



# The role of habitat features in patterns of population connectivity of two Mediterranean amphibians in arid landscapes of central Iberia

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## Abstract

**Context** Mediterranean wetland ecosystems are in continuous decline due to human pressure. Amphibians are key elements of biotic communities of Mediterranean temporary ponds and streams, and their persistence depends on the availability and inter-connectivity of breeding sites.

**Objectives** We investigated the role of different factors potentially driving functional connectivity patterns in two amphibian species at the landscape

and local scales. We focused on two Mediterranean endemic pond-breeding amphibians inhabiting semi-arid landscapes of central Spain, the common parsley frog (*Pelodytes punctatus*) and the common midwife toad (*Alytes obstetricans*).

**Methods** We genotyped 336 individuals of *P. punctatus* and 318 of *A. obstetricans* from 17 and 16 breeding populations at 10 and 17 microsatellite loci, respectively. We used remotely sensed vegetation/moisture indices and land use/cover data to derive optimized resistance surfaces and test their association with estimates of gene flow and migration rates across populations.

**Results** We found evidence for higher population connectivity in common midwife toads than in common parsley frogs, with a strong effect of water

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Jorge Gutiérrez-Rodríguez and João Gonçalves have contributed equally to this work.

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availability in patterns of population connectivity of both species. However, the two species differ in the role of landscape features on population connectivity, with the distance and spatial distribution of artificial land-use types positively influencing connectivity in *A. obstetricans* and meadows/pastureland favouring *P. punctatus*. This is in line with reported breeding site preferences for the two species, with *A. obstetricans* successfully breeding in artificial water bodies that *P. punctatus* generally avoid.

**Conclusions** This study highlights the importance of assessing species–habitat relationships shaping connectivity when developing and implementing conservation and management actions to benefit fragmented amphibian populations in the Mediterranean region. Our results show that amphibian species respond differently, even contrastingly to landscape features and thus require alternative, complementary strategies to improve population connectivity and ensure long-term viability.

**Keywords** Conservation · Remote sensing · Landscape resistance · Gene flow · Wetlands · Population genetics

## Introduction

The Mediterranean region is characterized by the presence of ecologically diverse types of wetlands (Pearce and Crivelli 1994; Médail and Quézel 1999). These ecosystems are abundant in southern Europe and North Africa and are listed as EU Priority Habitat (Natura 2000 code 3170, 92/43/CEE, 21 May 1992), but are in continuous decline due to the impact of human activities (Green et al. 2002; Bouahim et al. 2010). This decline is expected to be exacerbated by climate change, which will have a much greater impact on smaller, temporary water bodies (Parmesan 2006; Rhazi et al. 2012). Ephemeral wetlands in endorheic depressions are characterized by

high seasonality and fluctuating water levels linked to extremely variable precipitation patterns across seasons and years (Vanschoenwinkel et al. 2009; Sahuquillo et al. 2012). These habitats host diverse flora and fauna communities (Williams 2006), including many rare and threatened species (Keddy 2010), which are adapted to living under the stress associated with these extreme environmental conditions (Grillas et al. 2004).

Amphibians are key elements in the biotic communities of Mediterranean temporary ponds and streams (Gómez-Rodríguez et al. 2009). At the local scale, their breeding success and population dynamics directly depend on the availability of water bodies and their hydroperiod (Newman 1992; Jakob et al. 2003; Gómez-Rodríguez et al. 2010a, b, c; Cayuela et al. 2012). At the landscape scale, their persistence depends on the availability and connectivity among breeding sites, which is a function of the dispersal ability of the different species and the resistance of the terrestrial habitat matrix to movement.

The loss of Mediterranean temporary ponds and streams has had a significant impact on biodiversity (Cuttelod et al. 2009), and is one of the major threats causing amphibian declines in the region (Stuart et al. 2004, 2008). In areas where the availability of natural breeding sites is low because of changes in land use, aridization and desertification, like central Iberia, some amphibian species can take advantage of artificial constructions to hold water for irrigation, and breed successfully (Caballero-Díaz et al. 2020). However, little is known about patterns of connectivity in amphibian populations in these regions. Identifying factors shaping functional connectivity patterns (i.e., gene flow) in these areas is critical to guide management efforts to conserve amphibian populations and their associated biotic communities in view of the increasing fragmentation of their populations (Martínez-Solano 2006).

Landscape genetic tools allow linking genetic data with different potential response variables and explicitly quantify the effects of landscape composition, configuration and matrix quality on spatial patterns of genetic variation and gene flow (Storfer et al. 2007). Assessing comparative patterns of regional gene flow in co-distributed species can help develop cost-effective management practices with measures favouring a broader set of taxa (Gutiérrez-Rodríguez et al. 2017). Here, we focused on two Mediterranean endemic

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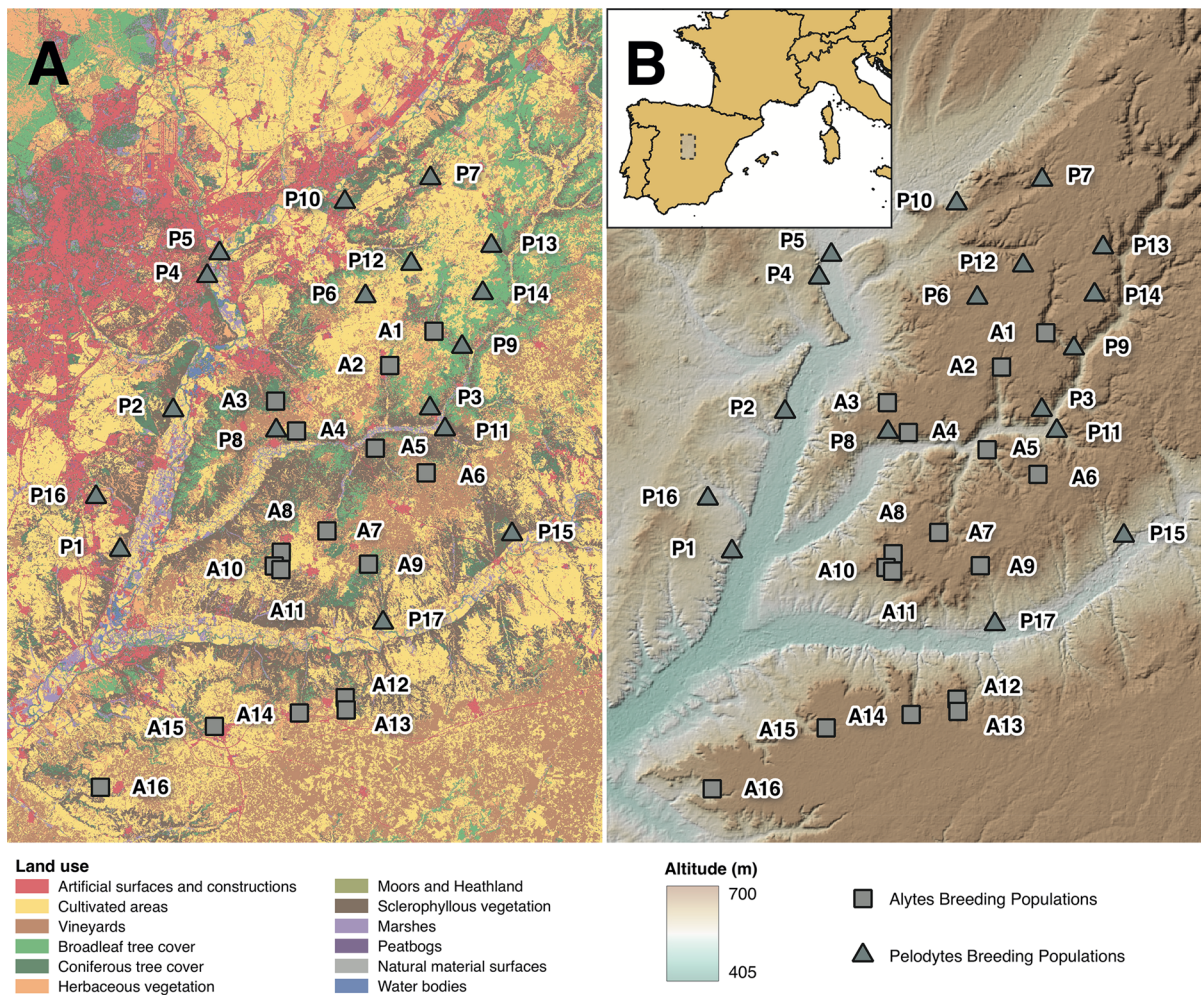
**Table 1** Locality information and genetic diversity estimates for sampled *Pelodytes punctatus* (P) and *Alytes obstetricans* (A) populations:  $N$ =sample size;  $N_A$ =mean number of allelesper locus;  $H_O$ : observed heterozygosity;  $H_E$ =expected heterozygosity;  $F_{IS}$ =inbreeding coefficient

ID	Locality	Longitude	Latitude	$N$	$N_A$	$H_O$	$H_E$	$F_{IS}$
P1	Madrid, Ciempozuelos, Altos de Palomero	40.128	-3.625	20	4.300	0.673	0.571	-0.178
P2	Madrid, San Martín de la Vega, La Boyeriza, camino de Polvorines	40.260	-3.561	20	6.300	0.660	0.704	0.063
P3	Madrid, Carabaña, Albercas “Los Huertos”	40.263	-3.243	20	3.800	0.835	0.644	-0.296
P4	Madrid, Rivas Vaciamadrid, La Yesera	40.388	-3.520	20	6.200	0.740	0.698	-0.061
P5	Madrid, San Fernando de Henares, La Guindalera	40.409	-3.504	20	4.100	0.735	0.636	-0.156
P6	Madrid, Pozuelo del Rey, Charcos m224	40.369	-3.324	20	3.100	0.799	0.613	-0.303
P7	Madrid, Santorcaz—Los Santos de la Humosa, Charcos m226	40.481	-3.244	20	8.600	0.770	0.803	0.041
P8	Madrid, Morata de Tajuña, Charcas aeródromo	40.242	-3.433	20	8.000	0.760	0.754	-0.008
P9	Madrid, Ambite, Arroyo de la Vega	40.322	-3.204	20	9.700	0.781	0.765	-0.020
P10	Madrid, Alcalá de Henares, El Gurugú	40.458	-3.350	20	5.200	0.703	0.631	-0.114
P11	Madrid, Carabaña, El Pradejón	40.244	-3.225	20	8.600	0.825	0.770	-0.071
P12	Madrid, Corpa, Fuente del Rey	40.400	-3.267	20	7.400	0.766	0.717	-0.069
P13	Madrid, Pezuela de las Torres, Las Cruces	40.417	-3.168	20	4.100	0.800	0.618	-0.294
P14	Madrid, Pezuela de las Torres, Monte Nuevo	40.372	-3.179	20	3.400	0.465	0.461	-0.008
P15	Madrid, Fuentidueña de Tajo, Castillejos	40.144	-3.142	20	5.600	0.730	0.685	-0.066
P16	Madrid, Valdemoro, Club de tiro	40.178	-3.655	20	2.600	0.700	0.542	-0.291
P17	Madrid, Villarejo de Salvanés, Valdepuercos	40.061	-3.300	16	3.300	0.825	0.607	-0.360
A1	Madrid, Villar del Olmo, Parque Forestal	40.336	-3.239	20	5.647	0.624	0.672	0.072
A2	Madrid, Valdilecha, El Rejal	40.304	-3.293	20	3.706	0.624	0.599	-0.041
A3	Madrid, Arganda del Rey, Fuente del Valle	40.270	-3.434	20	4.706	0.597	0.586	-0.020
A4	Madrid, Morata de Tajuña, Dehesa de Morata	40.241	-3.408	20	3.588	0.555	0.585	0.052
A5	Madrid, Tielmes, Fuente de Valdecañas	40.225	-3.311	20	6.471	0.685	0.707	0.030
A6	Madrid, Valdaracete, Las Pozas	40.202	-3.248	20	4.059	0.548	0.556	0.014
A7	Madrid, Valdelaguna, El Bosque	40.147	-3.370	20	6.471	0.656	0.708	0.073
A8	Madrid, Chinchón, Valquejigoso	40.127	-3.426	24	5.647	0.615	0.652	0.057
A9	Madrid, Villarejo de Salvanés, Fuente de los Perales	40.116	-3.319	20	7.824	0.750	0.725	-0.035
A10	Madrid, Colmenar de Oreja, Alberca Casa Dómine	40.114	-3.435	20	5.176	0.656	0.671	0.022
A11	Madrid, Colmenar de Oreja, Mingorrubio	40.110	-3.427	20	5.765	0.656	0.635	-0.033
A12	Toledo, Villarrubia de Santiago (Fuente Vieja)	39.990	-3.347	20	6.353	0.665	0.682	0.025
A13	Toledo, Villarrubia de Santiago (Fuente Nueva)	39.978	-3.345	20	7.235	0.635	0.709	0.104
A14	Toledo, Noblejas (albercas)	39.975	-3.403	20	7.294	0.675	0.690	0.021
A15	Toledo, Ocaña, Fuente Vieja	39.962	-3.507	20	5.647	0.638	0.662	0.036
A16	Toledo, Yepes	39.904	-3.647	14	2.882	0.443	0.465	0.046

pond-breeding amphibians, the common parsley frog (*Pelodytidae: Pelodytes punctatus*), and the common midwife toad (*Alytidae: Alytes obstetricans*). These species have broadly overlapping distributions but rarely share breeding sites. *Pelodytes punctatus* prefers shallow temporary ponds and streams, including those formed in flooded quarries and cultivated areas (Denoël et al. 2009; Caballero-Díaz et al. 2020), whereas *A. obstetricans* preferentially uses stagnant permanent ponds and pools, including water tanks and other artificial sites (Bosch et al. 2009;

Caballero-Díaz et al. 2020). Both species are listed as Least Concern (LC) by the IUCN, but their populations are in decline globally and locally in the study area due to a combination of factors, including habitat loss and the negative effect of alien species and emerging infectious diseases (Martínez-Solano 2006; Bosch et al. 2009; Denoël et al. 2009; Caballero-Díaz et al. 2020).

We investigated the role of different factors potentially driving functional connectivity patterns in both species at the landscape and local scales. We



**Fig. 1** Sampling locations for the two study species: *P. punctatus* and *A. obstetricans*. Population codes as in Table 1. **A** Land use map of the study area. **B** Topographic map of the study area. The inset in B shows the location of the study area in central Spain

genotyped a large sample of individuals of both species in a geographically comprehensive dataset and used remotely sensed vegetation/moisture indices and discrete land use/cover categories to derive optimized resistance surfaces to test their association with estimates of gene flow and migration rates across populations. We expected differences in the role of landscape features on connectivity patterns across species because they differ in their breeding site preferences, with *P. punctatus* usually occupying shallow temporary ponds and *A. obstetricans* generally using permanent water bodies, including artificial sites like water tanks used for irrigation. Finally, we discuss potential applications of our results to the design of

management actions that favour the persistence of viable population networks at the regional scale.

## Material and methods

### Study area and sampling

The study area is located in the centre of Iberian Peninsula, bounded by  $-3.784^{\circ}$  to  $3.024^{\circ}$  longitude and  $39.813^{\circ}$  to  $40.571^{\circ}$  latitude (Fig. 1). Elevation ranges from 470 m up to 940 m.a.s.l., and the Jarama, Tajuña and Tagus rivers shape a complex orography, characterized by paramos with loam, gypsum and limestone in calcareous soils. Paramos are dominated by olive

groves, vineyards and cereal crops, with corn in irrigated areas along river valleys. Typical Mesomediterranean vegetation occupies a reduced extension with the presence of pine forests, holm oaks (*Quercus ilex*), gallery forests and extensions of salt cedar (*Tamarix* sp.), with scrublands characterized by the presence of kermes oaks (*Quercus coccifera*) (Izco 1984). The two amphibian species selected for this study are broadly but discontinuously distributed in the region (Caballero-Díaz et al. 2020). We sampled 336 individuals of *P. punctatus* and 318 of *A. obstetricans* at 17 and 16 localities, respectively (Fig. 1, Table 1). Because both species are philopatric and tend to congregate in the vicinities of breeding sites, the sampling was focused on breeding populations, which are clearly delimited in the study area by the availability of water bodies and are largely isolated from each other by large extensions of unsuitable terrestrial habitat. Tissue samples were taken from tail clips of tadpoles of both species, which were collected using dip nets. In order to minimize the probability of sampling siblings, we captured tadpoles of different sizes (potentially representing different cohorts) and in different sections of the water bodies.

Average geographic distances across populations were  $4.97 \pm 3.25$  km in *Alytes* and  $7.12 \pm 3.99$  km in *Pelodytes* (Fig. 1; Supplementary material Tables S1 and S2). Tissue samples for molecular analyses were preserved in 100% ethanol prior to DNA extraction.

### Genetic analyses

Genomic DNA was extracted using NucleoSpin Tissue-Kits (Macherey–Nagel). A total of 10 previously characterized microsatellite markers for *P. punctatus* were amplified following published PCR conditions (Jourdan-Pineau et al. 2009; van de Vliet et al. 2009). These loci were grouped in three multiplex reactions (multiplex 1: PPU5, Ppu10, Ppu8, Ppu11; multiplex 2: PPU16, Ppu7, Ppu5; multiplex 3: Ppu14, Ppu15, Ppu9). For *A. obstetricans*, 17 previously published loci were grouped in five multiplex reactions (Maia-Carvalho et al. 2014), and PCR conditions and genotype calling followed Maia-Carvalho et al. (2014).

We tested for the presence of null alleles, stuttering and large allele dropout in microsatellite markers using MICROCHECKER v2.2.3 (van Oosterhout et al. 2004). Deviations from Hardy–Weinberg equilibrium (HWE) and evidence of linkage

disequilibrium (LD) were estimated with the software GENEPOP (Raymond and Rousset 1995; Rousset 2008), applying the sequential Bonferroni correction (Rice 1989) to adjust significance values for multiple tests. We calculated different estimates of genetic diversity for each population using GENALEX v6.5b5 (Peakall and Smouse 2012), including the number of alleles ( $N_A$ ), observed ( $H_O$ ) and expected heterozygosity ( $H_E$ ). We also calculated the inbreeding coefficient ( $F_{IS}$ ) because it is an indirect measure of philopatry (more philopatric species will, in principle, have higher  $F_{IS}$  values).

We applied three approaches to infer functional connectivity (= gene flow) from genetic data. First, we used BAPS v6 (Corander et al. 2008; Cheng et al. 2013) to characterize regional population genetic structure for both species. BAPS treats allele frequencies of molecular markers and the number of genetic clusters as random variables and uses Bayesian inference to characterize population genetic structure. We ran spatial genetic mixture analyses with ten independent runs and a maximum number of groups equal to the number of sampled localities. We compared genetic clusters resulting from each replicate run based on their likelihood score and identified the optimal clustering level based on a stochastic optimization algorithm (Corander et al. 2008).

Second, we calculated three indices of genetic differentiation between populations,  $F_{ST}$  (Weir and Cockerham 1984),  $G'_{ST}$  (Meirmans and Hedrick 2011) and  $D_{ST}$  (Jost 2008) using Genepop, GENODIVE v2.0b23 (Meirmans and van Tienderen 2004) and SMOGD v1.2.5 (Crawford 2010), respectively, to characterize patterns of genetic differentiation between populations of the two species. Measures of genetic differentiation provide indirect estimates of gene flow and may also reflect other processes, like genetic drift, but provide information about population structure at the landscape scale in philopatric organisms like amphibians. The three indices provide complementary information about population structure in different demographic and mutational scenarios (Jost et al. 2018).

Finally, we estimated recent migration rates between populations using BAYESASS v3.0 (Wilson and Rannala 2003). This program infers the proportion of recent migrants among populations from multilocus genotype data. We ran three different replicate analyses for each species with 50,000,000 iterations,

with a burn-in period of 2,000,000 and sampling pairwise migration rate estimates each 2,000 iterations. We assessed convergence of results across runs using software TRACER (Rambaut et al. 2018) and used those with the best likelihood in subsequent analyses.

#### Input data used for resistance surfaces and pre-processing

Connectivity is influenced by several factors operating at distinct spatial and temporal scales (Auffret et al. 2015; Gonçalves et al. 2016). To address this, we identified the most relevant factors impacting landscape connectivity and gene flow based on previous literature (e.g., Pérez-Espona et al. 2008; Gutiérrez-Rodríguez et al. 2017; Lourenço et al. 2019). This process allowed us to identify several landscape dimensions, including topography/geomorphology, vegetation amount and heterogeneity, water/moisture availability, linear elements (either natural, like rivers, or artificial, like roads or motorways) and land use/cover (LUC). Given the importance of the latter for explaining connectivity in complex landscape mosaics with marked urban/peri-urban gradients, we used fine-scale LUC data from the Spanish Land Cover Information System for 2015—reclassified for simplicity—(Supplementary Material Table S3; URL: <https://www.siose.es/>) to compute several types of resistance surfaces based on: (i) the distance to patches of specific classes (e.g. urban areas, water surfaces), (ii) binary surfaces denoting the presence or absence of a certain LUC class, and (iii) the percentage cover by LUC category. All resistance surfaces were calculated at a spatial resolution of 100 m (i.e., pixel size), considered adequate to represent landscape/environmental patterns and with enough detail to evaluate connectivity and gene flow of target species within reasonable computing times.

For topographic variables, we employed a 25 m Digital Elevation Model from the Spanish Centre of Geographic Information (URL: <http://centrodedescargas.cnig.es>) to derive an elevation resistance surface. This data was also used to calculate several indices, including the slope, the Topographic Ruggedness Index as a descriptor of topographic/elevation complexity (TRI; at two aggregation kernels,  $k=3\times 3$  and  $k=9\times 9$  pixels), and the Topographic Wetness Index (TWI) describing the tendency of a cell to accumulate water (Quinn et al. 1995).

We also calculated river density ( $m/Km^2$ ) using data on the hydrographic network from the European Catchment Database (URL: <https://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1>) for (i) all rivers in a 1000 m buffer, (ii) all rivers in a 2500 m buffer and, (iii) small rivers with Strahler class 1 or 2 in a 2500 m buffer.

Freely accessible Open Street Maps (OSM) data on the road network (URL: <https://download.geofabrik.de/europe/spain.html>) were employed to calculate: (i) road density, (ii) distance to roads and (iii) binary layers signalling the presence/absence of roads in each 100 m cell. To assess differences in connectivity response by road class and traffic volume, we approximated this based on OSM metadata, more specifically, the type of road. Motorways correspond to ‘high-traffic’ (typically with two or more lanes in each way) while other roads to ‘medium-traffic’ roads (typically with one lane per direction).

From Landsat-8 satellite data (USGS/NASA; URL: <https://earthexplorer.usgs.gov/>) with a spatial resolution of 30 m (16-day temporal resolution), we calculated resistance surfaces related to vegetation/greenness using the Normalized Difference Vegetation Index (NDVI; combining the red and near-infrared spectral bands), and related to water availability in soil/vegetation based on the Normalized Difference Water/Moisture Index (NDWI; combining spectral bands in the near-infrared and shortwave-infrared). Both NDVI and NDWI vary from  $-1$  to  $1$ , with lower values indicating, respectively, low vegetation cover (e.g., non-vegetated/artificial surfaces) or low water availability, and values closer to one the opposite (e.g. densely vegetated areas). Image data, covering the months of in-situ sampling, were aggregated to 100 m, thus using the same spatial resolution of other resistance surfaces through the average (capturing the amount of vegetation cover or water in soil/vegetation) and the standard deviation (related to the spatial heterogeneity of the same aspects). A total of 54 different resistance surfaces were tested, optimized and ranked using a modelling approach (the complete list of resistance surfaces is detailed in Table 2).

#### Statistical modelling and resistance surface optimization

To assess the relative support of each resistance surface (or variable) to explain differences in genetic

**Table 2** Summary list of resistance surfaces tested

Surface type/description	Resistance surface name/description	Data source description	
<b>Land cover/use</b>			
Binary (i.e., presence—1, or absence—0 of different land cover/use types per 100 m cell)	Agriculture areas presence/absence	SIOSE 2014—Land Cover Information System for Spain (resolution: ~50 m, map scale: 1:25 000) <a href="https://www.siose.es">https://www.siose.es</a>	
	Forest areas presence/absence		
	Heathlands/shrublands areas presence/absence		
	Meadows/pastureland areas presence/absence		
	Open soil areas presence/absence		
	Urban/artificial areas presence/absence		
	Distance (i.e., Euclidean distance in meters to features of each given land use/cover class)		Distance to agriculture crops
			Distance to all artificial surfaces
			Distance to forests
			Distance to heathlands/shrublands
Distance to meadows/pastureland			
Distance to open areas/bare soil			
Distance to urban habitational areas			
Coverage (i.e., percentage cover of each land cover/use type in 100 m cells)	Distance to vegetated areas		
	Distance to water/wetlands		
	% Agriculture crops		
	% Forest		
	% Heathlands/shrublands		
	% Meadows/pastureland		
<b>Roads (artificial linear features)</b>	% Open soil	Open Street Maps (OSM) for Spain <a href="https://download.geofabrik.de/europe/spain.html">https://download.geofabrik.de/europe/spain.html</a>	
	% Urban/artificial areas		
	Binary (i.e., presence—1, or absence—0 of roads per 100 m cell)		All road types presence/absence
			High traffic road presence/absence
			Medium traffic roads presence/absence
	Distance (i.e., Euclidean distance to each road type)		All road types distance
			High traffic road distance
			Medium traffic roads distance
	Density (i.e., total road length per area in 250 m, m/km <sup>2</sup> )		All road types density
			High traffic road density
Medium traffic roads density			
<b>Rivers (natural linear features)</b>			
<i>Density</i> (i.e., river length per area in 1000 m or 2500 m, m/km <sup>2</sup> )	All river types density (1000 m kernel)	European River Catchment Database (resolution: ~500 m, scale: 1:1,000,000) <a href="https://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1">https://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1</a>	
	All river types density (2500 m kernel)		
	Strahler order 1–2 rivers density (2500 m kernel)		

**Table 2** (continued)

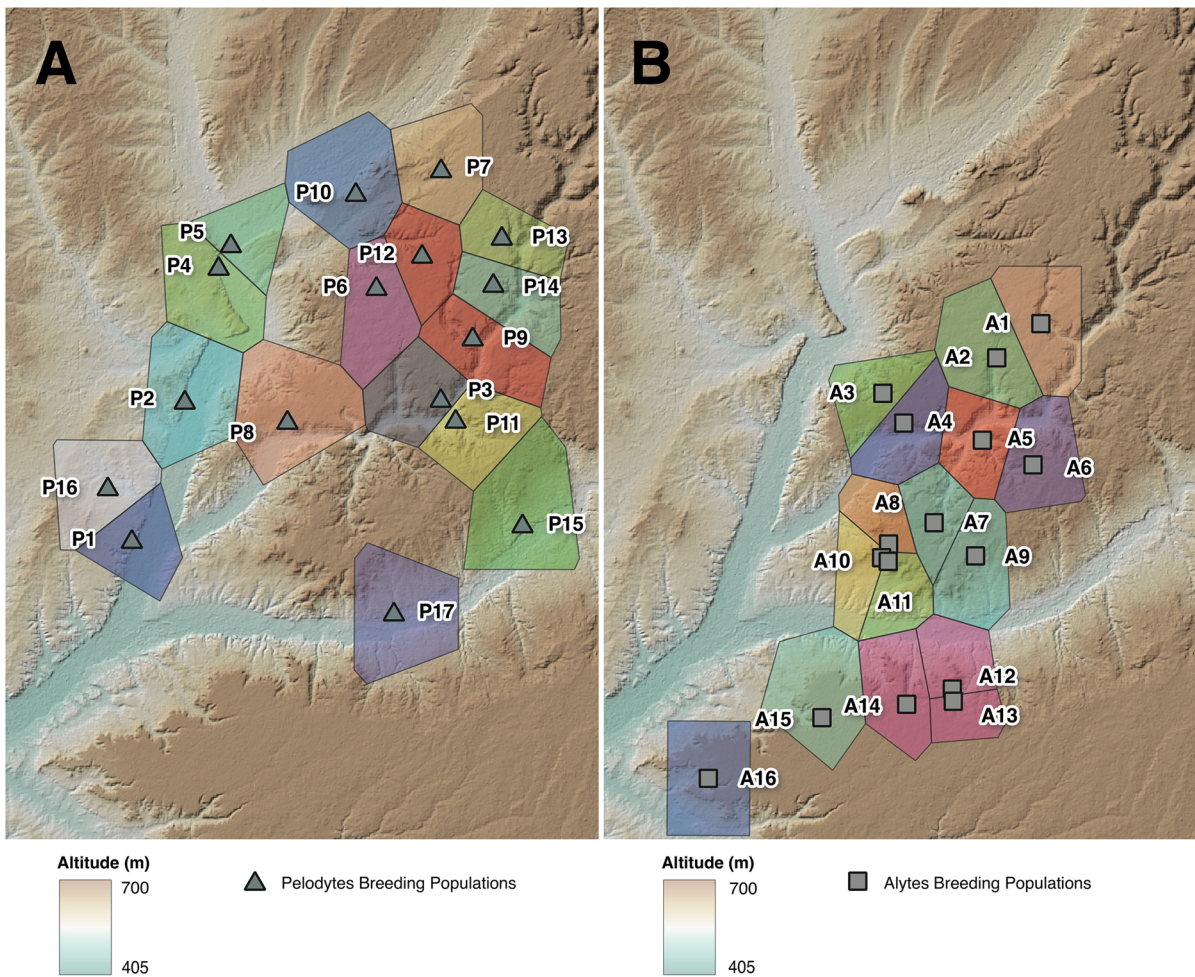
Surface type/description	Resistance surface name/description	Data source description
Satellite-based spectral indices of vegetation and water content		
Vegetation/ greenness indices (zonal statistics in 100 m cell)	Normalized Difference Vegetation Index average	Landsat-8 satellite imagery (surface reflectance, resolution: 30 m, date: 2013) <a href="https://earthexplorer.usgs.gov">https://earthexplorer.usgs.gov</a>
	Normalized Difference Vegetation Index std. dev	
	TCT 'Greenness' average	
	TCT 'Greenness' std. dev	
Soil/canopy water content or moisture (zonal statistics in 100 m cell)	Normalized Difference Water Index average	
	Normalized Difference Water Index std. dev	
	TCT 'wetness' index average	
	TCT 'wetness' index std. dev	
Surface brightness (zonal statistics in 100 m cell)	TCT 'brightness' average	
	TCT 'brightness' std. dev	
Geomorphology/topography		
Elevation and surface complexity (zonal average 100 m)	Elevation (average)	Digital Elevation Model for Spain (resolution: 25 m) <a href="https://centrodedescargas.cnig.es">https://centrodedescargas.cnig.es</a>
	Slope (% , average)	
	Topographic Rugdnes Index (3×3 cells)	
	Topographic Rugdnes Index (9×9 cells)	
Water accumulation (zonal average)	Topographic Wetness Index	

structure between sampled populations, we used the *ResistanceGA* package (Peterman et al. 2014; Peterman 2018) implemented in R v4.0 (R Core Team 2020). This R package implements an optimization approach based on genetic algorithms that selects the best numerical transformation type for a given resistance surface while also optimizing the equation parameters used in those transformations. *ResistanceGA* package starts with 'raw'/untransformed surfaces (which display different landscape features such as road density or the % cover of meadows) and ends up with actual resistance surfaces which are tailored to the response of each feature and species. Optimization allows bypassing the subjective assignment of resistance surface values based on expert opinion or by modelling individual movement patterns (Peterman 2018). The resulting optimized surface is then applied to iteratively calculate the cost-based distance between sampled locations using least-cost path analyses through the *gdistance* package (van Etten 2017). *ResistanceGA* then applies a linear mixed effects model with the maximum likelihood population effects

(MLPE) parameterization (Clarke et al. 2002) to relate the genetic and the cost-based distance matrices. Model inference and ranking of each resistance surface is based on the Akaike Information Criterion (corrected for finite sample size; AICc). For *ResistanceGA*, the AICc value is the fitness function output used for iteratively improving the relation between resistance surfaces and the response through the genetic algorithm. In addition, the MLPE allows accounting for the non-independence of values within pairwise distance matrices (Clarke et al. 2002; van Strien et al. 2012) and was fitted using maximum likelihood with the *LME4* package (Bates et al. 2015).

The algorithm implemented in R package *ResistanceGA* roughly follows these steps in an iterative fashion: (i) least cost path calculation, based on each hypothesized surface, is performed to determine the landscape-based distance between each subpopulation (i.e., cost-distance matrix); (ii) the MLPE model is used to relate the genetic distance matrix (i.e.,  $F_{ST}$ ,  $G''_{ST}$ , and  $D_{ST}$  as the response) to the cost-distance matrix (i.e., predictor); (iii) from





**Fig. 2** Optimal number of genetic clusters for *Pelodytes punctatus* (A) and *Alytes obstetricans* (B) according to BAPS analyses. Polygons represent Voronoi tessellations produced by the

spatial clustering module in BAPS; each cell corresponds to the physical neighborhood of an observed data point, and is colored according to genetic cluster membership

the model in step (ii) AICc is calculated to assess the level of support of each landscape surface; (iv) mathematical transformations are iteratively applied to the original surface, steps (i) to (iii) are recalculated, and each surface is compared and optimized through the AIC to achieve the best-performing resistance surface (i.e., by maximizing the explanatory power of the MLPE model on step (ii)).

Pairwise genetic distances  $F_{ST}$ ,  $G'_{ST}$ , and  $D_{ST}$  are the response variables, while optimized cost-based resistance distances between populations are independent/predictor variables. The genetic algorithm then determines the best parameters for transforming the original resistance surface, optimising AICc

model performance. Stopping criteria were set to a maximum of 250 rounds or 20 rounds without performance improvement.

To assess model performance, we compared AICc values between models generated for each optimized resistance surface to two baseline null models: (i) the isolation-by-distance (IBD) effect (which relies on the Euclidean distance between populations) and (ii) a neutral-landscape approach by simulating a gradient-like landscape (GL) using R package NLMR (Sciaini et al. 2018).

To determine which factors better explain gene flow, we ranked all models based on delta AICc values ( $\Delta AICc$ ). Models with  $\Delta AICc \leq 4$  were

considered with greater evidence/likelihood, thus entering the confidence set containing those models with substantial or good support for inference. We decided to use a less stringent threshold to identify potentially significant landscape-related factors explaining connectivity that would be missed if  $\Delta AICc < 2$  was used. We also calculated the Akaike weights ( $w_i$ , between 0 and 1) to characterize the evidence strength of each model quantitatively.

## Results

We amplified samples with a success rate of >99% for both species. Allele dropouts or stuttering were detected in loci Aobst $\mu$ 05 and Aobst $\mu$ 16 in several *Alytes* populations. In *Pelodytes* populations, potential null alleles were only detected in two populations for one locus in each of them, P2 (Ppu10) and P7 (Ppu11).

We did not detect significant deviations from HWE and LD in *Alytes*, except in loci Aobst $\mu$ 05 (A4, A5, A13, A16) and Aobst $\mu$ 16 (A6, A7, A11, A14, A15), and between Aobst $\mu$ 09 and Aobst $\mu$ 17 (A2, A6), respectively. In *Pelodytes*, deviations from HWE were detected in Ppu5 (P11), Ppu8 (P10), Ppu9 (P10), Ppu10 (P13, P16), Ppu14 (P2, P13) and Ppu15 (P9, P16), whereas significant LD was found between loci Ppu8 and Ppu9 in several populations (P3, P4, P6, P10, P12, P13, P14, P16).

Descriptive statistics of genetic diversity for *P. punctatus* and *A. obstetricans* are presented in Table 1. Estimates of genetic diversity were slightly higher in *P. punctatus*. The mean number of alleles per population ranged from 2.6 (P16) to 9.7 (P9) in *P. punctatus* and from 2.9 (A16) to 7.8 (A9) in *A. obstetricans*. The observed heterozygosity ranged from 0.46 (P14) to 0.83 (P3) in *P. punctatus*, and from 0.44 (A16) to 0.75 (A9) in *A. obstetricans*. Average population inbreeding coefficients ( $F_{IS}$ ) were higher in *A. obstetricans* (0.03) than in *P. punctatus* (−0.12).

The results of BAPS analyses supported optimal clustering levels at  $K = 16$  and  $K = 13$  for *P. punctatus* and *A. obstetricans*, respectively (Fig. 2). The number of clusters was consistent across replicate runs.

Pairwise estimates of  $F_{ST}$ ,  $G''_{ST}$ , and  $D_{ST}$  between species populations are presented in Supplementary material Tables S4–S9. Similar but slightly lower average values, indicating higher gene flow and greater population connectivity, were found in *A. obstetricans* ( $F_{ST} = 0.091$ ;  $G''_{ST} = 0.264$ ;  $D_{ST} = 0.209$ ) than in *P. punctatus* ( $F_{ST} = 0.094$ ;  $G''_{ST} = 0.326$ ;  $D_{ST} = 0.260$ ). Estimates of migration rates with BAYESASS were low across sites, except among geographically close localities (Supplementary material Tables S10 and S11), with slightly higher average migration rates between populations of *A. obstetricans* (mean = 0.0173) than in populations of *P. punctatus* (mean = 0.0133).

**Table 3** Model selection results with optimized resistance surfaces for *A. obstetricans* (the table includes only those variables with  $\Delta AICc < 10$ )

Genetic distance	Resistance surface/explanatory variable	$R2_m$	$R2_c$	AICc	$\Delta AICc$	$w_i$
$D_{ST}$	<b>Distance to water/wetlands</b>	0.38	0.77	− 292.15	<b>0.00</b>	0.57
	<b>Distance to all artificial surfaces</b>	0.38	0.74	− 291.53	<b>0.61</b>	0.42
	Distance to urban habitational areas	0.39	0.75	− 283.72	8.43	0.01
$F_{ST}$	<b>Distance to water/wetlands</b>	0.22	0.88	− 530.20	<b>0.00</b>	0.54
	<b>Distance to all artificial surfaces</b>	0.24	0.88	− 528.67	<b>1.53</b>	0.25
	<b>Distance to urban habitational areas</b>	0.24	0.88	− 528.20	<b>2.00</b>	0.20
	% of agriculture	0.20	0.86	− 522.19	8.01	0.01
$G''_{ST}$	<b>Distance to water/wetlands</b>	0.38	0.79	− 274.75	<b>0.00</b>	0.93
	Distance to urban habitational areas	0.34	0.76	− 269.14	5.61	0.06
	Distance to all artificial surfaces	0.39	0.77	− 265.83	8.92	0.01

Bold lettering indicates highly supported models i.e.  $\Delta AICc \leq 4$ .  $R2_m$  marginal R2 (fixed effects),  $R2_c$  conditional R2 (both for fixed and random effects), AICc Akaike Information Criterion (with finite sample size correction),  $\Delta AICc$  Delta AICc (difference between the ‘best’ model AICc and each model AICc),  $w_i$  Akaike weight (relative model likelihood/support across tested models for all resistance surfaces) for a given species and genetic distance matrix

**Table 4** Model selection results with optimized resistance surfaces for *P. punctatus* (the table includes only those variables with  $\Delta AICc < 10$ )

Genetic distance	Resistance surface/explanatory variable	$R2_m$	$R2_c$	AICc	$\Delta AICc$	$w_i$
$D_{ST}$	<b>Distance to meadows/pasturelands</b>	0.36	0.67	− 316.51	<b>0.00</b>	0.65
	<b>Topographic Wetness Index</b>	0.13	0.70	− 313.76	<b>2.75</b>	0.16
	Distance to urban habitational areas	0.32	0.69	− 310.51	6.00	0.03
	Distance to all artificial surfaces	0.31	0.69	− 310.05	6.46	0.03
	Distance to roads and railways	0.15	0.65	− 309.50	7.01	0.02
	Distance to heathlands/shrublands	0.16	0.63	− 309.49	7.03	0.02
	% Forest	0.21	0.64	− 309.35	7.16	0.02
	% Heathlands/shrublands	0.17	0.63	− 308.61	7.90	0.01
	Distance to water/wetlands	0.42	0.72	− 307.97	8.54	0.01
	River density—all rivers at 2500 m	0.23	0.68	− 307.86	8.66	0.01
	Topographic Ruggedness Index (K=9)	0.11	0.69	− 307.51	9.00	0.01
	Norm. Diff. Water Index average	0.17	0.66	− 307.34	9.17	0.01
	Norm. Diff. Vegetation Index average	0.23	0.69	− 306.99	9.52	0.01
	$F_{ST}$	<b>Topographic Wetness Index</b>	0.06	0.89	− 608.33	<b>0.00</b>
<b>River density—all rivers at 2500 m</b>		0.36	0.91	− 604.49	<b>3.84</b>	0.11
<b>Distance to meadows/pasturelands</b>		0.19	0.87	− 604.40	<b>3.93</b>	0.10
Slope (%)		0.05	0.88	− 599.63	8.70	0.01
Topographic Ruggedness Index (K=3)		0.05	0.88	− 598.06	10.27	0.00
Norm. Diff. Water Index average		0.11	0.89	− 597.99	10.34	0.00
$G''_{ST}$	<b>River density—all rivers at 2500 m</b>	0.47	0.83	− 305.50	<b>0.00</b>	0.43
	<b>Topographic Wetness Index</b>	0.11	0.75	− 304.33	<b>1.17</b>	0.24
	<b>Distance to meadows/pasturelands</b>	0.27	0.72	− 301.99	<b>3.51</b>	0.07
	Distance to urban habitational areas	0.27	0.74	− 301.43	4.08	0.06
	Distance to all artificial surfaces	0.31	0.76	− 301.12	4.38	0.05
	Distance to heathlands/shrublands	0.13	0.69	− 299.86	5.64	0.03
	% Forest	0.17	0.69	− 299.64	5.86	0.02
	Distance to roads and railways	0.12	0.70	− 299.42	6.08	0.02
	% Heathlands/shrublands	0.13	0.69	− 298.81	6.70	0.02
	Topographic Ruggedness Index (K=9)	0.09	0.74	− 298.58	6.93	0.01
	Norm. Diff. Vegetation Index average	0.23	0.75	− 298.55	6.96	0.01
	Distance to water/wetlands	0.38	0.75	− 297.81	7.70	0.01
	Topographic Ruggedness Index (K=3)	0.09	0.73	− 296.76	8.74	0.01
	Slope (%)	0.15	0.69	− 295.62	9.88	0.00
Norm. Diff. Water Index average	0.15	0.72	− 295.47	10.04	0.00	

$R2_m$  marginal R2 (fixed effects),  $R2_c$  conditional R2 (both for fixed and random effects), AICc Akaike Information Criterion (with finite sample size correction),  $\Delta AICc$  Delta AICc (difference between the ‘best’ model AICc and each model AICc),  $w_i$  Akaike weight (relative model likelihood/support across tested models for all resistance surfaces) for a given species and genetic distance matrix

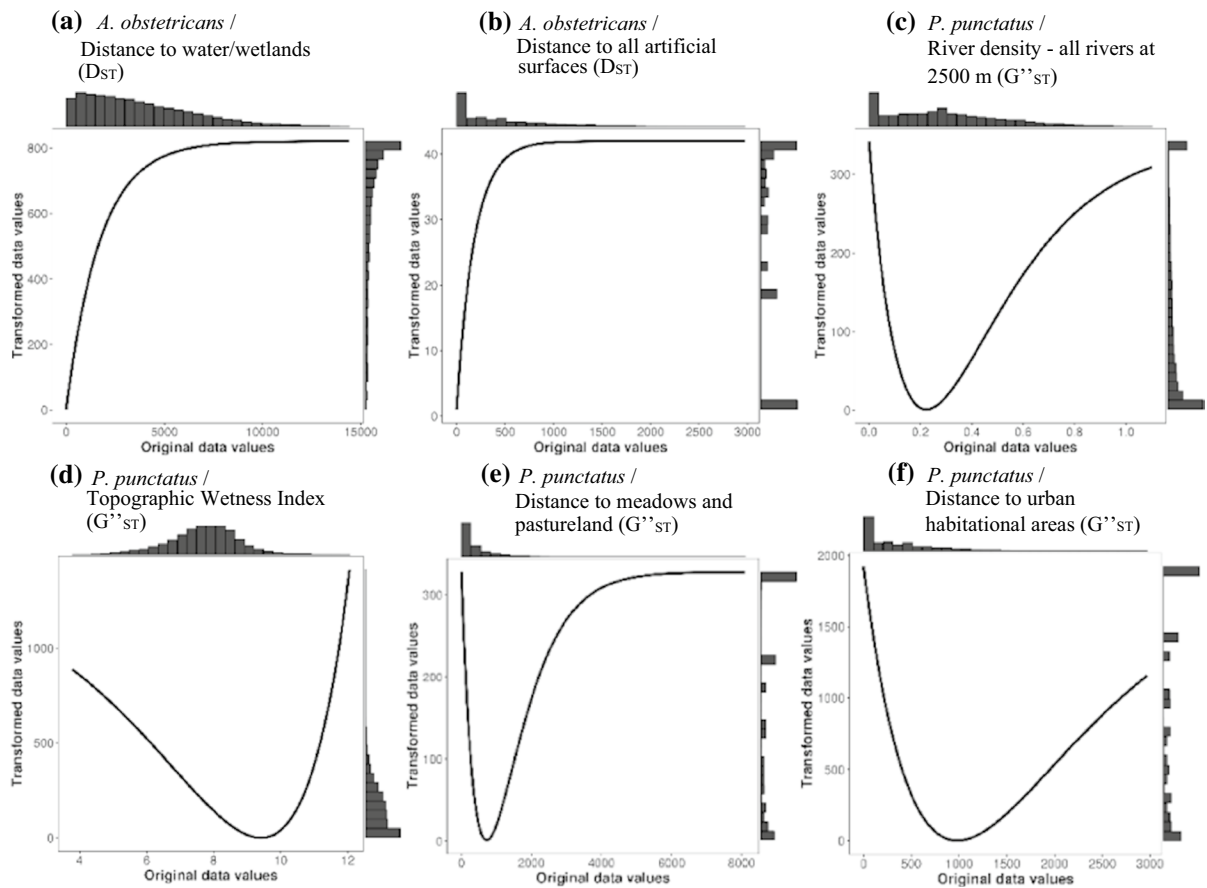
Bold lettering indicates highly supported models i.e.  $\Delta AICc \leq 4$

### Resistance surfaces and gene flow

Overall, model ranking based on AICc and optimized resistance surfaces (Tables 3 and 4, and Supplementary material Table S12) showed a high degree of

similarity for both species regarding the variables selected across different genetic distances.

Models for both species attained reasonably good performance values, as evidenced by the low rank of both null-models tested, the IBD and the simulated



**Fig. 3** Transformations applied to selected resistance surfaces (i.e. with greater model support) based on *ResistanceGA* results. Top labels indicate the species, resistance surface and the genetic distance metric with best results in the analysis. The x-axis (“Original data values”) represents the data in the

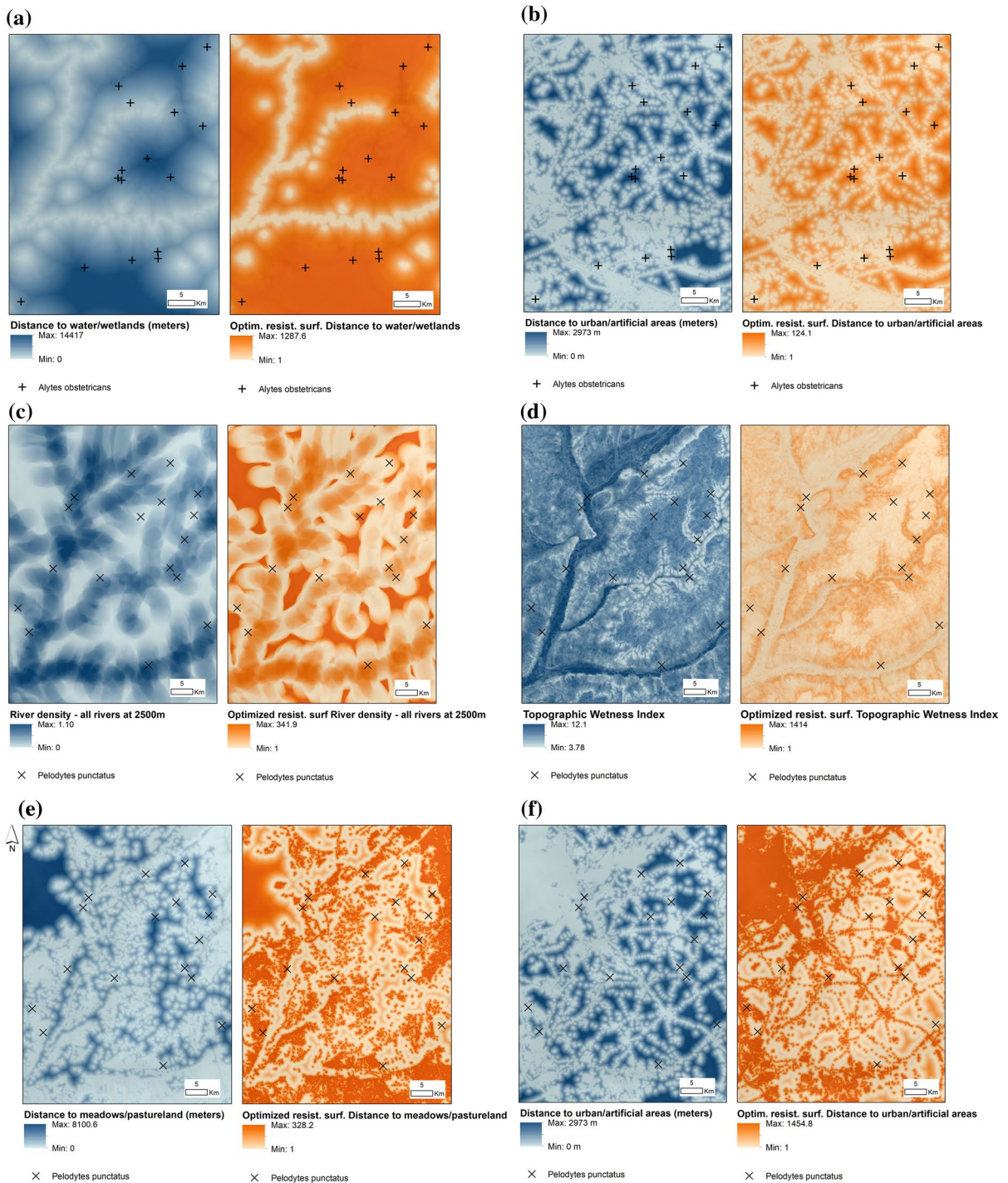
original scale/units while the y-axis (“Transformed data values”) shows the final resistance values after applying the optimized transformation. Top and lateral bars depict the histogram of each variable distribution

neutral GL. For *A. obstetricans*, a  $\Delta\text{AICc} \gg 4$  denotes no support for null-models, ranging from  $\Delta\text{AICc} = 19.96$  for  $\text{GL}/F_{\text{ST}}$  to  $\Delta\text{AICc} = 29.33$  for  $\text{IBD}/G''_{\text{ST}}$  (Table 3 and Supplementary material Table S12). For *P. punctatus*, despite slightly higher model uncertainty, null-models also attained no support, with  $\Delta\text{AICc} = 11.25$  for  $\text{IBD}/G''_{\text{ST}}$  to  $\Delta\text{AICc} = 15.95$  for  $\text{GL}/F_{\text{ST}}$  (Table 4 and Supplementary material Table S12). Marginal  $R^2$  values for selected models ( $\Delta\text{AICc} \leq 4$ ) ranged from 0.22 to 0.38 for *A. obstetricans* and from 0.06 to 0.47 for *P. punctatus*.

Overall, distance to water lines and water availability are the most important features for both species, especially in *A. obstetricans*, as evidenced by

the selection of variables related to the distance to rivers/wetlands or topographic water accumulation (Tables 3 and 4). Secondly, in terms of explanatory importance, is land cover/use, specifically (and in decreasing order of importance) the distance and the amount of artificial areas (housing and other types including roads, although with less spatial detail in comparison to road maps—See Fig. 3b, f), meadows and pastureland, and, with substantially less model support, agricultural areas, forest and heathland/shrubland (Supplementary material Table S13).

In general, distance-based resistance surfaces obtained greater explanatory importance than those depicting the percentage cover of different land use types (or their presence/absence) and continuous



**Fig. 4** Pairwise maps showing the original (blue) and transformed resistance surfaces (orange) through genetic algorithms optimization (based on  $G^*_{ST}$ ). Labels indicate the species and the resistance surface for: **a** *A. obstetricans*/Distance to water/wetlands, **b** *A. obstetricans*/Distance to all artificial surfaces,

**c** *P. punctatus*/River density—all rivers at 2500 m, **d** *P. punctatus*/Topographic Wetness Index, **e** *P. punctatus*/Distance to meadows and pasturelands, **f** *P. punctatus*/Distance to urban/artificial areas

remote sensing indices for vegetation and water. This result suggests that the proximity to particular landscape features is generally more relevant than the composition of landscape mosaics in explaining gene flow among populations.

Contrary to our expectations (see e.g. Gutiérrez-Rodríguez et al. 2017), finer-scale features captured by satellite Earth Observations related to vegetation and water availability (in terms of amount or spatial heterogeneity) did not comparatively attain relevant importance in the models of both species. In line with this, layers including linear elements like roads or motorways did not attain much explanatory power either (Tables 3 and 4, and Supplementary material Table S12). Nonetheless, the distance to artificial/urban areas was highly relevant for *Alytes obstetricans*, supporting a major role of the whole network of anthropogenic structures driving landscape resistance.

Despite some similarities, there are strong contrasts when comparing the relative importance of different explanatory variables between species and how these translate into landscape resistance to movement (Figs. 3 and 4). In *A. obstetricans* up to three variables entered the confidence set, including Distance to water/wetlands, Distance to all artificial surfaces, and Distance to urban habitational areas (Table 3). In *P. punctatus* a different set of variables was selected, including Distance to meadows/pasturelands, Topographic Wetness Index, and River density -all rivers at 2500 m (Table 4).

For *A. obstetricans*, the distance to water surfaces or lines is highly important for explaining gene flow, with resistance increasing non-linearly with increased distance to these areas (Figs. 3a and 4a). For *P. punctatus*, water is also important; however, its role manifests more in the density of the river network throughout the landscape, with resistance peaking at densities both very-low (absence of watercourses) and very-high (large rivers) (Figs. 3c and 4c). A minimum of resistance occurs for intermediate density of rivers and moderate wetness conditions as depicted by the topographic wetness index (Figs. 3d and 4d).

Overall, for *A. obstetricans*, distance and spatial distribution of artificial land-use types significantly influenced gene flow and connectivity patterns. In fact, the species seems to tolerate well urban/artificial elements in surrounding areas, with somewhat low resistance for smaller distances (i.e. within or close to

urban/artificial patches) and increasing with greater distances to these landscape features (Figs. 3b and 4b). Still, given the widespread presence of artificial structures throughout the landscape and the inter-persorption of anthropogenic land uses, large distances to artificial areas/lines are not often encountered in the study area (see lateral histogram for the x-axis in Fig. 3b). In contrast, for *P. punctatus*, artificial/urban landscape elements have less importance, but their effect is also opposed to that in *A. obstetricans*. For *P. punctatus*, higher resistance is found within or in the vicinity of urban/artificial areas, decreasing progressively towards a minimum of resistance around 1000 m from artificial areas (Figs. 3f and 4f). Also, in contrast with *Alytes*, the amount and spatial distribution of meadows and pastureland areas also seem to shape gene flow in *P. punctatus*, with resistance peaking at small distances (within or in the vicinity ~0-500 m) to these land use patches (Figs. 3e and 4e) and with a minimum of resistance for a distance range of 700-900 m. Moreover, for  $G''_{ST}$  (Table 4), other LUC types (forest and heathland/shrubland) as well as elevation complexity (slope and TRI) also attained some explanatory power (although low) for *P. punctatus*, showing that a more extensive set of factors shape gene flow patterns in this species.

## Discussion

We investigated the role of landscape features in shaping patterns of functional connectivity of two Mediterranean amphibian species in central Spain's semi-arid landscapes, where artificial irrigation structures provide critical breeding sites for some species. Understanding the role of different features in promoting or restricting gene flow is crucial to designing efficient management programs promoting the survival of amphibian communities in rural areas. Overall, our results provide evidence for higher population connectivity in common midwife toad (*Alytes*) than in common parsley frog (*Pelodytes*), with different landscape features shaping gene flow patterns for the two species. These differences should be considered when designing conservation actions targeted at each species separately, as suggested by studies on their breeding preferences (Caballero-Díaz et al. 2020). However, it should be kept in mind that our study is correlative, and while it provides an empirical

approach to infer landscape factors likely impacting connectivity in our target species, the actual factors and processes involved are unknown. Experimental approaches investigating species responses to landscape elements would provide valuable information but at present remain challenging in wild amphibian populations.

At the regional scale, our comparative study showed marked genetic structure in both species (Fig. 2). Clustering analyses detected a higher number of genetic clusters in *Pelodytes* than in *Alytes*, which showed lower  $D_{ST}/F_{ST}/G'_{ST}$  values (Supplementary material Tables S4 to S9) and overall higher migration rates (mean = 0.0173 vs 0.0133 in *Pelodytes*). Unfortunately, there is little information about the dispersal capacity of the two species (Ryser et al. 2003; Trochet et al. 2014) as for most amphibians (Pittman et al. 2014). Based on indirect (molecular) evidence, *Alytes* and *Pelodytes* seem to be poor dispersers, which could make them vulnerable to habitat fragmentation processes. This hypothesis needs to be further tested, for instance, with information on the frequency and distances covered by adult individuals of both species in capture-mark-recapture studies.

Landscape genetic analyses provide evidence for similarities and differences in how the two species interact with the landscape, based on resistance values of different habitat feature layers (Tables 3 and 4). Our criterion to consider models with  $\Delta AICc \leq 4$  is permissive, and inferences based on models with  $\Delta AICc$  values  $\geq 2 \leq 4$ , while providing better explanatory power than null models, should be taken with caution. In general, distance to some landscape features showed greater explanatory power than features based on the composition of landscape mosaics. The influence of landscape features on population connectivity at the regional scale revealed a strong effect of water availability (Tables 3 and 4). Both the distance to rivers/wetlands and the topographic water accumulation index are key variables explaining functional connectivity in *Alytes* and *Pelodytes*. This dependence on the presence of water is expected in Mediterranean semi-arid landscapes, where animal and plant communities are constrained by water availability (Noy-Meir 1973; Dodd and Lauenroth 1997). This effect is clearly the case in our study area, with an annual average precipitation of 415 mm, and almost no rainfall during the summer (between 250 and 600 mm; Romão and Escudero 2005; García et al.

2011). Climatic models have predicted a significant decrease in water availability in the Mediterranean basin (Houghton et al. 2001; Polade et al. 2017), which may have dramatic consequences for water-dependent species (Bates et al. 2008).

Beyond their shared dependence on water availability, our models provide evidence that landscape variables played a differential role on patterns of population connectivity in both species. In *P. punctatus*, the absence of watercourses and the presence of large rivers restricted gene flow among populations (Figs. 3c and 4c), with genetic connectivity increasing at intermediate river densities (Figs. 3d and 4d). In contrast, in *A. obstetricans*, distance to water shaped gene flow differently, with resistance increasing non-linearly with increased distance to these areas (Figs. 3a and 4a). These differences are probably associated with differences in the reproductive biology of the two species. The common parsley frog is very flexible in using aquatic habitats, usually occupying shallow temporary ponds in meadows and pastures for reproduction (Guyétant et al. 1999; Boix et al. 2001; Grillas et al. 2004; Salvidio et al. 2004; Tatin 2010; Escoriza 2017). In contrast, common midwife toads tend to choose long-hydroperiod or permanent water bodies because of their longer larval stage (Bosch et al. 2009). In the study area, Caballero-Díaz et al. (2020) found marked differences in the selection of breeding sites between the two species, with *Alytes* preferring artificial water bodies (fountains, water tanks) and *Pelodytes* breeding mostly in temporary ponds.

A second important difference is the role of variables related to land cover/use on patterns of gene flow in the two species. The distance and spatial distribution of artificial land-use types positively influenced connectivity in *A. obstetricans*, which seems to tolerate the presence of urban/artificial elements well. This effect is probably related to their ability to exploit artificial breeding sites successfully, many of which are located in peri-urban settings, including orchards and recreational areas (Caballero-Díaz et al. 2020). In contrast, in *P. punctatus*, which rarely breeds in those artificial water structures in urban areas, the amount and spatial distribution of meadows and pastureland areas were important positive predictors of gene flow.

This study highlights the importance of assessing species–habitat relationships shaping gene flow and population connectivity when developing and

implementing conservation and management actions to benefit fragmented amphibian populations in the Mediterranean region. As previously shown for other co-distributed Mediterranean amphibians (Gutiérrez-Rodríguez et al. 2017), our results show that amphibian species respond differently, even contrastingly to landscape features and thus require alternative, complementary strategies to improve population connectivity and ensure their long-term viability. For instance, the construction and maintenance of artificial ponds can be an efficient resource to create and sustain viable, well-interconnected populations of common midwife toads (*Alytes*), whereas measures directed to improve the conservation status of *Pelodytes* populations should focus on the conservation of natural habitats in low-resistance areas, including meadows and floodable pasturelands.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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