et al., 2014). The sexual reproduction of *Z. latifolia* could be one characteristic that makes natural and sexual selection as well as dispersal bias drive local adaptation and thus the observed pattern of IBE. However, the species also reproduces asexually through rhizomes and thus hybrids survival could be a tradeoff. The net effect of ecological processes targeting sexual reproduction and asexual reproduction, however, seems to favor local adaptation and hence the exclusive support of IBE by RCM.

In this study, IBE was further supported by the significant relationship between genetic diversity estimators and a number of environmental variables in the GLMs, although limited relationship in the spatial GLMMs (Table 5). Maximum temperature of the warmest month (bio_5) and precipitation seasonality (bio_15) were identified as the best environmental variables responsible for observed genetic differentiation based on VIF analysis. Temperature and precipitation are major drivers of plant adaptability through their influence on physiology and diversity (Hoffmann and Sgrò, 2011; Manel et al., 2012), forcing genetic change events and subsequent selection. Besides our study, influence of temperature and precipitation on genetic differentiation of both aquatic and terrestrial plants have been reported (Wang et al., 2016; Münzbergová et al., 2017). A significant relationship between precipitation seasonality (bio_15) and N_a was detected in our study. This indicates that the local adaptation, driven by interaction between precipitation and temperature, is responsible for *Z. latifolia* differentiation along the eastern China aquatic ecosystem. This could be due to the fact that the species is tolerant to a wide range of temperatures (Guo et al., 2007), but since it's an aquatic species, the differences in precipitation along the sampling sites and between seasons has profound influence. As a perennial and aquatic species, *Z. latifolia* reproduction and growth will be affected by temperature and precipitation fluctuations. Flowering, vegetative growth and reproductive success of plants have been reported to respond to temperature changes (De FRENNE et al., 2011). Similarly,

asynchronous flowering due to heterogenous rainfall patterns have been associated with IBE pattern (Garot et al., 2019).

IBR was the second best model explaining *Z. latifolia* genetic structuring. In partial Mantel, IBR had a positive correlation with genetic diversity when controlling for both geographical distance and environment. The model was partially supported by RCM (Table 4). Similarly, significant effect of cost distance on genetic differentiation has been reported in alpine ecosystem (Morente-López et al., 2018). Wetlands along eastern China are islands within terrestrial ecosystem interspersed with foothills and hills (An et al., 2007; Santamaria, 2002). Such natural barriers are important factors in the gene flow of flowering plants. Similar, albeit artificial barriers, have been reported to drive genetic differentiation in species that depend on wind, insects and birds for pollination and seed dispersal (Su et al., 2003). In the case of *Z. latifolia*, wind pollination seems to be inhibited by the complex landscape along the eastern China, and seed dispersal by lack of hydrological connectivity, which leads to genetic drift and thus population differentiation.

The exclusive IBE pattern detected contradicts Zhao et al. (2018; 2019) who reported a pattern of IBD along the eastern China populations of *Z. latifolia*. However, in their study, only IBD was tested using simple Mantel, for which we also found a strong positive correlation (r=0.5830; P=0.001). The observed direct correlation of genetic differentiation with geographical distance between populations could be due to the discrete pattern of *Z. latifolia* populations along the expansive geographical area. Moreover, rapid fragmentation and reclamation of the wetlands (An et al., 2007; Zhao et al., 2018) has increased population isolation, which inhibits wind- and water-mediated gamete dispersal. In our study, IBD was the least supported hypothesis in partial Mantel. When controlling for IBR, significant correlation between IBD and genetic differentiation was detected (Table 4). This could be because calculation of cost distance on DEM has an aspect of distance (Morente-López et al., 2018).

4.3 Implications for conservation and management

The genetic variation in the extant natural populations of *Z. latifolia* is relatively low and the high genetic divergence is shaped by environmental variation between groups of populations. The heterogenous landscape, discrete distribution of the natural populations coupled with wetland fragmentation and degradation has increased the isolation resulting in genetic isolation. Genetic diversity measures were sensitive to temperature and precipitation. Between 1956 and 2005, earth surface temperature has increased at an average of 0.65°C and might increase with further 1.8°C to 4°C by 2099 (IPCC, 2007). This is expected to affect the precipitation and together impact on species diversity and survival. Therefore, further extensive genetic and morphological studies need to be carried out to identify the role of the environment in shaping local adaptation of the *Z. latifolia*, besides the aquatic ecosystem response assessment. The use of a common garden approach in combination with genome-wide association studies (GWAS) could help elucidate patterns of local adaptation (de Villemereuil et al., 2016).

Our results show that gene flow is critically inhibited among the populations. Therefore, management and conservation approaches should include both *in-situ* and *ex-situ* considerations. Populations in the central and southeast China wetland (e.g. CH, LZX, and DT) that showed the highest genetic variability should be prioritized for germplasm collection and conservation. The genetic profiles of the sampled populations within each of the five clusters could constitute valuable traits that require conservation as well. Geographic barriers showed positive correlation to genetic structuring of *Z. latifolia*. This is related to topographical complexity, which impedes pollen

dispersal. While nothing can be done about the topography, seed and somatic propagule dispersal through water can be enhanced. Where landscapes are disconnected, revegetation with broad genotypes can be used to promote gene flow, which may also include deliberate movement of propagules across fragmented landscapes (Reviewed by Sexton et al., 2014). Chen et al. (2017) proposed dredging the watercourses to achieve hydrological connectivity within each wetland. Populations along the Yangtze and Yellow river exchange gametes through periodical floods, which shows that this approach is feasible in other wetlands.

5 Data accessibility

All datasets for this study are included in the article's Supplementary Material

6 Authors' contribution

YC conceived the idea and designed the research project. WL gave suggestions to the design of the study. YC, XF and WF collected the samples and assembled experiment materials. GKW, XF performed the experiment. GKW analyzed the data and wrote the manuscript. All authors contributed to the revision and final editing of the manuscript prior to submission.

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8 Supplementary material

Supplementary Table S1. Details of SSR primer sequences used in this study

Supplementary Table S2. Genetic diversity measures, geographical variables and bioclimatic variables used in in this study

Supplementary Table S3. F_{ST} pairwise matrix, all significant at $P = 0.05$

9 References

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695 **Tables**

696 **Table 1.** Geographical information and summary measures of clonal diversity and genetic variation
697 for each of the 28 wild populations of *Zizania latifolia* along the five latitudinal regions starting from
698 the north towards the south.

Population	Location	Regions	*N*	*G*	N_a	N_e	H_O	H_E	F_{IS}
BS	Baihilazi, Heilongjiang Province		22	21	1.880	1.509	0.303	0.261	-0.135**
XXT	Xiaoxintun, Heilongjiang Province		21	21	1.560	1.398	0.219	0.183	-0.172**
HDY	Hadayan, Heilongjiang Province		20	16	1.400	1.202	0.113	0.111	0.021
KEB	Kuerbin River, Heilongjiang Province	I	22	19	1.480	1.223	0.149	0.138	-0.057
LQQ	Lanqitun, Heilongjiang Province		20	19	1.920	1.549	0.282	0.297	0.077
HW	Hongwei, Heilongjiang Province		20	19	1.920	1.684	0.278	0.286	0.055
YLZ	Yihaoyuliangzi, Heilongjiang Province		20	18	1.920	1.514	0.178	0.190	0.091
JH	Jinhua, Liaoning Province		20	20	2.160	1.627	0.292	0.280	-0.017
HR	Huanren, Liaoning Province		20	17	1.640	1.386	0.198	0.175	-0.100
ZD	Zhangdang, Liaoning Province	II	20	20	1.560	1.358	0.204	0.175	-0.138**
DG	Donggang, Liaoning Province		20	20	1.800	1.361	0.234	0.193	-0.186***
LZX	Liaozhong, Liaoning Province		20	20	3.320	2.262	0.448	0.434	-0.007

HXD	Huanxiangdian, Shandong Province		20	20	2.040	1.548	0.316	0.288	-0.071
DP	Dongpinghu, Shandong Province		20	20	3.120	2.188	0.470	0.436	-0.053
LQ	Luqiao, Shandong Province	III	20	20	3.200	2.169	0.442	0.436	0.012
MK	Mankou, Shandong Province		20	20	3.200	2.104	0.440	0.428	-0.003
CH	Changhu Lake, Hubei Province		21	21	4.000	2.874	0.488	0.484	0.016
DT	Dongting Lake, Hunan Province		21	21	3.480	2.468	0.450	0.475	0.077**
HH	Honghu Lake, Hubei Province		20	20	3.600	2.498	0.470	0.464	0.012
LZ	Liangzi Lake, Hubei Province	IV	21	21	3.320	2.296	0.451	0.456	0.034
LG	Longgan Lake, Hubei Province		21	21	1.560	1.275	0.200	0.160	-0.224***
SJ	Shengjin Lake, Anhui Province		21	21	3.600	2.394	0.430	0.468	0.104***
BD	Baidang Lake, Anhui Province		22	22	3.280	2.169	0.476	0.458	-0.017
NM	Nama, Guangxi Province		21	19	1.600	1.302	0.173	0.165	-0.020
FC	Fangchenggang, Guangxi Province		21	21	1.600	1.405	0.236	0.205	-0.130*
BL	Beiliu City, Guangxi Province	V	23	22	1.560	1.294	0.175	0.150	-0.138**
WC	Wuchuan City, Guangdong Province		21	21	1.800	1.459	0.223	0.216	-0.008
DC	Dongcheng, Guangdong Province		20	20	1.640	1.292	0.166	0.157	-0.029
Mean			20.643	20.000	2.327	1.743	0.304	0.292	-0.036

Note: N, number of individual plants; N_a, observed alleles number; N_e, effective allele number; H_O, observed heterozygosity; H_E , expected heterozygosity; F_{IS}, inbreeding coefficient; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 2. Genetic diversity found at 25 microsatellite loci in *Zizania latifolia*

Locus	*A*	H_O	H_E	F_{ST}	F_{IS}	*Nm*
ZM4	7	0.278	0.304	0.602	0.111	0.060
ZM25	6	0.192	0.183	0.762	-0.022	0.045
ZM26	9	0.281	0.278	0.641	0.016	0.058
ZM28	4	0.114	0.114	0.818	0.026	0.037
ZM35	12	0.459	0.463	0.451	0.034	0.062
ZM44	11	0.495	0.510	0.386	0.050	0.059
RM14233	12	0.379	0.339	0.599	-0.090	0.060
RM20118	6	0.410	0.342	0.556	-0.170	0.062
RM28090	7	0.529	0.435	0.431	-0.197	0.061
Zt1	7	0.290	0.250	0.692	-0.130	0.053
Zt23	6	0.187	0.187	0.718	0.027	0.051
ZL1	30	0.706	0.607	0.292	-0.136	0.052
ZL3	29	0.413	0.480	0.476	0.167	0.062
ZL4	28	0.396	0.444	0.512	0.131	0.062
ZL5	28	0.416	0.454	0.511	0.108	0.062
ZL9	5	0.211	0.212	0.560	0.095	0.062
ZL10	7	0.276	0.277	0.510	0.028	0.062
ZL31	7	0.346	0.305	0.427	-0.109	0.061
ZL32	8	0.315	0.291	0.482	-0.056	0.062
ZL36	5	0.213	0.193	0.739	-0.085	0.048
ZL42	5	0.105	0.110	0.445	0.079	0.062
ZL43	4	0.117	0.115	0.432	0.014	0.061
ZL55	4	0.072	0.072	0.875	0.016	0.027
ZL56	5	0.344	0.273	0.649	-0.236	0.057
ZL57	4	0.067	0.059	0.908	-0.125	0.021
Mean	10.240	0.304	0.292	0.579	-0.016	0.061

Note: A, average number of alleles per locus; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{ST}, coefficient of genetic differentiation; *Nm*, gene flow

Table 3. AMOVA design and results (average over 25 loci)

Source of variation	d. f.	Sum of squares	Variance components	Percentage variation	Statistics	*P*
			Among population			
Among populations	27	5718.302	5.20096	57.88		<0.001
Within populations	1092	133.310	3.78508	42.12	$Ø_{pt}$=0.5787	<0.001
			Among clusters			
Among clusters	4	391.365	3.50502	36.44		<0.001
Within clusters	23	2226.957	2.32734	24.20	$Ø_{pt}$=0.6064	<0.001

706
707
708 **Table 4.** Reciprocal causal modeling, partial and simple Mantel results for IBD, IBR and IBE

		IBD	IBR	IBE
		Geo	Res	Env
I: Reciprocal causal modeling matrix				
IBD	Geo	0	0.0851	**0.4961**
IBR	Res	-0.0851	0	**0.0392**
IBE	Env	**-0.4961**	**-0.0392**	**0**
II: Simple and Partial Mantel correlation matrix				
IBD	Geo	0.5830***	0.2684***	0.3063***
IBR	Res	0.1833**	0.6051***	0.2724***
IBE	Env	-0.1898	0.2332***	0.6160***

709 *Note:* IBD, isolation by distance; IBR, isolation by resistance; IBE, isolation by environment; Geo, geographic distance;
710 Res, resistance distance; Env, environmental distance.
711 **(I)** Reciprocal causal modeling matrix; columns indicate test model and rows indicate alternative models. Each value
712 represents the relative support of the test model
713 **(II)** Simple and Partial Mantel correlation matrix. Columns indicates test model and rows indicate partialed-out models.
714 Values are r values for correlations, diagonal values are the simple Mantel test r of a variable. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P$
715 < 0.001.
716

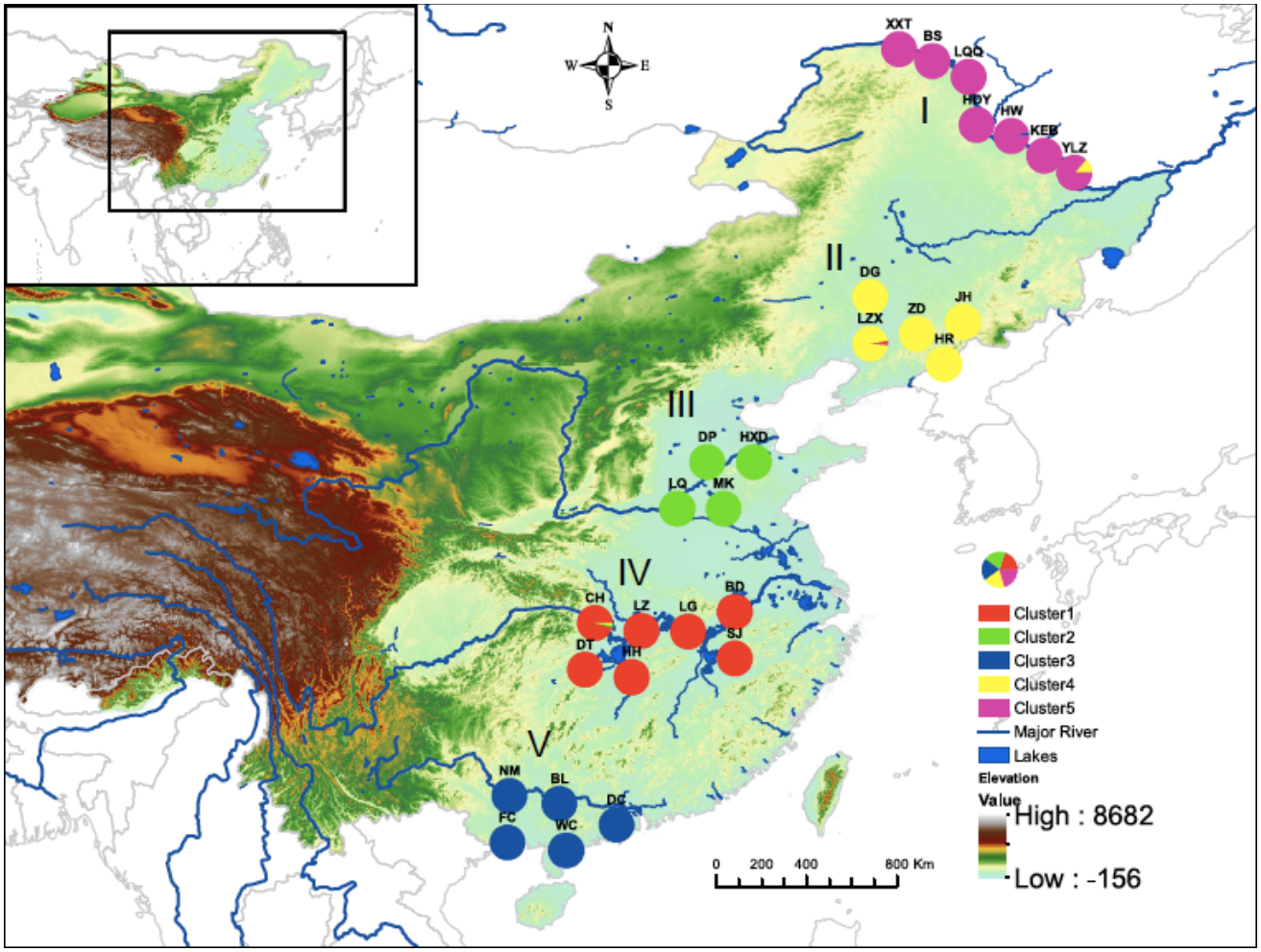
717 **Table 5.** Generalized linear model for the influence of environmental variables on genetic diversity
718 measures. Values are significant at *P*-values ≤ 0.05

Independent variables	Dependent variables		
	H_E	H_O	N_a
bio_4	–	–	0.012
bio_5	**0.024**	**0.017**	–
bio_10	–	0.046	0.030
bio_14	–	–	0.032
bio_15	**0.032**	**–**	**0.0090**
bio_17	–	–	0.020
bio_19	–	–	0.043

719

720 **Figures**

721



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723
724 **Figure 1**: Geographical distribution of wild rice samples across the five (I to V) latitudinal regions in
725 China and their respective genetic clusters each denoted by a different color.
726
727

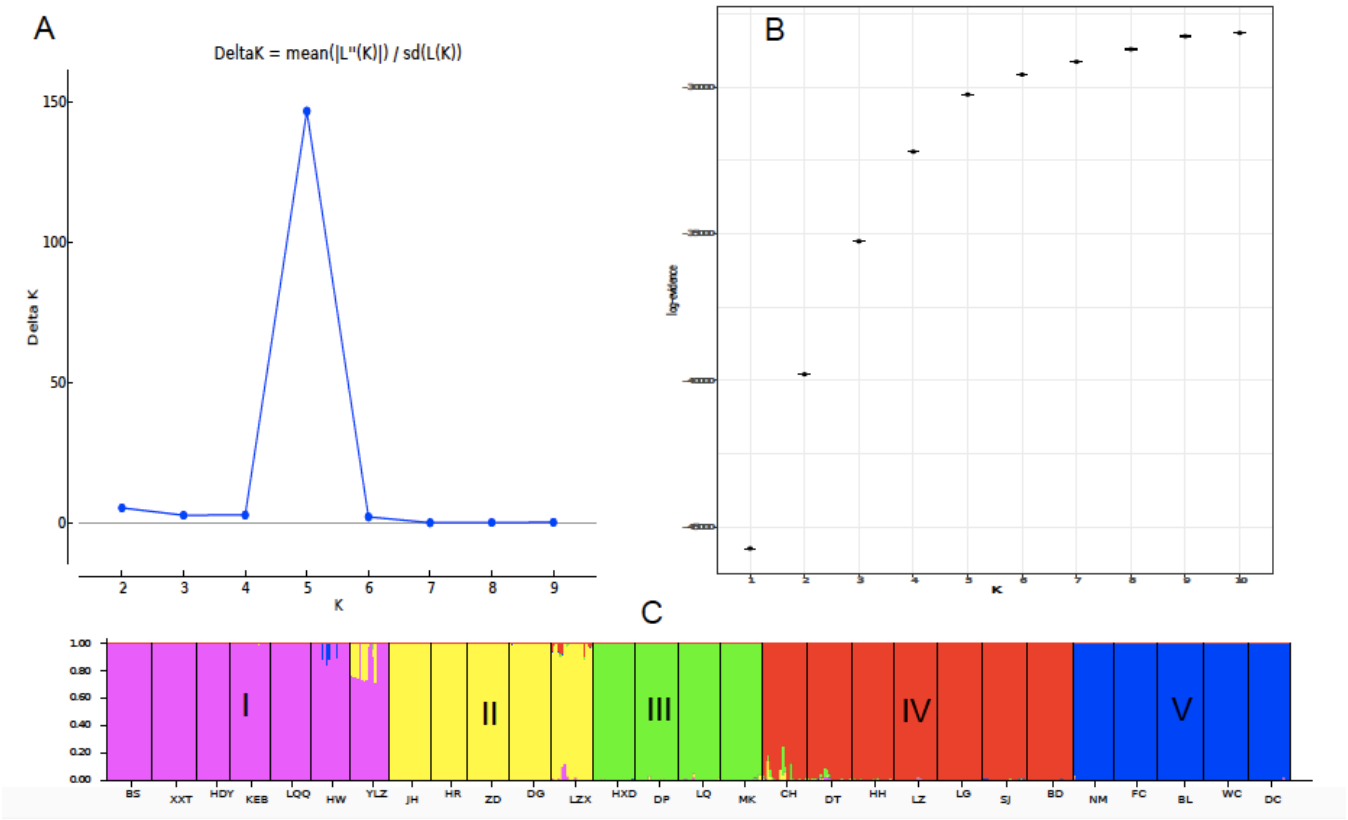
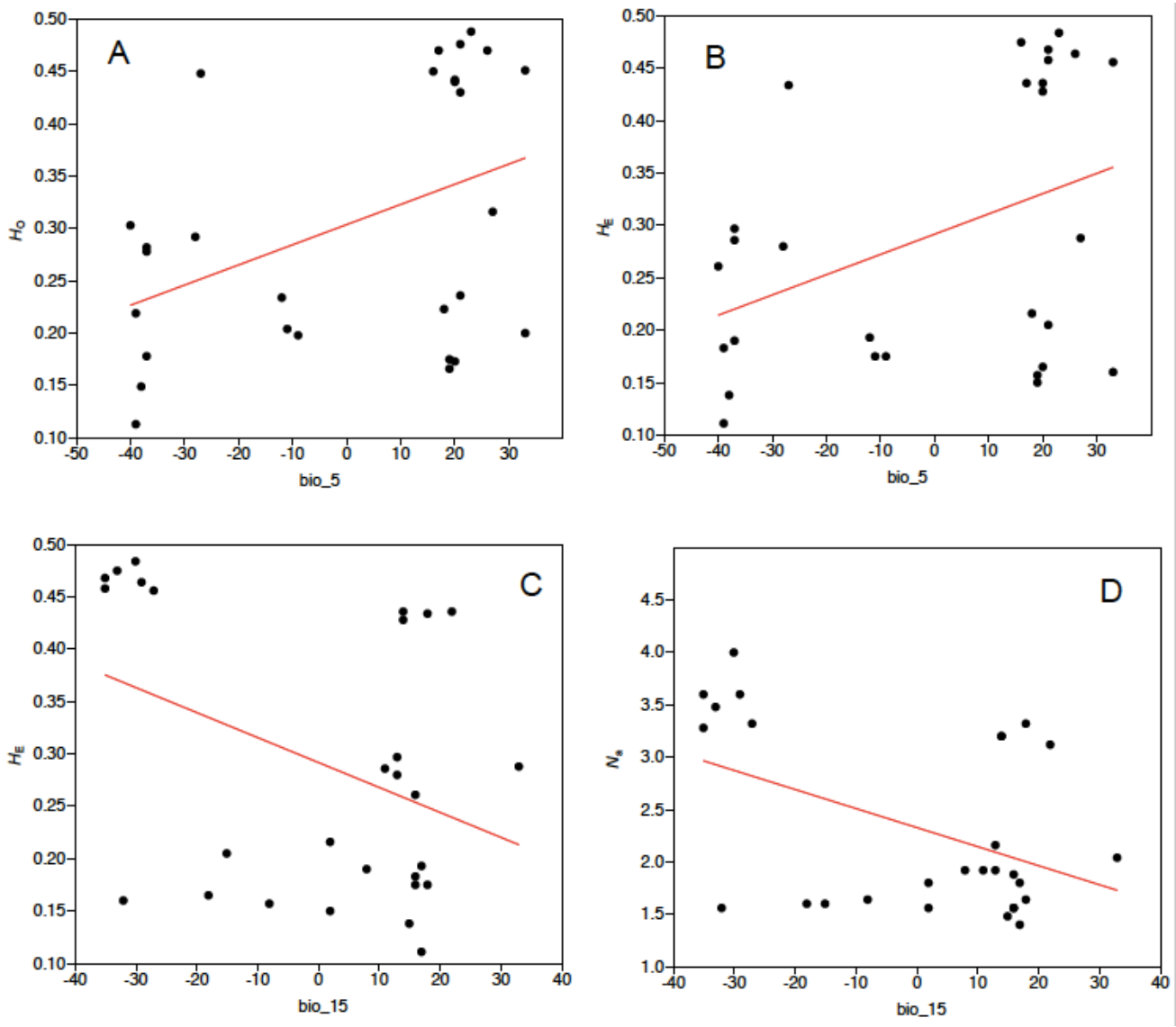


Figure 2: Bayesian analysis of population structure (STRUCTURE) for $K = 5$ inferred by DISTRUCT as calculated by: **(A)**, Evanno's delta K and; **(B)**, log-evidence in thermodynamic integration. **(C)** STRUCTURE composition of individuals, with each of the clusters corresponding to the five latitudinal regions and color-coded similar to Figure 1.



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736 **Figure 3**: Significant relationships between genetic diversity estimators and the two most important
737 environmental variables, based on variance inflation factor analysis. **(A)** H_O, observed
738 heterozygosity; **(B and C)** H_E, expected heterozygosity; **(D)** N_a, number of alleles per population.
739 bio_5; Maximum temperature of warmest month, bio_15; precipitation seasonality.

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