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RESEARCH ARTICLE

Reconciling direct and indirect estimates of functional connectivity in a Mediterranean pond-breeding amphibian

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Abstract

Understanding demographic dynamics and functional connectivity among demes is essential to prevent, identify and reverse amphibian population declines. Attempts to characterize connectivity among amphibian populations have largely relied on the use of molecular markers to assess patterns of genetic structure at the landscape scale and identify factors that promote or restrict gene flow. However, few studies have integrated population size estimates and direct records of dispersal rates (e.g. based on capture-mark-recapture data) to help interpret molecular-based inferences. We conducted such an integrative, long-term monitoring program of a metapopulation of the sharp-ribbed newt (*Pleurodeles waltl*), a pond-breeding salamander endemic to the Iberian Peninsula and North Africa. Over 12 breeding seasons, we compiled individual capture histories to estimate population sizes and document patterns of dispersal, and inferred migration rates and pairwise relatedness based on 22 microsatellite loci. Direct (based on capture-mark-recapture) estimates revealed low levels of contemporary dispersal between the two major breeding sites in the study area, whereas indirect (molecular) estimates provided evidence for asymmetric gene flow, more intense from the larger to the smaller population, without consistent differences between sexes. Direct and indirect estimates can be reconciled based on the species' generation time, and support a scenario of density-dependent dispersal, with recent colonization of the smaller pond inferred from differences in the relative proportions of ancestry categories across ponds. Our integrative approach can be applied to design conservation actions aiming to improve functional connectivity among amphibian populations based on empirical evidence.

Keywords Capture-mark-recapture · Connectivity · Dispersal · Gene flow · Iberian Peninsula · Integrative demography · *Pleurodeles waltl*

Introduction

Habitat fragmentation and loss is the main cause of amphibian population declines and extinctions in temperate latitudes (Wake and Koo 2018). Understanding functional connectivity among demes in population networks is thus paramount to prevent and reverse negative population trends (Petranka et al. 2004; Leung et al. 2017). Attempts to characterize patterns of connectivity among amphibian populations at the landscape scale have largely relied on molecular markers to identify factors that promote or restrict gene flow (Newman and Squire 2001; Wang 2009a; Gutiérrez-Rodríguez et al. 2017a). However, in the absence of information on the frequencies and distances of individual displacement events, it is difficult to relate gene flow estimates with the actual dispersal capacity of individuals, which is a critical parameter to design and implement conservation actions (Sinsch et al. 2012; Sinsch 2014; Trochet et al. 2017). This calls for integrative studies combining individual-based capture-mark-recapture data providing direct records of dispersal and indirect (molecular-based) estimates of gene flow.

Because dispersal is often a density-dependent process (Cayuela et al. 2020), accounting for the size of populations is also important to fully understand patterns of functional connectivity among demes. Population size can be approximated by estimating the census size or abundance of individuals (N) using capture-mark-recapture or N-mixture methods

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(Ficetola et al. 2018). Alternatively, the effective population size (N_e) is proportional to the genetic contribution of individuals to the next generation, and thus accounts for the size of the population in terms of its capacity to maintain genetic diversity (Frankham et al. 2014; Wang et al. 2016). Both estimates can be used to calculate the effective to census size ratio (N_e/N), a measure of the relative vulnerability of the population to demographic stochasticity (Palstra and Fraser 2012). Altogether, dispersal, local abundance and effective size estimates provide invaluable information to characterize amphibian population dynamics.

The Mediterranean region is one of the worlds' hotspots of biodiversity and hosts many endemic amphibian species (Myers et al. 2000; Çiçek and Cumhuriyet 2017). Historically, ecosystems in this region have experienced high anthropogenic pressure and extensive changes in land use. Natural habitats for amphibians are thus fragmented to different degrees, including the spatial dissociation of their preferred aquatic (temporary ponds and streams, Díaz-Paniagua 1990) and terrestrial habitats. Many amphibian communities in this region are structured as networks of spatially structured populations or metapopulations which strongly rely on connectivity among breeding nuclei to counteract local declines or extinctions (Mazerolle 2001; Smith and Green 2005; Capellà-Marzo et al. 2020). Conservation of amphibian communities in the Mediterranean region thus requires detailed knowledge of their spatial and temporal dynamics, including robust estimates of local abundance and functional connectivity among demes (Semlitsch 2008).

In this study, we estimate local abundance and reconcile direct and indirect estimates of functional connectivity in a spatially structured population of the sharp-ribbed newt (*Pleurodeles waltl* Michahelles, 1830), a common inhabitant of Mediterranean temporary ponds, listed as Near Threatened in IUCN's Red List based on inferred population decline caused by habitat loss and the negative effects of invasive species (Beja et al. 2009). Our study area is located in Sierra de Guadarrama (Madrid, Central Spain), where previous studies have reported female-biased dispersal in the species (Gutiérrez-Rodríguez et al. 2017b), and showed that vegetation heterogeneity plays an important role in promoting population connectivity at the landscape level (Gutiérrez-Rodríguez et al. 2017a). Here we extend previous studies and further characterize the demographic and spatial dynamics of a *P. waltl* metapopulation by estimating population size and assessing functional connectivity between the two main breeding ponds in the study area in relation to local abundance and sex-biased dispersal. We combine capture history records from a 12-year capture-mark-recapture monitoring program (2009–2020) with molecular-based estimates of migration rates and pairwise relatedness. We reconcile direct and indirect inferences of dispersal behaviour in *P. waltl* in light of its life history and discuss the role of density effects and pond colonization history in inferred connectivity patterns.

Materials and methods

Study area and monitoring program

A general description of the study area can be found in Sánchez-Montes and Martínez-Solano (2011) and Capellà-Marzo et al. (2020). Two temporary ponds are the main breeding sites for *P. waltl* in the area (Fig. 1): Laguna, a natural, seasonal pond with a surface of 12,000 m^2 that fills up with the winter rains and dries up in late spring or early summer; and Gravera, an abandoned, naturalized gravel pit

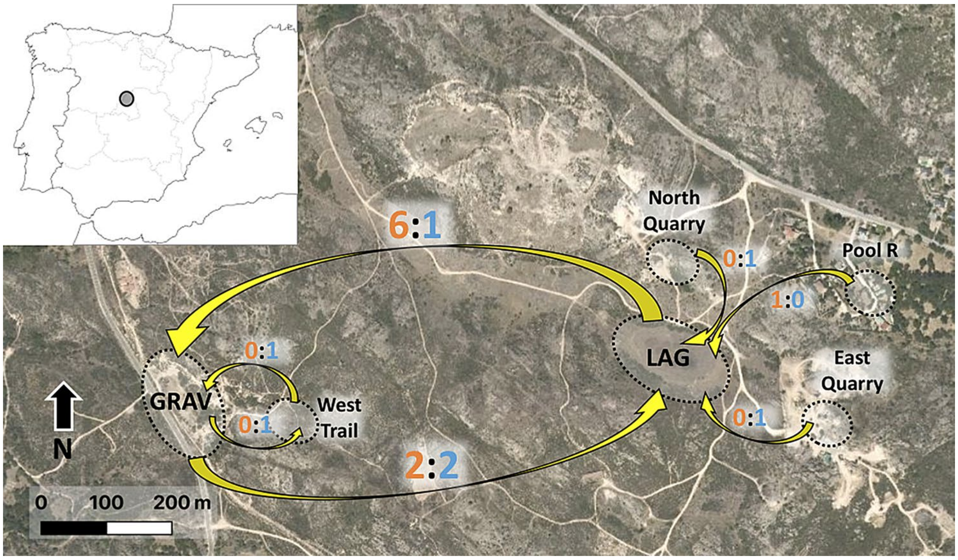

Fig. 1 Number of displacements detected in the study area during the 12-year monitoring period (2009–2020). Females: orange; males: blue. *GRAV* Gravera, *LAG* Laguna. Other locations where individuals of *P. waltl* were captured are also shown

of 3200 m^2 of surface area, maintaining water from early winter until August in most years. The two ponds are 700 m apart, with the topography gently sloping down from Laguna (1050 m above sea level -a.s.l.-) to Gravera (1000 m a.s.l.).

The *P. waltl* metapopulation inhabiting the study area has been subject to a capture-mark-recapture monitoring program since 2009. Details on sampling procedures are provided in Gutiérrez-Rodríguez et al. (2017b). Briefly, we performed several nocturnal surveys every year during the breeding season of *P. waltl*, mainly focusing on adult individuals. We captured newts using baited funnel traps and dip nets. We then sexed all captured individuals based on external morphological characters, marked them with PIT-tags with an unique alphanumeric code, clipped the 3rd toe of the right hind limb as a tissue sample for genetic analyses, and released the newts in the place of capture after processing. All procedures were approved by the Ethics Committee of Consejo Superior de Investigaciones Científicas, Spain (ref.: 710/2018) and Comunidad de Madrid (ref.: PROEX 040/19). In addition to Laguna and Gravera, we extended nocturnal surveys to terrestrial habitat (trails connecting the two ponds) and to four minor aquatic sites in the study area: two quarries presenting small temporary ponds and an abandoned swimming pool (Fig. 1). We registered the site of capture of each individual and recorded the geographic coordinates using a GPS in the case of terrestrial locations.

Genotyping with microsatellites

We genotyped tissue samples from 187 adult individuals of *P. waltl* captured in the study area during the 2018 breeding season (Gravera: 89 individuals, 47 females and 42 males; Laguna: 98 individuals, 51 females and 47 males). We used commercial kits (DNeasy® 96 Blood & Tissue Kit, Qiagen) for DNA extraction according to the manufacturer's protocol and genotyped individuals with 22 microsatellite loci (van de Vliet et al. 2009; Gutiérrez-Rodríguez et al. 2014) combined in 6 multiplex reactions using Type-it Microsatellite PCR kits (Qiagen). DNA fragment sizes were analysed with an ABI PRISM 3730 sequencer and, based on this information, we manually scored alleles for each individual using GeneMapper v4.0 (Applied Biosystems).

Local abundance (N) and effective size (N_e)

We estimated the local abundance of females and males in Gravera and Laguna in the 2018 breeding season by analysing the individual capture history records using the POPAN formulation (Schwarz and Arnason 1996) in program MARK v9.0 (White and Burnham 1999). This formulation is well-suited for the analysis of open populations and models four parameters: (1) the population size (N), considering all individuals present in each breeding pond during the analysed period, (2) the probability of survival of individuals across sampling sessions (ϕ), (3) the probability of capture of each individual at each sampling session (p), and (4) the proportion of N that enters the population before each sampling session (*pent*). We estimated N separately for each sex and breeding pond, and built a set of models assuming parameters ϕ, p and *pent* as either constant (.) or variable depending on sex (g), time (t) or both factors and their interaction (g*t). Therefore, the maximum number of parameters in each analysis (corresponding to the saturated model) was the sum of 2*(n – 1) ϕ, 2*n p, 2*n *pent* and 2 N parameters, with n being the number of sampling sessions in the dataset. Due to strong differences in activity periods between sexes, we ran separate analyses for females and males in Laguna to avoid model overparameterization. We assessed the likelihood of all constructed models and ranked them based on Akaike's information criterion corrected for small sample sizes (AICc) (Akaike 1974).

We used the software COLONY v2.0.6.5 (Jones and Wang 2010) to estimate N_e for Gravera and Laguna, separately. All the available genotypes of adult individuals captured at each pond were included as the corresponding 'offspring sample' in each analysis to estimate N_e from the inferred frequency of sibs (Wang 2009b). We accounted for the possibility of a polygamous mating system in both sexes and assumed random mating within each breeding pond. We ran five replicate analyses with medium run lengths and high precision. We used N_e estimates from Colony and N estimates from MARK to calculate the effective to census population size ratio as N_e/N for each breeding site separately.

Genetic structure and functional connectivity

We calculated the summary statistics for each locus and the multilocus estimates of allelic richness, observed and expected heterozygosity and inbreeding coefficient F for Gravera and Laguna with the R package *adegenet* (Jombart 2008), and used GenAlEx v.6.503 (Peakall and Smouse 2012) to estimate F_{ST} between the two breeding populations. We then estimated bi-directional migration rates per generation using BayesAss v3.0.4 (Wilson and Rannala 2003). This program assumes migration rates to be small and constant over short periods of time (Faubet et al. 2007). We ran ten independent analyses to check for convergence of results. Each analysis was run for 5,000,000 iterations, with the first 1,000,000 iterations discarded as burn-in, and mixing parameters for allelic frequencies, inbreeding coefficients and migration rates set to 0.4, 0.6 and 0.1, respectively. These values provided acceptance rates between 20 and 60% in MCMC chains, which are optimal according to the software manual. We assessed convergence of migration rate estimates across replicate runs by checking logfiles with Tracer v1.7.1 software

(Rambaut et al. 2018). The probability of ancestry for each individual was divided into three categories: non-migrant (resident), first-generation migrant, and second-generation migrant.

As a complementary, indirect approach, we compared values of pairwise genetic relatedness between individuals from the same and from different ponds (to detect possible differences in the net immigration/emigration balance across sites) and among sexes (to test for sex-biased dispersal). We hypothesize that individuals from the pond receiving more migrants should show significantly lower average pairwise relatedness values, and that the more philopatric sex should show significantly higher average pairwise relatedness values. We used the software COANCESTRY v1.0.1.9 (Wang 2011) to estimate pairwise relatedness between all pairs of individuals with five moment estimators (Lynch 1988; Lynch and Ritland 1999; Queller and Goodnight 1989; Ritland 1996; Wang 2002) and two maximum likelihood estimators (Milligan 2003; Wang 2007). We tested for differences between groups (Gravera vs. Laguna and females vs. males) by fitting linear models in R (R Core Team 2019), using the values of each relatedness estimator as the response variable and either the sex or the sampling pond as the grouping factor. We assessed the normality of residuals by Shapiro–Wilk tests and visual inspection of qqplots and used non-parametric Wilcoxon tests (Wilcoxon 1945) in cases of non-normality of residuals.

Results

During our 12-year monitoring of the *P. waltl* metapopulation, we recorded a total of 4562 captures representing 1423 different individuals (696 females and 727 males). A large proportion of these individuals (70.5% overall: 72.3% of females and 68.8% of males) were captured on multiple occasions, with one to 19 recaptures per individual. Considering 1009 individuals with two or more captures throughout the 12-year period, we only recorded 16 displacements (i.e. captures of marked individuals in a different location from that of the previous capture). These displacements were performed by 13 different individuals (1.3%), including eleven dispersal events between Gravera and Laguna (Fig. 1). In some individuals we recorded more than one dispersal event, including two females that travelled from Gravera to Laguna and then back to Gravera in a single year. During the 2018 breeding season (when samples for molecular analyses were collected) we recorded 1032 captures, corresponding to 575 different individuals (345 females and 230 males). The recapture rate in this breeding season was 44.1% for females and 29.1% for males.

Table 1 Comparison of POPAN models for Laguna and Gravera in the 2018 breeding season, ranked by their AICc weight

Model	AICc	AICc weight	Param	Deviance
Gravera				
$\phi(.)p(g*t)pent(g*t)$	1183.72	0.902	51	−387.5
$\phi(g)p(g*t)pent(g*t)$	1188.46	0.098	52	−385.8
$\phi(t)p(g*t)pent(g*t)$	1203.40	< 0.001	62	−398.9
$\phi(g^{*}t)p(g*t)pent(g*t)$	1235.28	0	74	−403.5
Laguna females				
$\phi(.)p(t)pent(t)$	860.55	1	32	−481.0
$\phi(t)p(t)pent(t)$	892.11	0	46	−487.6
Laguna males				
$\phi(.)p(t)pent(t)$	765.27	1	32	−604.1
$\phi(t)p(t)pent(t)$	798.75	0	46	−609.5

The number of parameters (Param.) of the saturated model in each locality can be calculated as $s*(n-1)\ \phi + s*n\ p + s*n\ pent + s\ N$, minus the number of confounding parameters, being s the number of different sexes and n the number of sampling sessions accounted for in the analysis

Table 2 Estimates (and 95% confidence intervals) of the number of females ($N_{♀}$) and males ($N_{♂}$) of *P. waltl* and the effective population size (N_e) in Gravera and Laguna

Parameter	Gravera	Laguna
$N_{♀}$	190 (161–250)	265 (218–357)
$N_{♂}$	138 (114–179)	340 (276–445)
Sex-ratio	1.38	0.78
N_e	60 (42–89)	64 (46–94)
N_e/N	0.18	0.11

Sex-ratio is calculated as $N_{♀}/N_{♂}$. The effective to census size ratio is calculated as $N_e/(N_{♀}+N_{♂})$

Local abundance (N) and effective size (N_e)

The best models according to AICc values showed that apparent survival (ϕ) was approximately constant throughout the 2018 breeding season, whereas the probability of capture (p) and the probability of entrance of individuals ($pent$) showed variation across sexes and sampling sessions (Table 1 and Supplemental Tables S1-S3). The estimated abundance of *P. waltl* in Laguna was almost double that of Gravera, with the sex-ratio slightly biased towards males in the former and towards females in the latter (Table 2). Estimates of N_e were similar in both ponds (Gravera: mean 60, 95% CI 42–89; Laguna: mean 64, 95% CI 46–94). The corresponding effective to census population size ratios were 0.18 in Gravera and 0.11 in Laguna (Table 2).

Genetic structure and functional connectivity

Based on abundance estimates, the genotyped sample represents between 14 and 30% of the females and males active during the 2018 breeding season at each breeding pond. We found no significant differences in allelic richness between ponds (allelic richness Laguna = 4.955, Gravera = 4.727; Wilcoxon rank test, W = 234, p = 0.856) (Table S4, Figure S1). Genetic differentiation between the two breeding populations was significantly > 0 ($p < 0.0001$ based on 9999 permutations), with an estimated F_{ST} of 0.009. Most BayesAss replicate analyses (8/10) converged on likelihood scores around −7419, while two replicates apparently got stuck on a local maximum (likelihood scores around −7449). Based on the runs with the best likelihood, migration rates were asymmetric: 31.83% from Laguna to Gravera and 5.24% in the opposite direction, that is, higher migration from the larger to the smaller population. Most individuals from Laguna (90.81%) were assigned to the resident ancestry class, whereas the vast majority of individuals from Gravera (98.87%) were classified as 1st or 2nd generation migrants from Laguna (Fig. 2).

We found significant differences in four out of seven relatedness estimators calculated with Coancestry in pooled samples from Gravera versus Laguna, with higher relatedness in individuals from Laguna in three cases (Table S5). Females showed significantly higher genetic relatedness than males overall (i.e., pooling samples from both ponds) in three out of seven estimators (Table S5). These differences between sexes were mainly driven by females from Laguna, which showed significantly higher relatedness than males in three estimators, whereas only one estimator showed significant differences among sexes in Gravera (Table S5).

Discussion

Despite the importance of functional connectivity in the dynamics of spatially structured populations, very little is known about this process in pond-breeding amphibians (Cayuela et al. 2020). Specifically, few studies have used capture-mark-recapture data in combination with molecular-based estimates of gene flow to quantify typical dispersal rates and distances across species (Schmidt et al. 2006). Our integrative approach benefits from this combination, resolving some of the shortcomings of each approach when implemented independently. For instance, capture-mark-recapture data can provide misleading inferences about connectivity, because of the heterogeneous probabilities of detection of individuals (which can be accounted for in some models), and because not all recorded dispersal events result in successful reproduction and thus in gene flow (Gamble

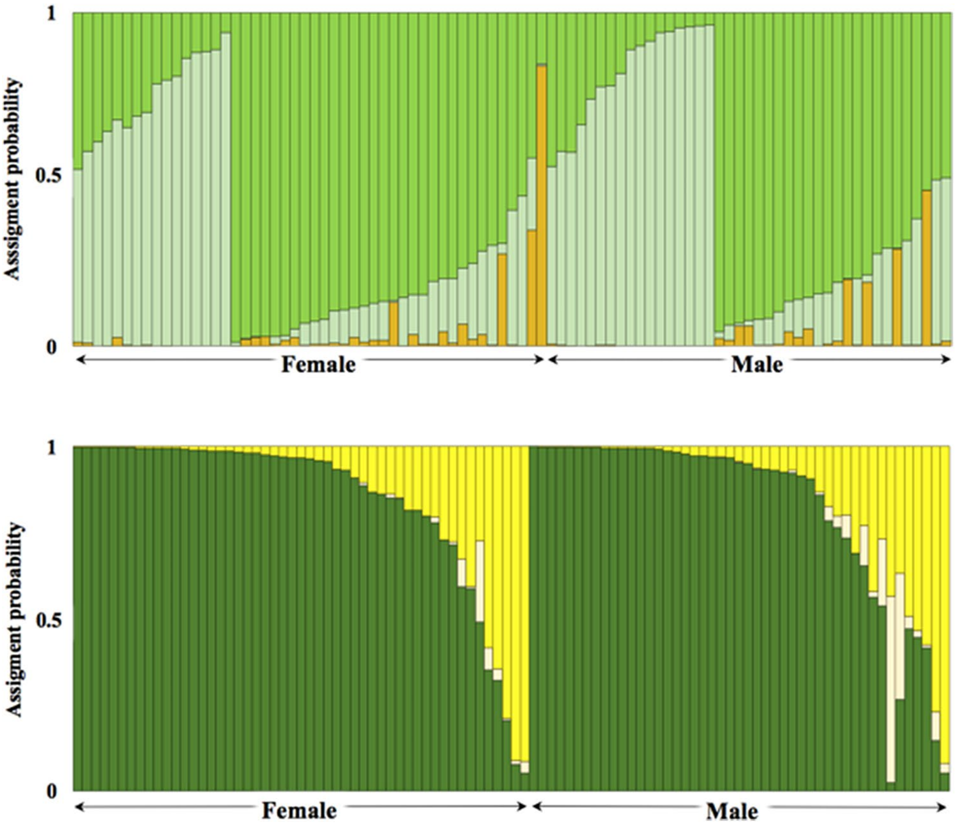

Fig. 2 Probability of ancestry estimated for each male and female of *P. waltl* genotyped in Gravera (top) and Laguna (bottom). Ancestry categories include: resident from Gravera (orange) or Laguna (dark green), or migrant (first-generation migrant from Gravera: light yellow; second-generation migrant from Gravera: bright yellow; first-generation migrant from Laguna: light green; second-generation migrant from Laguna: intermediate green)

et al. 2007; Sinsch 2014). In turn, migration rate estimates based on molecular data rely on analytical assumptions that are often not met in natural populations and thus cannot be directly equated with those based from capture-mark-recapture data (Jehle et al. 2005; Lowe and Alledorf 2010). Moreover, it is difficult to ascertain the timeframe to which genetic estimates apply (Waples 2005). Taken together, with consideration of the species natural history, direct and indirect estimates can be reconciled, producing robust insights on functional connectivity in natural populations.

Direct estimates of dispersal rates and recorded distances during our 12-year monitoring program suggest that dispersal is a rare event in this *P. waltl* metapopulation, with only 1.3% of recaptured individuals moving from their place of initial capture, even lower than in other European urodeles (Schmidt et al. 2006). This low estimate contrasts with that estimated for a syntopic species (*Pelophylax perezi*), which shows high dispersal rates between the same breeding sites (20.1%, Capellà-Marzo et al. 2020). A possible bias may result from juvenile-mediated dispersal (Sjögren-Gulve 1998; Pittman et al. 2014; Sinsch 2014). However, over 12 years of monitoring we marked a large number of juveniles, most of which (244 out of 245, 99.5%) were recaptured again in the pond where they were first captured. Thus, our results can be taken as representative of the dispersal behaviour of *P. waltl* in the study area and suggest strong site-fidelity in the species.

The relatively long distance between the two major breeding sites in the study area does not seem to be the limiting factor to dispersal, because shorter dispersal events were also rare, although they may have been underestimated because secondary sites (trails, quarries, abandoned pool) were not sampled as frequently as the two main breeding sites. Regardless, dispersal rates are probably higher in areas where breeding pond density is higher. While the number of observations is limited, more marked individuals moved from the larger (Laguna) to the smaller population (Gravera) than in the opposite direction (Fig. 1), suggesting density-dependent dispersal. An alternative explanation is that dispersal is instead shaped by topography, with animals moving preferentially downslope from Laguna to Gravera. This is consistent with landscape genetics studies showing a significant effect of slope on resistance to gene flow (Gutiérrez-Rodríguez et al. 2017a).

Low observed dispersal between Laguna and Gravera is in agreement with molecular-based estimates showing significant genetic differentiation between the two major breeding sites. This differentiation is in the lower range of that reported for other urodeles at similar spatial scales ($F_{ST}=0$–0.046, Schmidt et al. 2006). However, we also verified gene flow between ponds, as inferred from BayesAss analyses. Although low genetic differentiation in a two-population system is a challenging scenario for the inference of migration rates with this program (Meirmans 2014), most runs, including those with the best likelihood scores and lowest deviance, supported asymmetric migration, with a preferred direction (Laguna to Gravera) consistent with direct observations based on capture-recapture data. The lower overall relatedness among individuals from Gravera is also in agreement with this interpretation. Considering that, from a genetic point of view, more than a single effective migrant per generation is considered to be enough to homogenize populations (Wright, 1931, 1940; Mills and Allendorf, 1996), our results call for the joint management of the two ponds as a single management unit (currently, only Laguna is protected by regional legislation).

Contemporary low dispersal and indirect evidence of high genetic connectivity between the two breeding sites can be reconciled based on the sharp differences in the composition of ancestry classes in each pond. Most genotyped individuals in Gravera were inferred to be first- or second-generation migrants from Laguna, suggesting that the former population was founded by individuals migrating from Laguna in the recent past (within two–three generations). Current patterns of genetic structure result from both historical and present-day processes (Newman and Squire 2001; Zellmer and Knowles 2009). Considering the longevity of *P. waltl*, which attains sexual maturity in the second or third post-metamorphic year, but can live well over ten years in the wild (based on our own capture-recapture data, unpublished), these results are consistent with the timeframe associated with the origin of this pond, formed in the 1973–1986 period (Figure S2). Mining activities ceased after this period and the area is now naturalized. Other possible scenarios include local extinction-recolonization dynamics, where frequent local extinctions are offset by recolonization from nearby populations (McCauley 1993). However, the stability of the two breeding ponds and the longevity of the species suggest local extinctions are infrequent. Colonization of the newly available habitat in Gravera probably resulted in high biological success of migrant individuals, leading to both high reproductive success of adult migrants and the successful establishment of juvenile migrants (Semlitsch 2008). This initial dispersal event from Laguna to Gravera might have been driven by density effects. Subsequently, as the Gravera population increased in size, successful establishment of migrants may have been less frequent, with occasional migrants maintaining functional connectivity thereafter (Harrison and Hastings 1996). While some rare alleles present in Laguna are not found in Gravera, overall both populations have similar levels of genetic diversity (Figure S1, Table S4), which is consistent with sustained migration over time from the onset of first colonization to the present, rather than with a strong founder effect.

We did not find conclusive evidence of sex-biased dispersal in *P. waltl*. On the one hand, most individuals with recorded displacements between Gravera and Laguna were females, although absolute frequencies were low. On the other hand, three out of seven estimators showed significantly higher relatedness among females, thus suggesting male-biased dispersal, whereas in a previous study of the Laguna population, Gutiérrez-Rodríguez et al. (2017b) found evidence for the opposite pattern. These discrepancies suggest that males and females may be equally philopatric, with a minor proportion of individuals of both sexes migrating away from their natal ponds, a common pattern in urodeles (Schmidt et al. 2006). Philopatric behaviour probably evolved to exploit stable breeding habitats (Johnson and Gaines 1990), which are highly fragmented in the Mediterranean region due to both natural causes and human activities, thus favouring this behaviour (Smith and Green 2005).

In conclusion, we found direct and indirect evidence that functional connectivity among *P. waltl* breeding demes relies on a low number of migrants per generation at the spatial scale considered (< 1 km). This low migration rate may be, however, essential to allow colonization of new breeding habitats and avoid the deleterious effects of genetic erosion and inbreeding which threaten isolated populations (Frankham et al. 2014). In view of the low effective population sizes estimated (which are below the recommended thresholds for conservation in Frankham et al. 2014), and considering the rapid loss of temporary ponds across its range (Beja et al. 2009), maintaining functional connectivity across breeding sites seems critical for the viability of population networks at the landscape scale. The high longevity of the species probably explains its resilience in heavily altered habitats, and its capacity to maintain gene flow between breeding sites with a very low number of dispersers per year. Our study provides new data about life history traits that are relevant for the management of the species, especially migration rates, which are key to assess patterns of functional connectivity. By integrating population size estimates and direct records of dispersal rates (based on long-term capture-mark-recapture data), we were able to link molecular-based inferences and actual individual movement patterns. Our results can be applied to the design of conservation actions aimed at improving functional connectivity; specifically, the creation and maintenance of networks of ponds in a stepping-stone scheme, with habitat corridors that take into account the frequency and spatial scale at which dispersal events occur in the species.

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Data availability Genotypes of all individuals are available from the authors upon request.

Declarations

Conflict of interest The authors declare no competing interests.

Informed consent All authors consent to submitting this article to Conservation Genetics.

Research involving human and animal rights All procedures were approved by the Ethics Committee of Consejo Superior de Investigaciones Científicas, Spain (ref.: 710/2018) and Comunidad de Madrid (ref.: PROEX 040/19).

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Contr 19:716–723

Beja P, Bosch J, Tejedo M, Edgar P, Donaire-Barroso D, Lizana M, Martínez-Solano Í, Salvador A, García-París M, Recuero Gil E, Slimani T, El Mouden EH, Geniez P (2009) *Pleurodeles waltl*. IUCN Red List Threat Species 2009:e.T59463A1192633

Capellà-Marzo B, Sánchez-Montes G, Martínez-Solano I (2020) Contrasting demographic trends and asymmetric migration rates in a spatially structured amphibian population. Integr Zool 15:482–497

Cayuela H, Valenzuela-Sánchez A, Teulier L, Martínez-Solano Í, Léna JP, Merilä J, Muths E, Shine R, Quay L, Denoël M, Clobert J, Schmidt BR (2020) Determinants and consequences of dispersal in vertebrates with complex life cycles: a review of pond-breeding amphibians. Q Rev Biol 95:1–36

Çiçek K, Cumhuriyet O (2017) Amphibians and reptiles of the Mediterranean basin. In: Fuerst-Bjelis B (ed) Mediterranean identities: environment, society, culture. InTech, Rijeka, pp 203–237

Díaz-Paniagua C (1990) Temporary ponds as breeding sites of amphibians at a locality in Southwestern Spain. Herpetol J 1:447–453

Faubet P, Waples R, Fourier UJ (2007) Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. Mol Ecol 16:1149–1166

Ficetola GF, Barzaghi B, Melotto A, Muraro M, Lunghi E, Canedoli C, Carretero MA (2018) N-mixture models reliably estimate the abundance of small vertebrates. Sci Rep 8:1–8

Frankham R, Bradshaw CJ, Brook BW (2014) Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol Conserv 170:56–63

Gamble LR, McGarigal K, Compton BW (2007) Fidelity and dispersal in the pond-breeding amphibian, *Ambystoma opacum*:

implications for spatio-temporal population dynamics and conservation. Biol Conserv 139:247–257

Gutiérrez-Rodríguez J, González EG, Martínez-Solano Í (2014) Development and characterization of twelve new polymorphic microsatellite loci in the Iberian ribbed newt, *Pleurodeles waltl* (Caudata: Salamandridae), with data on cross-amplification in *P. nebulosus*. Amphibia-Reptilia 35:129–134

Gutiérrez-Rodríguez J, Gonçalves J, Civantos E, Martínez-Solano Í (2017a) Comparative landscape genetics of pond-breeding amphibians in Mediterranean temporal wetlands: the positive role of structural heterogeneity in promoting gene flow. Mol Ecol 26:5407–5420

Gutiérrez-Rodríguez J, Sánchez-Montes G, Martínez-Solano Í (2017b) Effective to census population size ratios in two Near Threatened Mediterranean amphibians: *Pleurodeles waltl* and *Pelobates cultripes*. Conserv Genet 18:1201–1211

Harrison S, Hastings A (1996) Genetic and evolutionary consequences of metapopulation structure. Trends Ecol Evol 11:180–183

Jehle R, Wilson GA, Arntzen JW, Burke T (2005) Contemporary gene flow and the spatio-temporal genetic structure of subdivided newt populations (*Triturus cristatus, T. marmoratus*). J Evol Biol 18:619–628

Johnson ML, Gaines MS (1990) Evolution of dispersal: theoretical models and empirical tests using birds and mammals. Annu Rev Ecol Evol Syst 21:449–480

Jombart T (2008) *adegenet*: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555

Leung B, Greenberg DA, Green DM (2017) Trends in mean growth and stability in temperate vertebrate populations. Divers Distrib 23:1372–1380

Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? Mol Ecol 19:3038–3051

Lynch M (1988) Estimation of relatedness by DNA fingerprinting. Mol Biol Evol 5:584–599

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. Genetics 152:1753–1766

Mazerolle MJ (2001) Amphibian activity, movement patterns, and body size in fragmented peat bogs. J Herpetol 35:13–20

McCauley DE (1993) Evolution in metapopulations with frequent local extinction and recolonization. Oxf Surv Evol Biol 9:109–134

Meirmans PG (2014) Nonconvergence in Bayesian estimation of migration rates. Mol Ecol Resour 14:726–733

Milligan BG (2003) Maximum-likelihood estimation of relatedness. Genetics 163:1153–1167

Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. Conserv Biol 10:1509–1518

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858

Newman RA, Squire T (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). Mol Ecol 10:1087–1100

Palstra FP, Fraser DJ (2012) Effective/census population size ratio estimation: a compendium and appraisal. Ecol Evol 2:2357–2365

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research: an update. Bioinformatics 28:2537–2539

Petranka JW, Smith CK, Scott AF (2004) Identifying the minimal demographic unit for monitoring pond-breeding amphibians. Ecol Appl 14:1065–1078

Pittman SE, Osbourn MS, Semlitsch RD (2014) Movement ecology of amphibians: a missing component for understanding population declines. Biol Conserv 169:44–53

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol 67:901–904

Ritland K (1996) Estimators for pairwise relatedness and individual inbreeding coefficients. Genet Res (Camb) 67:175–185

Sánchez-Montes G, Martínez-Solano Í (2011) Population size, habitat use and movement patterns during the breeding season in a population of Perez's frog (*Pelophylax perezi*) in central Spain. Basic Appl Herpetol 25:81–96

Schmidt P, Weddeling K, Thomas M, Rottscheidt R, Tarkhnishvili DN, Hachtel M (2006) Dispersal of *Triturus alpestris* and *T. vulgaris* in agricultural landscapes–comparing estimates from allozyme markers and capture-mark-recapture analysis. In: Vences M, Köhler J, Ziegler T, Böhme W (eds.) Herpetologia Bonnensis II: Proceedings of the 13th Congress of the Societas Europaea Herpetologica, Bonn, pp 139–143

Schwarz CJ, Arnason AN (1996) A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics 52:860–873

Semlitsch RD (2008) Differentiating migration and dispersal processes for pond-breeding amphibians. J Wildl Manag 72:260–267

Sinsch U, Oromi N, Miaud C, Denton J, Sanuy D (2012) Connectivity of local amphibian populations: modelling the migratory capacity of radio-tracked natterjack toads. Anim Conserv 15:388–396

Sinsch U (2014) Movement ecology of amphibians: from individual migratory behaviour to spatially structured populations in heterogeneous landscapes. Can J Zool 92:491–502

Sjögren-Gulve P (1998) Spatial movement patterns in frogs: differences between three *Rana* species. Ecoscience 5:148–155

Smith MA, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28:110–128

Trochet A, Le Chevalier H, Calvez O, Barthe L, Isselin-Nondedeu F, Picard D, Debelgarric M, Pégourie N, Rocher R, Ribéron A (2017) Postbreeding movements in marbled newts (Caudata, Salamandridae): a comparative radiotracking study in two habitat types. Herpetologica 73:1–9

van de Vliet MS, Diekmann OE, Serrao EA, Beja P (2009) Isolation of highly polymorphic microsatellite loci for a species with a large genome size: sharp-ribbed salamander (*Pleurodeles waltl*). Mol Ecol Resour 9:425–428

Wake DB, Koo MS (2018) Amphibians. Curr Biol 28:R1237–R1241

Wang IJ (2009a) Fine-scale population structure in a desert amphibian: landscape genetics of the black toad (*Bufo exsul*). Mol Ecol 18:3847–3856

Wang J (2002) An estimator for pairwise relatedness using molecular markers. Genetics 160:1203–1215

Wang J (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. Genet Res (Camb) 89:135–153

Wang J (2009b) A new method for estimating effective population sizes from a single sample of multilocus genotypes. Mol Ecol 18:2148–2164

Wang J (2011) COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol Ecol Resour 11:141–145

Wang J, Santiago E, Caballero A (2016) Prediction and estimation of effective population size. Heredity 117:193–206

Waples R (2005) Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? Mol Ecol 14:3335–3352

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird Study 46:S120–S139

Wilcoxon F (1945) Individual comparisons by ranking methods. Biometrics 1:80–83

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97–159

Wright S (1940) Breeding structure of populations in relation to speciation. Am Nat 74:232–248

Zellmer AJ, Knowles LL (2009) Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. Mol Ecol 18:3593–3602

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