# Dispersal in Time- the role of seed banks in patchy population dynamics of an aquatic plant

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## Abstract

Progressive habitat fragmentation increasingly threatens plant species with narrow habitat requirements. While local environmental conditions largely define population growth rates and recruitment success at the patch level, dispersal is critical for population viability at the landscape scale. However, identifying dynamics of plant metapopulations in habitat networks is often confounded by the uncertainty of the extent of dormant population compartments. Here, we combined a landscape-scale assessment of an amphiphytic species' population structure with detailed measurements of dispersal complexity in time to reveal past and present dispersal events and putative shifts in functional connectivity. Using 13 polymorphic 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 subpopulations in both time and space. Considerable spatial genetic structure and isolation-by-distance (IBD) patterns suggest limited gene flow between population sites. In contrast, patch level measures of isolation and habitat availability showed minor effects on genetic diversity levels. Low differentiation along the time gradient highlights the superior role of temporal over spatial dispersal. Though some instances of recent colonisation were observed, our results suggest the actual genetic structure of kettle hole populations to result from multiple local seed rain events. Our findings uncover stepping-stone dynamics with source-sink effects based primarily on dispersal from long-term-persistent local populations to adjacent populations. Overall, spatio-temporal connectivity patterns provide support for metapopulation dynamics in our studied system and highlight the importance of persistent seed banks as long-term source of genetic diversity.


## Introduction

Continuous habitat has become a scarce resource – many populations are scattered across habitat patches resulting from progressive fragmentation and degradation of natural areas (Haddad et al., 2015; Lindenmayer & Fischer, 2013). In plants, a landscape-scale fragmentation may particularly disadvantage species with propagules less adapted to long distance dispersal if patch distances exceed several hundred meters, thereby drastically limiting spatial gene flow (Aguilar et al., 2008; Bohrer et al., 2005; Schleicher et al., 2011; Vellend, 2008). As a consequence, spatially isolated populations are expected to exhibit higher levels of divergence and to hold lower levels of genetic variation with limited potential to respond to changing selection pressures (Lande & Shannon, 1996; Hill & Mackay, 2004). However, population differentiation may be homogenized and local extinction risks reduced, if the landscape configuration and the availability of mobile vectors allow gene flow to be maintained. Accordingly, in a net of linked populations constituting a metapopulation, an effective propagule dispersal maintains genetic connectivity that is crucial for long-term persistence under intensive disturbance regimes (Hanski & Gaggiotti, 2004; Ouborg & Eriksson, 2004). Regarding the relevance of metapopulation 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 (Freckleton & Watkinson, 2002; Honnay et al., 2005, 2008; Ellstrand, 2014). Especially in systems with drastic short-term environmental shifts, a considerable proportion of genetic variants may enter dormancy and remain in soil seed banks over years and even decades to overcome periods of unfavorable conditions (Willis et al., 2014).

Although this bet-hedging strategy known as 'storage effect' can create a reservoir of genetic diversity (Warner & Chesson, 1985), rates of germination, seed mortality, and recruitment may widely differ among patches, further complicating the estimation of local extinction risks and colonisation probabilities (Bekker et al., 1997, 2003; Thompson, 2000). Tellier (2019) postulated that selection effects on plant fitness components may be unevenly enhanced or attenuated 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 (Fréville et al., 2013). 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 maintenance of adaptive variation by balancing selection and reduces effects of background selection (Levin, 1990; Schulz et al., 2018; Tellier, 2019). Indeed, the dormant genotype fraction can prevent a loss of genetic diversity across multiple reproductive seasons, resulting in an overall increased effective population size (Lundemo et al., 2009). This is particularly relevant in highly dynamic systems where local populations are expected to undergo strong demographic fluctuations with alternating presence/absence in the above ground population. For instance, field experiments on five plant species in highly unpredictable desert environments identified dormancy and increased local reproduction as superior strategies for population persistence with negligible contribution of spatial dispersal to seedling recruitment (Siewert & Tielbörger, 2010). This pattern has been repeatedly reported in studies with high environmental

stochasticity, whereas more constant conditions have been found to favour spatial dispersal or both strategies similarly (Dostál, 2005; Frisch, 2002). Yet, in a review based on 43 studies, Buoro & Carlson (2014) found no consistent pattern of negative co-variation between spatial and temporal dispersal, contrary to theoretical predictions. They outlined the substantial need for studies that simultaneously measure spatial and temporal dispersal in nature. Disentangling the relative prevalences 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 (Kremer et al., 2012). Especially strongly structured populations that primarily track favourable conditions through recruitment from local seed rains (“dispersal in time”) are prone to extinction if the magnitude of environmental shift exceeds their niche breadth (Weiss-Lehman & Shaw, 2020). On the other hand, recurrent spatial dispersal may counteract local adaptation to conditions at the tolerance limit and thus suppress potential niche expansion (Bolnick & Nosil, 2007). Consequently, climate change effects are likely to unbalance the forces of spatial and temporal rescue effects in future (Bohonak & Jenkins, 2003).

To date, only a few studies have addressed the spatio-temporal patterns of plant metapopulations since this requires long-term data on patch occupancy, a series of discrete habitat patches, and the assessment of potentially long-lived seed banks (Alexander et al., 2012). Here, we use series of isolated wetlands as a well-suited study system to assess metapopulation processes by examining above- and below-ground population structures in different cohorts of the early successional hapaxanth 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 (Simpson, 1989; Brendonck & De Meester, 2003; Summers et al., 2018; Weider et al., 2018). Therefore, well-stratified local soil layers (Kleeberg et al., 2016), populations restricted to clearly defined patches and species’ intrinsically high seed production allow to examine the chronology of connectivity through a combination of population genetic analyses from resurrection experiments and field surveys. Based on the assumptions above we aim to test (i) whether the degree of genetic differentiation and diversity in extant *O. aquatica* populations can be related to past population dynamics, (ii) to what extent the spatial configuration of the habitat network influences the genetic diversity of *O. aquatica* populations, and (iii) if the variation of functional connectivity in a long-term-scattered landscape follows metapopulation dynamics.

# 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. The site is characterised by a mosaic of wide open areas of cropland, grassland, and mixed forest. Most agricultural land has been conventionally farmed for decades, resulting in 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 (Kalettka & Rudat, 2006).

### Study species

Fine-leaved water Dropwort, *Oenanthe aquatica* (Apiaceae) is a hapaxanth, hydrochorous amphiphyte plant species growing at disturbed eutrophic stagnant waters and ditches on muddy sediments (Jensch & Poschlod, 2008; Westberg & Kadereit, 2014). *Oenanthe aquatica* populations can build-up dominant stands in environments with regular recurrent flooding events. Seed production is high and provides seed accumulation in a soil bank (Hroudová et al., 1992). As typical in Apiacean species, flowers are protandric and pollinated by a range of insects covering different taxonomic orders. The species is predominantly outcrossing, however, few incidences of selfing have also been observed (Favre-Bac et al., 2016).

### Sampling and molecular analyses

In spring 2019, ten pairs of spatially proximate wetland islands (ie, kettle holes) were selected for tissue and soil core sampling of *O. aquatica* populations, including two locations 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. 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 $m^2$ sites, along an intra-site spatial vegetation gradient of size-related length from the ruderal riparian 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 size of the local kettle hole. Four samples were taken from any 1 $m^2$ spot. Subsequently, the upper and lower 5 cm soil layers were separated, and each combined for any 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 (Kleeberg et al., 2016). In the greenhouse, core samples were germinated under a randomised flooding treatment at natural light regime and day and night temperature of 23-30 and 18 °C, respectively, to induce germination at natural water-logged habitat conditions. Seedling tissue was sampled opportunistically at the two-leaf-stage, revealing a total of 194 samples from viable soil populations in both layers at 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. (2018). Each individual was genotyped for 13 polymorphic microsatellite loci described in Favre-Bac et al. (2014) (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-labelled (FAM,VIC, NED, PET) and assembled in 6 Polymerase Chain Reactions (PCR) (Supplementary Table S1). 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). Additionally, genotype data of the same microsatellites of a 2016 cohort from 10 partially overlapping locations at the focal study site (Lozada-Gobilard et al., 2021) were calibrated and included to complement our sampling and increase temporal resolution (for methodological details, see Lozada-Gobilard et al., 2021). We further consider all emerged progeny sampled within one season or from one seed bank stratum in our study area as a distinct *cohort* while any local population sampled from a single cohort will be referred to as a *temporal population*.

## 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 each site and each year. For each population and locus, we tested for significant deviation from Hardy-Weinberg-Equilibrium using the hw.test function from the *pegas* package (Paradis, 2010) with 1,000 permutations. To assess multilocus linkage disequilibrium (LD) for each population we computed the standardized index of association $r_d$ (Agapow & Burt, 2001) calling the ia() function from the poppr package (Kamvar et al., 2015). Significant deviation from the expectation of linkage equilibrium among loci was tested using 1000 permutations for each temporal population with n>4 followed by a Bonferroni-correction of significance levels. Observed and expected heterozygosity (Ho, He) as well as rarefied Allelic richness (Ar), total allele number and inbreeding coefficient (Fis) were calculated using the *hierfstat* package (Goudet, 2005). Clones were identified using the mlg.id function implemented in the *poppr* package (Kamvar et al., 2015). Effects of patch area, nearest-patch distance, and number of neighbouring patches within a 500 m radius – the distance that was found to correlate with plant and genetic diversity measures (Lozada-Gobilard et al., 2019, 2021) – on population estimates of rarefied allelic richness (Ar), expected and observed heterozygosity (He, Ho), and Inbreeding coefficient (Fis) per cohort were determined using Spearman-correlation in the stat_cor function from the R package *ggpubr*.

### Spatial genetic structure

Interindividual genetic distances were calculated using principal component analysis (PCA) of allele occurrence matrices for each cohort separately . The obtained genetic distances were based on retaining the number of principal components explaining 75% of the total genetic variance. To test whether PCA based genetic distances and geographic Euclidean distances are correlated, a Manteltest for isolation by distance (IBD) was conducted using the R package *adegenet* (Jombart, 2008). A correlation significance test was carried out using the mantel.randtest() function with 10,000 permutations. Additionally, linear regression models were applied for each correlation by the lm() function in the R. To assess levels of temporal population structuring based on variation in local allele frequencies between years, we estimated pairwise G'st (Hedrick, 2005) of temporal populations with n>4 and tested significance (10,000 permutations) using the *diveRsity* package (Keenan et al., 2013). To identify potential genetic barriers, a spatial analysis of principal components (sPCA) was performed based on Moran's I index of spatial autocorrelation, thus taking into account the difference in allele

frequencies among sites, conditional on geographic distance. This multivariate analysis was performed for each cohort with the R package *adegenet* (Jombart, 2008) using the neighbourhood by distance method to weight network connectivity. To check for statistical significance of global and local spatial structures, Monte Carlo tests were carried out separately for each cohort with 10,000 iterations. The scores of the first eigenvectors resulting from sPCA were mapped according to their corresponding geographic coordinates.

### 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 (Pritchard et al., 2000). The likelihood for the number of putative genetic clusters (K) was estimated by setting K from 2 to the maximum number of locations per respective cohort data set (10 iterations each) 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. 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 (Evanno et al., 2005) in the *Pophelper* package (Francis, 2017) in R. Clustering results of individual membership probabilities were visualised with the implemented *pophelperShiny* App. The clustering procedure was repeated under similar conditions for a fused dataset comprising samples of all cohorts to also reveal structuring among years. The relative contribution of each inferred genetic cluster per population and cohort was illustrated as piechart on a physical map. More detailed information about spatial and temporal population connectivity was gained by computing a spatial population graph for fused data across all cohorts using the R package *graph4lg* (Savary et al., 2021) and *popgraph* (Dyer, 2017). Accordingly, a minimum spanning network was created with nodes representing populations and edges representing mean weight of links based on inverse PCA distances. For genetic graph construction, populations of each cohort were treated as distinct locations. To determine the graph lay-out we used the Fruchterman-Reingold algorithm as implemented in *popgraph* package. Additionally, an analysis of molecular variance (AMOVA) was performed in *Arlequin* Version 3.5 (Excoffier et al., 2005) using 20,000 permutations to test for partitioning of variation among locations and between cohorts. We therefore selected a subset of populations with minimum size of four plant specimens sampled, present in at least three of the four cohorts (2019, 2016, S1, S2), grouped according to location. Recent migration events were inferred using the first-generation-migrant detection in *GENECLASS2* under the Bayesian Criterion of Rannala & Mountain (1997), calculating the respective likelihood of individuals to belong to their local gene pool or to external ones. For this analysis, we pooled the data originating from the two sampled soil strata for each location to avoid misassignment due to small sample sizes. We used a resampling method with 10,000 simulations (Paetkau et al., 2004) and a Type I error set to 0.01, reducing the probability for false positives (i.e., incorrect identification of residents as immigrants; Rannala & Mountain, 1997; Berry et al., 2004).

## Results

# Genetic diversity

We observed significant, but low levels of Multilocus Linkage Disequilibrium in 14 out of 44 temporal populations (rd= 0.022- 0.059) predominantly in extant 2016 and 2019 cohorts (Supplementary Table S3). Significant deviations from Hardy-Weinberg equilibrium were detected in at least one population at 11 out of 13 loci in each of the two extant cohorts and at 8 out of 13 loci in each seed bank cohort (Supplementary Figure S2). 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. Average Fis values were generally positive across all cohorts (Table 2, Supplementary Figure S4). Estimates of genetic diversity averaged across populations for each cohort are summarized in Table 2. Over all 13 loci, a total of 210 alleles were scored ranging between 6-32 per locus. Pairwise comparisons of the genetic diversity among contemporary, recent and two soil-born cohorts did not reveal significant temporal differences in either of the parameters examined (Supplementary Table S5). Average population allelic richness varied between 1.615 and 2.665 (mean=2.415) with consistent values among temporal population samples. Overall expected heterozygosity (He) was 0.632 (mean) with cohort averages between 0.621 and 0.648 and site-specific cohort values ranging from 0.384 to 0.712. Overall observed heterozygosity (Ho) was smaller than He (mean=0.568), a pattern also frequently observed across populations, with Ho ranging from 0.269 to 0.692 and cohort averages between 0.565 and 0.583. Fis was found to vary considerably among spatial samples with mostly moderate positive, but also some slightly negative Fis values ranging from -0.096 to 0.343 (Supplementary Figure S4; mean=0.091), indicating prevalent heterozygote deficiencies for most populations. Extreme temporal fluctuations of Fis found in a few outlier populations are likely biased by the small size of some soil layer samples. For five incidences, clonal (i.e., genetically identical) individuals were detected, all in cohort 2016. Over the four sampled cohorts, variation of population-level genetic diversity and inbreeding did not follow any directional pattern.

# Spatio-temporal connectivity

Despite the comparably small spatial sampling scale, global G'st among local sites remained relatively high in all cohorts (0.3755–0.4039; Table 1), indicating substantial steady population structuring. Nested AMOVA with temporal samples grouped according to location evidenced high among site/population variation, suggesting pronounced spatial population differentiation (accounting for 11.9% of the total allelic variation, $p<0.001$), but comparably low variation among the temporal populations (2019, 2016, S1, S2) within sites (1.24%, $p<0.001$) (Table 3). Most of the allelic variation (86.86% , $p<0.001$) was based on intra-population variation. Further detailed examination of temporal variation through pairwise G'ST comparisons among distinct temporal populations within each location also revealed rather marginal differences. Exceptions were found in above- and below-ground-population-pairs of P04 (G'st=0.0939), P11 (G'st=0.063), P14 (G'st=0.072, 0.076), and P25 (G'st=0.0655) with low, but statistically significant temporal structure (Supplementary Table S6).

*STRUCTURE* analysis inferred 14 distinct genetic clusters in a pooled sample set including all cohorts. The individual assignment of posterior membership probabilities confirmed stable genetic constitution through time, with only slightly different assignments to clusters across cohorts at a particular site. At the same time IBD is indicated, as clusters aggregated not only temporal populations from the same site, but in several cases also from nearby sites, which is illustrated by the similar colour distribution in seed bank and extant cohorts (Figure 1). However, the single populations P10 and P05 include many intermediate genotypes that mainly share two clusters of neighboring populations, indicating local source sink dynamics. P01 could not be clearly assigned to a separate dominant or intermediate local cluster, suggesting colonisation from external genetic sources, which is further corroborated by the absence of a soil seed bank at this site. Moreover, we observed four patches without viable seed bank samples, where individuals were assembled in single separate clusters (Figure 1), presumably indicating a recent colonisation with recruitment from near surface layers.

Consistent with the cluster-analysis, genetic connectivity derived from the PCA-based minimum spanning network showed substantial substructuring of populations according to population origin (Figure 2). Temporal populations of the same location are partitioned along discrete branches, in many cases as indicated by sequential ID, near temporal populations of adjacent locations. Highest genetic affinity indicated by thickest edges was observed between temporal populations. Populations with mixed individual *STRUCTURE* cluster assignment are arranged as connective nodes (indicating admixture originated from adjacent sites; P 05, P10), or as single end node (compatible with colonisation from unsampled sites; P01). In addition, the genetic graph provides some support for regionally enhanced gene flow with higher connectivity within southern, northern and centered populations with few exceptions. Still the observed connectivity pattern does not fully reflect latitudinal clines in allele variation, as northern populations connect southern and centered populations. These results generally point towards limited migration between patches and strong among-cohort-connectivity within a site, an interpretation further corroborated by the relatively low number of first-generation migrants inferred in the

assignment analysis in *GENECLASS2* (Figure 3). Consistent with *STRUCTURE* analyses in population P01 with a mixed cluster assignment, we inferred one recent migrant from a population of the nearby patch P02, while a recent migration from P08 to P02 was detected indicating colonisation potential and putative stepping stone dynamics even on intermediate scales. Similar movement patterns were observed among northern and central populations.

Migrations observed in 2016 occurred primarily at local to intermediate scales, whereas some long-distance north-south migration was observed in 2019 and in seed bank populations. Interestingly, of the

limited number of recent migrants inferred, a considerable proportion were long distance migrants. Overall, the obtained pattern provides some tendency for a predominant westward migration.

# Spatial genetic structure

In all four cohorts examined, individual level pairwise genetic distances increased moderately with geographic distance indicating spatially restricted gene flow (Figure S8). Accordingly, Mantel tests of correlation evidenced an isolation-by-distance pattern ($p<0.001$) for all cohorts, with strongest effects in the 2016 extant and recent soil seed bank cohort

($r=0365/R^2=0.136$, $r=0.265/R^2=0.094$) and less pronounced effects of spatial isolation in the 2019 extant and S2 cohort ($r=0.177/R^2=0.037$, $r=0.196/R^2=0.056$). The conducted sPCA revealed significant global and local spatial genetic structure in all cohorts. Similarly, significant local structures were evidenced in the two aboveground and the lower layer (S2) seed bank data sets.

Individual genotypes showed rather homogeneous local distribution of first global scores and a pronounced latitudinal separation at a large-scale: With a few exceptions, individuals of all cohorts were partitioned from the Northeast and Southwest along the first global Eigenvalue (Figure 4). A heterogeneous score distribution was found in populations located at the geographical center of the sampling area suggesting a transition zone characterised by intermediate genotypes (Figure 4). Landscape configuration effects on genetic diversity The impact of landscape and local habitat characteristics on measures of genetic diversity was found to be cohort-specific (Figure 5). Inbreeding and observed heterozygosity showed significant correlations in relation to patch area only in one cohort. With rising size of habitat, observed heterozygosity showed a significant decline in the recent cohort 2016, which is also indicated by the concomitant increase of inbreeding in these populations (Figure 5 A,C). Spatial isolation measures had no significant effect on either of the genetic diversity parameters (Figure 5 E-L), except for distance to the nearest neighbouring patch having a marginally significant (p=0.065) effect on Fis in the 2019 cohort, the data set comprising the largest range of distances (up to 600m). Here, an increase of the distance to nearest neighbor patch appears to result in higher local inbreeding. This trend can be observed also in the other cohorts, albeit not yielding statistical significance (Figure 5 E). Putative available habitat, as measured by the number of adjacent patches, did not correlate with any of the measures of genetic diversity (Figure 5 I,J,K,L). Finally, neither allelic richness nor expected heterozygosity were affected by the regional habitat configuration (Figure 5 B,F,J,D,H,L).

## Discussion

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

The results are significant in two major respects: first, we found considerable genetic differentiation among sampling sites in all cohorts, and second, the general connectivity patterns and levels of genetic differentiation in our study system persist over time. 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. Likewise, enhanced extinction risks due to genetic Allee effects following a change in population structure are not apparent at our sites, given the relatively high genetic diversity, variable inbreeding and weak, occasional Multilocus Linkage Disequilibrium in populations across cohorts (Luque et al., 2016).

STRUCTURE clustering and genetic graph analyses did not only prove a general increase of connectivity between spatially adjacent patches but also a (re-)colonisation 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). The latter is exemplified in the colonisation of population P01 from recent immigration of at least two nearby patches sampled in 2019 and 2016. Despite repeated recruitment from local soil seed banks, which could cause local erosion and inbreeding, the genetic variability in populations remains stable through the time period covered here (presumably several decades). An explanation could be sufficient outcrossing with variants from nearby populations, as well as rare, but detectable, immigration from distant populations. This can potentially contribute to adaptive genetic variation, but in established populations a marked shift in allele frequencies due to immigration of new variants is likely to be mitigated by the predominance of resident genotypes (Falahati-Anbaran et al., 2014). Overall, most populations store a long-term viable, sufficiently diverse seed bank and therefore are not driven by extinction/recolonization dynamics as assumed under the conceptual framework of metapopulation theory (Husband & Barrett, 1996; Hanski, 1998). However, the variation of functional connectivity in the historically scattered landscape exhibit some features of metapopulation dynamics. In particular, the occurrence of *O. aquatica* at sites with no detectable seedbank could reflect extinction/recolonization dynamics. Alternatively, these incidences may comprise previously unoccupied patches. Their recent colonization could have two implications: Current environmental conditions enable increased dispersal along stepping stone corridors, hence promoting regional connectivity in the long term. This would require some recent temporal synchronisation of favourable conditions among donor and recipient patches (Larroque et al., 2019) with typically asynchronous water regimes, as could be caused, for example, by an intense recent spring flooding in a range of patches, followed by a simultaneous dry-fall. Among such flooded patches, seeds of *O. aquatica* may be repeatedly transported by mobile linkers, such as waterbirds (Green et al., 2016; Kleyheeg et al., 2017), and may accumulate over the course of the season. More transient local populations could occasionally emerge based on randomly occurring mass effects, as some patches may provide less suitable conditions for seed longevity (Bekker et al., 2003) and prevent the formation of a soil seed bank in the long run . These ephemeral populations, which could be found in patches less frequented by mobile linkers and characterized by prolonged dry periods with higher risks of seed or seedling death, may arise as a result of 'recolonization rescue' as described by Hanski (1999). Sudden short-term emergence is likely, since *O. aquatica* as early successional species can quickly form monotypic stands from only a few individuals, if conditions are suitable (Hroudová et al., 1992). A community study conducted in 2015 at the same study area suggests mass effect processes to be prevalent in non-flooded patches

(Lozada-Gobilard et al., 2019). Both mass effect scenarios could have different genetic consequences. We may observe a higher genetic diversity in populations emerged from diverse migrant seed variants accumulated in standing waters as found in P01, whereas large populations arisen from recent immigration of a few variants in drier environments will show reduced genetic diversity, resulting from a founder effect/bottleneck (Wright, 1981), as observed in the most isolated population P17. However, this effect might be quickly mitigated if gene flow via pollen is maintained between patches (Aavik & Helm, 2018) which could apply to patch P19 (figure 2,3). In the most recent aboveground cohort, any of these scenarios could apply to those local populations where we could not detected soil seed banks. Both scenarios would generally fit a stepping stone dynamic in our study system, in line with our generally observed isolation-by-distance pattern, i.e., an increase of genetic differentiation with geographic distance. Identification of factors limiting seed viability and a continuous seed bank monitoring could contribute to a deeper understanding of the persistence of these recently established connectivity patterns observed in the cohort 2019.

# 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 (Vasić et al., 2020), as confirmed by the inferred isolation-by-distance pattern. In our study system, populations with a long-lived seed banks represent starting points for recolonisation rescue, from where local variation slowly propagates across the landscape. In line with this, the spatially explicit analysis detected a separation between the most northern and southern samples with a geographically centered zone of intermediate genotypes/increased admixture, further evidencing stepping stone processes. 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 (Noël et al., 2013; Kayler et al., 2018). Examination of small-scale patch configuration effects showed that low spatial isolation and large patch areas enhance diversity patterns in plant metacommunities (Plue & Cousins, 2018; Schöpke et al., 2019). We expected to find a similar pattern at the population level, however, there was no impact of isolation measures on population genetic diversity at the patch level. Still, the trend toward increased inbreeding with increasing distance from the nearest neighbor patch suggests that noticeable effects of isolation on local genetic diversity may occur, at least at large distances, another indication of the relative importance of disperser movement (Williams et al., 2010; Lozada-Gobilard et al., 2021). Limited pollinator availability in isolated patches (Lozada-Gobilard et al., 2021) may lead to increased levels of selfing and therefore elevated inbreeding at these sites. A reduced primary seed dispersal causing clustered occurrences of siblings close to their parent plant could 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 or even negative. This may be due to increased inter-specific competition as the emergence of more competitive species in late successional stages may suppress seedling recruitment in *O. aquatica*, a process likely more pronounced in larger patches (Schöpke et al., 2019; Lozada-Gobilard et al., 2021) ultimately resulting in ephemeral population built-up. Hence, unlike in populations that are resulting from progressive fragmentation of continuous habitat, the extent of genetic variation and connectivity in our study system is less a matter of patch size (Plue et al., 2017) than a matter of small-scale environmental conditions and historical population establishment in the naturally scattered landscape.

## Conclusion

We here assessed the spatio-temporal connectivity dynamics in a presumed aquatic plant metapopulation system. Our combined approach of spatial and temporal sampling allowed us to track recent colonisation 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 recolonisation rescue effects as predicted by metapopulation theory (Van Schmidt & Beissinger, 2020). We showed a significant spatial genetic structure and evidenced a large contribution of seed banks to local recruitment in *O. aquatica* populations. Stepping-stone processes are likely to maintain population viability with waves of dispersal occurring in response to temporary environmental synchrony of favourable patch conditions. Our study further highlights the crucial role of soil seed banks in serving as source to spread variation and ensure the long-term persistence of *O. aquatica* in the kettle hole system. A future drawback of populations with large seed banks could be the likely delay of adaptive responses in the face of harsh environmental shifts in the context of climate change (Tellier, 2019). Therefore, it remains to be elucidated whether recurrent dispersal, which may promote adaptive potential by introducing adaptive variation, is sufficient to compensate for potential maladaptation faced by locally adapted populations in the course of a rapid environmental perturbation.

# Declarations


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### Author contributions statement

MT conducted the field study as well as the greenhouse and laboratory surveys. MT further analysed data and interpreted results under supervision of FJ and RT. SLG contributed data and valuable comments to the manuscript. FJ and RT conceived the study, provided critical feedback on the theoretical framework, and contributed to writing the manuscript. All authors read and approved the final manuscript.

### Conflict of Interest

The authors declare no competing interests.

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## Tables

**Table 1** Location labels of sampled Oenanthe aquatica populations grouped by geographical region, coordinates (WGS84) and sample size (n) of local populations from two extant cohorts of 2019 and 2016 as well as from upper layer (recent) and lower layer (historical) seed banks (S1, S2), '*' no sampling performed, '–' absence record of O. aquatica. Locations of surveys with neither established nor resurrected samples are not shown. 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 | - | - | - |
| Agroscapelab | P02 | 53.407186 | 13.641936 | * | 25 | * | * |
| | P03 | 53.400064 | 13.671076 | 20 | * | 7 | 21 |
| | P04 | 53.397381 | 13.665745 | 20 | 25 | 4 | 9 |
| | P05 | 53.394665 | 13.662017 | * | 20 | * | * |
| | P06 | 53.385815 | 13.699426 | 20 | - | 14 | 2 |
| | P07 | 53.385975 | 13.709487 | * | 25 | * | * |
| | P08 | 53.384092 | 13.706979 | * | 25 | * | * |
| | P09 | 53.383518 | 13.708530 | 20 | * | 7 | 10 |
| | P10 | 53.382468 | 13.707032 | * | 27 | * | * |
| Central | P11 | 53.353470 | 13.618264 | 15 | 25 | 1 | 15 |
| Agroscapelab | P12 | 53.352202 | 13.623910 | 18 | * | - | - |
| | P13 | 53.355701 | 13.619981 | 2 | * | - | - |
| | P14 | 53.345009 | 13.631840 | 20 | * | 9 | 12 |
| | P15 | 53.335577 | 13.584429 | 20 | * | - | - |
| | P16 | 53.342272 | 13.564552 | 20 | * | 11 | 10 |
| | P17 | 53.334885 | 13.566890 | 17 | * | - | - |
| Southwestern | P18 | 53.328058 | 13.524727 | 20 | * | 10 | 8 |
| Agroscapelab | P19 | 53.326380 | 13.523601 | 19 | * | - | - |
| | P20 | 53.319181 | 13.539932 | 20 | * | 5 | 16 |
| | P21 | 53.317754 | 13.528872 | * | 25 | * | * |
| | P22 | 53.317162 | 13.532987 | 20 | * | - | - |
| | P23 | 53.308486 | 13.552990 | 17 | 19 | 2 | - |
| | P24 | 53.306179 | 13.558765 | * | 13 | * | * |
| | P25 | 53.306886 | 13.553263 | 20 | * | 2 | 9 |

**Table 2** Summary of cohort-specific sample size (n), observed and expected heterozygosity (Ho,He), allelic richness (Ar), total number of alleles (n.all), and inbreeding coefficient (Fis) averaged over all locations (± SD). Global G'st and Fst as well as mean inter-patch Euclidean distance (D_interloc in km) among the sites/local populations for each cohort are listed for the annual samples from 2019, 2016 as well as upper layer (more recent) and lower layer (historical) seed banks (S1, S2).

| *Cohort* | *n* | *Ho* | *He* | *Ar* | *n.all* | *Fis* | *Fst* | *G'st* | *D_interloc* |
|---|---|---|---|---|---|---|---|---|---|
| *2019* | 317 | 0.568 ±0.125 | 0.622 ±0.073 | 2.387 ±0.226 | 63.556 ±13.509 | 0.105 ±0.090 | 0.136 | 0.405 | 6.207 ±4.349 |
| *2016* | 225 | 0.577 ±0.114 | 0.621 ±0.079 | 2.400 ±0.*223* | 69.700 ±10.635 | 0.078 ±0.061 | 0.149 | 0.403 | 6.434 ±5.012 |
| *S1* | 71(+1)* | 0.565 ±0.119 | 0.645 ±0.047 | 2.429 ±0.114 | 49.200 ±11.213 | 0.083 ±0.128 | 0.139 | 0.386 | 6.631 ±4.631 |
| *S2* | 122 | 0.538 ±0.100 | 0.648 ±0.073 | 2.460 ±0.110 | 59.800 ±13.240 | 0.070 ±0.077 | 0.126 | 0.374 | 6.444 ±4.466 |

**Table 3** AMOVA results of spatial and temporal variation. Groups consist of temporal populations grouped according to patch location. Significance level is based on 20,000 permutations.

| *Source of variation* | *df* | *Sum of squares* | *Variance components* | *% variation* | *Fixation indices* | *p* |
|---|---|---|---|---|---|---|
| Among locations | 7 | 396.071 | 0.563 | 11.90 | FCT 0.119 | p<0.001 |
| Among populations within locations | 17 | 96.237 | 0.059 | 1.24 | FSC 0.014 | p<0.001 |
| Within populations | 691 | 2840.305 | 4.110 | 86.86 | | |
| Total | 715 | 3330.613 | 4.732 | | FST 0.131 | p<0.001 |

# Figures

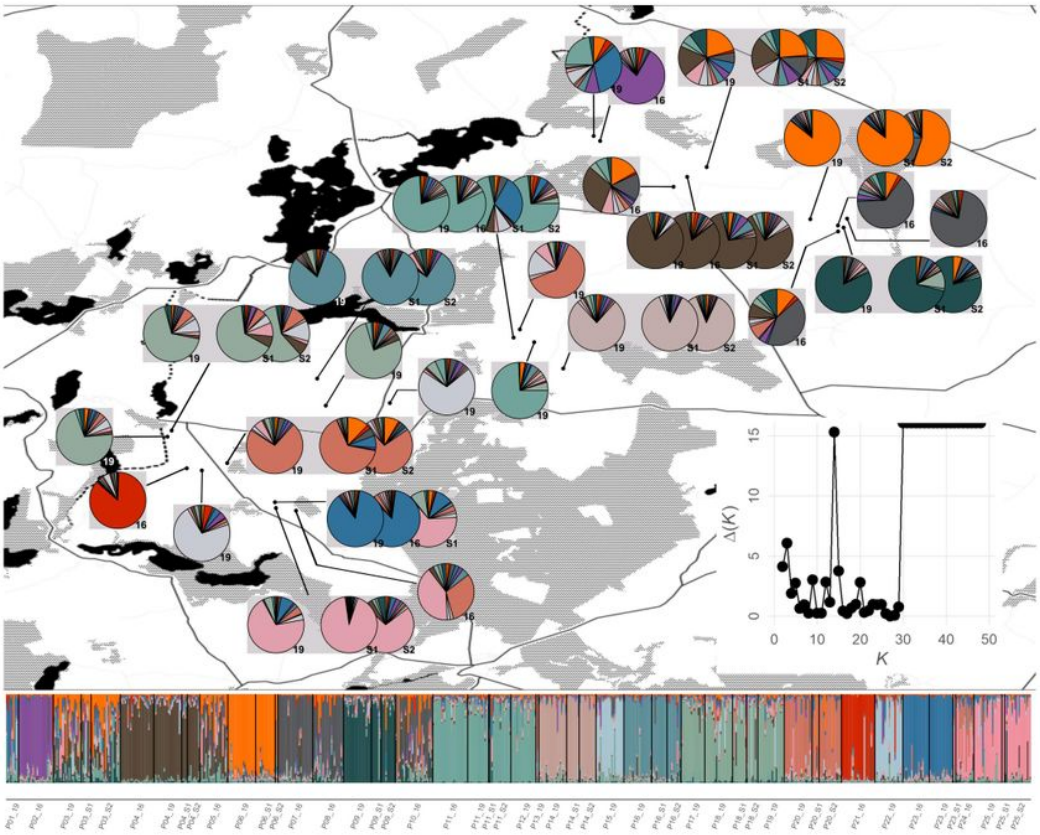



**Figure 1**

Results of STRUCTURE analysis (K=14). bottom: Each bar represents an individual genotype with relative membership probability illustrated by distinct colours; top: Geographical origin of two extant and two seed bank cohorts of *Oenanthe aquatica*. Each Pie-chart represents a single cohort's population (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= 14, illustrated by the Evanno-plot. Grey rectangles aggregate temporal populations of the same location.

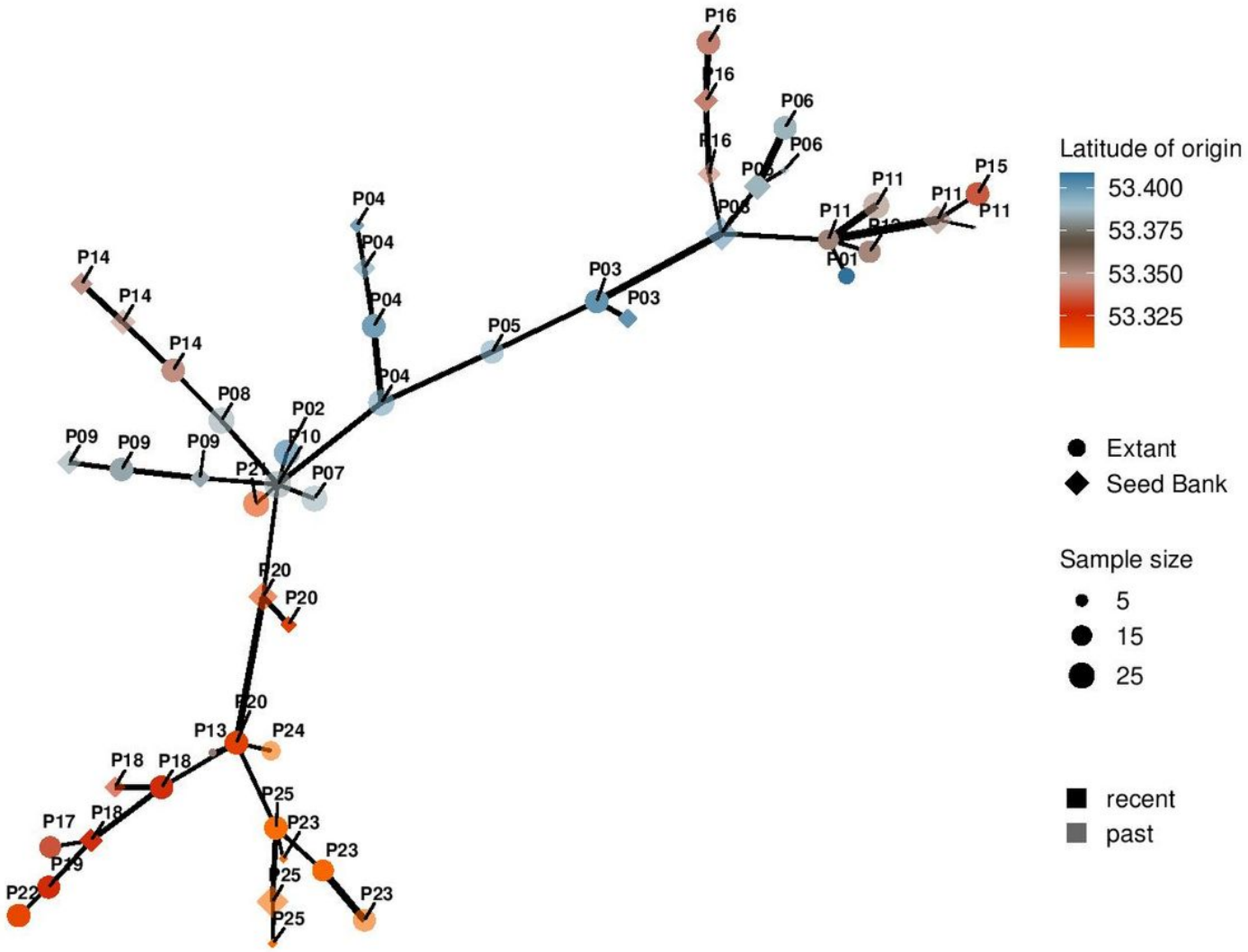
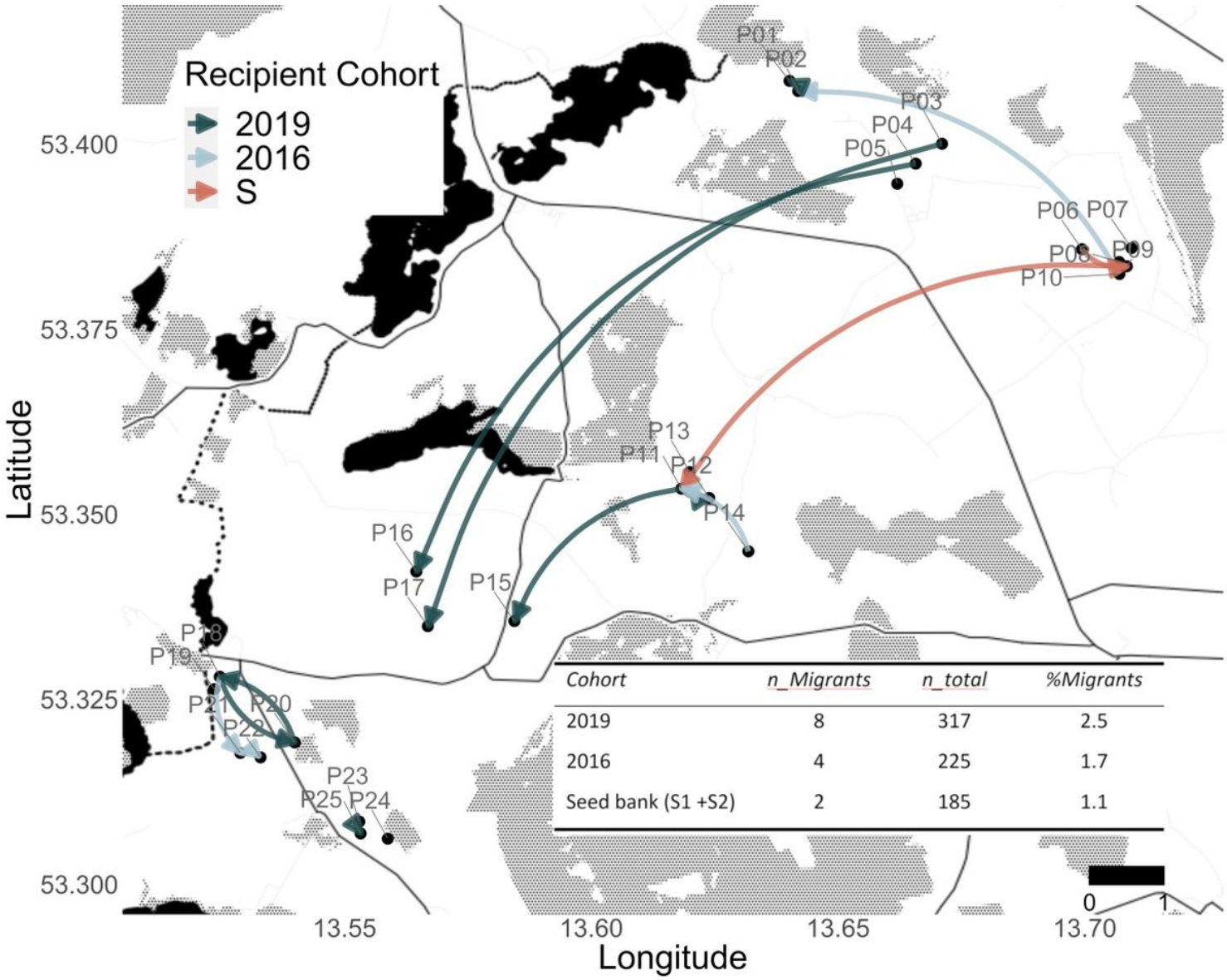



**Figure 2**

PCA- based minimum spanning network with each node representing a population. Colours depict latitudinal order of sampling locations, radii represent sample sizes, and transparency indicates chronological range of cohorts within extant (circle) or seed bank (diamond). Edges represent genetic connections with thickness proportional to genetic affinity while edge length is arbitrary.




| Cohort | n_Migrants | n_total | %Migrants |
|---|---|---|---|
| 2019 | 8 | 317 | 2.5 |
| 2016 | 4 | 225 | 1.7 |
| Seed bank (S1 +S2) | 2 | 185 | 1.1 |

**Figure 3**

Map of study site showing individual first generation migrants of Oenanthe aquatica between different habitat patches as determined by GENECLASS2, with arrows illustrating migration routes and colours coding for recipient cohort; inset: Summary of absolute and relative numbers of 1st generation migrants detected by GENECLASS2 in two extant and one pooled seed bank cohorts.

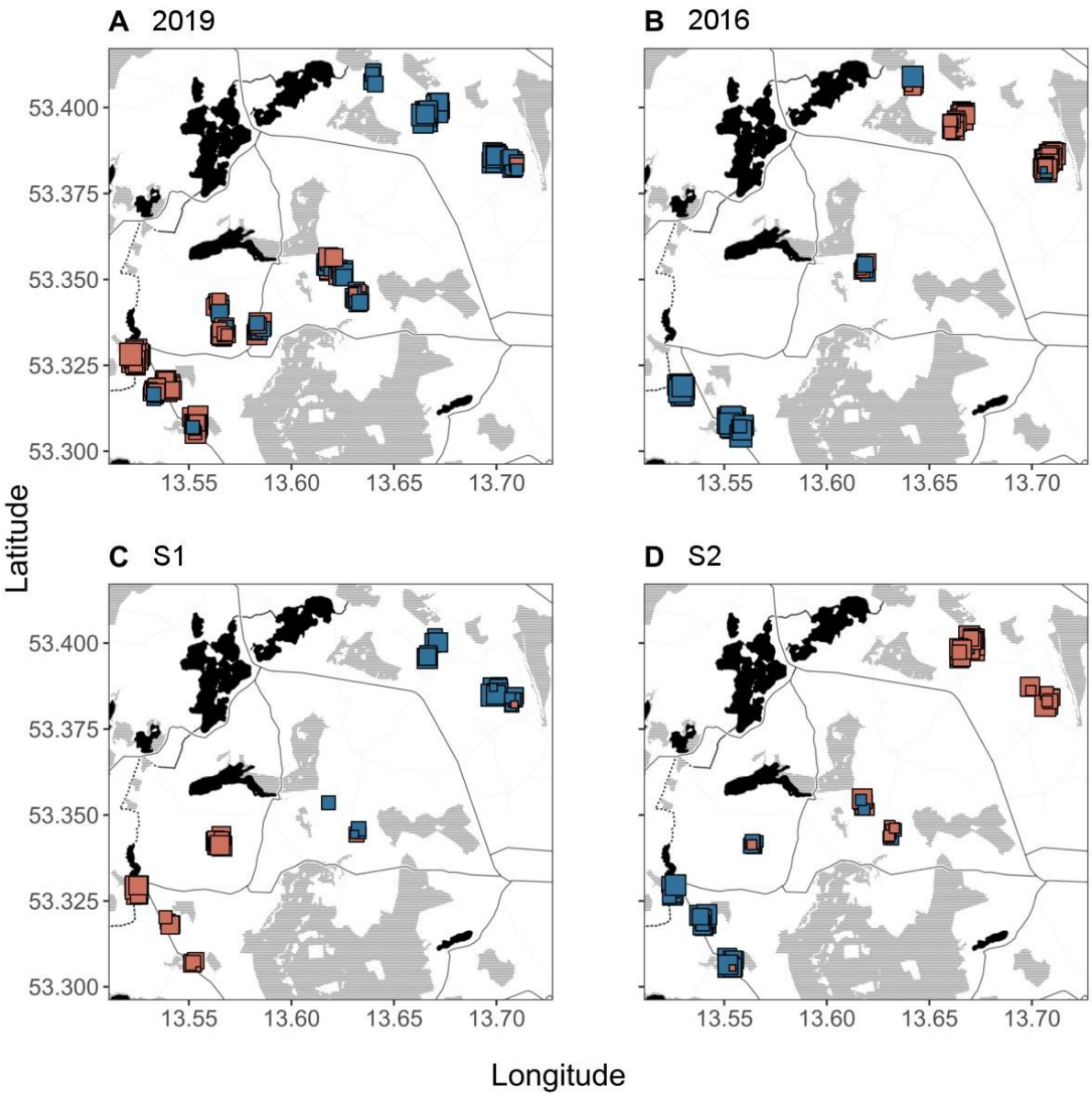



**Figure 4**

Map of the first global scores of the sPCA for each individual of cohort 2019 (A), 2016 (B), recent seed bank (C), and historical seed bank (D). The size of the squares represents the score of individual genotypes (blue–positive, grey red–negative) which are depicted according to their location.

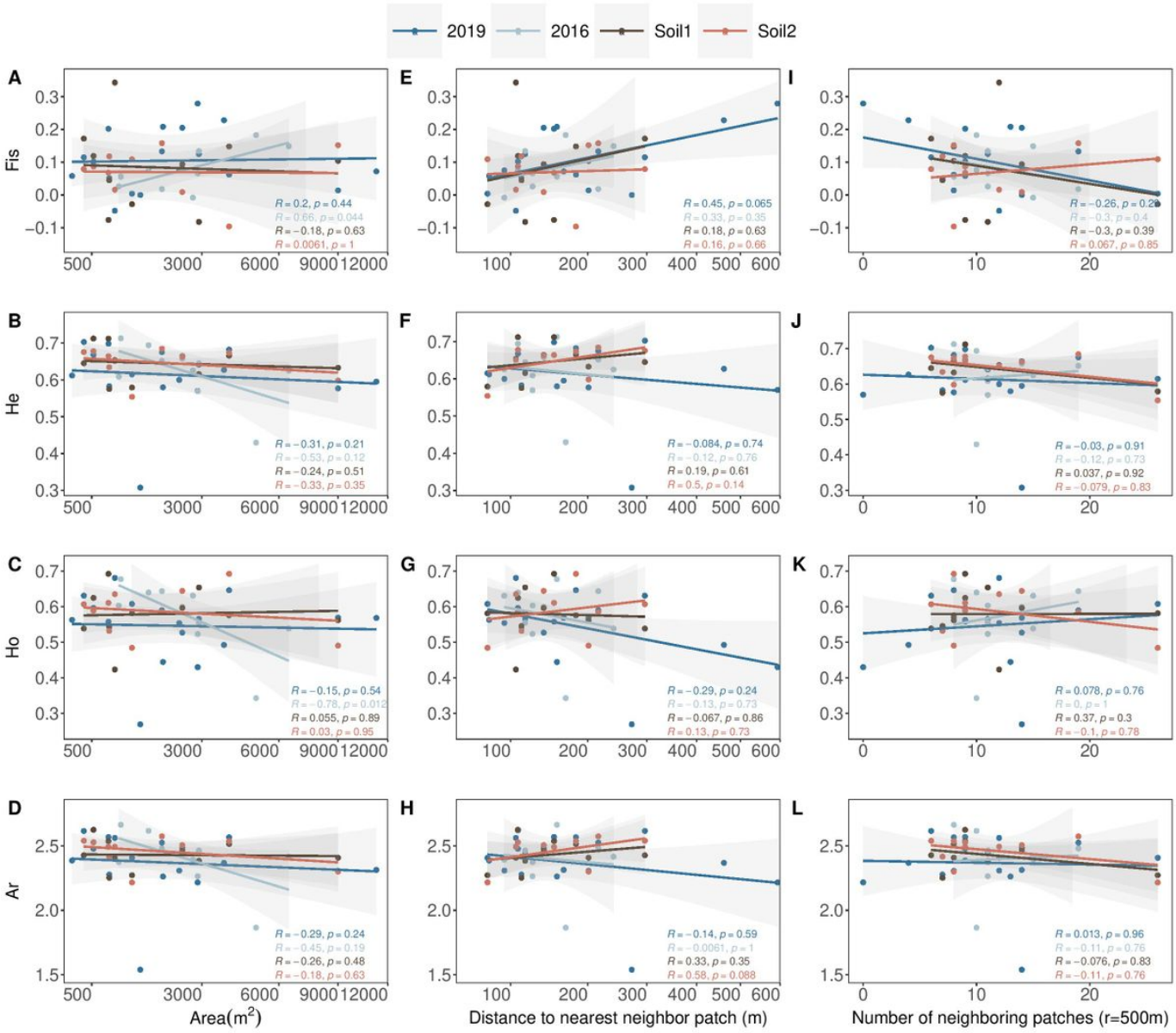



**Figure 5**

Relationship between allelic richness (Ar), expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (Fis) with patch area (A,B,C,D), and measures of degree of isolation depicted as distance to nearest neighbor patch (E,F,G,H) and the number of neighboring patches within a 500m buffer (I,J,K,L). Estimates of Spearman statistics (R) and P-values (p) are shown separated by cohort of origin.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TomowskiHeredOenantheSIGuide.docx
- TomowskiHeredOenantheSI.pdf