Open areas associated with traditional agriculture promote functional connectivity among amphibian demes in Mediterranean agrosystems

Luis Albero · Íñigo Martínez-Solano · Miguel Hermida · Manuel Vera · Pedro Tarroso · Eloy Bécares

Abstract

Context Functional connectivity across fragmented habitat patches is essential for the conservation of animal populations in humanized landscapes. Given their low dispersal capacity, amphibians in the Mediterranean region are threatened by habitat fragmentation and loss due to changes in land use, including agricultural intensification.

Objectives We assessed patterns of functional connectivity of a Near Threatened Mediterranean amphibian, the sharp ribbed newt (*Pleurodeles waltl*), in an agricultural landscape matrix in NW Spain subject to different intensification regimes.

Methods We sampled newts in 17 ponds embedded in a terrestrial habitat matrix dominated by agricultural land uses. Genome-wide molecular markers (1390 SNPs) were used to assess patterns of genetic diversity and gene flow among ponds. We tested the role of landscape features on functional connectivity using isolation by resistance models incorporating information on Normalized Difference Vegetation Index (NDVI) data.

Results We found low levels of genetic diversity in all sampled populations. Global F_{ST} estimates and cluster analyses revealed shallow but significant genetic structure in the study area, with NDVI-based resistance models showing that open areas (rainfed crops and grasslands) offer lower resistance to gene

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flow and thus promote functional connectivity among demes.

Conclusions Our study highlights the important role of landscape features, such as open areas resulting from traditional rainfed agriculture, in promoting functional connectivity between amphibian populations in Mediterranean agrosystems. Conservation policies must adopt a functional network strategy and protect groups of inter-connected temporary ponds across the traditional agricultural matrix to efficiently preserve their associated biotic communities.

Keywords Agricultural intensification · Conservation · Landscape genomics · Pond-breeding amphibians · RAD-seq · SNPs

Introduction

Habitat destruction and fragmentation are among the main drivers of global biodiversity loss (Newbold et al. 2015; Fardila et al. 2017). Fragmentation causes deleterious effects in spatially-structured populations resulting from decreased connectivity (Haddad et al. 2015), which can be counteracted via demographic rescue if connectivity is restored (Córdova-Lepe et al. 2018). Functional connectivity has been defined as the actual movement of individuals among local populations through the landscape matrix and which successfully reproduce in the receiving populations (Taylor 2006). As such, functional connectivity can be measured by the amount of gene flow (Storfer et al. 2010). When functional connectivity is low, demographic isolation results in reduced population viability, especially in small populations, because of inbreeding and genetic drift (Spurgin and Gage 2019). Therefore, conservation efforts should be directed to assess patterns of inter-patch connectivity, including the identification of suitable habitat corridors and features and configurations of the landscape matrix that facilitate dispersal and prevent the isolation of animal populations.

Agriculture is one of the main drivers of habitat loss and fragmentation, especially in temperate and Mediterranean areas, where mostly primary, pristine habitats are lost (Foley et al. 2005). Traditional agricultural landscapes are compositionally heterogeneous, with different crop types and interspersed patches of natural habitats. However, modern agriculture often tends to promote monocultures to increase yields, resulting in landscape homogenization and biodiversity loss (Benton et al. 2003; Fahrig et al. 2011). For species inhabiting habitat patches within an agricultural matrix, the composition and configuration of croplands within the matrix, as well as the use of agrochemicals and the presence of alien invasive species, represent the main factors potentially affecting the amount of habitat use and inter-patch dispersal, and thus the vulnerability of local populations to extinction due to stochastic events. Reduced connectivity (i.e., gene flow) between populations leads to a concomitant loss of genetic diversity, including beneficial alleles, due to inbreeding and genetic drift (Halverson et al. 2006). In this context, small populations will be more rapidly depleted of genetic diversity, which can be assessed by calculating their effective population size (N_e), an important conservation parameter defined as the size of an 'ideal' population that experiences the same rate of change of allele frequencies or heterozygosity as the target population (Wright 1931). Genomic studies at the landscape level can provide estimates of effective population sizes and patterns of gene flow among demes, identifying smaller and/or isolated populations of conservation concern and landscape features promoting or restricting functional connectivity (Taylor 2006; Smith et al. 2009; Marchi et al. 2013).

Pond-breeding amphibians are suitable model systems in landscape genomics studies because their populations are naturally spatially structured due to the discontinuous presence of freshwater breeding habitats in the landscape. Patterns of inter-deme connectivity are shaped by features of the terrestrial habitat and their interaction with dispersal capacity, which is generally assumed to be low but is unknown for most species (Vos et al. 2007; Youngquist et al. 2017; Cayuela et al. 2020). Amphibians in agricultural areas in the Mediterranean region mostly use temporary ponds as preferred breeding sites. The increase in the transformation of rainfed into irrigated crops, as well as the continuous efforts to concentrate crops in larger plots in the agricultural landscape, are favoring the isolation of these wetlands and their biotic communities, potentially compromising their viability in the long term (Beja and Alcazar 2003; Ficetola and De Bernardi 2004; Ferreira and Beja 2013).

Agricultural intensification also leads to the loss and degradation of terrestrial and aquatic habitats used by adult amphibians for both foraging and dispersal, such as non-cropped patches, streams, temporally flooded ditches or traditional irrigation channels (Gray et al. 2004). Despite the potential for agricultural intensification to disrupt connectivity among amphibian demes, few studies have used genomic tools to assess patterns of gene flow in these systems in the Mediterranean region. Wetlands in this region are smaller and more temporary and isolated than those in northern Europe (Bolle et al. 2003; Bekioglu et al. 2007). These differences may impact patterns of amphibian abundance, dispersal and survival, especially in less mobile species like urodeles (salamanders and newts). In particular, small, more isolated populations tend to harbor lower levels of local genetic diversity and display high genetic differentiation among demes at the landscape level, which makes them more sensitive to further habitat fragmentation and loss.

We assessed the influence of agricultural intensification on functional connectivity in an amphibian model, the sharp ribbed newt *Pleurodeles waltl* Michahelles, 1830. This Ibero-Maghrebian endemic is the largest salamandrid newt in the world and is listed as Near Threatened (NT) by the International Union for the Conservation of Nature (IUCN) because of inferred declines associated with widespread habitat loss and the negative effects of invasive species (Beja et al. 2009). Adult newts are mostly aquatic but can disperse among ponds through the terrestrial habitat matrix during humid or rainy nights (Salvador 2015), although these dispersal events seem to be very rare (Fernández de Larrea et al. 2021; Reyes-Moya et al. 2022). Capture-mark-recapture studies have documented low dispersal rates between breeding sites located up to 700 m apart (Gutiérrez-Rodríguez et al. 2017a; Fernández de Larrea et al. 2021; Reyes-Moya et al. 2022) and found evidence of genetic structure at small spatial scales in natural landscapes (Gutiérrez-Rodríguez et al. 2017c), suggesting the species is highly sensitive to habitat fragmentation. Therefore, we expected to find stronger genetic structure among demes in our study area, where changes in the landscape matrix caused by agricultural intensification are hypothesized to result in reduced dispersal and gene flow among demes, especially considering the low dispersal potential of the species.

We developed a genome-wide panel of molecular markers (single nucleotide polymorphisms, SNPs) to characterize genetic diversity and fine-scale patterns of connectivity among 17 breeding populations (demes) of *P. waltl* in an agricultural area and explored the relationship between genetic structure and landscape habitat features using Normalized Difference Vegetation Index (NDVI) data. Specifically, we tested the effect on connectivity of land uses related to agricultural intensification *vs* natural habitat patches, including forested areas and vegetated streams. We discuss the implications of our results for the conservation and management of amphibian populations in agricultural areas in the Mediterranean region.

Materials and methods

Sampling

The study area (Fig. 1) is located in southeast León province (Spain), part of the historical region of "Tierra de Campos" in the Iberian northern plateau, between the Esla and Cea rivers, in the Duero basin. The climate is Mediterranean with continental influence. Landscape cover is mostly composed of cereal rainfed crops, with some amount of natural vegetation comprising oak forests (*Quercus pyrenaica*) and grasslands. The amount of natural vegetation cover increases northwards, and irrigated crops, mostly corn, are more frequent on the western part of the area. The lithological features of this area favor the formation of numerous water bodies, mostly temporary ponds (Fernández Aláez et al. 1999).

The study area represents the northern range limit for *P. waltl*, which was found to be widespread in the area during our surveys. We sampled 7–14 adult newts from 17 ponds (total sample size: 185 individuals) along a north–south gradient (Fig. 1; Table S1). While modest, these sample sizes (N) have been proven to be sufficient in other amphibian studies using SNP markers (McCartney-Melstad et al. 2018; Rödin-Mörch et al. 2021). Sampled ponds can be grouped in two major wetland complexes, Payuelos in the north (ponds 1–13), and Oteros in the south (ponds 15, 16 and 17), with one pond in an intermediate position (pond 14). We selected ponds where *P. waltl* was locally abundant,
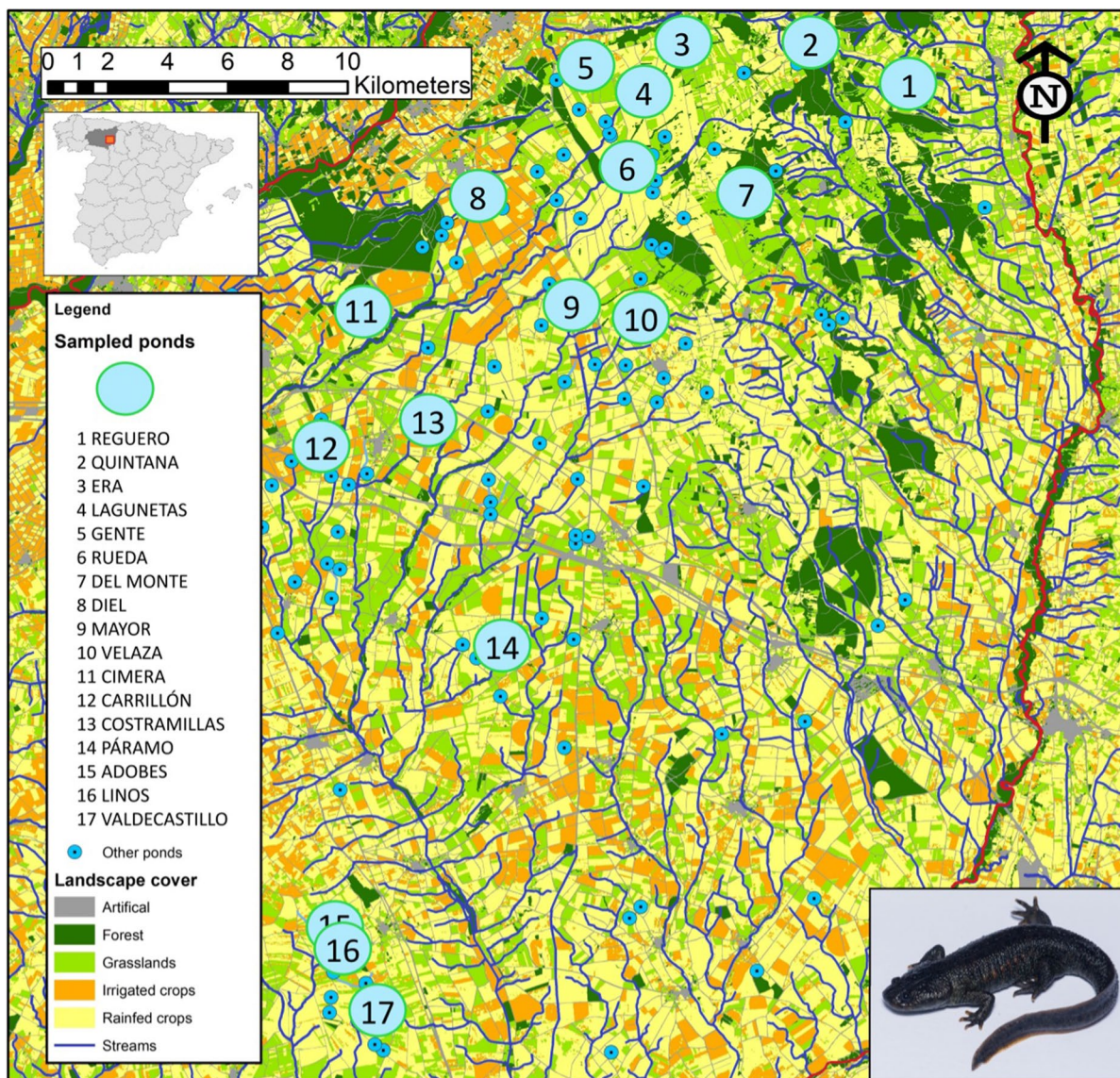
Fig. 1 Map of the study area showing main land cover types and the location of sampled ponds. Landscape cover data was obtained from the database of "Mapa de Cultivos y Superfi-cies Naturales de Castilla y León" (MCSNCyL, Junta de Castilla y León). The main rivers of Esla (west) and Cea (East) are marked in red

and that were located in habitats representing the three main land uses in the study area (i.e., rainfed crops, irrigated crops, and natural areas). Distances among ponds ranged from 1 to 35 km (Table S2). Adult newts were captured in their aquatic stage during nocturnal surveys, using funnel traps or by hand, and released immediately after tissue sampling (toe clips, which were preserved in 100%

ethanol prior to DNA extraction). All experimental protocols were approved by the regional authority (Consejería de Medio Ambiente, Junta de Castilla y León; permit code AUES/CYL/693/2019).

DNA extraction, SNP calling and genotyping

DNA was extracted from tissue samples using the DNeasy Blood and Tissue kit (Qiagen), following

the standard protocol provided by the manufacturer and using RNase. Prior to genomic analyses, we performed a preliminary test of polymorphism using 18 microsatellite markers used in previous studies (e.g., Gutiérrez-Rodríguez et al. 2017b) to genotype 93 individuals from five ponds. This preliminary screening showed a large proportion of monomorphic markers in the sample (9/18, 50%), with no evidence of genetic differentiation among ponds based on Structure runs under different values of K (i.e., genetic clusters).

The 2b-RAD protocol (Wang et al. 2012) was used to obtain a reliable SNP collection across the entire *P. waltl* genome, following the protocol of Maroso et al. (2019), treating DNA with the 2b-type restriction enzyme *AlfI*. DNA from the 185 sampled individuals was pooled in three genomic libraries (60–65 individuals each) for sequencing in an Illumina NextSeq500 System. Reads were cleaned using the *process_radtags* function in the software STACKS v2.4 (Catchen et al. 2013), retaining reads with a size of 36 bp (the expected length of the fragments after digestion with *AlfI*) and with Phred quality scores > 20 in at least 75% of nucleotides. Reads where the *AlfI* recognition site was missing were removed. After cleaning, sequences were aligned to the *P. waltl* genome (Elewa et al. 2017) using the software BOWTIE v1.2 (Langmead et al. 2009) and allowing a maximum of two mismatches (-v 2 option in BOWTIE). Due to the large size of this genome (≈ 20 Gb), fragments containing the *AlfI* recognition site were extracted using the script *ExtractSites.pl* from E. Meyer's lab (Oregon University, USA), prior to alignment. RADtags matching against different regions of the genome were removed to filter out potential paralogs (-m 1 option in BOWTIE). Aligned loci were processed with the STACKS module *gstacks* with default parameters (model marukilow and alpha threshold 0.05). Then, the *populations* module was used without any filtering parameter to obtain an initial SNP catalogue. Finally, the software PLINK v1.9 (Chang et al. 2015) was used to implement the following filtering steps and obtain the final catalogue of SNPs: (i) genotyped in at least 75% of individuals (flag –geno 0.25 in PLINK), (ii) minimum allele count (MAC) of 3 (–mac 3 in PLINK), and (iii) consistent with Hardy–Weinberg (HW) equilibrium (p-value > 0.05) in at least 75% of the demes (–hwe 0.05 in each deme

and manual editing). Loci not passing these filters were removed prior to downstream analyses.

Identification of outlier loci

SNPs linked to genomic regions under natural selection may bias inferences of neutral genetic structure. Therefore, we used two complementary approaches to identify loci putatively under selection (i.e., outlier loci). First, we applied the Bayesian F_{ST}-based method used by BAYESCAN v2.01 (Foll and Gaggiotti 2008), with default parameters (i.e., 20 pilot runs; prior odds value of 10; 100,000 iterations; burn-in of 50,000 iterations and a sample size of 5000). Loci with a False Discovery Rate (FDR) lower than 5% were considered as outliers. Second, we used the R package PCADAPT v4.0 (Luu et al. 2017; Prive et al. 2020), which implements a principal components-based method not requiring a priori population assignment. This method renders low false-positive rates by using individual information. We selected the optimal number of principal components (PC) with the "chooseK" option. For outlier identification we used the q-values, again using as a cut-off a FDR of 5% (q-value < 0.05). We considered as outliers the loci identified by at least one of the two methodologies applied.

Genetic diversity and population structure

Basic population genetics statistics, including the mean number of alleles per locus (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E), and the inbreeding coefficient (F_{IS}), were calculated for each sampled pond (breeding demes) using the software GENEPOP v4.7 (Rousset 2008), ARLEQUIN v3.5 (Excoffier and Lischer 2010), and R package diveRsity (Keenan et al. 2013). In addition to PLINK filtering, conformance to Hardy–Weinberg (HW) expectations for each deme was also assessed with the probability test implemented in GENEPOP. The effective population size (N_e) for each deme was estimated using the linkage disequilibrium method implemented in NeESTIMATOR v2.1 (Do et al. 2014), using the default threshold values for allele frequencies (PCrits) of 0.01, 0.02 and 0.05. Global and pairwise population differentiation (F_{ST}) values were calculated using the R package StAMPP (Pembleton et al. 2013), with 10,000 bootstrap replicates to

assess their significance, and applying the Bonferroni correction for multiple testing to p-values.

For population structure analyses, we excluded outlier loci (neutral SNP dataset, see Results), but we also report results based on the full SNP catalogue (full SNP dataset, see Supplemental Material). The Bayesian clustering method implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000) was used to assess the number of genetically homogeneous population units (K). For each K value (from one to 17), five replicates were run using an admixture model with correlated allele frequencies, with a burn-in of 50,000 steps and 200,000 post-burn-in iteration steps. Due to the weak structure found, we incorporated information about sampling locations by using the *LOCPRIOR* option. Results from the STRUCTURE runs were processed with the software STRUCTURE HARVESTER v0.6 (Earl and Von Holdt 2012) to infer the optimal value of K based on the ΔK method (Evanno et al. 2005). The software CLUMPAK (Kopelman et al. 2015) was used for graphical representation of individual cluster assignment probabilities across K values. As an alternative and complementary description of genetic structure, we also performed a discriminant analysis of principal components (DAPC) with the R package ADEGENET (Jombart 2008). Genotypes were transformed via principal components (PCA) and the Bayesian information criterion (BIC) was used to find the optimal number of clusters via the k-means procedure (function *find.clusters*), retaining 60 principal components (PCs) for the analysis, less than a third of the number of individuals (N/3), as higher numbers of PCs can render membership assignment probabilities unstable (Jombart 2012). DAPC ordination analyses were run for all individuals separately and for the optimal number of clusters returned by the program.

We also ran spatially explicit analyses of population structure in TESS3 (Caye et al. 2016), as implemented in the R package *tess3r*. The clustering algorithm implemented in TESS uses genetic and geographic data simultaneously, providing better results than non-spatial clustering algorithms when genetic divergence among populations is low. We ran the algorithm to estimate ancestry coefficients for values of K from 1 to 17 with 200 replicates for each. The optimal value of K was assessed using cross-validation scores.

Landscape genomic analyses

Isolation by distance (IBD) was tested by assessing the correlation between the matrices of geographical Euclidean distances between ponds (Table S2) and genetic distances (F_{ST}) with a Mantel test, using the *mantel* function in the R package VEGAN (Oksanen et al. 2017). To test for IBD, we applied the correction of Rousset (1997) for a two-dimensional geographic distribution, using $F_{ST}/(1 - F_{ST})$ and the logarithm of geographic distances. IBD tests were carried out using the neutral SNP dataset, but also for the full SNP dataset. The proportion of shared alleles (DPS), as calculated by the R package GRAPH4LG, was also used as a finer and complementary measure of genetic distance to test for IBD.

Due to the semi-aquatic biology of the focal species, and the lack of other major topographical features in the study area, we focused on the potential influence of agricultural *vs.* forested areas on gene flow and developed a model of landscape resistance between sampled ponds. We obtained values of the Normalized Difference Vegetation Index (NDVI) for the study area, a parameter related to vegetation cover and wetness and which has been identified as a predictor of gene flow in landscape genetics studies involving amphibians (Antunes et al. 2018; Sinai et al. 2019; Velo-Antón et al. 2021), including *P. waltl* (Gutiérrez-Rodríguez et al. 2017c). A temporal series of NDVI values covering the last 20 years (2002–2022) was used to incorporate information of land use changes. The NDVI time series was obtained from the MOD13Q1 product (version 6.1) based on the MODIS Terra satellite and downloaded from the Earth Data website (www.earthdata.nasa.gov). A harmonic regression to produce a set of five coefficients representing different seasonalities in the NDVI time series (Estrada-Peña et al. 2014) was utilized. The linear combination of these coefficients was optimized with Genetic Algorithms with 500 iterations and values between − 10 to 10, to derive a model of landscape resistance that maximizes connectivity. A Generalized Least Squares (GLS) model between pairwise genetic dissimilarities and the pairwise resistance distances obtained from the optimized resistance surface was then applied. A correlation structure was used based on the maximum likelihood populations effect model (MLPE) to control for the lack of independence resulting from using pairwise

distances. Three different models of isolation (Isolation by Distance, Isolation by Resistance, and a model incorporating both predictors) were tested using genetic and distance matrices. These models hypothesize that geographic distances, landscape factors (as described by NDVI values), or their combination, explain genetic distances among populations, respectively. Models were ranked according to the Akaike Information Criterion (AIC) and compared with a null model. All landscape genetic analyses were performed in R with packages RASTER, GDISTANCE, GA and CORMLPE. For landscape genomics analyses, genetic distance matrices were calculated with the neutral SNP dataset, but results obtained with the full SNP dataset are also presented for reference in the Supplemental Material.

Results

Sampling and sequencing

The final dataset contained 184 individuals, for which the *populations* module from STACKS returned an initial catalogue of 198,897 variable sites (i.e., SNPs). After filtering, a final list of 1528 highly reliable SNPs was retained, with a mean genotyping rate of 151 genotyped individuals per locus (range 139–182, see Tables S8–S10), and a mean percentage of missing genotyped loci per individual of 17.9% (range 4.5–49%).

Identification of outlier loci

Three loci were identified as outliers by Bayescan, and 138 loci were identified by PCADAPT (Table S11). All of them were inferred to be under divergent selection. The three outlier loci identified by Bayescan were included in the 138 loci set identified by PCADAPT; therefore, these markers were removed from downstream analyses, resulting in a neutral SNP dataset of 1390 markers.

Genetic diversity

We found very low values of genetic diversity across all demes (Tables 1, S3), with mean observed heterozygosity (H_O) = 0.138 and mean expected heterozygosity (H_E) = 0.106 in the neutral SNP dataset. The mean number of alleles per locus (N_a) was also low (1.37). Genetic diversity (H_E), using the neutral dataset, was not correlated with sample size (R^2 = 0.022; p-value = 0.571), and the least diverse deme was that with the largest sample size (Pond 11-Cimera, N = 14). Global F_{IS} values for all demes were negative (i.e., excess of heterozygotes); however, these demes were in accordance with HW expectations, with negative F_{IS} values probably resulting from the low number of individuals analyzed in each deme and/or small effective population sizes (but see below). Diversity values obtained with the complete dataset were similar to those in the neutral SNP dataset (H_O = 0.138, H_E = 0.112, N_a = 1.48; see Table S4).

Estimates of N_e for the different datasets (Tables 1, S4) were mostly consistent across all PCrit values used; when inconsistency among estimates was found, we retained the estimate obtained under the highest threshold (0.05). Reliable estimates could not be obtained for most ponds with the neutral SNP dataset, suggesting high population sizes in most ponds (except Pond 3, Table 1). Estimates based on the full SNP dataset were lower, with no reliable estimates for seven ponds and low values in most of the others (< 100, except for ponds 5 and 16, Table S4).

Population structure

Global F_{ST} as estimated with the neutral SNP dataset was 0.0233 (p-value = 0.001), and higher with the full SNP dataset (0.0052, p-value: 0.001), showing evidence of population structure in the study area. Focusing on the neutral SNP dataset, some pairwise F_{ST} values were significantly different from zero in comparisons involving certain ponds, especially ponds 3, 7, 11 and 14 (Tables S5–S6). Both STRUCTURE and DAPC analyses identified K = 2 as the optimal number of population units. At this clustering level, most demes had average assignment probabilities > 75% to a major genetic cluster widespread across the study area (Fig. S1). Similar results were obtained for the full SNP dataset (Fig. S2). DAPC results (Figs. S3–S6) were largely congruent with STRUCTURE, with individual assignment probabilities at K = 2 also showing a widespread genetic cluster (Cluster 1), with individuals from all populations, and a second cluster (Cluster 2) with, on average, higher assignment probabilities in the northernmost part of the study area (ponds 1–8) (Figs. S3–S4). Most ponds

Table 1 Genetic diversity in sampled *Pleurodeles waltl* demes (using the neutral SNP dataset)

	N	N_a	N_a (s.d.)	H_O	H_E	F_{IS}	N_e (95% CI)
1 REGUERO	10	1.399	0.402	0.147	0.115	−0.164	Infinite
2 QUINTANA	12	1.407	0.383	0.15	0.117	−0.161	Infinite
3 ERA	12	1.353	0.399	0.135	0.106	−0.165	9.7 (8.9–10.5)
4 LAGUNETAS	14	1.367	0.361	0.138	0.107	−0.147	630.8 (186.6-Inf.)
5 GENTE	10	1.397	0.422	0.142	0.11	−0.187	Infinite
6 RUEDA	8	1.384	0.436	0.146	0.11	−0.219	Infinite
7 DEL MONTE	14	1.403	0.384	0.153	0.119	−0.161	Infinite
8 DIEL	11	1.383	0.409	0.145	0.111	−0.184	Infinite
9 MAYOR	7	1.347	0.447	0.131	0.097	−0.227	Infinite
10 VELAZA	11	1.38	0.387	0.145	0.11	−0.179	Infinite
11 CIMERA	14	1.32	0.366	0.122	0.095	−0.143	Infinite
12 CARRILLÓN	11	1.339	0.394	0.13	0.099	−0.179	Infinite
13 COSTRAMILLAS	10	1.335	0.397	0.129	0.098	−0.187	Infinite
14 PARAMO	12	1.338	0.381	0.132	0.1	−0.18	Infinite
15 ADOBES	10	1.373	0.406	0.138	0.105	−0.184	Infinite
16 LINOS	7	1.352	0.462	0.13	0.097	−0.234	Infinite
17 VALDECASTILLO	11	1.365	0.403	0.14	0.107	−0.182	Infinite
ALL	184	1.37	0.402	0.138	0.106		

N sample size, N_a mean number of alleles (*s.d.* standard deviation), H_O observed heterozygosity, H_E expected heterozigosity, F_{IS} inbreeding coefficient, N_e effective population size (Infinite: no reliable estimates could be obtained)

with lower assignment proportions to Cluster 2 are from the central (ponds 6 and 9) or southern (ponds 10–17) part of the study area, which is dominated by intensive agricultural landscapes. The scatterplot of DAPC results with ADEGENET showed little structure, with demes of ponds 1, 5, 6 and 17 being the most differentiated along the first two axes based on the neutral SNP dataset (Fig. S5).

The spatial clustering analysis with TESS provided finer resolution of genetic structure among ponds (Figs. 2, S7–9). Using the neutral SNP dataset, likelihood scores did not show either a clear minimum value or a plateau (Fig. S7), but 95% confidence intervals for runs with K > 6 were very broad, so results using K = 2 to K = 7 are presented (Figs. 2, S8). For K = 2, two genetic clusters are recovered showing some geographic structure, with a trend of decreasing average assignment probabilities to the minor genetic cluster towards the south of the study area. Results for other K values also suggest some differences between northern and southern population groups, with the intermediate Pond 14 showing some differentiation from its closest neighbors (Fig. 2). Results were similar with the full SNP dataset (Figure S9).

Landscape genomics

Based on Mantel test results, no evidence for IBD was found, neither with the neutral nor with the full SNP dataset and using F_{ST} (as $F_{ST}/1 − F_{ST}$) or DPS as measures of genetic distance, with no significant correlation between pairwise genetic and geographic distances (Figs. S10–S12). Regarding landscape genomics analyses, lower NDVI values in the study area were associated with low vegetation cover, as in traditional rainfed crops or grasslands, whereas high NDVI values corresponded to streams, which in the study area are mostly covered by riparian vegetation, and forests. The optimization of resistance surfaces using the neutral SNP dataset showed open areas associated with traditional agriculture, such as rainfed crops and grasslands, to be the land cover classes with higher conductance (Figs. 3, S13). Forest was the landscape feature showing higher resistance according to the optimized surface, with irrigated crops and artificial surfaces having intermediate conductance (Fig. S13). Both resistance optimization and model selection were similar in analyses with the full SNP dataset (Fig. S14–S15). Although all models other than the null were significant, the model including only isolation by resistance (IBR) had better explanatory power regarding patterns of connectivity among
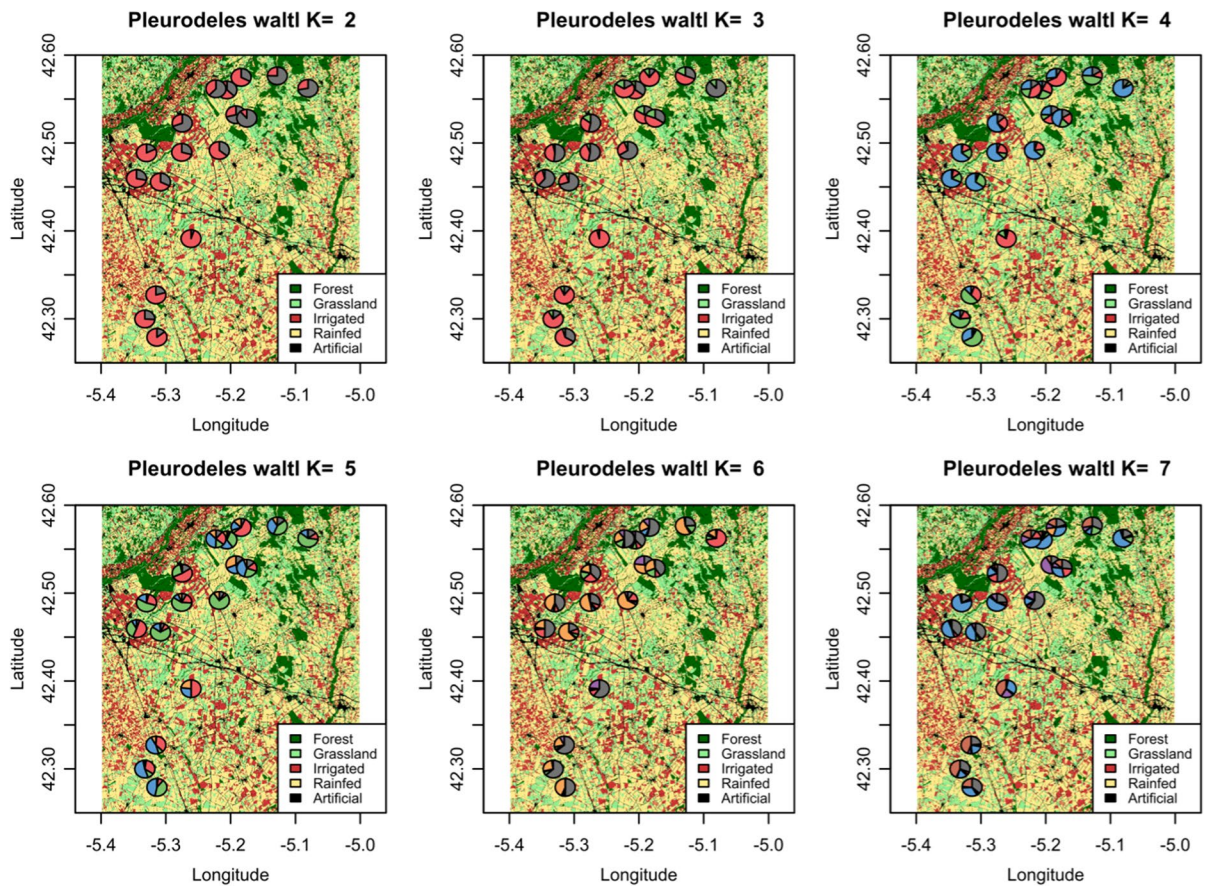
Fig. 2 Map of ancestry proportions for each *Pleurodeles waltl* deme, based on TESS analyses for K = 2 to K = 7, using the neutral SNP dataset

demes than models including IBD or the null model (Tables 2, S7).

Discussion

Landscape genomics studies can take advantage of large SNP panels to answer questions on the relative roles of local demography and landscape features in shaping patterns of genetic diversity and population structure. Based on a set of 1390 neutral markers, we estimated low H_O and H_E values in all demes, raising concerns about the long-term viability of *P. waltl* populations in the study area. Low genetic diversity in these demes may be a consequence of both histori- cal and recent processes. Our study area is located at the northern range limit for the species, with popula- tions that are part of a genetic lineage that colonized

the Iberian North Plateau relatively recently (in the last Inter-Glacial, about 120,000 years ago). This colonization process probably involved sequential bottleneck events, causing sharp reductions in several genetic diversity indices in comparison with popula- tions located closer to historical refugia (Gutiérrez- Rodríguez et al. 2017b). Additional support for this hypothesis comes from the fact that preliminary screening of microsatellite markers in five ponds from our study area showed a large proportion of them to be monomorphic, although they were shown to be polymorphic in central Iberian populations. However, effective population sizes seem to be high, suggesting populations are not threatened by inbreed- ing depression and retain their evolutionary potential (Frankham et al. 2014), provided functional connec- tivity is maintained.
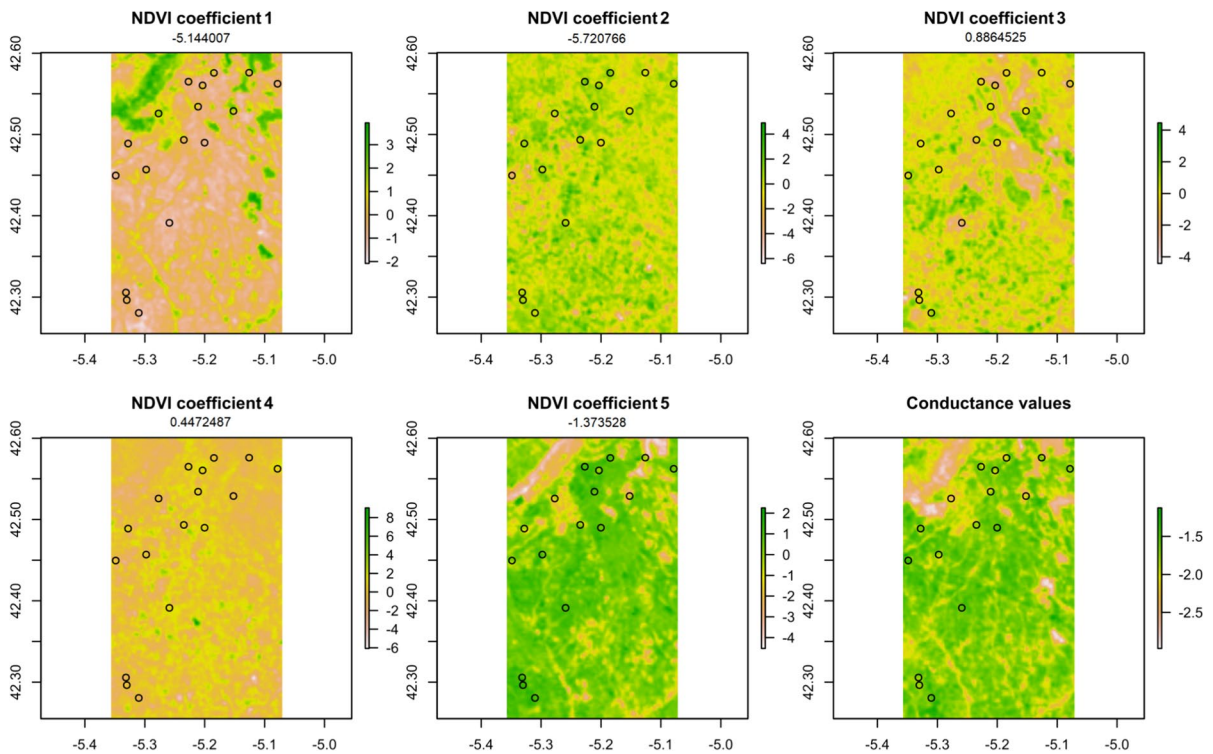
Fig. 3 NDVI values for the five coefficients used for optimization of resistance surfaces, and final conductance values after optimization with Genetic Algorithm with the goal of maximizing the connectivity using genetic data, with the neutral SNP dataset. The coefficient weights resulting from the optimization are shown for each NDVI coefficient

Table 2 Model selection results for the IBD and IBR (NDVI-based) analyses, using F_{ST} values calculated using the neutral SNP dataset

Models	Df	logLik	AIC	ΔAIC	AICw	L.Ratio	p-value	stdErrRes
IBR	4	600.269	− 1192.538	0.000	0.417	7.084	0.0077	0.0036
IBD	4	600.205	− 1192.411	0.127	0.391	6.957	0.0083	0.0037
IBD + IBR	5	600.300	− 1190.601	1.936	0.158	7.147	0.0075	0.0037
Null	3	596.726	-1187.454	5.084	0.032	0.000	1.0000	0.0036

Df degrees of freedom, *logLik* log likelihood, *AIC* Akaike information criterion, *ΔAIC* delta AIC value, *AICw* Akaike weight, *L.Ratio* likelihood ratio, *stdErrRes* standarized residual error, *IBD* isolation by distance model, *IBR* isolation by resistance model

We found shallow but significant genetic structure among *P. waltl* demes in the study area, with no evidence of isolation by distance at the spatial scale considered, although landscape genomics analyses supported a role for geographic distance in shaping patterns of genetic differentiation (see below). Previous studies of the species showed low dispersal potential and strong genetic structure at small spatial scales (Gutiérrez-Rodríguez et al. 2017a, c; Fernández de Larrea et al. 2021; Reyes-Moya et al. 2022).

Our results show less pronounced population differentiation than other amphibian studies conducted at similar or even smaller spatial scales (Oliver et al. 2017; Antunes et al. 2018; Sinai et al. 2019; Winiarski et al. 2019; Haugen et al. 2020; Van Buskirk and Van Rensburg 2020), even in environments with no apparent barriers to dispersal, including agricultural landscapes (Sotiropoulos et al. 2013; Hauguen et al. 2020; Unglaub et al. 2021).

Our landscape genomic analyses shed light on the main factors shaping genetic structure in the study area. Whereas IBR provided the best fit to the data, IBD (alone or in combination with IBR) also contributed to increase explanatory power, suggesting a role for geographic distance on differentiation. In addition, IBR results show higher landscape resistance at higher values of NDVI (Figs. 3, S15), supporting that open areas associated with traditional agricultural areas (including rainfed crops and grasslands) promote connectivity to a greater extent than forested areas. In addition to the flat topography and positive effect of open areas on gene flow in the study area, long distance movements of newts can be facilitated by some landscape features, especially wetlands and water courses (Da Fonte et al. 2019; Cayuela et al. 2020). During our field surveys, we observed individuals of *P. waltl* using streams and irrigation channels, suggesting that these linear water structures can act as corridors for amphibian dispersal, as reported in other studies (Emel and Storfer 2014; Haugen et al. 2020; Parsley et al. 2020). Information about dispersal in *P. waltl* is still incomplete, but it likely takes place mostly during their terrestrial stage and in juvenile phases, as in other urodeles (Perret et al. 2003; Roe and Grayson 2008). Therefore, flat and open areas, such as crops, may be preferred by the species during its dispersal phase. On the other hand, streams and areas with dense vegetation (high NDVI values) may be used as refuges during dry or cold periods but, based on our analyses, do not seem to be used as landscape corridors for dispersal in the study area.

An intensively managed zone, with mainly irrigated corn, is located in the study area between ponds 8 and 9, separating the traditionally managed rainfed area (ponds 1–7 and 10) and a heterogeneous cropland area comprising rainfed and irrigated crops (ponds 11–14). Different amphibian communities have been previously documented in these two areas (Albero et al. 2021), with less diversity and structure in the communities from the irrigated area, and *P. waltl* being less abundant than in the rainfed area. The irrigated area showed higher values of NDVI and lower conductance than traditional rainfed areas (Fig. 3), and TESS results show differences in genetic clustering assignments associated with this intensively managed area (for K = 2, see Fig. 2), suggesting it may act as a soft barrier restricting dispersal between the two major groups of ponds in the study area. These results are consistent with other studies reporting higher genetic differentiation in amphibian populations in intensive agricultural landscapes compared to natural habitats (Lenhardt et al. 2017; Gauffre et al. 2022; but see Youngquist et al. 2017). Nevertheless, given the long generation time of *P. waltl* (Cayuela et al. 2022), the effects of the recent shift to irrigation in this area on patterns of genetic structure may not be fully appreciable yet.

The shallow genetic structure documented in the present study contrasts with the results of Gutiérrez-Rodríguez et al. (2017c), who assessed the genetic structure of *P. waltl* populations in central Spain at a similar geographic scale, finding strong differentiation over short distances (5–10 km). Apart from differences in the historical demography of their sampled populations, which are closer to historical glacial refugia and thus are more genetically diverse than those in our study, and in the type of markers used (microsatellites vs. SNPs), landscape features differ substantially between both studies and have probably contributed to shape patterns of connectivity among demes differently, as shown by our landscape genomics analyses. For instance, the dominant land cover class in Gutiérrez-Rodríguez et al. (2017c) is "dehesa" woodland and scrub, with a minor proportion of agricultural land cover classes, which dominate our study area. More importantly, their study was conducted in the foothills of Sierra de Guadarrama, a hilly area, with slope and altitude being recovered as two of the major factors restricting gene flow, in agreement with studies in other amphibian species (Sánchez-Montes et al. 2018; Cayuela et al. 2020). In contrast, our study area is part of a flat plateau with no significant topographic features, which may favor gene flow. Gutiérrez-Rodríguez et al. (2017c) also found a positive role of the spatial heterogeneity of vegetation amount (NDVI st.-dev), rather than of vegetation cover itself. These contrasting results highlight the fact that the relationship between landscape features and gene flow is often complex and can vary throughout species' ranges, depending on the availability and spatial configuration of different types of terrestrial habitat patches as well as on historical factors. Another important feature potentially contributing to differences between studies in inferred patterns of connectivity among demes is the higher pond density in our study area (~1 pond/10 km^2, see Fig. 1). Most of these ponds are suitable breeding habitats

for the target species, and most of them are surrounded by agricultural plots instead of forest, which can partly explain why agricultural areas showed lower resistance to gene flow. In this context, climate change projections for the Mediterranean basin predict a decrease in precipitation and an increasing incidence of heat waves, which will likely result in more frequent and severe drought periods (Giorgi and Lionello 2008) and a concomitant decrease in pond hydroperiod and density at the landscape scale. Understanding the role of different factors in promoting/restricting connectivity among demes is therefore fundamental to design and implement conservation plans for Mediterranean amphibian communities in the face of climate change.

Few studies have compared patterns of genetic structure of passive vs. active dispersing organisms in aquatic metacommunities. Using presence/absence data, ecological studies have found a stronger spatial effect, linked to dispersal limitations, for both passive dispersers (macrophytes, zooplankton) and large-bodied active dispersers (amphibians), in contrast to flying insects, which show no dispersal limitation (Van De Meutter et al. 2007; De Bie et al. 2012). Previous studies on a passive-dispersal aquatic plant species (*Myriophyllum alterniflorum*) in the same ponds sampled in this study revealed a marked genetic discontinuity between the pond groups of Payuelos and Oteros, with low levels of gene flow and a strong IBD pattern (García-Girón et al. 2019), in agreement with this expectation. Our results show overall higher connectivity in *P. waltl* than in *Myriophyllum*, highlighting that under favorable conditions, amphibian populations can remain functionally connected, even in humanized landscapes.

In conclusion, our study highlights the important role of landscape features, such as open areas resulting from traditional rainfed agriculture, in promoting functional connectivity of amphibian populations in Mediterranean agrosystems. Temporary ponds are unique ecosystems that are increasingly threatened by urban and agricultural development and the introduction of alien species (Beja and Alcazar 2003). Conservation guidelines and policies such as the Common Agricultural Policy or the Water Framework Directive (both from the European Union) should adopt a functional network strategy, protecting clusters of inter-connected temporary ponds throughout the agricultural matrix and landscape features that favor connectivity, such as traditional rainfed crops,

to ensure the conservation of the diverse amphibian communities of Mediterranean ecosystems.

Declarations

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