Dispersal versus local recruitment - the central role of seed banks for meta-populaton dynamics in an aquatic plant

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Abstract

Progressive habitat fragmentation threatens plant species with narrow habitat requirements. While local environmental conditions define population growth rates and recruitment success at the patch level, dispersal is critical for population viability at the landscape scale. Identifying the dynamics of plant meta-populations is often confounded by the uncertainty about dormant population compartments. We combined a landscape-scale assessment of an amphiphytic species' population structure with measurements of dispersal complexity in time to track dispersal and putative shifts in functional connectivity. Using 13 microsatellite markers, we analyzed the genetic structure of extant *Oenanthe aquatica* populations and their soil seed banks in a kettle hole system to uncover hidden connectivity among populations in time and space. Considerable spatial genetic structure and isolation-by-distance (IBD) patterns suggest limited gene flow between sites. Spatial isolation and size of patches showed minor effects on genetic diversity. Local recruitment was prevalent, despite some evidence for spatial migration and recent colonization. Our findings uncover stepping-stone dynamics with source-sink effects based primarily on dispersal from persistent local to adjacent populations. Overall, spatiotemporal connectivity patterns provide support for meta-population dynamics in our system and highlight the importance of persistent seed banks as a long-term source of genetic diversity.

Introduction

Continuous habitat has become a scarce resource – many organisms are scattered across habitat patches resulting from progressive fragmentation and degradation of natural areas [1,2]. In plants that lack long-distance dispersing propagules, landscape-scale fragmentation may limit spatial gene flow among distant patches [3–6]. Therefore, spatially isolated populations may diverge more and harbor less genetic variation, which can ultimately limit their adaptive response to selection [7,8]. However, population genetic structure may be homogenized and local extinction risks reduced if the landscape configuration and vector availability maintain gene flow. Accordingly, effective propagule dispersal in a meta-population increases genetic connectivity which is crucial for long-term persistence. Increased immigration may particularly prevent long-term extinction of local populations in areas subject to intensive disturbance with presumably increased mortality rates [9–11]. Regarding meta-population theory in plants, inference of connectivity and genetic diversity of above-ground population structures may be misleading, as only a fraction of the actual viable population and history of local selective forces may be assessed from such single snapshot data [12–15]. Especially in systems with drastic short-term environmental shifts, dormancy may evolve if plants with delayed seed germination produce more surviving offspring than those whose seeds all germinate in their first year [16].

Although this bet-hedging strategy known as 'storage effect' can create a reservoir of genetic diversity [17], rates of germination, seed mortality, and recruitment may widely differ among patches, further complicating the estimation of local extinction risks and colonization probabilities [18–20]. Tellier [21] postulated that selection effects on plant fitness components may be amplified or mitigated by

stochastic events when viable seed bank fractions are small. Consequently, in highly dynamic patch networks, the persistence of a plant metapopulation critically depends on whether a species is equipped to maintain the exchange of genes among subdivided habitats and across successive generations [22]. Reconstructing current and past dispersal events can therefore unravel the dynamics of connectivity and cross-generational source-sink effects between spatially separated populations. Recent research emphasizes the role of persistent soil seed banks and their potential to decrease rates of genetic drift. Moreover, under environmental perturbation on short time scales, dormancy enables the maintenance of adaptive variation by balancing selection and reduces the effects of background selection [21,23,24]. Indeed, the dormant genotype fraction can prevent a loss of genetic diversity across multiple reproductive seasons, resulting in an overall increased effective population size [25]. This is particularly relevant in highly dynamic systems, such as arid environments, where local populations are expected to undergo strong demographic fluctuations with varying presence/absence in the above-ground population [26–29].

Theoretical predictions suggest both seed dormancy and dispersal to increase with environmental variability in meta-populations [30–32]. Yet, in a review based on 43 empirical and simulation studies, Buoro & Carlson [33] found mixed trends of co-variation between spatial and temporal dispersal [26,27,31]. These authors outlined the substantial need for studies that simultaneously measure spatial and temporal dispersal in nature. Disentangling the relative prevalence of these risk-spreading mechanisms may also enhance the predictability of the potential for evolutionary rescue in populations under climate change. Although contributing to the resilience of fragmented populations, both dispersal strategies pose different risks and chances in the context of range shifts and niche expansion [29]. Especially strongly structured populations that primarily track favorable conditions through recruitment from local seed rains (“dispersal in time”) are prone to extinction if the magnitude of environmental shift exceeds their niche breadth [34]. On the other hand, recurrent spatial dispersal may counteract local adaptation to conditions at the tolerance limit and thus suppress potential niche expansion [35]. Consequently, climate change effects are likely to unbalance the forces of spatial and temporal rescue effects in the future [36].

To date, only a few studies have addressed the spatiotemporal patterns of plant meta-populations since this requires long-term data on patch occupancy, a series of discrete habitat patches, and the assessment of potentially long-lived seed banks [37]. Here, we use series of isolated wetlands as a well-suited study system to assess meta-population processes by examining above- and below-ground population structures in different cohorts of the early successional monocarpic annual amphiphyte *Oenanthe aquatica* (L.) Poir. This landscape genetic case study further allows us to determine the relative contribution of spatial gene flow versus seed bank recruitment in shaping local genetic diversity. The target populations are distributed across small spatially distinct temporary wetlands embedded in an area of intensive agriculture. These glacially formed "kettle holes" are habitat islands characterized by short-term changes in local hydrologic conditions, ranging from flooded to completely dry within a season, and longer-term dry periods or periods of standing water. We expect the extent of gene flow between these discrete habitats to be reflected in the genotype composition of the soil-stored viable seed pool [38–41]. By population genetic analyses of both the extant populations and the soil seed bank, we infer

local population chronology as well as connectivity shifts among kettle holes. Our target patches and their soil strata are spatially distinct and the seed production of our target species is high. Taking advantage of these preconditions we aim to test (i) whether *O. aquatica* population structure changes over time, (ii) how the spatial configuration of habitat patches affects the genetic diversity of local populations and (iii) if meta-population theory can explain observed population genetic patterns.

Results

Genetic diversity

Of the twenty surveyed patches, two (2a,12a) were excluded from further analyses as no *O. aquatica* individuals were obtained in extant or seed bank communities. Two of the remaining 18 populations were absent in the survey conducted in 2016 but present in 2019, none of which was shown to have a viable seed bank (Table 1). Among 580 genotyped individuals, we detected no clones. Low levels of Multilocus Linkage Disequilibrium r_d were observed in four populations of seed bank and extant cohorts ranging from 0.029 to 0.06 (Supplementary Table s1). We detected significant deviations from Hardy-Weinberg equilibrium in at least one population at eleven out of 13 loci in each of the two extant cohorts and at eight out of 13 loci in each seed bank cohort (Supplementary Figure s1). Nevertheless, none of the loci consistently deviated from HWE proportions (as would be expected with abundant null alleles/allelic dropouts), hence all loci were included in further analyses. Over all 13 loci, a total of 198 alleles were scored, ranging between 6–31 alleles per locus. Genetic diversity measures varied among populations and across cohorts, while mean diversity values across cohorts varied only slightly (Fig. 1, Supplementary Table s1).

Table 1
Sampled *Oenanthe aquatica* populations grouped by geographic region in the *Agroscapelab* research area in northeastern Germany (see Methods), coordinates (WGS84), and sample size (n) of local populations from extant cohorts of 2019 and 2016 as well as from upper (recent; S1) and lower soil layer (historical; S2) seed banks, '0' absence record of *O. aquatica*, '()' recorded, but not genetically analyzed, '-' unvisited. Note that sample sizes varied among soil samples due to differential germination yield.

				n			
Region	***Location ID***	***Latitude***	***Longitude***	***2019***	***2016***	***S1***	***S2***
Northeastern	P01	53.408561	13.640170	9	0	0	0
Agroscapelab	P02a	53.405311	13.639557	0	0	0	0
	P02	53.400064	13.671076	20	-	7	21
	P03	53.397381	13.665745	20	25	4	9
	P04	53.385815	13.699426	20	(2)	14	2
	P05	53.383518	13.708530	20	-	7	10
Central	P06	53.353470	13.618264	15	25	1	15
Agroscapelab	P07	53.352202	13.623910	18	0	0	0
	P08	53.355701	13.619981	2	-	0	0
	P09	53.345009	13.631840	20	-	9	12
	P10	53.335577	13.584429	20	-	0	0
	P11	53.342272	13.564552	20	-	11	10
	P12	53.334885	13.566890	17	-	0	0
	P13a	53.321288	13.570545	0	-	0	0
Southwestern	P13	53.328058	13.524727	20	-	10	8
Agroscapelab	P14	53.326380	13.523601	19	-	0	0
	P15	53.319181	13.539932	20	-	5	16
	P16	53.317162	13.532987	20	-	0	0
	P17	53.308486	13.552990	17	19	2	0
	P18	53.306886	13.553263	20	-	2	9

Except for one recent seed bank cohort (P09_S1), F_{IS} values were generally positive indicating prevalent heterozygote deficiencies in most populations. Pairwise comparisons of the genetic diversity between extant (cohort 2019) and soil (cohort S2) populations revealed no significant differences over time in any

of the parameters examined (Supplementary Table s2). A_R varied between 3.743 and 5.846 (mean = 4.617) with consistent values among cohorts of the same population. Overall average H_E was 0.637 with values ranging from 0.554 to 0.702. H_O was generally lower than H_E (mean = 0.571), with values ranging from 0.269 to 0.692 (Supplementary Table s1). *NeEstimator* returned infinite effective population sizes in some populations' cohorts. Most obtained estimates of effective population size were low with estimates ranging from 13 (CI = 6.1–43.3) to 201.7 (CI = 42.4–inf). Over the sampled cohorts, variation of population-level genetic diversity and inbreeding revealed no directional pattern.

Spatio-temporal Connectivity

A nested Analysis of Molecular Variance (AMOVA) with cohort samples grouped according to their respective population revealed most genetic variation (87.32%) within cohorts, while only 1.31% was attributable to differentiation between cohorts within populations. Genetic differentiation among the local populations accounted for 11.38% of the observed genetic variance (Table 2).

Table 2
AMOVA results of spatial and temporal variation. Significance level is based on 20,000 permutations.

Source of variation	*df*	*Sum of squares*	*Variance components*	*% variation*	*Fixation indices*	*p*
Among populations	10	505.632	0.548	11.90	FCT 0.114	p < 0.001
Among cohorts within populations	16	98.877	0.063	1.31	FSC 0.015	p < 0.001
Within cohorts	861	3625.109	4.210	87.32		
Total	887	4229618	4.822		FST 0.131	p < 0.001

STRUCTURE analysis inferred 16 distinct genetic clusters in a pooled sample set including all cohorts. Individual assignment of posterior membership probabilities confirmed stable genetic composition over time, which is illustrated by the shared colour distribution in seed bank and extant cohorts within local populations (Figure 2), whereas clusters were highly segregated among populations/sampling sites. At the same time, *STRUCTURE* indicated local dispersal as several populations showed genetic impact on neighboring populations. In one instance, two adjacent southwestern populations were assigned to the same genetic cluster (grey-brown-P13, P14). We observed five kettle holes without viable seed banks (as inferred from our germination experiment; see methods for details), yet individuals were assembled to separate kettle-hole-specific clusters (Figure 2). This separation argues against recent colonization from one of the other populations studied.

Consistent with the cluster-analysis, the NJ-tree inferred from Edwards distances[42,43] over all cohorts showed substantial structuring with cohorts of the same population grouped together, indicated by sequential ID (Figure 3). The geographical configuration of populations is not fully reflected by the tree topology, as spatially adjacent populations were not consistently grouped together.

In the individual-based NJ- tree, the overall population structure is less well resolved, but individuals are largely grouped by their population of origin and showed no clear sub-structuring by cohort (Figure 3). Furthermore, *GENECLASS2* determined a high self-assignment rate of 89.6%, as 512 of 570 individuals could be assigned to their respective sampling location. Of those individuals not assigned to their population of origin, 21 had a low probability of descent from any of our populations, indicating immigration from unsampled populations, while 37 were assigned to other sampled populations from both, adjacent and distant patches (Supplementary Table s3). Ten first-generation migrants (0.018%) were identified by the L_{home} approach with six of them in accordance with the assignment test (Figure 4). Half of the recent migrations occurred between patches less than 1.5km (160 to 1400 m) apart, while the other half were found over distances of more than 5km (6km to 12km). On average 92% of the specimens of the extant cohort 2019 descend from the previous cohorts of the same population. These results generally point towards strong among-cohort-connectivity and limited dispersal between patches. Finally, pairwise G'st comparisons between populations (pooled across cohorts) were all significant (mean=0.330), suggesting considerable population divergence (Supplementary Table s4), indicating substantial steady spatial population structure.

Spatially Explicit Genetic Structure

A significant increase of individual pairwise genetic distances with geographic distance was observed in the aboveground and in seedbank cohorts indicating consistent spatial restriction of gene flow (Supplementary Figure s2). Accordingly, Mantel tests of correlation revealed an isolation-by-distance pattern ($p < 0.001$) for all cohorts, with strongest effects in the soil seed banks ($r_{(S1)} = 0.482$, $r_{(S2)} = 0.394$) and a less pronounced effect in extant populations ($r = 0.119$). The sPCA showed significant global spatial genetic structure among populations in three analysed cohorts ($p_{19} < 0.001$, $p_{S1} < 0.001$, $p_{S2} = 0.019$). Significant fine-scale structures were only obtained in the aboveground ($p_{19} = 0.039$) and the lower layer (S2) seed bank data set ($p_{S2} = 0.022$). We detected a latitudinal separation at larger scales: With a few exceptions, individuals of all cohorts were partitioned from the Northeast and Southwest along the first global component (Fig. 5). A heterogeneous distribution of genotype scores was observed in populations located at the geographical center of the study area and along the southwestern locations, suggesting a transition zone with increased admixture (Fig. 5). This is corroborated by the analysis of resistance to dispersal based on IBD-residuals which delineates a dispersal corridor along southwestern localities (Fig. 5). Putative dispersal barriers are inferred in areas of increased urbanization and along large forests (Fig. 5).

Landscape Configuration Effects On Genetic Diversity

An impact of spatial isolation, i.e. nearest neighbor distance, and habitat size on measures of local genetic diversity was found only in extant cohorts 2019 (Fig. 6). Increased distances to the nearest neighbor patch result in a decrease in H_0 and increased local inbreeding. None of the genetic diversity measures were affected by the local habitat size, measured as patch area (Supplementary Table s5). Finally, neither allelic richness nor expected heterozygosity were related to any habitat configuration measure or their interaction.

Discussion

Spatio-temporal connectivity of O. aquatica in a dynamic landscape

Our results on spatiotemporal connectivity of *O. aquatica* across kettle holes are important in two major respects: first, we found genetic differentiation and restricted gene-flow among sampling sites in all cohorts, both in the extant populations and the soil seed bank, and across varying spatial scales from a few hundred meters up to several kilometers. And second, the general connectivity patterns and levels of genetic differentiation in our study system persist over time. This is supported by the pronounced isolation by distance pattern observed in all cohorts and a clear separation of the majority of local populations (across both extant plants and seed banks) into unique clusters. Accordingly, the spatial structuring observed in the viable dormant propagule pool is well reflected in aboveground *O. aquatica* populations. In particular, long-established local populations exhibit nearly unchanged allele frequencies over the time period studied here. However, variance partitioning, population structure analyses, and inferred migration events suggest some admixture across sites.

STRUCTURE clustering and GENECLASS2 assignment tests showed an increase of connectivity between spatially adjacent patches in a few cases, but also indicate a (re-) colonization of patches with presumably limited or absent seed banks (i.e., those sites where no germinations were observed in any of the soil bank samples). Interestingly, of the limited number of recent migrants inferred, a considerable proportion were long-distance migrants.

Individual sites harbor genetically distinct populations with repeated local recruitment and formation of persistent soil seed banks. At individual sites, genetic diversity stochastically varies among cohorts, presumably reflecting varying degrees of drift due to local environmental fluctuations. However, genetic diversity measures do not exhibit any consistent trend over the time period covered here (presumably several decades). Genetic variation partitioning reveals substantial divergence among local populations, despite occasional outcrossing with variants from nearby sites, as well as rare, but detectable, long-distance dispersal (Table 2). Overall, many populations in the habitat network examined store a long-term viable, sufficiently diverse seed bank and therefore are not primarily driven by recurrent extinction/recolonization dynamics as assumed by metapopulation theory in its strict sense [44,45]. However, variation in functional connectivity, including short-term changes in the occurrence of local

populations (as, e.g., in P01, P07) and genetically inferred dispersal across some kettle holes comprise features of metapopulation dynamics in this historically scattered landscape. In particular, the occurrence of *O. aquatica* at patches with no detectable seed bank is pointing to recruitment from transient seedbanks or recent colonization.

These incidences may comprise previously unoccupied patches, where recent immigration could be facilitated by contemporary environmental conditions that increase dispersal along stepping-stone corridors, as shown by the dispersal paths revealed by the resistance analyses. Such colonization would require some recent temporal synchronization of favorable conditions among donor and recipient patches [46]. Water regimes among individual kettle holes are typically asynchronous. However, a plausible scenario for a synchronization in the water regime could be an intense recent spring flooding in a range of patches, followed by a simultaneous dry fall in summer. Kettle holes in this region were reported to overflow after snowmelt and to dry up during the summer period [47,48]. Among such flooded patches, seeds of *O. aquatica* may be repeatedly transported by mobile linkers, such as water birds [49,50], and accumulate throughout the season. Such processes would promote regional connectivity in the long term. An alternative scenario is that transient local populations emerge occasionally based on randomly occurring mass effects, given that some patches may provide less suitable conditions for seed longevity [19]. This would prevent the formation of a soil seed bank in the long run. Such ephemeral populations (as represented by our grey brown genetic cluster–P13,P14; cf. Figure 2) may emerge as a result of a 'recolonization rescue' as described by Hanski [51]. A sudden short-term emergence of *O. aquatica* is likely since the species can form monotypic stands from only a few individuals if conditions are favorable [52]. A community study conducted in 2015 in the same study area suggests mass effect processes to be prevalent in non-flooded patches [53], as these patches may experience (1) temporarily elevated mobile link activity and/or (2) prolonged dry periods associated with higher seed or seedling mortality for amphibic plant species. However, the genetic consequences of these two mass effect scenarios will differ. With elevated mobile link activity after sudden flooding, we may observe a higher genetic diversity in populations emerged from diverse migrant seed variants accumulated in short-term standing waters, as, e.g., at P14 and P16. Contrary, when recovering from high mortality, large populations may have arisen from recent immigration of only a few variants in drier environments. Here, founder effects/bottlenecks may cause reduced genetic diversity [54], as observed in the most spatially isolated populations, i.e., P12 and P10 (Supplementary Table s1). Still, this effect might be mitigated if gene flow via pollen is maintained between patches [55]. Any of these scenarios could apply to those local populations where we could not detect soil seed banks.

Both scenarios would be in line with the observed isolation-by-distance (IBD) pattern, i.e., an increase of genetic differentiation with geographic distance. This IBD population structure suggests a stepping-stone dynamic in our study system, which is further corroborated by contemporary dispersal being predominantly inferred among adjacent sites.

With our methodology, we cannot fully exclude that scarce seeds in the soil remained undetected. Specifically, if a population without a detected seed bank forms a unique cluster, it could originate from such scarce seeds bearing a local genotype. However, the high seed production of our studied species and our small-scale sampling of both soil and extant populations make this interpretation less probable. Therefore, we consider it more likely that those populations which form their own genetic cluster but do not hold a detectable seed bank have been colonized by specimens from a population not sampled in our study.

In future studies, it would be worth to monitor the longevity of seed banks in different hydrological regimes to identify factors that limit seed viability. Such information could improve the reliability of predictions about how stable the observed population connectivity may be under altered environmental conditions.

Consequences Of Spatial Isolation And Local Habitat Features

Taking into account that aquatic plants cannot colonize the surrounding agriculturally utilized matrix, kettle holes play a key role as connective landscape structures with stepping-stone functions[56], as confirmed by the inferred isolation-by-distance pattern in our study system. Populations with a long-lived seed bank represent starting points for recolonization rescue, from where local variation slowly propagates across the landscape. This is in agreement with the significant latitudinal cline detected by spatially explicit analyses, separating northern and southern populations with an increased admixture at the geographical center. As precipitation is a major driver of kettle hole hydrological conditions, increased levels of connectivity between adjacent habitat patches are likely to be related to local environmental synchrony [57,58]. Small-scale patch configuration effects on local diversity were already shown in an earlier study on plant metacommunities reporting a decline in species richness with increasing spatial isolation and decreasing patch area [59,60]. We confirmed noticeable effects of isolation on local genetic diversity at the population level (Fig. 6) and found inbreeding to increase with distance from the nearest neighbor patch, pointing towards the relative importance of mobile link movement [61,62]. Limited pollinator availability in isolated patches[74] may lead to increased levels of selfing and therefore elevated inbreeding at these sites. A reduced primary seed dispersal distance causing clustered occurrences of siblings close to their parent plant would also explain enhanced inbreeding at remote habitat patches likely less frequented by large mobile linkers. The effect of patch size on local genetic diversity was negligible. As a result of increased inter-specific competition, seedling recruitment in *O. aquatica* is likely to be suppressed at late successional stages when more competitive species emerge. This process is likely more pronounced in larger patches [60,63] and ultimately results in an ephemeral population-built-up. Hence, unlike in populations that undergo progressive fragmentation of continuous habitat, the extent of genetic variation and connectivity in our study system is less a matter of patch size [59] than a matter of small-scale environmental conditions and historical population establishment in the naturally scattered landscape.

Conclusion

We here assessed the spatiotemporal connectivity dynamics in a presumed aquatic plant meta-population system. Our combined approach of spatial and temporal sampling allowed us to track recent colonization events and to uncover stepping-stone dynamics with source-sink effects. These were primarily based on dispersal from local long-term persistent to spatially adjacent ephemeral populations, pointing to recolonization rescue effects as predicted by meta-population theory [64]. We showed a significant spatial genetic structure and found repeated local recruitment to largely shape the current *O. aquatica* population structure. Stepping-stone processes are likely to maintain population viability and gene flow with waves of dispersal occurring in response to temporary environmental synchrony of favorable patch conditions. Our study further highlights the crucial role of soil seed banks in serving as a source to maintain and spread variation and hence to ensure the long-term persistence of *O. aquatica* in the kettle hole system. In the face of climate change, a future drawback to population connectivity may be regional droughts that increase the distances among patches to a level that pollen and seeds cannot easily travel. A lack of immigrant variants may cause genetic erosion, further limiting the ability of local populations to adjust to harsh environmental changes [21].

It remains to be elucidated whether recurrent spatial dispersal is sufficient to maintain population connectivity under potentially increasing population separation in the course of long-term environmental change.

Methods

Study area

The study was conducted in the 'AgroscapeLab Quillow' (www.zalf.de/de/struktur/eip/Seiten/AgroScapeLab.aspx), an open research platform covering an area of approximately 268 km^2 in Brandenburg, Northeast-Germany (cf. Figure 2). The site is characterized by a mosaic of wide open areas of cropland, grassland, and mixed forest. Most agricultural land has been conventionally farmed for decades, resulting in a seasonal rotation of large monotypic stands. Several hundred small glacial wetlands ("kettle holes") ranging in size from 0.01 to 3 ha are heterogeneously scattered throughout the area and form a regional resource pool for natural communities. Periodic changes of local water regimes vary asynchronously depending on size, steepness, and successional stage, and occur at different time scales driven by seasonal precipitation [48].

Study species

Fine-leaved water Dropwort, *Oenanthe aquatica* (Apiaceae) is a monocarpic, hydrochorous amphiphyte plant species growing at disturbed eutrophic stagnant waters and ditches on muddy sediments [65,66]. *Oenanthe aquatica* populations can build-up dominant stands in environments with regular recurrent flooding events. Seed production is high and seeds are accumulated in a soil bank [52]. As typical in

Apiacean species, flowers are protandrous and pollinated by a range of insects covering different taxonomic orders. *O. aquatica* is predominantly outcrossing, however, incidences of selfing have also been observed [67].

Sampling and molecular analyses

Integrated with a soil community study, 20 kettle holes, comprising 8 pairs of spatially proximate sites and four intermediate (putative stepping stone) sites, were selected for tissue and soil core sampling of *O. aquatica* populations in spring 2019, including two sites without *O. aquatica* in the extant plant community (Table 1). For 18 kettle holes, leaf material of their above-ground populations was collected for genotyping. Sampled individuals were separated by at least 1.5 meters. For locations with n<10 extant individuals, we sampled the whole population. As local abundances varied considerably, sample sizes ranged from two to 22 individuals (mean=15.1±7.4) per kettle hole, resulting in a total of 317 individuals. In parallel, soil cores were taken with a 15-cm drill at four 1 m^2 squares, along an intra-site spatial vegetation gradient of size-related length from the ruderal edge to the central aquatic zone to account for local variation in seed bank stock at each kettle hole. The extent of the gradient varied depending on the diameter of the local kettle hole. Four samples were taken from each 1 m^2 spot. Subsequently, the upper and lower 5 cm soil layers were separated, and each combined for each 1 m^2 spot, resulting in four upper and four lower composite soil core samples per location. Core chronologies of kettle holes in the focus research region suggest that the upper soil layers reflect recent seed rains, while the lower sample seed fraction dates back several decades in the past [68]. In the greenhouse, a 2 cm thick layer (~480 ml) of each composite core sample was spread on a sandy substrate layer (2 cm ~480 ml) in a plastic tray. Using the emergence method of van der Valk & Davis (1978), a randomized flooding treatment was applied to induce germination at natural water-logged habitat conditions. This method has proven effective for assessing both persistent and transient seed banks in ephemeral wetlands [69]. Samples were exposed to natural light regime and day and night temperature of 23-30 and 18 °C, respectively. After morphological identification, seedlings other than *O. aquatica* were removed to reduce competition. Leaf tissue was sampled opportunistically when reaching two-leaf-stage, yielding a total of 194 samples from viable soil populations in both layers of ten study sites, while for one further site, a viable population was found only in the upper layer. All leaf samples including soil bank seedling tissue were preserved in 96% EtOH at -20 C until further processing.

Genomic DNA of extant *O. aquatica* populations was extracted using a modified CTAB protocol as described by Inglis et al. [70]. Each individual was genotyped for 13 polymorphic microsatellite loci described in Favre-Bac et al. [71] (O_01, O_03, O_10, O_13, O_13, O_17, O_18, O_20, O_21, O_28, O_32, O_37, O_38, O_47). For multiplexing, forward primers of each primer pair were differently fluorescent-labeled (FAM, VIC, NED, PET) and assembled in 6 Polymerase Chain Reactions (PCR) (Supplementary Table s6). PCR products were separated via electrophoresis using a 3500 Genetic Analyser Sequencer (Applied Biosystems Hitachi). Allelic peak sizes were identified from resulting electropherograms using GeneMapper software (Version 5.0, Applied Biosystems). For three of our study sites, genotype data for

the same microsatellites were available for the 2016 extant population [63]. To enable their inclusion for comparison, these data were calibrated (for laboratory methodological details, see [63]). Allele size data for a total of 579 individuals is provided in the supplementary Table s7. In subsequent analyes, we considered all emerged plants sampled within one season or seedlings emerging from one seed bank stratum as a distinct cohort, resulting in two aboveground cohorts (‘2019’, ‘2016’) and two belowground cohorts (‘Soil1’, ‘Soil2’).

Data Analyses

Genetic diversity and patch-network configuration

Measures of genetic diversity were estimated in R (Version 4.1.1) and R studio (Version 1.4.1717) for all cohorts with n³8 of each local population. For each population’s cohort and locus, we tested for significant deviation from Hardy-Weinberg-Equilibrium using the hw.test function from the *pegas* package [72] with 1,000 permutations. To assess multilocus linkage disequilibrium (LD) we computed the standardized index of association r_d [73] calling the ia() function from the *poppr* package [74]. Significant deviation from the expectation of linkage equilibrium among loci was tested using 1000 permutations, followed by a Bonferroni-correction of significance levels. Observed and expected heterozygosity (H_O, H_E) as well as rarefied allelic richness (A_R), total allele number, and inbreeding coefficient (F_{IS}) were calculated for population cohorts using the *hierfstat* package [75]. The mlg.id function implemented in the *poppr* package [74] was called to identify putative clones. We used NeEstimator v2.1[76] under the linkage disequilibrium model and random mating assumption to infer effective population sizes (*Ne*) of seed bank and aboveground cohorts (cohorts with n³8 only), excluding allele frequencies below a threshold of 0.05 [77]. Effects of patch area, nearest-neighbor patch distance and their interaction on diversity estimates A_R, H_E , H_O, and F_{IS} of cohorts (n³8) were assessed in R running generalized linear models (GLMs) with Gaussian distributions and z-transformed variables. We performed a stepwise backward selection to reveal best-fit models (minimum AIC) excluding variables iteratively from the full model. Patch area and distance to nearest-neighbor patch were determined using Google Earth Pro 7.3.6.9345 (Supplementary Table s1).

Spatial genetic structure

Inter-individual genetic distances were calculated with the R package poppr [74] using Edwards chord distance for seed banks and standing cohorts separately. A Mantel test for isolation by distance (IBD) was conducted to test whether genetic distances and geographic Euclidean distances are correlated, using the R package *adegenet* [78]. Significance of correlations was assessed using the mantel.randtest() function with 10,000 permutations. Additionally, linear regression models were applied for each correlation by the lm() function in R using log-transformed and original geographic distance data. The best-fit model based on R^2 was chosen for IBD calculation. To identify potential barriers to gene flow, a spatial analysis of principal components (sPCA) was performed which integrates geographic

configuration using Moran's I index of spatial autocorrelation, to detect spatial structure in allele frequency data. This multivariate analysis was performed separately for each cohort with the R package *adegenet* [78], using the *Inverse distances* method to weight network connectivity. Statistical significance of global and local spatial structures was tested using the Monte Carlo resampling method with 10,000 permutations. We mapped the individual scores of the first principal component according to their sampling locality with geographic positions being slightly jittered for improved visualization. Moreover, we conducted a resistance analyses based on the deviation from IBD for the aboveground population 2019 to identify recent dispersal corridors and barriers, using the *ResDisMapper* package[79]. For this analysis, we obtained residuals from the isolation by distance method (see above) for each pair of individuals to calculate landscape resistance. We mapped interpolated significant resistance scores as well as high certainty corridors and barriers. This method requires no prior information about dispersal-limiting environmental characteristics and has been shown to produce accurate resistance values at small spatial scales [79,80].

Spatio-temporal genetic variation

To test whether individuals can be assigned to distinct genetic groups following increased differentiation among sampled locations, Bayesian genetic clustering was performed using *STRUCTURE* Version 2.3.4 [81]. Using a fused dataset comprising samples of all cohorts, the likelihood for the number of putative genetic clusters (K) was estimated to evaluate structuring among years and sites. We ran ten simulations for each K from two to 42 (the total number of local cohorts across all populations) under the admixture model without prior population information. Parameter settings involved a burn-in period of 100,000 iterations followed by 500,000 Markov Chain Monte Carlo (MCMC) repetitions. The optimal number of clusters was determined by plotting the likelihood of K for each value of K ($LnPr[X|K]$) using the $\triangle K$ method [82] in the *Pophelper* package [83] in R. Clustering results of individual membership probabilities were visualized with the implemented *pophelperShiny* App.

The relative contribution of each inferred genetic cluster per population and cohort was illustrated as piechart on a physical map. To test for partitioning of variation among populations and between cohorts, an analysis of molecular variance (AMOVA) was performed in *Arlequin* Version 3.5 [84] using 20,000 permutations. We therefore selected a subset of populations, for which at least two of four cohorts (2019, 2016, S1, S2) were available with a minimum sample size n=8 per cohort. Levels of spatial population structuring were further examined using pairwise G'st [85] among populations. Given the low variability across cohorts within populations (see Results), cohorts ($n^3 8$) were pooled for any population prior to the calculation of G'st. We tested for significance of G'st values by estimating 95% confidence interval based on 1,000 bootstraps over loci using the *diveRsity* package [86]. Recent dispersal and signals of immigration were inferred by a stepwise assignment procedure in *GENECLASS2* [87]. First, we conducted the implemented self-assignment test to assign or exclude reference populations as possible origins for each individual. Therefore, we calculated individual assignment probabilities by the Monte Carlo resampling method proposed by Paetkau et al. (2004) [88] with 10,000 simulations and assessed source population

log-likelihood under the Bayesian Criterion of Rannala & Mountain [89]. As a baseline for individual assignment, we pooled the data from all cohorts per population [90]. A probability threshold of 0.01 was set to exclude individuals likely originating from unsampled sources. Additionally, we detected potential first-generation-migrants under the Bayesian Criterion of Rannala & Mountain [89] using the same resampling alghorithm to calculate the likelihood of individuals belonging to their local gene pool or external ones. A probability threshold of a=0.01 was set to reduce the probability of false positives (i.e., incorrect identification of residents as immigrants) [89,91]. We further examined dispersal in time by determining the proportion of the most recent cohorts (2019) classified as descendants of earlier cohorts. For this, we created representative reference data for each local population by removing putative immigrant samples detected during the first two steps[92] resulting in ten local datasets. 2019 population data and reference data from previous cohorts (2016, S1, S2; only cohorts with n³5 considered) were selected to perform an assignment test as earlier (a=0.01). A neighbor-joining tree (NJ- tree) based on Edwards genetic distance with 1,000 bootstrap replicates was constructed in *ape* for population level (n³5) and individual level to assess spatial and temporal divergence simultaneously. NJ-trees were visualized using *ggtree* [93].

Declarations

Declaration on ethics and research permits

We hereby declare that our experimental research and field studies on cultivated and wild plants, including the collection of plant material, are in accordance with relevant institutional, national and international guidelines and legislation. Leaf samples of *Oenanthe aquatica* were collected under a sampling permit issued by the State environmental authority LfU Brandenburg (Germany).

Data Availability

All datasets generated and analyzed during this study are available in the main text and supplementary material. Additional information can be obtained from the corresponding author on request. Reference plant material (species identification by Maxi Tomowski) is deposited at the Unit of Evolutionary Biology, Institute of Biochemistry and Biology, University of Potsdam, Germany.

Author contributions statement

MT was responsible for conducting the field study as well as the greenhouse and laboratory surveys analyzing data, interpreting results. SLG contributed to writing the Manuscript, collection of data and consultation, FJ and RT provided valuable feedback on the theoretical framework of the study and contributed to writing the manuscript, RT provided critical feedback on data analyses and interpretation and supervised this project. All authors reviewed and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Figures

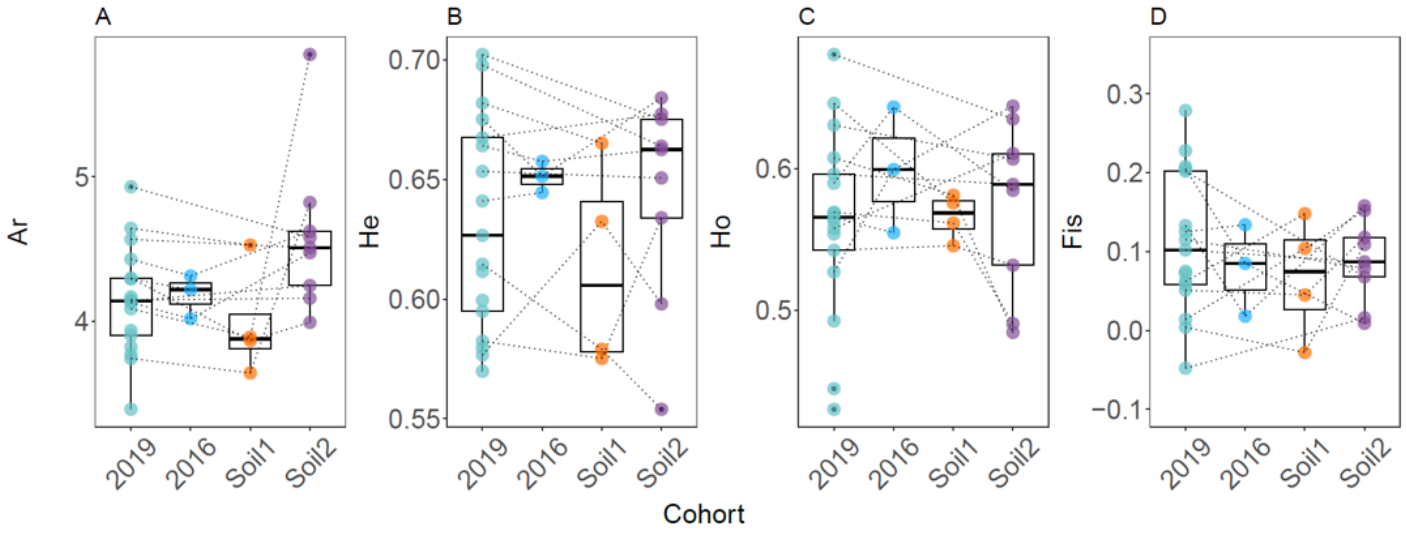

Figure 1

Mean levels and variation of genetic diversity measures: allelic richness (Ar), expected heterozygosity (He), observed heterozygosity (Ho) and inbreeding coefficient (Fis) between Oenanthe aquatica cohorts in reverse chronological order, dots represent population values, between-box lines illustrate population level dynamics in genetic diversity by connecting values of the same location across multiple cohorts.

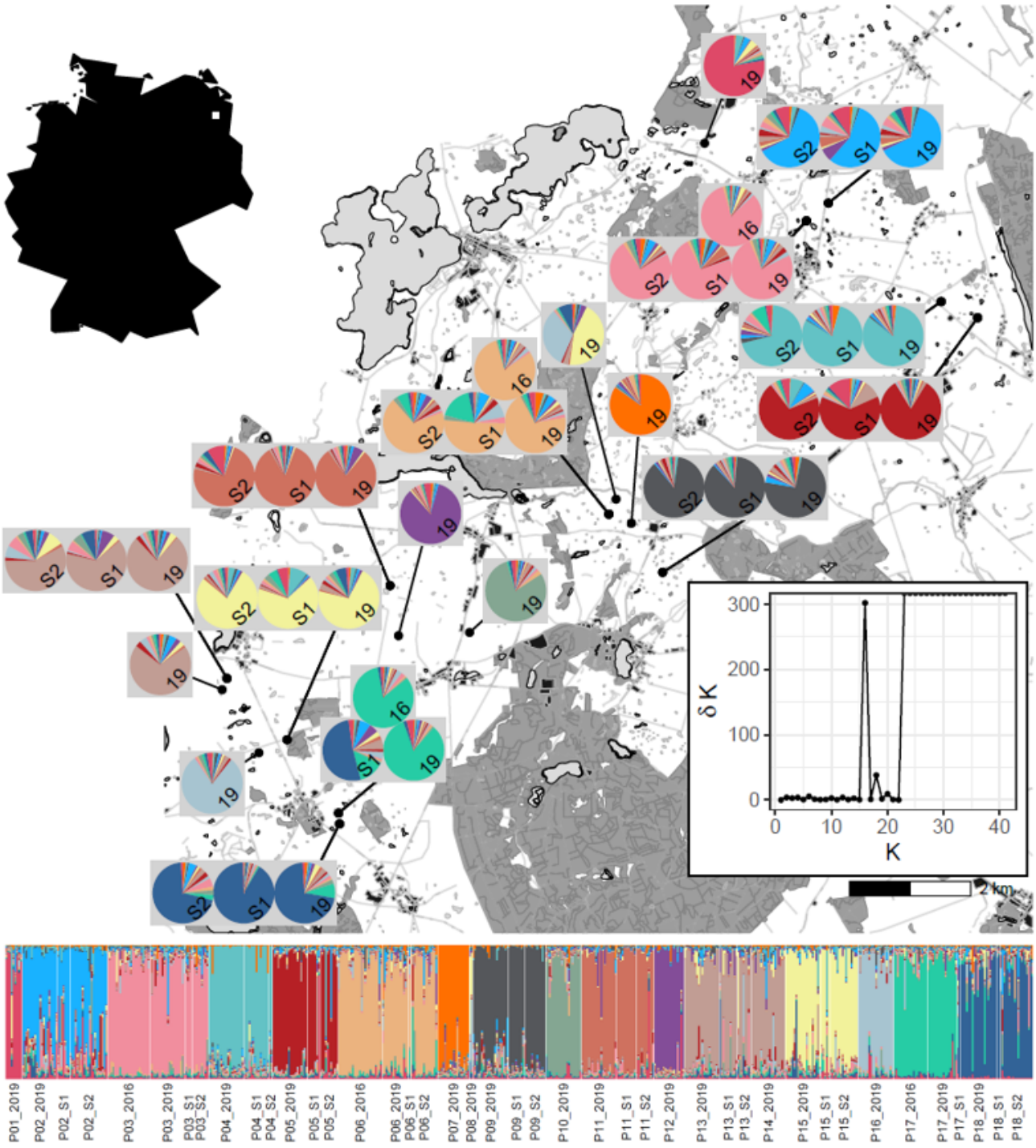

Figure 2

Resul*ts of STRUCTURE analysis (K=16)*. *top-left*: Map sketch shows Germany with sampling site indicated by a white square. *bottom*: Each bar represents an individual genotype with relative membership probability illustrated by distinct colours; *right*: Geographical origin of two extant and two seed bank cohorts of *Oenanthe aquatica*. Each pie-chart represents a single cohort (S1,S2: upper and lower layer seed bank, respectively; 16: 2016 above-ground and 19: 2019 above-ground annual samples)

showing average relative proportion of cluster assignment revealed by STRUCTURE with optimal K= 16, illustrated by the Evanno-plot. Grey rectangles aggregate cohorts of local populations. Note that not all cohorts are present in all populations.

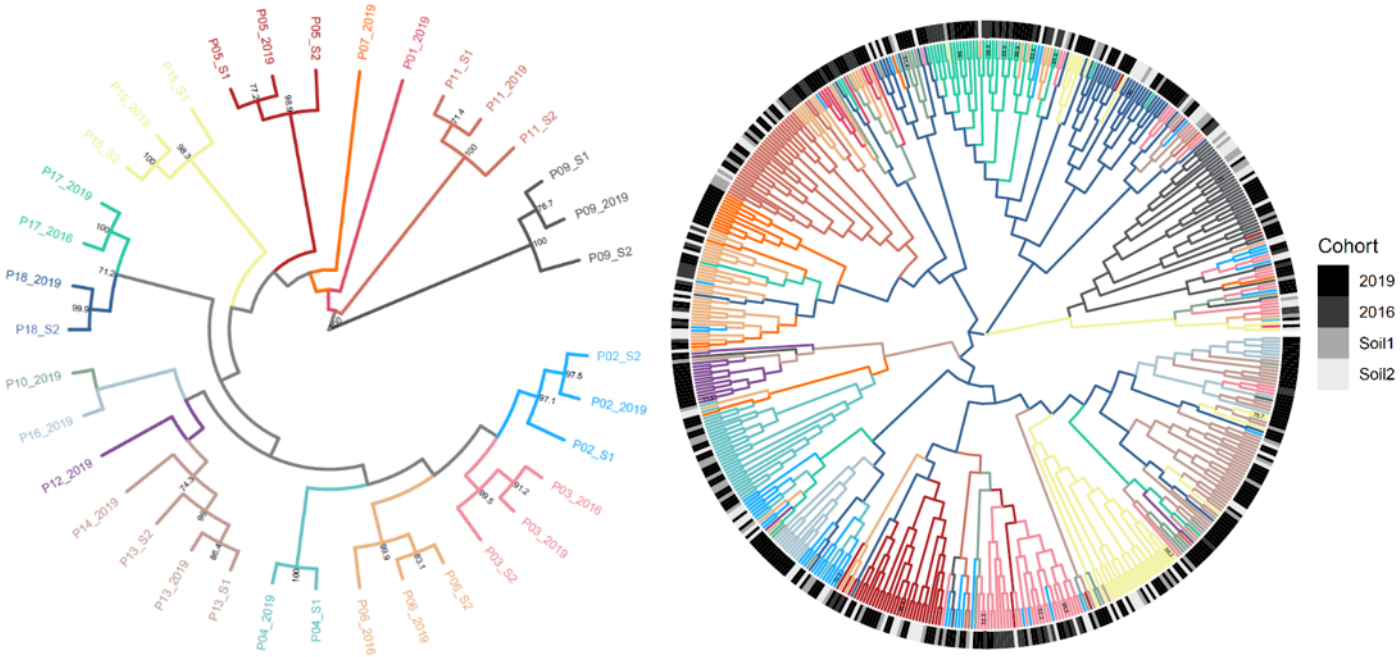

Figure 3

Neighbour-joining trees based on Edwards' chord distances between population-specific cohorts (left) and individuals (right) of all cohorts of O. aquatica. Numbers at branches indicate confidence values >50% revealed by 1000 bootstrap replications. Colors of branches delineate the 16 genetic clusters identified by STRUCTURE. Outer bars (right) show the individuals' cohort of origin.

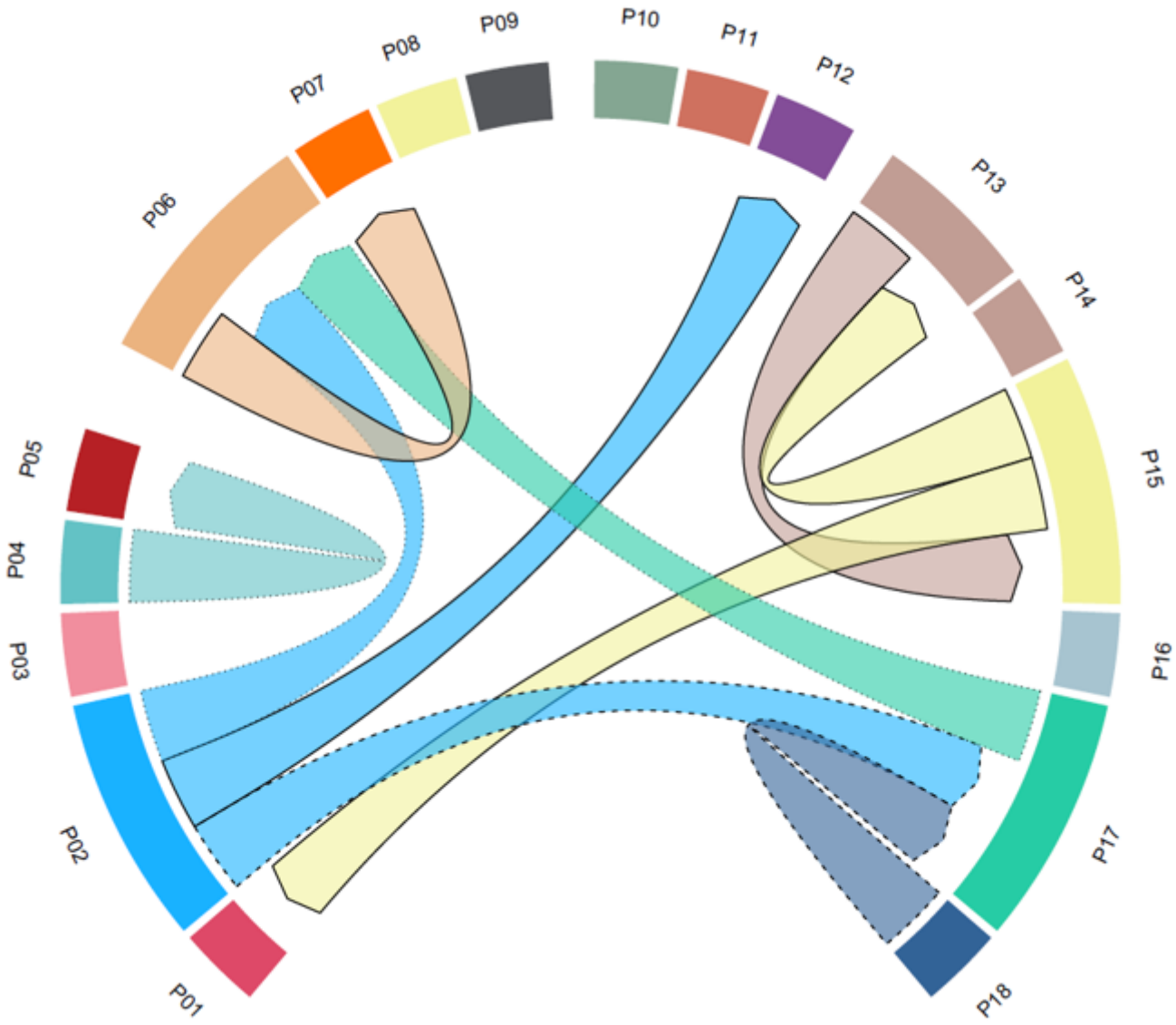

Figure 4

Chord diagram showing individual first-generation migrants of Oenanthe aquatica between habitat patches as determined by GENECLASS2, with chord size proportional to the number of migrants detected and arrows illustrating single migration events. Colors delineate the genetic clusters revealed by STRUCTURE analyses. Solid, dashed, and dotted lines depict recipient cohorts 2019, upper and lower seed bank, respectively.

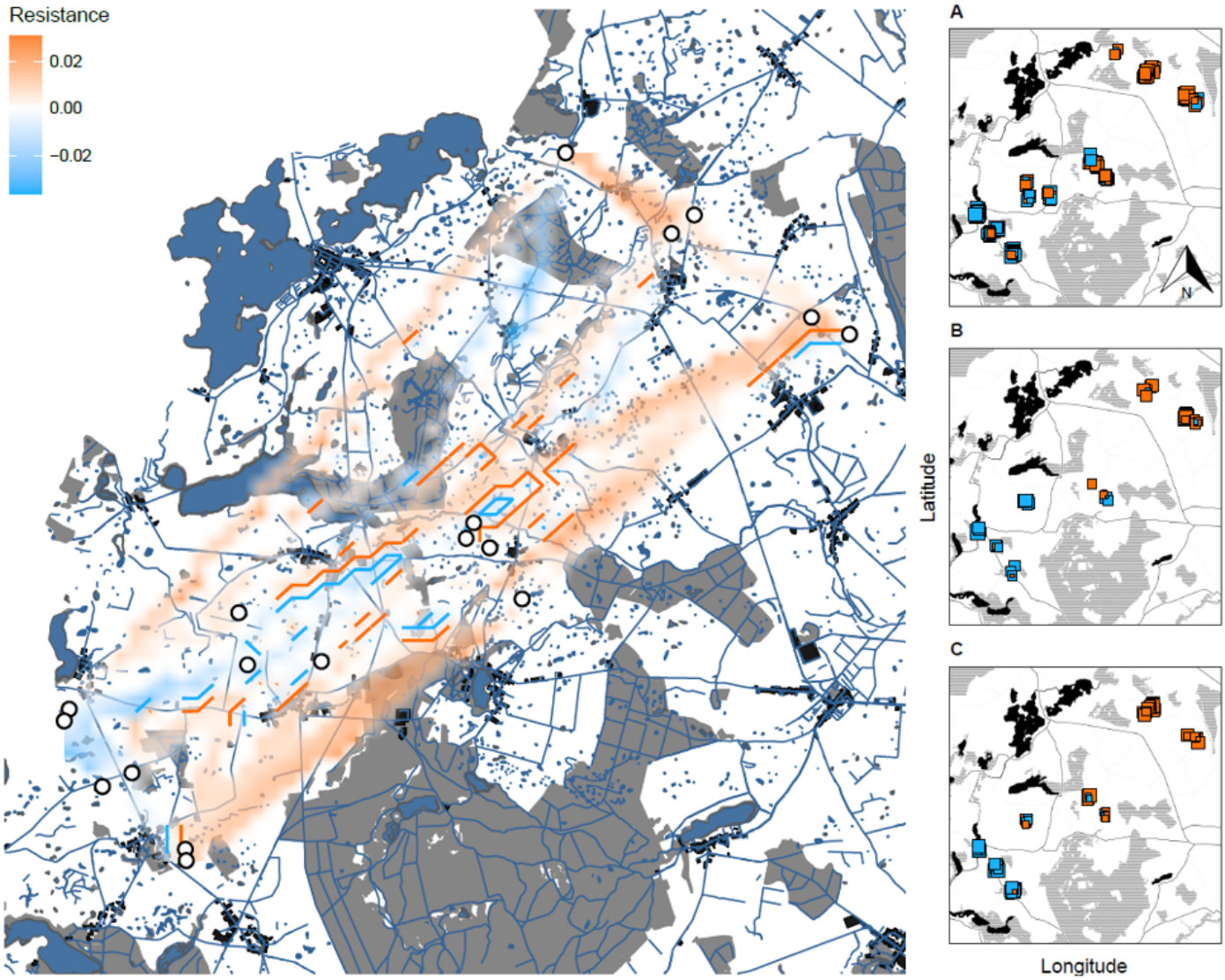

Figure 5

Left: Map displaying resistance values estimated by ResDisMapper with orange and bright-blue areas of high and low resistance, respectively. Contour lines indicate areas of high or low resistance with high certainty; cells lacking statistical significance are not shown. White circles are sampling sites; grey, black and dark blue areas depict woodland, urban sites and wetland, respectively. right: Maps of the spatial genetic structure inferred by sPCA for t 2019 (A), recent seed bank (B), and historical seed bank (C) cohorts. The squares represent the first global score of individual genotypes (bright-blue–positive, orange–negative) with size proportional to score value.

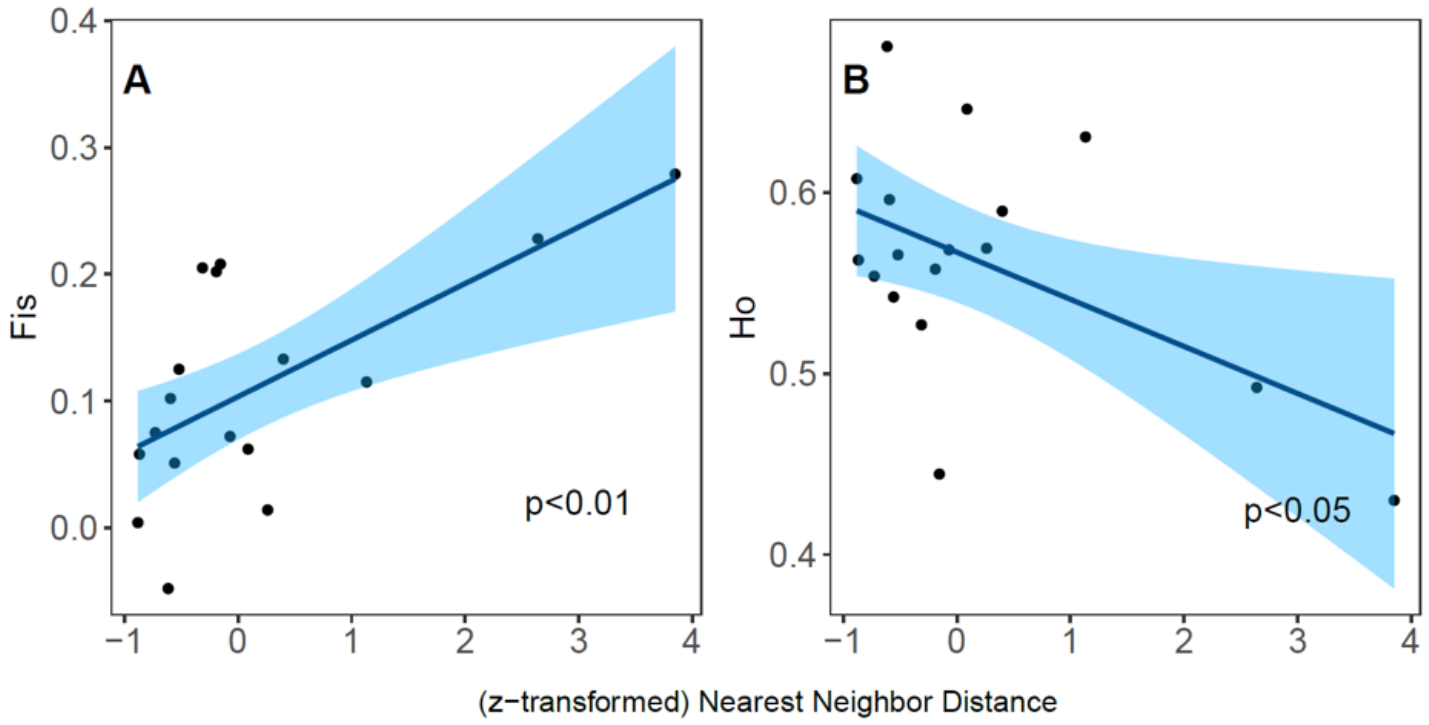

Figure 6

GLM regressions between patch isolation measured as **(z-transformed)** nearest neighbor distance and population inbreeding coefficient *Fis* (**A**) and expected heterozygoszity H_O (**B**). Depicted are **models with significant effects of isolation on genetic diversity measures** based on the backward **selection** procedure (see Supplementary Table s5 for details)**.**

Supplementary Files

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