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ORIGINAL PAPER

Fine-scale genetic structure in a salamander with two reproductive modes: Does reproductive mode affect dispersal?

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Abstract
Reproduction is intimately linked with dispersal, but the effects of changes in reproductive strategies on dispersal have received little attention. Such changes have occurred in many taxonomic groups, resulting in profound alterations in life-history. In amphibians, many species shifted from oviparous/larviparous aquatic reproduction (deposition of eggs or pre-metamorphic larvae in water) to pueriparous terrestrial reproduction (parturition of terrestrial juveniles). The latter provides greater independence from water by skipping the aquatic larval stage; however, the eco-evolutionary implications of this evolutionary step have been underexplored, largely because reproductive modes rarely vary at the intraspecific level, preventing meaningful comparisons. We studied the effects of a transition to pueriparity on dispersal and fine-scale genetic structure in the fire salamander (*Salamandra salamandra*), a species exhibiting two co-occurring reproductive modes: larviparity and pueriparity. We performed genetic analyses (parentage and genetic spatial autocorrelation) using 11 microsatellite loci to compare dispersal and fine-scale genetic structure in three larviparous and three pueriparous populations (354 individuals in total). We did not find significant differences between reproductive modes, but in some larviparous populations movement patterns may be influenced by site-specific features (type of water bodies), possibly due to passive water-borne dispersal of larvae along streams. Additionally, females (especially larviparous ones) appeared to be more philopatric, while males showed greater variation in dispersal distances. This study also points to future avenues of research to better understand the eco-evolutionary implications of changes in reproductive modes in amphibians.

Keywords Dispersal · Larviparity · Pueriparity · Transition in reproductive mode · *Salamandra salamandra* · Spatial autocorrelation

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Introduction

Dispersal influences many ecological (e.g. tracking optimal conditions), demographic (e.g. regulating population density), and evolutionary (e.g. gene flow) processes, contributing to the long-term persistence of populations (Bowler and Benton 2005; Cosgrove et al. 2018). Dispersal is a trait with multi-causality, governed by a complex interplay between intrinsic (phenotype-dependent) and extrinsic (environment-dependent) factors (Clobert et al. 2009; Ousterhout and Semlitsch 2018), which often promote high variability in dispersal-related traits (e.g. physical capacity for dispersal and movement behaviour) not only among but also within species (Stevens et al. 2010). The effects of environmental variation (including landscape composition and configuration, climate, and topography) between patches on animal movement have been shown to influence patterns of dispersal to a large extent (e.g. Velo-Antón et al. 2013; Wang 2013; Ousterhout and Semlitsch 2018). Besides extrinsic factors, a multitude of intrinsic traits (e.g. body size, age, sex, physiology, behaviour and genetic) are known to covary with dispersal-related traits (Ronce and Clobert 2012; Saastamoinen et al. 2018). In particular, sex is commonly associated with differences in dispersal in various taxa, with individuals of one sex usually moving farther due to unequal social (e.g. mating system) and/or ecological (e.g. competition for mates or resources) pressures acting upon them (Trochet et al. 2016).

Because dispersal is the primary mechanism driving gene flow, dispersal-related traits and other co-evolving traits are often intimately linked with reproductive biology, evolving in a way to increase fitness and reproductive success (Bowler and Benton 2005; Bonte et al. 2012). Hence, species that have undergone transitions in reproductive modes may have also experienced evolutionary changes in dispersal-related traits, but this topic has remained largely underexplored. A transition from an egg-laying reproductive mode to a live-bearing (viviparous) one has occurred more than 150 times in a wide array of vertebrates (mostly in reptiles and, to a lesser extent, in amphibians; reviewed in Blackburn 2015), entailing profound morphological, physiological, life-history, behavioural, ecological, and genetic changes, especially in females (e.g. Buckley et al. 2007; van Dyke et al. 2014; Shine 2015; Helmstetter et al. 2016; Haliwell et al. 2017). Previous work has shown that a transition to viviparity may affect dispersal capacity. For instance, in reptiles and fishes, viviparous females incur greater energetic costs and, consequently, have lower dispersal abilities due to the physical burden of carrying offspring for a longer period compared to egg-laying congeners (see Shine 1980, 2015; Banet et al. 2016). Additionally, dispersal behaviour in these examples is expected to be governed to a much lesser extent by the surrounding environment, because the shift in reproductive mode (e.g. live-bearing) entailed a greater independence from habitat features required for successful reproduction (e.g. suitable nests for egg deposition in reptiles and water bodies for development of embryos and larvae in amphibians; see Russell et al. 2005; Shine 2015). This subject, in particular, has received very little attention, although a couple of studies on reptiles have suggested that low availability of nesting sites promotes longer movements of oviparous, compared to viviparous females, because they must seek suitable sites for egg deposition (see Shine 2015).

In the three amphibian orders, there are examples of species shifting from ancestral oviparous or larviparous aquatic reproduction (delivery of eggs or larvae in water, respectively) to pueriparous terrestrial reproduction (parturition of terrestrial juveniles; Blackburn 2015), possibly triggered by the lack of surface water in drier environments, as proposed by Velo-Antón et al. (2015) in *Salamandra*. The larval stage in pueriparous amphibians is absent, conferring a fully terrestrial lifestyle and total independence from

water when depositing offspring. Given that the dispersal ecology of aquatic-breeding amphibians is intrinsically linked to the distribution and availability of water sources for reproduction (Russell et al. 2005; Semlitsch 2008), a shift from aquatic reproduction to terrestrial is expected to bring changes in dispersal behaviour. Specifically, terrestrial modes of reproduction putatively allow individuals to expand home ranges and colonize new areas to exploit more resources without relying on proximity to suitable water bodies (Liedtke et al. 2017; Lourenço et al. 2017). Based on this premise, previous landscape studies have suggested that terrestrial reproduction in amphibians may promote higher connectivity in heterogeneous, fragmented landscapes, given their ability to thrive in water-limited environments (direct-developer Dwarf squeaker frog, *Arthroleptis xenodactyloides*, Measey et al. 2007; pueriparous Nimba toad, *Nimbaphrynoides occidentalis*, Sandberger-Loua et al. 2018). Conversely, the lungless plethodontid salamanders (Plethodontidae), in which most species exhibit terrestrial reproduction (direct-developing), have very limited dispersal capacity (typically <60 m; reviewed in Smith and Green 2005) and significant genetic differentiation over fine spatial scales mostly due to their high susceptibility to desiccation (e.g. *Batrachoseps attenuates*, Martínez-Solano et al. 2007; *Plethodon albagula*, Peterman et al. 2014).

Previous studies, however, have not included direct comparisons between aquatic and terrestrial breeding amphibians with similar traits and inhabiting analogous landscape contexts and, therefore, do not provide strong inferences about the effects of terrestrial reproduction on dispersal. Studies including species with multiple reproductive modes (aquatic versus terrestrial reproduction) at the intraspecific level are crucial for performing comparative analyses that provide quantitative assessments of the effects of reproductive mode on dispersal. Such systems can reduce the potential bias arising from comparisons between closely related species, in which confounding factors such as differences in other phenotypic traits and the environments they inhabit may prevent robust conclusions. However, variation in reproductive modes within species is rare (Blackburn 2015; Velo-Antón et al. 2015).

In amphibians, intraspecific variation in reproductive strategies involving live-bearing has been reported in only two sister urodele species, the fire salamander (*Salamandra salamandra*, Linnaeus 1758; Velo-Antón et al. 2015) and the North-African fire salamander (*S. algira*, Bedriaga 1883; Dinis and Velo-Antón 2017). To better understand the influence of terrestrial reproduction on movement, we used *S. salamandra* as a model system. Two distinct reproductive strategies co-occur in *S. salamandra*: larviparity, in which females deliver up to ca. 90 larvae in water bodies (e.g. streams and ponds) after a gestation period of approximately 90 days; and pueriparity, in which females deliver ca. 1–35 fully metamorphosed terrestrial juveniles after the same gestation period (Buckley et al. 2007; Velo-Antón et al. 2015). Throughout most of its range, *S. salamandra* females are larviparous (the ancestral trait), but pueriparity is present in three Iberian subspecies (*S. s. bernardezi*, *S. s. fastuosa* and *S. s. gallaica*; Velo-Antón et al. 2015). While pueriparity in *S. s. bernardezi* likely arose during the Pleistocene in the Cantabrian Mountains (north of Spain), later introgressing eastwards with *S. s. fastuosa* populations during subsequent cycles of warm and cold climates (García-París et al. 2003), pueriparity in *S. s. gallaica* originated independently in only two insular populations of northwestern Spain (Velo-Antón et al. 2007, 2012). Moreover, not only because shifts in reproductive strategies can cause greater changes in the biology and ecology of females, but also because sex itself is a major factor influencing dispersal within species, testing for sex-biased dispersal in *S. salamandra* may contribute additional insights into the role of terrestrial reproduction on dispersal and its evolutionary consequences. Whether there are sex-specific differences in dispersal in *S.*

salamandra is unclear. While Schulte et al. (2007) did not observe sex-specific differences in movement patterns in *S. s. terrestris*, several studies suggested male-biased dispersal as a potential driver of the mito-nuclear discordances observed in *S. salamandra* across different regions and different subspecies (northern Spain, García-París et al. 2003; central Spain, Pereira et al. 2016). Moreover, Helfer et al. (2012) used ecological and molecular methods to confirm male-biased dispersal in an alpine *Salamandra* species (*S. atra*).

Here, we use multilocus nuclear genetic data (microsatellites) to compare dispersal and fine-scale genetic structure between larviparous and pueriparous populations in *S. salamandra*. We hypothesize that (H1) pueriparous females will exhibit higher genetic similarity at greater distances (genetic autocorrelation), due to dispersal behaviour promoting longer dispersal movements, compared to larviparous females. This pattern is expected to arise due to greater dependency on proximity to water for delivery of larvae in larviparous females. Furthermore, given the mito-nuclear discordances found across the range of *S. salamandra,* likely due to male-biased dispersal, together with available evidence of such mechanism in other *Salamandra* species, we expect (H2) that males move farther than females.

Materials and methods

Study area and sampling

Our study focused on two *S. salamandra* Iberian subspecies—*S. s. gallaica* and *S. s. bernardezi*—co-distributed across the western Iberian Peninsula (Fig. 1). The subspecies *S. s. gallaica* exhibits exclusively larviparity in mainland populations, whereas *S. s. bernardezi* exhibits pueriparous reproduction (Velo-Antón and Buckley 2015). The two known pueriparous insular populations of *S. s. gallaica* were not included in this study due to their specific characteristics (isolated populations, low genetic diversity, and differentiated behaviour; Velo-Antón et al. 2012; Velo-Antón and Cordero-Rivera 2017). The environmental niche of *S. s. gallaica* (which includes Mediterranean and Atlantic ecosystems) is wider than in *S. s. bernardezi* (only occurring in Atlantic ecosystems; Velo-Antón and Buckley 2015). Because environmental variation may influence patterns of dispersal (Cosgrove et al. 2018), we restricted sampling of *S. s. gallaica* to northwestern Spain, where climate (Atlantic influence) and vegetation (e.g. predominance of deciduous forests of *Quercus* spp.) are similar to the Cantabrian region (Amigo et al. 2017). Both subspecies also share a contact zone in this region (Fig. 1), where substantial genetic and phenotypic admixture takes place (Galán 2007; Velo-Antón unpublished data), and thus we did not include populations from this hybrid zone.

We sampled during rainy nights in the months of April and November of 2016–2017, coinciding with the periods of highest activity of adult salamanders in northern Spain. We collected a total of 354 toe clip samples from individual adults (180 males and 174 females) in six localities (three per reproductive mode), with a sample size per locality of 53–67 (Fig. 1; Table 1). The impact of this procedure on the animals is expected to be minimal, as fire salamanders are capable of regenerating limbs within a few weeks (see Blaustein et al. 2018). The sampled localities exhibit favourable habitat conditions for *S. salamandra* at the local scale, comprising humid deciduous woodlands (e.g. *Quercus* spp. and *Fagus* spp.) with a high availability of shelter (e.g. fallen logs and rocks; Velo-Antón and Buckley 2015). In most localities, adjacent streams (ca. < 1 m width) and rivers (ca.

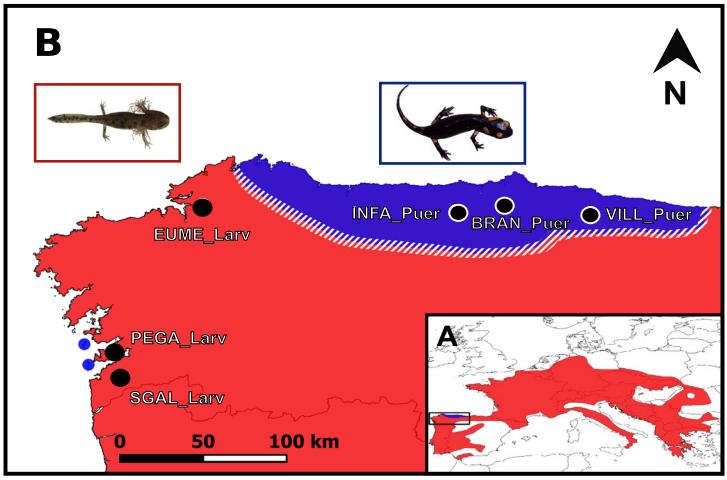


Fig. 1 Study area. **a** Distribution of *Salamandra salamandra* in Europe, with larviparous and pueriparous populations highlighted in red and blue, respectively. **b** Sampled localities and respective acronyms illustrated in our study area. The two blue dots in the northwestern coast of Spain correspond to the two pueriparous insular populations of *S. s. gallaica* not included in this study (see main text). The white dashed polygon illustrates roughly the contact zone between larviparous and pueriparous populations

Table 1 Population genetic statistics from the studied localities

Pop	Lat	Long	Hab	n	nm	nf	M_A	N_A	H_O	H_E	A_R	F	R
PEGA_Larv	42.3210	− 8.7151	D-S	55	30	25	4	12.18	0.78	0.79	11.07	0.01	0.03
EUME_Larv	43.4063	− 8.0852	D-R	53	26	27	6	9.27	0.65	0.72	8.19	0.02	0.05
SGAL_Larv	42.1342	− 8.6859	M	56	26	30	6	12.73	0.77	0.80	11.15	0.03	0.03
INFA_Puer	43.3612	− 6.2632	D-R	61	32	29	5	12.82	0.76	0.82	11.60	0.02	0.03
BRAN_Puer	43.4089	− 5.9202	D-R	62	30	32	4	11.55	0.76	0.81	10.52	0.04	0.04
VILL_Puer	43.3446	− 5.2987	D-S	67	36	31	7	11.91	0.78	0.84	11.10	0.02	0.04

The name of each population (Pop) depicts the associated reproductive mode (*Larv* larviparity; *Puer* pueriparity). Latitude (Lat) and longitude (Long) coordinates are also displayed, along with a brief description of the surrounding habitat in each locality (Hab; D—deciduous forest; M—mixed coniferous and deciduous forests; S—adjacent stream of width < 1 m; R—adjacent river of width ca. 10–30 m). Genetic diversity statistics are: n—number of samples collected; nm—number of sampled males; nf—number of sampled females; M_A—minimum number of allele mismatches among individuals; N_A—mean number of alleles per locus; H_O—observed heterozygosity; H_E—expected heterozygosity; A_R—allelic richness; F—population mean inbreeding coefficient; R—population average relatedness

10–30 m width) were present, the only exception being the locality of SGAL_Larv, in which a small stream is located more than 200 m away from the sampled site. We chose to sample in putative good quality habitat to avoid the presence of unsuitable habitat or anthropogenic features that could potentially disrupt standard dispersal ecology in this species, thus assuring a continuous distribution of individuals. In each locality, we sampled individuals along a ca. 1-km long transect. This length was deemed adequate based

on previous long-term (≥ 2 years) mark-recapture studies that showed dispersal distances less than 500 m for most individuals of *S. salamandra* (Rebelo and Leclair 2003; Schulte et al. 2007; Hendrix et al. 2017). We also attempted to sample individuals on the same side of the stream or river to avoid potential barrier effects to dispersal. This condition was not met in the localities of PEGA_Larv and VILL_Puer, in which 19 and 18 salamanders, respectively, were sampled on the opposite side of the stream along one stretch of the transect due to steep terrain and/or very dense vegetation. These individuals were still kept for downstream analyses because (1) in both localities, the opposite sides of the streams were connected by small (ca. 1.5-m long) wood bridges, which we observed many fire salamanders crossing (AL and GVA personal observations), and (2) kinship analyses carried out in COLONY showed that many pairs of relatives comprised individuals sampled on opposite sides of these streams (seven out of ten in PEGA_Larv and four out of 20 in VILL_Puer; see Fig. 2a, f). Additionally, we sexed all individuals through inspection of the cloaca (Velo-Antón and Buckley 2015) and recorded their locations with a handheld GPS. A roughly even number of males and females were sampled in each locality to prevent biases in spatial autocorrelation analyses arising from uneven sample sizes, though we avoided clustering samples from the same sex to have adequate representations along the transects of both sexes (Fig. 2). Because some localities were sampled during multiple nights, we carried out two procedures to avoid resampling previously captured individuals. First, we inspected toes from all encountered individuals. Second, in some localities, samples were collected seven months apart (e.g. April and November of 2016). Because fire salamanders are capable of regenerating their toes, we used the option *Multilocus Matches* implemented in GENALEX 6.5 (Peakall and Smouse 2012) to check for genotype matches.

Laboratory procedures and genotyping

Genomic DNA was extracted from fresh tissue using the Genomic DNA Tissue Kit (EasySpin), following the manufacturer's protocol. The quantity and quality of extracted products were assessed in a 0.8% agarose gel. A total of 14 microsatellites (Steinfartz et al. 2004; Hendrix et al. 2010), distributed in four optimized multiplexes (panels Ssal1, Ssal2, Ssal3 and Ssal4), were amplified through polymerase chain reactions (PCR; see Appendix 1 for PCR conditions and Appendix 2 for multiplex details). PCR products were verified on a 2% agarose gel and run on an ABI3130XL capillary sequencer (Applied Biosystems). Alleles were scored in GENEMAPPER 4.0 (Applied Biosystems; see Appendix 1 for more details concerning allele scoring).

Population genetic analyses

We tested whether microsatellite loci deviated from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium (LE) by performing exact tests in GENEPOP 4.2 (Rousset 2008; dememorization = 5000, batch length = 10,000, batch number = 1000). We applied the false discovery rate (Benjamini and Hochberg 1995) to correct p values from HWE and LE multiple exact tests. Because the inclusion of related individuals may introduce significant biases in these tests (Sánchez-Montes et al. 2017), we excluded individuals sharing familial relationships from these analyses (see "Parentage analyses" section). The presence of null alleles was investigated in INEST 2.0 (Chybicki and Burczyk 2009) with a total of

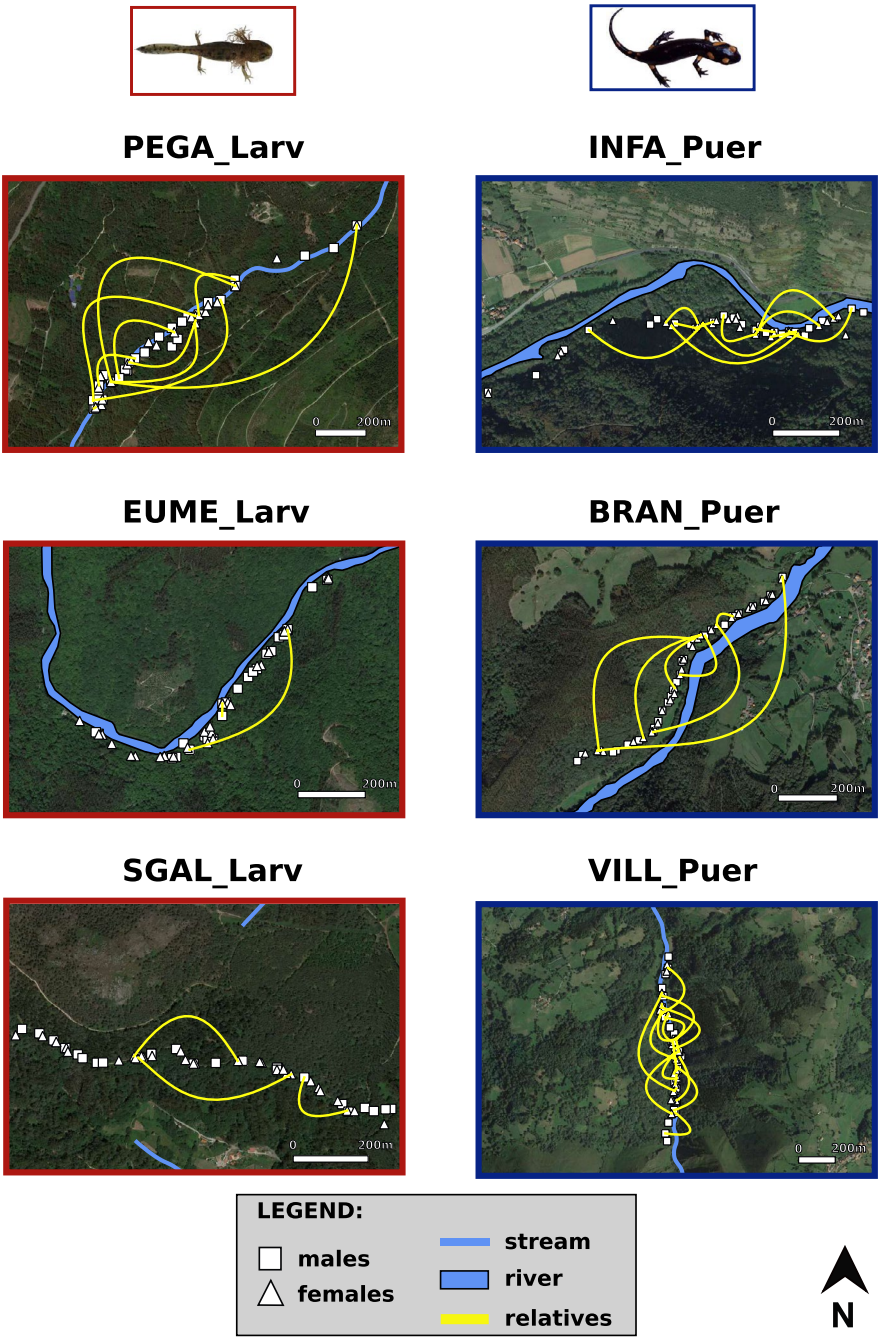


Fig. 2 Aerial photographs of sampled localities. Each panel illustrates the spatial distribution of males and females along with their kinship relationships identified in COLONY (posterior probability ≥ 0.80). Water bodies (large rivers and streams) are also displayed. Left and right panels correspond to localities in which the reproductive mode of females is larviparity (PEGA_Larv, EUME_Larv, and SGAL_Larv) and pueriparity (INFA_Puer, BRAN_Puer, and VILL_Puer), respectively

200,000 iterations, thinned every 200 iterations and with a burn-in of 10% for the individual inbreeding model.

We estimated the mean number of alleles per locality (N_A), observed (H_O) and expected heterozygosity (H_E), and allelic richness (A_R) with the R (R Development Core Team 2017) package *diveRsity* (Keenan et al. 2013). The A_R metric was corrected for the smallest locality's sample size in our data set (n = 53). Population mean inbreeding coefficient (F) was calculated in INEST 2.0, while population average relatedness (R) was estimated using the triadic likelihood estimator implemented in COANCESTRY (Wang 2011).

Comparing patterns of dispersal between reproductive modes and sexes

Long-term radio tracking and mark-recapture approaches have provided important tools to measure dispersal in *S. salamandra* populations (e.g. Rebelo and Leclair 2003; Schulte et al. 2007; Schmidt et al. 2014; Hendrix et al. 2017). Although these methods provide detailed dispersal and demographic data (e.g. survival rates), they are generally time-consuming and usually restricted to single populations. Molecular data alone have been found to be consistent with radio-tracking and mark-recapture estimates in amphibians and shown to be reliable for examining dispersal (Liebgold et al. 2011; Banks and Peakall 2012; Wang and Shaffer 2017), while enabling the simultaneous study of multiple populations with lower sampling effort.

We employed a genetic spatial autocorrelation approach (Smouse and Peakall 1999), as implemented in GENALEX 6.5 (option *Spatial*) to examine the influence of reproductive mode and sex on fine-scale genetic structure and, thus, infer dispersal from these genetic patterns (Peakall et al. 2003; Banks and Peakall 2012). This method has been applied widely to infer variation in patterns of dispersal between sexes based on genetic structure (Banks and Peakall 2012), including in the direct-developing red-back salamander (*Plethodon cinereus*), in which it was shown consistent with mark-recapture estimates of dispersal (Liebgold et al. 2011). Additionally, genetic spatial autocorrelation was successfully used to infer variation in dispersal between groups with another phenotypic trait, body colouration, in *Plethodon cinereus* (Grant and Liebgold 2017), further demonstrating its utility for our study. This multivariate distance-based method uses pairs of genetic and geographic distance matrices as input and calculates an autocorrelation coefficient (r; bounded by [−1, 1]) as a measure of genetic similarity between pairs of individuals for each distance class, with results summarized in a correlogram. Distance classes displaying positive r values indicate that pairs of individuals within that class are more genetically similar than average. If positive values are found within the shortest distance classes, it may indicate that individuals are philopatric to their natal areas, while positive r values at farther distance classes may indicate dispersal of many related individuals over a specific range of distances.

We first built two multilocus data sets per sampled locality (12 in total): one for males and one for females. Pairwise between-individual matrices of Euclidean geographic distances and genetic distances were calculated for each multilocus data set (option *Distance*), except for the locality of EUME_Larv. The path of the stream in this locality did not allow for sampling along a straight transect, so we calculated stream-distances between individuals rather than Euclidean distances (Fig. 2c; see details in Appendix 3). To test whether reproductive mode (H1) and sex (H2) influence fine-scale genetic structure (and dispersal), we generated four "combined" correlograms comparing patterns of genetic structure between the following subsamples: (1) larviparous males versus pueriparous males; (2) larviparous females versus pueriparous females; (3) larviparous males versus larviparous

females; and (4) pueriparous males versus pueriparous females (see below). We used the option *Multiple Pop Subsets* to generate these "combined" correlograms in which an overall r value is estimated for each distance class based on the individual r estimates of each population included in the subsample (e.g. the overall r values for the subsample "larviparous males" are calculated from r estimates obtained from males sampled at PEGA_Larv, EUME_Larv, and SGAL_Larv; see Peakall et al. 2003 and Banks and Peakall 2012 for more details about this method). Additionally, because patterns of genetic structure may vary significantly among sampled localities, we generated six additional within-locality correlograms comparing males versus females to provide complementary insights into the effects of reproductive mode and sex on dispersal. The latter six correlograms were computed using the option *Multiple Pops* (i.e. males and females were treated as separate "populations"; Banks and Peakall 2012). We estimated overall r values for the "combined" correlograms at eight distance classes for a total length of 1 km in each subsample (100-m classes up to 700 m, and a distance class of 701–1000 m). The size and number of distance classes were chosen based on the dispersal ecology of *S. salamandra* (Schulte et al. 2007; Hendrix et al. 2017) and as a trade-off between resolution and sample size in each class (i.e. number of pairs of individuals binned into each class). We decided to pool all pairs separated by > 700 m in one class (701–1000 m) due to small sample sizes. Also because of small sample sizes, within-locality correlograms were computed only for six distance classes in each sex (0–100 m, 101–200 m, 201–300 m, 301–500 m, 501–700 m, and 701–1000 m). Lastly, in some localities, very few pairs of individuals were separated by a distance greater than 1000 m. We decided to exclude these observations from these analyses, not only to avoid estimating r values based on low sample sizes for distance classes > 1000 m but also to make correlograms directly comparable.

We first assessed patterns of fine-scale genetic structure at both global (i.e. whole correlogram) and individual distance class levels. These analyses were performed to quantify the degree of genetic structure of the groups being compared (i.e. subsamples in the case of "combined" correlograms, and males and females in the case of within-locality correlograms). To test for deviations from the null hypothesis of no genetic structure (i.e. a "flat" correlogram) for each group at the global scale, we employed the heterogeneity Omega test (ω; Smouse et al. 2008). Estimates of ω, as well as other heterogeneity tests (see below), were regarded as significant at $p < 0.01$, as recommended by Banks and Peakall (2012). Additionally, we performed 9999 permutations of the data to generate a null distribution of no spatial genetic structure (i.e. $r = 0$) for each distance class. The r values estimated from our multilocus data for each distance class were then compared to this null distribution with a one-tailed t test ($p < 0.05$), allowing us to determine whether r values were significantly higher or lower than expected by chance. The 95% confidence intervals (CIs) of each r value were computed through 10,000 bootstrap resamplings.

Additional statistical tests were carried out to test explicitly our two hypotheses. For the "combined" correlograms, to test for general differences in fine-scale genetic structure (and infer dispersal differences) between reproductive modes (H1; larviparous males versus pueriparous males and larviparous females versus pueriparous females), we employed the heterogeneity Omega group test (ω_{groups}; $p < 0.01$; Smouse et al. 2008) to assess global differences in r patterns among subsamples and the heterogeneity t^2 test ($p < 0.01$; Smouse et al. 2008) to quantify differences in r values for each distance class. To test for differences in dispersal between sexes at distance class and global scales (H2; larviparous males versus larviparous females and pueriparous males versus pueriparous females), these same heterogeneity tests were also used.

For within-locality correlograms, we compared *r* patterns between each pair of sampled localities at global and distance class levels to test our hypothesis that pueriparous females will disperse farther, on average, than larviparous females (H1). We expected that pairwise comparisons involving the sampled localities with different reproductive modes will exhibit greater differences in *r*. These pairwise comparisons were restricted to individuals of the same sex (i.e. males versus males and females versus females from different sites; total of 15 comparisons per sex). Pairwise heterogeneity Omega group tests (ω_{groups}; $p < 0.01$; Smouse et al. 2008) were employed to assess global differences in *r* patterns among pairs of localities, whereas differences in *r* values for each distance class were assessed through pairwise heterogeneity t^2 tests ($p < 0.01$; Smouse et al. 2008). Finally, to test our hypothesis that males disperse farther than females (H2), we performed the same heterogeneity tests, but comparisons of *r* values between males and females were performed only within each sampled locality to avoid potential bias arising from environmental variation between localities.

Parentage analyses

For each sampled locality, we performed parentage analyses using COLONY 2.0.6.1 (Jones and Wang 2010) to identify putative pairs of relatives and evaluate the spatial distribution of related individuals. COLONY requires the input of three subsamples: (1) putative fathers; (2) putative mothers; and (3) putative offspring. *Salamandra salamandra* is an iteroparous species, meaning that multiple cohorts coexist contemporaneously. Because precise age determination in the field was unfeasible and because fire salamanders are known to live for more than 20 years (Rebelo and Caetano 1995), our data set may contain parent–offspring pairs even though our sampling was restricted to adult salamanders. Accordingly, males and females were distributed in the candidate father and mother samples, respectively, and all individuals were pooled in the candidate offspring sample. The inclusion of individuals both in parent and offspring subsamples decreases the statistical power of this method to identify relatives, although COLONY has been shown to perform satisfactorily under these conditions (Wang and Santure 2009). A total of three runs per sampled locality with different seed numbers were performed. In each run, we set the full-likelihood method, with high likelihood precision and long run length under a scenario of polygamy for both sexes. Sibship scaling was deactivated, and no a priori information regarding known parents was provided. We identified pairs of individuals as related if they exhibited a posterior probability higher than 0.8 in at least two runs. This probability threshold is lower than those employed in previous studies (e.g. 0.95; Richards-Zawacki et al. 2012; Carvalho et al. 2018). However, we were not interested in determining the exact familial relationships (e.g. parent-offspring, full-siblings or half-siblings) but rather in identifying the most related pairs of individuals within our sample (hereafter "relatives"). To assess the spatial distribution of relatives, we calculated the proportion of relatives separated across three distance intervals: (1) $\leq$200 m (most individuals [ca. 80–90%] move <200 m; Schulte et al. 2007; Hendrix et al. 2017); (2) 201–500 m (the scale at which some individuals disperse among breeding localities; Ficetola et al. 2012); and (3) >500 m (rare long-distance dispersal events; Ficetola et al. 2012; Hendrix et al. 2017). Finally, we calculated the Probability of Identity (PI) in GENALEX, which is the probability that two individuals drawn at random from a given population share identical genotypes at all loci, to assess the power of our multilocus data for discriminating individuals. The PI when accounting for the presence of relatives (PISibs) was also estimated.

Results

Population genetic analyses

All sampled individuals in a transect exhibited a minimum of four to seven allele mismatches with each other (Table 1). Therefore, we concluded that no genotyped salamander comprised a recapture, and all individuals were kept for downstream analyses. Three loci showed consistent deviations from HWE (heterozygote deficits) and clear evidence for null alleles, one (SalE2) in all larviparous populations and two (SST-C3 and SalE06) in all pueriparous populations. We excluded these three loci, and all downstream analyses were performed with the remaining 11 loci. There was no evidence for deviations from LE. Both larviparous and pueriparous groups showed similar and very high levels of genetic diversity (range N_A: 9.27–12.82; range H_O: 0.65–0.78; range H_E: 0.72–0.84; A_R: 8.19–11.60), while inbreeding (range F: 0.01–0.04) and relatedness (range R: 0.03–0.05) were both very low (Table 1).

Comparison of dispersal patterns between reproductive modes and sexes

At the global level, both larviparous ($\omega = 38.4$, $p < 0.01$; Fig. 3b, 4a) and pueriparous females ($\omega = 44.3$, $p < 0.01$; Fig. 3b, 4b) exhibited significant positive genetic structure according to the heterogeneity ω test, while analyses within each sampled locality showed that only females in two larviparous populations (PEGA_Larv, $\omega = 30.9$,

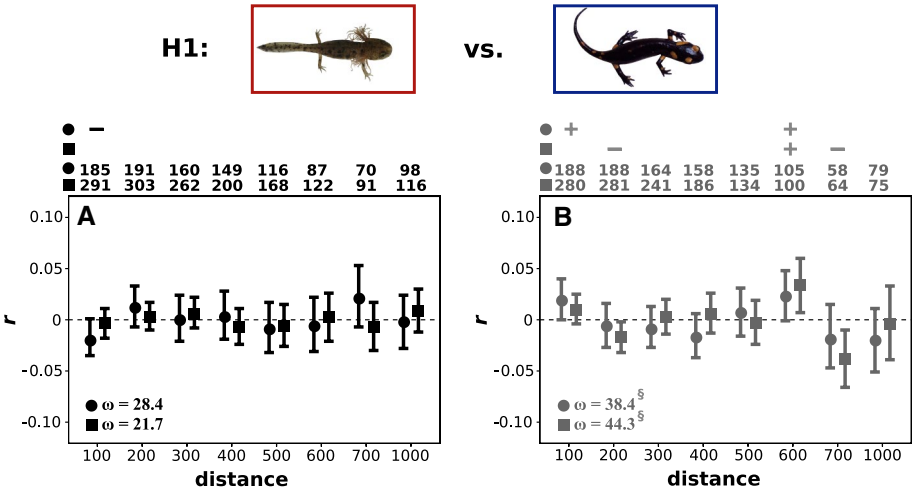


Fig. 3 "Combined" correlograms of the autocorrelation coefficient, *r*, comparing the following subsamples: **a** larviparous males (black circles) versus pueriparous males (black squares); and **b** larviparous females (grey circles) versus pueriparous females (grey squares). These correlograms were generated to test explicitly our hypothesis of differences in genetic structure between reproductive modes (H1). Omega (ω) statistics at the whole correlogram (global) level are shown for each analysed subsample, and significant ω values ($p < 0.01$) are denoted by the symbol (§). The numbers above the plot indicate the number of pairs analysed per subsample (represented by the symbols at left of these numbers) for each distance class. The symbols "+" and "−" denote distance classes for which *r* values are significantly higher or lower than zero (dashed line; $p < 0.05$), respectively, based on one-tailed tests for a particular subsample (represented by the symbols at left)

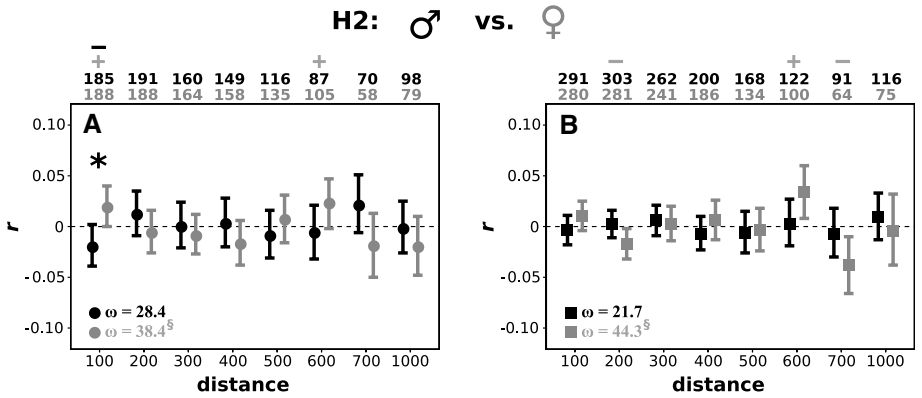


Fig. 4 "Combined" correlograms of the autocorrelation coefficient, *r*, comparing the following subsamples: **a** larviparous males (black circles) versus larviparous females (grey circles); and **b** pueriparous males (black squares) versus pueriparous females (grey squares). These correlograms were generated to test explicitly our hypothesis of differences in genetic structure between sexes (H2). Omega (ω) statistics at the whole correlogram (global) level are shown for each analysed subsample, and significant ω values ($p < 0.01$) are denoted by the symbol (§). The numbers above the plot indicate the number of pairs of males (black) and females (grey) analysed for each distance class. The symbols "+" and "−" (males, black; females, grey) denote distance classes for which *r* values are significantly higher or lower than zero (dashed line; $p < 0.05$), respectively, based on one-tailed tests. Black asterisks denote distance classes for which *r* values between analysed subsamples are significantly different ($p < 0.01$) according to t^2 tests

$p < 0.01$; EUME_Larv, $\omega = 30.9$, $p < 0.01$) and males from one larviparous population (SGAL_Larv, $\omega = 31.9$, $p < 0.01$) exhibited strong genetic structure (Fig. 5). At the distance class level, significant genetic structure was found at 0-100 m for larviparous females ($r = 0.020$, $p = 0.02$) and at 501-600 m for both larviparous ($r = 0.024$, $p = 0.03$) and pueriparous females ($r = 0.034$, $p < 0.01$) in "combined" correlograms (Fig. 3; Appendices 4, 5, 6, 7). Analyses within each sampled locality revealed strong genetic structure only for a total of four distance classes (two in females for distances ≤ 200 m and two in males for distances ≥ 300 m; Fig. 5 and Appendices 8, 9).

The heterogeneity ω_{groups} tests performed in the "combined" correlograms did not show significant differences between neither reproductive modes nor sexes at the global level (Appendix 10). We also did not find significant differences in genetic structure between reproductive modes at the distance class level (Appendix 11). However, between sexes, we found that larviparous females showed a much higher genetic similarity than larviparous males at a distance class of 100 m ($t^2 = 7.03$, $p < 0.01$; see Fig. 4 and Appendix 11). Although pueriparous females exhibiting also a higher *r* than pueriparous males at 100 m, this difference was not statistically significant.

Pairwise comparisons involving within-locality correlograms revealed no significant differences between reproductive modes at both the global (see Appendices 12, 13) and distance class levels (see Appendices 14, 15). Only the larviparous population pair PEGA_Larv/EUME_Larv showed significant differences in *r* at a distance of 101–200 m ($t^2 = 6.95$, $p < 0.01$; Appendix 15). We also did not find statistical support for sex-biased dispersal within any locality. Nevertheless, for distances up to 100 m, females always exhibited higher *r* values than males, and for the 701–1000 m distance class, males and females in most study sites exhibited non-significant positive and negative *r*, respectively. One exception is that females in SGAL_Larv showed a non-significant positive *r* at 701–1000 m.

Parentage analyses

COLONY identified a total of 54 pairs of relatives (Table 2; Fig. 2). The number of pairs of relatives identified per locality varied between two (EUME_Larv) and 20 (VILL_Puer). The minimum (8.3 m) and maximum (1162.8 m) distances between relatives were both recorded in PEGA_Larv. Overall, the frequency of pairs of relatives was higher in pueriparous populations up to 200 m, whereas larviparous and pueriparous populations showed similar frequencies for distances of 201–500 m (Fig. 6; Table 2). At distances > 500 m, larviparous populations showed a higher proportion of relatives compared to pueriparous populations (Fig. 6). The population of Pega_LARV, in which four pairs of relatives were identified more than 500 m apart, primarily accounted for this pattern (Table 2). Both PI (range: 1.8×10^{-16}–1.6×10^{-11}) and PISibs (range: 6.4×10^{-6}–6.3×10^{-5}) were very low.

Discussion

Do pueriparous females disperse farther than larviparous ones?

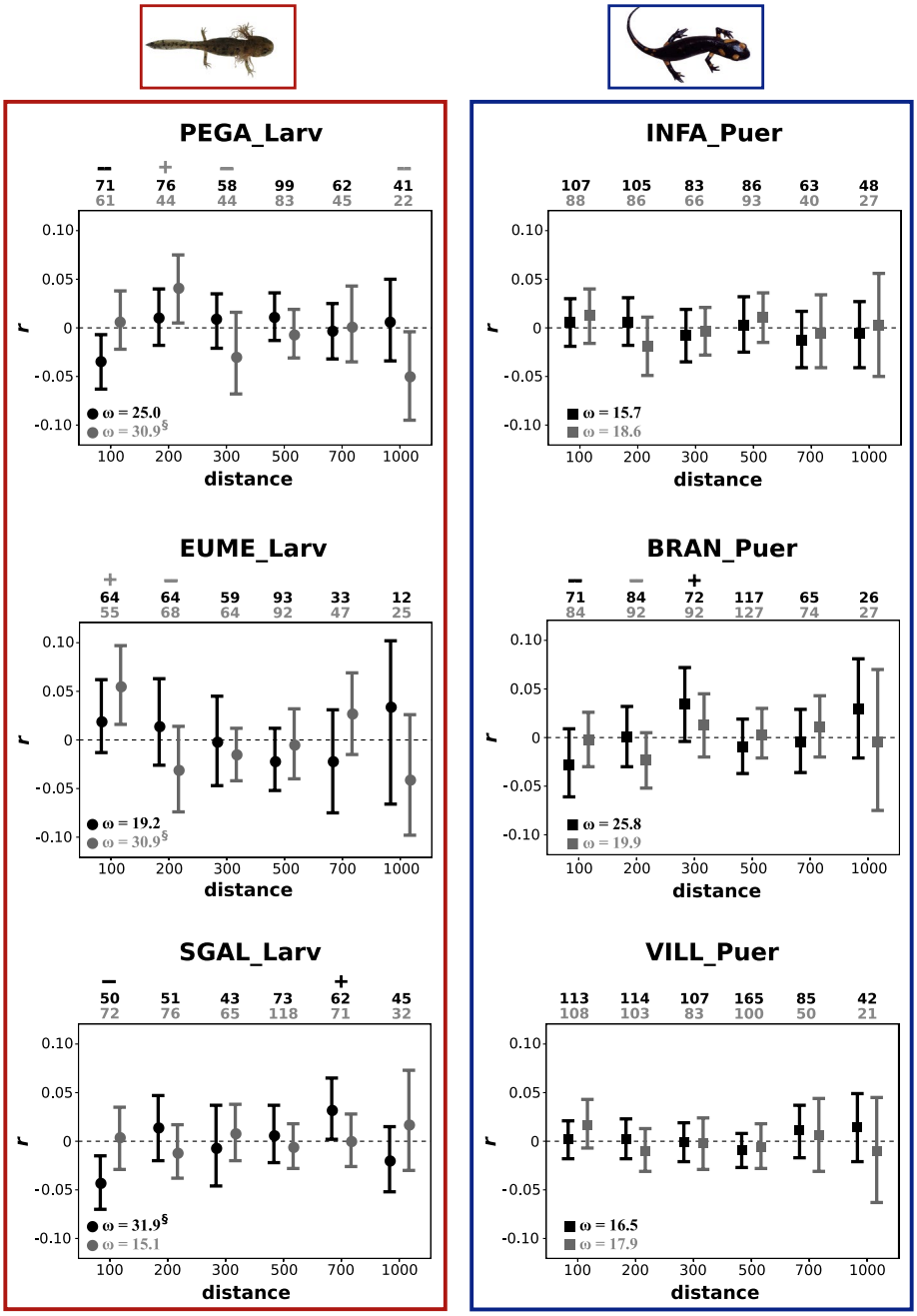
Here, we took advantage of one of the very few species exhibiting both aquatic and terrestrial reproduction to perform comparisons between reproductive modes and infer their effects on dispersal based on patterns of genetic structure. Contrary to our predictions, we did not find significant differences in patterns of genetic structure (and therefore dispersal) between larviparous and pueriparous females (or males) at fine spatial scales. Previous studies on pueriparous and direct-developing amphibians indicated that terrestrial reproduction allows individuals to survive, disperse, and reproduce in a wider range of suboptimal habitats due to higher independence from surface water compared to species with aquatic reproduction (Gibbs 1998; Marsh et al. 2004; Liedtke et al. 2017; Lourenço et al. 2017). This greater independence from water potentially promotes higher genetic connectivity in heterogeneous and fragmented landscapes at the population level (Measey et al. 2007; Sandberger-Loua et al. 2018). For instance, based on allozyme data sets and patterns of isolation-by-distance, Tilley (2016) observed higher genetic divergence between populations of aquatic-breeding salamanders (*Desmognathus*) compared to those of direct-developing lungless salamanders (*Plethodon*), suggesting this pattern could be due to the higher dependence of *Desmognathus* salamanders on aquatic breeding habitats (headwaters of streams).

Environmental conditions are a major driver of differentiation in dispersal strategies within species due to divergent selective pressures acting on individuals with different traits (Bowler and Benton 2005; Cote et al. 2017). Selection for dispersal will often occur if the benefits of emigrating outweigh those of remaining in the natal patch (Ousterhout and Semlitsch 2018). Within *S. salamandra*, Hendrix et al. (2017) used mark-recapture techniques and telemetry to compare movement patterns between two larviparous subpopulations of *S. salamandra*, in which individuals of each subpopulation deliver larvae either in ponds or streams. The authors found that individuals reproducing in ponds dispersed farther and exhibited higher variation in dispersal distances than salamanders reproducing in streams, possibly to cope with the spatio-temporal availability of pond habitats. In our study system, the potential benefits of terrestrial breeding for dispersal and population connectivity in heterogeneous and fragmented habitats may not be expressed, at least in

Fig. 5 Correlograms of the autocorrelation coefficient, *r*, comparing males (larviparous, black circles; pueriparous, black squares) and females (larviparous, grey circles; pueriparous, grey squares) sampled in larviparous (left panel; PEGA_Larv, EUME_Larv, and SGAL_Larv) and pueriparous populations (right panel; INFA_Puer, BRAN_Puer, and VILL_Puer). Omega (ω) statistics at the whole correlogram (global) level are shown for each analysed population, and significant ω values ($p < 0.01$) are denoted by the symbol (§). The numbers above the plot indicate the number of pairs of males (black) and females (grey) analysed for each distance class. The symbols "+" and "−" (males, black; females, grey) denote distance classes for which *r* values are significantly higher or lower than zero (dashed line; $p < 0.05$) based on one-tailed tests, respectively

S. salamandra, at small spatial scales and in regions with largely intact, suitable habitats. In situ observations of the habitat (mostly deciduous forests), together with high observed population density and genetic diversity, suggest that our study sites contained favourable conditions for survival and reproduction in both larviparous and pueriparous populations. These conditions may have resulted in similar dispersal tendencies between larviparous and pueriparous salamanders, as the key resource (water) that may be responsible for any dispersal asymmetry between reproductive modes is not a limiting factor in our study sites (except possibly in SGAL_Larv; see below). Because the lineages of pueriparous *S. salamandra* are both relatively recent, we also cannot rule out the possibility that they retained ancestral dispersal traits; essentially, the shift in reproductive mode might not have been followed by a behavioural adaptation to the derived pueriparous condition in *S. s. bernardezi*, though dispersal-related traits can show low conservation at the intraspecific level (Stevens et al. 2010). Moreover, although *S. salamandra* comprises a good model to test our hypotheses because it has multiple reproductive modes, there are potentially important differences between larviparous and pueriparous fire salamanders. Specifically, larviparous *S. s. gallaica* individuals are generally larger (body size up to 250 mm) than *S. s. bernardezi* individuals (body size up to 180 mm; Velo-Antón and Buckley 2015; Velo-Antón et al. 2015), and previous studies have shown larger salamanders usually show higher dispersal capacity (Bennett et al. 1989; but see also Denton et al. 2017). Hence, if pueriparity and larger body size (found in larviparous populations) both lead to greater dispersal distances, then we may not observe differences between different reproductive modes. Complementing our genetic analyses with mark-recapture or radio tracking data at our study sites may help clarify these results.

Despite the lack of major differences in dispersal and fine-scale genetic structure between larviparous and pueriparous salamanders according to genetic spatial autocorrelation, parentage analyses in larviparous salamanders at the PEGA_Larv locality revealed a disproportionately higher number of pairs of relatives farther apart (> 500 m) compared to the other populations (Fig. 4; Table 2). This may suggest that water-borne long-distance dispersal (active or passive due to strong discharges after heavy rain) along the stream during the larval stage may have contributed to these patterns, as reported previously in larvae of *S. salamandra* (Thiesmeier and Schuhmacher 1990; Reinhardt et al. 2018). Water-borne dispersal is unlikely to occur in large rivers similar to the one located in EUME_Larv, as its strong current and drifting objects probably causes high mortality rates due to physical damage, thus preventing successful dispersal (see Segev and Blaustein 2014). Additionally, we cannot entirely discount rare long-distance movements undertaken by adults over single or multiple generations in explaining the patterns obtained in PEGA_Larv. Hendrix et al. (2017) found that stream-adapted individuals did not move beyond 500 m, while 90% of the studied pond-adapted salamanders moved up to 700 m, with a few pond-adapted individuals performing long-distance movements up to 1.9 km possibly driven by the limited availability of ponds in



their study area, as hypothesized by the authors. However, we find those types of movements unlikely in our study, because (1) many ecological studies in adult larviparous fire salamanders have systematically reported small home ranges and dispersal distances below 500 m in suitable environments (Schulte et al. 2007; Ficetola et al. 2012; Schmidt

Table 2 Summary statistics of kinship relationships identified in COLONY for each population (Pop)

Pop	N_kin	Range (m)	≤ 200 [p]	201-500 [p]	> 500 [p]
PEGA_Larv	10	8.3–1162.8	2 [0.20]	4 [0.40]	4 [0.40]
EUME_Larv	2	9.9–465.7	1 [0.50]	1 [0.50]	–
SGAL_Larv	3	145.8–410.1	1 [0.33]	2 [0.67]	–
INFA_Puer	12	55.6–377.8	8 [0.67]	4 [0.33]	–
BRAN_Puer	7	61.0–873.5	2 [0.29]	3 [0.42]	2 [0.29]
VILL_Puer	20	9.0–579.8	12 [0.6]	7 [0.35]	1 [0.05]

The following statistics are displayed: N_kin (number of pairs of relatives identified), and Range (distance range between pairs of relatives identified in a population). The number and proportion (*p*; within square brackets) of pairs of relatives found within 3 distance classes (≤ 200, 201–500, and > 500) are also displayed

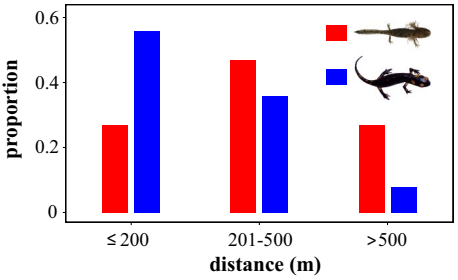


Fig. 6 Proportion of pairs of relatives identified in COLONY from larviparous (red) and pueriparous (blue) populations of *Salamandra salamandra* across three distance classes (200 m, 201–500 m, and > 500 m)

et al. 2014), and (2) if long-distance movements of adults were common, then we would expect to observe similar patterns in other studied larviparous populations.

Interestingly, unlike the other larviparous and pueriparous populations, females in SGAL_Larv showed a relatively elevated relatedness for a distance of > 700 m (Fig. 3). This contrasting pattern may be related to the lack of nearby aquatic systems along this transect (Fig. 2e). Dispersal behaviour, particularly in female amphibians, is often driven by the availability of breeding resources (water bodies) within their perceptual range (Russell et al. 2005; Semlitsch 2008; Wang et al. 2012). For instance, Wang et al. (2012) showed that female-biased dispersal in a frog was favoured in islands containing a lower density of breeding sites compared to those with abundant reproductive resources. The lower abundance of nearby water bodies in SGAL_Larv potentially prompted females to adjust their dispersal behaviour, increasing dispersal distances to increase the likelihood of encountering water bodies to deposit larvae. Increasing the number of sampled larviparous populations at varying distances from aquatic systems (both streams and rivers), as well as incorporating field ecological approaches (e.g. mark-recapture or telemetry) is crucial to providing further insights on how salamanders navigate these landscapes.

Do males disperse farther than females?

Larviparous females had significantly higher relatedness than larviparous males at a distance class of < 100 m, suggesting females exhibit more philopatric behaviour. Additionally, the higher (although non-significant) *r* values of females compared to males at < 100 m in both "combined" (Fig. 4b) and within-locality (Fig. 5) correlograms also seem

to support a marked philopatric behaviour of females. However, the underlying causes cannot be determined from our data, though Helfer et al. (2012) have suggested that philopatry in *S. atra* females may be driven by resource-defence mechanisms. Additionally, many pueriparous and larviparous females moved long distances, up to ca. 500–600 m (Fig. 3b). In high-quality environments, there is a trade-off between resource availability and competition among individuals that governs the probability of dispersal (e.g. Bowler and Benton 2005; Liebgold et al. 2011). For some females, it is possible that very high intraspecific competition forced them to disperse. Indeed, a distance of 500–600 m has been observed directly (movement data, Schulte et al. 2007; stream-adapted fire salamanders in Hendrix et al. 2017) and indirectly (ecological spatial autocorrelation analyses; Ficetola et al. 2012) as the maximum dispersal distance for many fire salamander individuals, with occasional long-distance movements (pond-adapted fire salamanders; Hendrix et al. 2017). On the other hand, males often exhibited higher, though not statistically significant, genetic relatedness compared to females at 700–1000 m, with the exception of females in SGAL_Larv. This may suggest that males in the studied localities exhibit greater variation in dispersal distances than females, partially supporting the male-biased dispersal hypothesis proposed by biogeographic studies to justify the mito-nuclear discordances found throughout the distribution of this species (García-París et al. 2003; Pereira et al. 2016). Nevertheless, longer transects with a higher number of sampled individuals across more sites are needed to confirm this and further elucidate the underlying drivers of dispersal in males and females in this system.

Conclusions

To our knowledge, empirical studies addressing the eco-evolutionary implications of shifts in reproductive modes for dispersal and fine-scale genetic structure are scarce. Our study, which focused on fire salamander populations exhibiting contrasting reproductive strategies (aquatic versus terrestrial reproduction), revealed no obvious differences in movement patterns between reproductive modes across the studied landscapes, which were largely composed of contiguous suitable habitat. However, our results raise the possibility that the intrinsic dispersal behaviour of larviparous salamanders may be associated with site-specific landscape features (i.e. abundance and type of water bodies), which under particular environmental contexts may translate to marked differences in dispersal-related traits compared to pueriparous salamanders, although further work is needed to validate this hypothesis. Female fire salamanders (especially larviparous ones) also appeared more philopatric than males, although patterns of dispersal in females deserve further investigation under scenarios with low water body availability. This study also provides avenues for future research on the outcomes of shifts in reproductive modes in amphibians. Specifically, *S. salamandra* comprises a good system to explicitly test the hypothesis that pueriparous amphibians should show higher population connectivity than larviparous populations in fragmented landscapes. Additionally, the association between larval and adult dispersal ecology and water dependence in larviparous populations requires further investigation, which will help to elucidate how the spatiotemporal availability of aquatic breeding resources contributes to patterns of sex-biased dispersal and regional connectivity in this species.

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Data availability The microsatellite genotype data set generated during the current study will be deposited on Dryad upon acceptance of this manuscript.

Appendix 1: Detailed PCR conditions and allele scoring procedures

Each PCR reaction contained a total volume of 10–11 µl: 5 µl of Multiplex PCR Kit Master Mix (QIAGEN), 3 µl of distilled water, 1 µl of primer multiplex mix and 1–2 µl of DNA extract (~ 50 ng/µl). To identify possible contaminations, a negative control was employed. PCR touchdown cycling conditions were equal in all multiplexes: the reaction started with an initial step at 95 °C for 15 min, 19 cycles at 95 °C for 30 s, 90 s of annealing at 65 °C (decreasing 0.5 °C each cycle), 72 °C for 40 s, followed by 25 cycles of 95 °C for 30 s, 56 °C for 60 s, 72 °C for 40 s, and ended with a final extension of 30 min at 60 °C.

Prior to allele scoring in GENEMAPPER, allele fragment length binning was performed on a set of tissue samples of very high quality (ca. 50 samples) collected across northern Spain. The DNA Size Standard LIZ 500 DSMO-100 (MCLAB) was employed to determine the relative size of fragments. Following binning procedures, genotypes were checked and corrected by two persons to avoid potential erroneous scoring of alleles. Additionally, to reduce the potential influence of allele dropout and false alleles, we scored only alleles exhibiting clear fluorescence peaks higher than 100 relative fluorescent units. Microsatellite markers that failed to amplify or exhibited dubious allelic profiles (e.g. with high prevalence of peak artefacts) in samples containing more than 25% of missing data were reamplified in uniplex reactions (i.e. for a single microsatellite locus) to increase the likelihood of amplification. Each uniplex PCR contained a total volume of 10–11 µl: 5 µl of Multiplex PCR Kit Master Mix (QIAGEN), 2.8 µl of distilled water, 0.4 µl of forward primer (1 µM), 0.4 µl of reverse primer (10 µM), 0.4 µl of the respective fluorescently labelled oligonucleotides (10 µM; see Appendix 2), and 1–2 µl of DNA extract. Cycling conditions are the same as those described for multiplexes.

Appendix 2: Details of the 14 microsatellites used in this study

Information regarding multiplex arrangement, original published primers and fluorescently labelled oligonucleotides used as template for modified forward primers is displayed. The primer volume used to create a multiplex with a total volume of 100 µl (distilled H_2O plus the volumes of the unlabelled and fluorescently labelled primers) is also represented (PVM). The forward and reverse primers were concentrated at 10 µM and 100 µM,

respectively. This table is adapted from Supplementary Material 2 of Álvarez et al. (2015) and Table S2 of Lourenço et al. (2017).

Locus	Multiplex	Label*	Primer forward (5′–3′)	Primer reverse (5′–3′)	PVM (µl)
SST-A6-I[2]	Ssal1	NED	TTCAGTGCTCTTGCA GGTTG	AGTCTGCAAGGATAG AAAGATCG	2.0
SST-A6-II[2]	Ssal1	PET	ATTCTCTCTGACAAG GATTGTGG	GGTAGACAGACATCA AGGCAGAC	1.2
SalE14[1]	Ssal1	VIC	GCTGCCCTCTCTGCC TACTGACCAT	GCCAAGACATGGAAC ACCCTCCCGC	0.8
Sal29[1]	Ssal2	6-FAM	CTCTTTGACTGAACC AGAACCCC	GCCTGTCGGCTCTGT GTAACC	8.0
SST-B11[2]	Ssal2	PET	TCAAACGGTGCCAAA GTTATTAG	TTAATTGGCAGTTTTCTT TCCAG	2.0
SalE12[1]	Ssal2	VIC	CTCAGGAACAGTGTG CCCCAAATAC	CTCATAATTTAGTCTACC CTCCCAC	0.8
SST-C3[2]	Ssal3	PET	CCGTTTGAGTCACTT CTTTCTTG	TTGCTTTACCAACCA GTTATTGTC	1.4
SalE7[1]	Ssal3	NED	TTTCAGCACCAAGAT ACCTCTTTTG	CTCCCTCCATATCAA GGTCACAGAC	0.8
SalE5[1]	Ssal3	6-FAM	CCACATGATGCCTAC GTATGTTGTG	CTCCTGTTTACGCTT CACCTGCTCC	0.6
SalE2[1]	Ssal3	VIC	CACGACAAAATACAG AGAGTGGATA	ATATTTGAAATTGCC CATTTGGTA	3.0
SalE06[1]	Ssal4	VIC	GGACTCATGGTCACC CAGAGGTTCT	ATGGATTGTGTCGAA ATAAGGTATC	1.2
Sal3[1]	Ssal4	6-FAM	CTCAGACAAGAAATC CTGCTTCTTC	ATAAATCTGTCCTGT TCCTAATCAG	1.2
SalE8[1]	Ssal4	NED	GCAAAGTCCATGCTT TCCCTTTCTC	GACATACCAAAGACT CCAGAATGGG	0.8
SST-G9[2]	Ssal4	NED	CCTCGTCAGGGGTTG TAGG	CTTTCCAGGAAGAAA CTGAGATG	0.8

*An extra number of base pairs were added at the 5′ end of the original sequence of forward primers in order to allow binding of four different fluorescent labelled oligonucleotides (6-FAM—TGT AAA ACG ACG GCC AGT; VIC—TAA TAC GAC TCA CTA TAG GG; NED—TTT CCC AGT CAC GAC GTT G; PET—GAT AAC AAT TTC ACA CAG G)

[1]Steinfartz et al. (2004)

[2]Hendrix et al. (2010)

Appendix 3: Details of the calculation of pairwise between-individual matrices of geographic distances in EUME_Larv

The two geographic distance matrices obtained in locality EUME_Larv were subjected to additional pre-treatment procedures. This is because not all individuals along the transect were sampled in a straight path, particularly the westernmost individuals (Fig. 2c). Since a river that may comprise a barrier to dispersal is located adjacently to sampled individuals, the pairwise Euclidean distances involving these westernmost individuals are likely underestimated. To circumvent this issue, we digitized a shapefile adjacent to the river in QGIS (QGIS Development Team 2017). Then, we employed the R package *gdistance* (van Etten 2017) to rasterize the

shapefile and calculate a pairwise "least-cost" distance that accounted for the river as a barrier to dispersal involving those individuals and remaining sampled individuals.

Appendix 4: Summary statistics of spatial autocorrelation analyses comparing larviparous males and pueriparous males

Summary statistics of spatial autocorrelation analyses comparing larviparous males and pueriparous males (see respective correlogram in Fig. 3a) aimed at testing H1. The Omega test value (ω) and respective *p* value (*p*) for each subsample is displayed. Significant ω values were declared when $p < 0.01$ (values in bold; Smouse et al. 2008). Remaining parameters were estimated for each of the eight distance classes tested: 100 (0–100 m), 200 (101–200 m), 300 (201–300 m), 400 (301–400 m), 500 (401–500 m), 600 (501–600 m), 700 (601–700 m), and 1000 (701–1000 m). These parameters are: N (number of pairs of individuals analysed), *r* (autocorrelation coefficient) and respective lower (*r* lower 95% CI limit) and upper (*r* upper 95% CI limit) bounds of the 95% CIs. The *p* values of one-tailed tests to determine if *r* values were significantly higher (p (*r-rand* ≥ *r-obs*)) or lower (p (*r-obs* ≥ *r-rand*)) than expected for a given distance class are also displayed, with significant *p* values ($p < 0.05$) in bold and underlined.

Data set and parameters	ω (p_ω)	100	200	300	400	500	600	700	1000
Larviparous males	28.4 (0.03)								
N		185	191	160	149	116	87	70	98
r		−0.019	0.013	0.001	0.004	−0.008	−0.005	0.022	−0.001
r lower 95% CI limit		−0.035	−0.007	−0.021	−0.019	−0.032	−0.031	−0.007	−0.028
r upper 95% CI limit		0.001	0.033	0.024	0.028	0.017	0.022	0.053	0.024
p (*r-rand* ≥ *r-obs*)		0.979	0.068	0.446	0.336	0.733	0.662	0.079	0.551
p (*r-obs* ≥ *r-rand*)		**0.021**	0.932	0.554	0.664	0.267	0.338	0.921	0.449
Pueriparous males	21.7 (0.72)								
N		291	303	262	200	168	122	91	116
r		−0.003	0.003	0.006	−0.007	−0.006	0.003	−0.007	0.009
r lower 95% CI limit		−0.018	−0.010	−0.008	−0.024	−0.026	−0.021	−0.030	−0.012
r upper 95% CI limit		0.011	0.017	0.022	0.011	0.015	0.026	0.017	0.030
p (*r-rand* ≥ *r-obs*)		0.699	0.319	0.186	0.806	0.744	0.382	0.699	0.187
p (*r-obs* ≥ *r-rand*)		0.301	0.681	0.814	0.194	0.256	0.618	0.301	0.813

Appendix 5: Summary statistics of spatial autocorrelation analyses comparing larviparous females and pueriparous females

Summary statistics of spatial autocorrelation analyses comparing larviparous females and pueriparous females (see respective correlogram in Fig. 3b) aimed at testing H1. The Omega test value (ω) and respective *p* value (*p*) for each subsample is displayed. Significant ω values were declared when $p < 0.01$ (values in bold; Smouse et al. 2008). Remaining parameters were estimated for each of the eight distance classes tested: 100 (0–100 m), 200 (101–200 m), 300 (201–300 m), 400 (301–400 m), 500 (401–500 m), 600 (501–600 m), 700 (601–700 m), and 1000 (701–1000 m). These parameters are: N (number of pairs of individuals analysed), *r* (autocorrelation coefficient) and respective lower (*r* lower 95% CI limit) and upper (*r* upper 95% CI limit) bounds of the 95% CIs. The *p* values of one-tailed tests to determine if *r* values were significantly higher (p (*r-rand* ≥ *r-obs*)) or lower (p (*r-obs* ≥ *r-rand*)) than expected for a given distance class are also displayed, with significant *p* values ($p < 0.05$) in bold and underlined.

Data set and parameters	ω (p_ω)	100	200	300	400	500	600	700	1000
Larviparous females	**38.4 (< 0.01)**								
N		188	188	164	158	135	105	58	79
r		0.020	−0.005	−0.008	−0.016	0.008	0.024	−0.018	−0.019
r lower 95% CI limit		0.000	−0.027	−0.027	−0.037	−0.016	−0.001	−0.047	−0.051
r upper 95% CI limit		0.040	0.016	0.013	0.006	0.031	0.048	0.015	0.011
p (*r-rand* ≥ *r-obs*)		**0.017**	0.714	0.782	0.941	0.210	**0.031**	0.858	0.917
p (*r-obs* ≥ *r-rand*)		0.983	0.286	0.218	0.060	0.790	0.970	0.142	0.083
Pueriparous females	**44.3 (< 0.01)**								
N		280	281	241	186	134	100	64	75
r		0.010	−0.017	0.003	0.006	−0.003	0.034	−0.038	−0.004
r lower 95% CI limit		−0.004	−0.032	−0.014	−0.013	−0.024	0.007	−0.066	−0.039
r upper 95% CI limit		0.025	−0.002	0.020	0.026	0.019	0.060	−0.010	0.033
p (*r-rand* ≥ *r-obs*)		0.077	0.992	0.354	0.242	0.602	**0.003**	0.994	0.612
p (*r-obs* ≥ *r-rand*)		0.923	**0.008**	0.646	0.758	0.398	0.997	**0.007**	0.389

Appendix 6: Summary statistics of spatial autocorrelation analyses comparing larviparous males and larviparous females

Summary statistics of spatial autocorrelation analyses comparing larviparous males and larviparous females (see respective correlogram in Fig. 4a) aimed at testing H2. The Omega test value (ω) and respective *p* value (*p*) for each subsample is displayed. Significant ω values were declared when $p < 0.01$ (values in bold; Smouse et al. 2008). Remaining

parameters were estimated for each of the eight distance classes tested: 100 (0–100 m), 200 (101–200 m), 300 (201–300 m), 400 (301–400 m), 500 (401–500 m), 600 (501–600 m), 700 (601–700 m), and 1000 (701–1000 m). These parameters are: N (number of pairs of individuals analysed), *r* (autocorrelation coefficient) and respective lower (*r* lower 95% CI limit) and upper (*r* upper 95% CI limit) bounds of the 95% CIs. The *p* values of one-tailed tests to determine if *r* values were significantly higher (p (*r-rand* ≥ *r-obs*)) or lower (p (*r-obs* ≥ *r-rand*)) than expected for a given distance class are also displayed, with significant *p* values ($p < 0.05$) in bold and underlined.

Data set and parameters	ω (p_ω)	100	200	300	400	500	600	700	1000
Larviparous males	28.4 (0.03)								
N		185	191	160	149	116	87	70	98
r		−0.019	0.013	0.001	0.004	−0.008	−0.005	0.022	−0.001
r lower 95% CI limit		−0.035	−0.007	−0.021	−0.019	−0.032	−0.031	−0.007	−0.028
r upper 95% CI limit		0.001	0.033	0.024	0.028	0.017	0.022	0.053	0.024
p (*r-rand* ≥ *r-obs*)		0.979	0.068	0.446	0.336	0.733	0.662	0.079	0.551
p (*r-obs* ≥ *r-rand*)		**0.021**	0.932	0.554	0.664	0.267	0.338	0.921	0.449
Larviparous females	**38.4 (< 0.01)**								
N		188	188	164	158	135	105	58	79
r		0.020	−0.005	−0.008	−0.016	0.008	0.024	−0.018	−0.019
r lower 95% CI limit		0.000	−0.027	−0.027	−0.037	−0.016	−0.001	−0.047	−0.051
r upper 95% CI limit		0.040	0.016	0.013	0.006	0.031	0.048	0.015	0.011
p (*r-rand* ≥ *r-obs*)		**0.017**	0.714	0.782	0.941	0.210	**0.031**	0.858	0.917
p (*r-obs* ≥ *r-rand*)		0.983	0.286	0.218	0.060	0.790	0.970	0.142	0.083

Appendix 7: Summary statistics of spatial autocorrelation analyses comparing pueriparous males and pueriparous females

Summary statistics of spatial autocorrelation analyses comparing pueriparous males and pueriparous females (see respective correlogram in Fig. 4b) aimed at testing H2. The Omega test value (ω) and respective *p* value (*p*) for each subsample is displayed. Significant ω values were declared when $p < 0.01$ (values in bold; Smouse et al. 2008). Remaining parameters were estimated for each of the eight distance classes tested: 100 (0–100 m), 200 (101–200 m), 300 (201–300 m), 400 (301–400 m), 500 (401–500 m), 600 (501–600 m), 700 (601–700 m), and 1000 (701–1000 m). These parameters are: N (number of pairs of individuals analysed), *r* (autocorrelation coefficient) and respective lower (*r* lower 95% CI limit) and upper (*r* upper 95% CI limit) bounds of the 95% CIs. The p-values of one-tailed tests to determine if *r* values were significantly higher (p (*r-rand* ≥ *r-obs*)) or lower (p (*r-obs* ≥ *r-rand*)) than expected for a given distance class are also displayed, with significant *p* values ($p < 0.05$) in bold and underlined.

Data set and parameters	ω (p_ω)	100	200	300	400	500	600	700	1000
Pueriparous males	21.7 (0.72)								
N		291	303	262	200	168	122	91	116
r		−0.003	0.003	0.006	−0.007	−0.006	0.003	−0.007	0.009
r lower 95% CI limit		−0.018	−0.010	−0.008	−0.024	−0.026	−0.021	−0.030	−0.012
r upper 95% CI limit		0.011	0.017	0.022	0.011	0.015	0.026	0.017	0.030
p (*r-rand* ≥ *r-obs*)		0.699	0.319	0.186	0.806	0.744	0.382	0.699	0.187
p (*r-obs* ≥ *r-rand*)		0.301	0.681	0.814	0.194	0.256	0.618	0.301	0.813
Pueriparous females	**44.3 (< 0.01)**								
N		280	281	241	186	134	100	64	75
r		0.010	−0.017	0.003	0.006	−0.003	0.034	−0.038	−0.004
r lower 95% CI limit		−0.004	−0.032	−0.014	−0.013	−0.024	0.007	−0.066	−0.039
r upper 95% CI limit		0.025	−0.002	0.020	0.026	0.019	0.060	−0.010	0.033
p (*r-rand* ≥ *r-obs*)		0.077	0.992	0.354	0.242	0.602	**0.003**	0.994	0.612
p (*r-obs* ≥ *r-rand*)		0.923	**0.008**	0.646	0.758	0.398	0.997	**0.007**	0.389

Appendix 8: Summary statistics of spatial autocorrelation analyses comparing males and females in each sampled larviparous population

Summary statistics of spatial autocorrelation analyses comparing males and females in each sampled larviparous population (PEGA_Larv, EUME_Larv, and SGAL_Larv; see respective correlograms in Fig. 5). The Omega test value (ω) and respective *p* value (*p*) for each sampled locality is displayed. Significant ω values were declared when $p < 0.01$ (values in bold; Smouse et al. 2008). Remaining parameters were estimated for each of the six distance classes tested: 100 (0–100 m), 200 (101–200 m), 300 (201–300 m), 500 (301–500 m), 700 (501–700 m), and 1000 (701–1000 m). These parameters are: N (number of pairs of individuals analysed), *r* (autocorrelation coefficient) and respective lower (*r* lower 95% CI limit) and upper (*r* upper 95% CI limit) bounds of the 95% CIs. The p-values of one-tailed tests to determine if *r* values were significantly higher (*p* (*r-rand* ≥ *r-obs*)) or lower (*p* (*r-obs* ≥ *r-rand*)) than expected for a given distance class are also displayed, with significant *p* values ($p < 0.05$) in bold and underlined.

Data set and parameters	ω (p_ω)	100	200	300	500	700	1000
PEGA_Larv							
Males	25.0 (0.01)						
N		71	76	58	99	62	41
r		−0.035	0.010	0.009	0.011	−0.003	0.006
r lower 95% CI limit		−0.063	−0.018	−0.021	−0.013	−0.032	−0.034
r upper 95% CI limit		−0.007	0.040	0.035	0.036	0.025	0.050

Data set and parameters	ω (p_ω)	100	200	300	500	700	1000
p (*r-rand* ≥ *r-obs*)		0.997	0.210	0.277	0.153	0.597	0.372
p (*r-obs* ≥ *r-rand*)		**0.003**	0.790	0.723	0.847	0.403	0.628
Females	**30.9 (< 0.01)**						
N		61	44	35	83	45	22
r		0.007	0.042	−0.029	−0.006	0.002	−0.049
r lower 95% CI limit		−0.022	0.005	−0.068	−0.031	−0.035	−0.095
r upper 95% CI limit		0.038	0.075	0.016	0.019	0.043	−0.004
p (*r-rand* ≥ *r-obs*)		0.264	**0.009**	0.950	0.713	0.447	0.988
p (*r-obs* ≥ *r-rand*)		0.736	0.991	**0.049**	0.288	0.553	**0.013**
EUME_Larv							
Males	19.2 (0.11)						
N		64	64	59	93	33	12
r		0.020	0.015	−0.001	−0.021	−0.021	0.035
r lower 95% CI limit		−0.013	−0.026	−0.047	−0.052	−0.075	−0.066
r upper 95% CI limit		0.062	0.063	0.045	0.012	0.031	0.102
p (*r-rand* ≥ *r-obs*)		0.157	0.210	0.514	0.910	0.779	0.214
p (*r-obs* ≥ *r-rand*)		0.844	0.790	0.486	0.091	0.221	0.786
Females	**30.9 (< 0.01)**						
N		55	68	64	92	47	25
r		0.056	−0.030	−0.014	−0.004	0.028	−0.040
r lower 95% CI limit		0.016	−0.074	−0.042	−0.040	−0.015	−0.098
r upper 95% CI limit		0.097	0.014	0.012	0.032	0.069	0.026
p (*r-rand* ≥ *r-obs*)		**0.006**	0.950	0.771	0.625	0.098	0.919
p (*r-obs* ≥ *r-rand*)		0.994	**0.049**	0.230	0.375	0.902	0.081
SGAL_Larv							
Males	**31.9 (< 0.01)**	50	51	43	73	62	45
N		−0.042	0.015	−0.006	0.007	0.033	−0.019
r		−0.070	−0.020	−0.046	−0.022	0.002	−0.052
r lower 95% CI limit		−0.015	0.047	0.037	0.037	0.065	0.015
r upper 95% CI limit		0.996	0.169	0.613	0.301	**0.012**	0.884
p (*r-rand* ≥ *r-obs*)		**0.004**	0.831	0.387	0.699	0.988	0.116
p (*r-obs* ≥ *r-rand*)							
Females	15.1 (0.24)	72	76	65	118	71	32
N		0.005	−0.011	0.009	−0.005	0.001	0.018
r		−0.029	−0.038	−0.020	−0.028	−0.026	−0.030
r lower 95% CI limit		0.035	0.017	0.038	0.018	0.028	0.073
r upper 95% CI limit		0.353	0.788	0.264	0.678	0.479	0.173
p (*r-rand* ≥ *r-obs*)		0.647	0.212	0.736	0.322	0.521	0.827
p (*r-obs* ≥ *r-rand*)		50	51	43	73	62	45

Appendix 9: Summary statistics of spatial autocorrelation analyses estimated and comparing males and females in each sampled pueriparous population

Summary statistics of spatial autocorrelation analyses comparing males and females in each sampled pueriparous population (INFA_Puer, BRAN_Puer, and VILL_Puer; see respective correlograms in Fig. 5). The Omega test value (ω) and respective *p* value (*p*) for each sampled locality is displayed. Significant ω values were declared when $p < 0.01$ (values in bold; Smouse et al. 2008). Remaining parameters were estimated for each of the six distance classes tested: 100 (0–100 m), 200 (101–200 m), 300 (201–300 m), 500 (301–500 m), 700 (501–700 m), and 1000 (701–1000 m). These parameters are: N (number of pairs of individuals analysed), *r* (autocorrelation coefficient) and respective lower (*r* lower 95% CI limit) and upper (*r* upper 95% CI limit) bounds of the 95% CIs. The p-values of one-tailed tests to determine if *r* values were significantly higher (*p* (*r-rand* ≥ *r-obs*)) or lower (*p* (*r-obs* ≥ *r-rand*)) than expected for a given distance class are also displayed, with significant *p* values ($p < 0.05$) in bold and underlined.

Data set and parameters	ω (p_ω)	100	200	300	500	700	1000
INFA_Puer							
Males	15.7 (0.22)						
N		107	105	83	86	63	48
r		0.006	0.006	−0.008	0.003	−0.013	−0.006
r lower 95% CI limit		−0.019	−0.018	−0.035	−0.025	−0.041	−0.041
r upper 95% CI limit		0.030	0.031	0.019	0.032	0.017	0.027
p (*r-rand* ≥ *r-obs*)		0.245	0.293	0.751	0.380	0.834	0.653
p (*r-obs* ≥ *r-rand*)		0.755	0.707	0.250	0.620	0.166	0.347
Females	18.6 (0.12)						
N		88	86	66	93	40	27
r		0.013	−0.019	−0.003	0.011	−0.006	0.003
r lower 95% CI limit		−0.016	−0.049	−0.028	−0.015	−0.041	−0.050
r upper 95% CI limit		0.040	0.011	0.021	0.036	0.034	0.056
p (*r-rand* ≥ *r-obs*)		0.140	0.943	0.584	0.163	0.632	0.444
p (*r-obs* ≥ *r-rand*)		0.860	0.058	0.416	0.837	0.369	0.556
BRAN_Puer							
Males	25.8 (0.02)						
N		71	84	72	117	65	26
r		−0.028	0.001	0.035	−0.010	−0.004	0.029
r lower 95% CI limit		−0.061	−0.030	−0.004	−0.037	−0.036	−0.021
r upper 95% CI limit		0.009	0.032	0.072	0.019	0.029	0.081
p (*r-rand* ≥ *r-obs*)		0.962	0.467	**0.014**	0.795	0.606	0.133
p (*r-obs* ≥ *r-rand*)		**0.038**	0.533	0.987	0.205	0.394	0.867
Females	19.9 (0.14)						
N		84	92	92	127	74	27
r		−0.002	−0.023	0.013	0.003	0.011	−0.004
r lower 95% CI limit		−0.030	−0.052	0.020	−0.021	−0.020	−0.075
r upper 95% CI limit		0.026	0.005	0.045	0.030	0.043	0.070

Data set and parameters	ω (p_ω)	100	200	300	500	700	1000
p (*r-rand* ≥ *r-obs*)		0.538	0.953	0.183	0.388	0.220	0.575
p (*r-obs* ≥ *r-rand*)		0.462	**0.047**	0.817	0.612	0.780	0.425
VILL_Puer							
Males	16.5 (0.18)						
N		113	114	107	165	85	42
r		0.002	0.002	−0.001	−0.009	0.011	0.014
r lower 95% CI limit		−0.018	−0.018	−0.021	−0.027	−0.017	−0.021
r upper 95% CI limit		0.021	0.023	0.019	0.008	0.037	0.049
p (*r-rand* ≥ *r-obs*)		0.414	0.414	0.542	0.895	0.173	0.184
p (*r-obs* ≥ *r-rand*)		0.586	0.586	0.458	0.105	0.827	0.816
Females	17.9 (0.14)						
N		108	103	83	100	50	21
r		0.017	−0.010	−0.002	−0.006	0.006	−0.010
r lower 95% CI limit		−0.007	−0.031	−0.029	−0.028	−0.031	−0.063
r upper 95% CI limit		0.043	0.013	0.024	0.018	0.044	0.045
p (*r-rand* ≥ *r-obs*)		0.057	0.843	0.583	0.703	0.360	0.674
p (*r-obs* ≥ *r-rand*)		0.943	0.157	0.417	0.298	0.641	0.326

Appendix 10: Matrix of pairwise ω_{groups} values (below diagonal) and respective *p* values (above diagonal) between the compared subsamples

Matrix of pairwise ω_{groups} values (below diagonal) and respective p-values (above diagonal) between the compared subsamples (LM—larviparous males; LF—larviparous females; PM—pueriparous males; PF—pueriparous females) in the "combined correlograms" (see Figs. 3 and 4). These analyses aimed at testing both of our hypotheses (i.e. differences in fine-scale genetic structure between reproductive modes [LM versus PM and LF versus PF] and sexes [LM versus LF and PM versus PF]) at global level. Comparisons between males and females were performed only within reproductive mode (NA—not applicable). No pairwise comparison was significant ($p < 0.01$; Banks and Peakall 2012).

Subsample	LM	LF	PM	PF
LM	0	0.02	0.73	0.14
LF	30.00	0	NA	0.57
PM	12.19	NA	0	NA
PF	NA	14.35	22.22	0

Appendix 11: Pairwise t^2 values and respective p-values between the analysed subsamples

Pairwise t^2 values and respective p-values (p_{t2}) between the analysed subsamples (LM—larviparous males; LF—larviparous females; PM—pueriparous males; PF—pueriparous females) for the eight distance classes evaluated: 100 (0–100 m), 200 (101–200 m), 300

(201–300 m), 400 (301–400 m), 500 (401–500 m), 600 (501–600 m), 700 (601–700 m), and 1000 (701–1000 m). These analyses aimed at testing both of our hypotheses (i.e. differences in fine-scale genetic structure between reproductive modes [LM versus PM and LF versus PF] and sexes [LM versus LF and PM versus PF]) at distance class level. Significant pairwise comparisons ($p_{t2}<0.01$; Banks and Peakall 2012) are in bold and underlined.

Comparison	100	200	300	400	500	600	700	1000
Hypothesis 1								
LM vs. PM								
t^2	1.22	0.53	0.12	0.53	0.01	0.20	2.12	0.30
p_{t2}	0.27	0.47	0.74	0.48	0.91	0.66	0.15	0.58
LF vs. PF								
t^2	0.61	0.75	0.59	1.93	0.41	0.29	0.85	0.37
p_{t2}	0.43	0.39	0.45	0.17	0.52	0.59	0.36	0.55
Hypothesis 2								
LM vs. LF								
t^2	**7.03**	1.37	0.35	1.51	0.83	2.33	3.20	0.72
p_{t2}	**≤0.01**	0.24	0.56	0.22	0.37	0.13	0.07	0.39
PM vs. PF								
t^2	1.53	3.38	0.09	0.98	0.04	2.76	2.70	0.37
p_{t2}	0.21	0.07	0.77	0.32	0.84	0.10	0.10	0.55

Appendix 12: Matrix of pairwise ω_{groups} values (below diagonal) and respective *p* values (above diagonal) between males sampled from different sampled localities

Matrix of pairwise ω_{groups} values (below diagonal) and respective p-values (above diagonal) between males sampled from different sampled localities. These analyses aimed at testing if males from pairs of populations exhibiting the same or different (italics) reproductive modes showed significant differences in fine-scale genetic structure at global level. No pairwise comparison was significant ($p<0.01$; Banks and Peakall 2012).

population	PEGA_Larv	EUME_Larv	SGAL_Larv	INFA_Puer	BRAN_Puer	VILL_Puer
PEGA_Larv	0	0.18	0.74	*0.56*	*0.75*	*0.49*
EUME_Larv	16.29	0	0.04	*0.78*	*0.30*	*0.83*
SGAL_Larv	8.56	21.96	0	*0.28*	*0.16*	*0.39*
INFA_Puer	*10.69*	*8.09*	*14.29*	0	0.19	0.85
BRAN_Puer	*8.43*	*14.13*	*16.80*	16.01	0	0.54
VILL_Puer	*11.37*	*7.38*	*12.81*	7.17	10.87	0

Appendix 13: Matrix of pairwise ω_{groups} values (below diagonal) and respective *p* values (above diagonal) between females sampled from different sampled localities

Matrix of pairwise ω_{groups} values (below diagonal) and respective p-values (above diagonal) between females sampled from different sampled localities. These analyses aimed at testing if females from pairs of populations exhibiting the same or different (italics) reproductive modes showed significant differences in fine-scale genetic structure at global level. No pairwise comparison was significant ($p < 0.01$; Banks and Peakall 2012).

Population	PEGA_Larv	EUME_Larv	SGAL_Larv	INFA_Puer	BRAN_Puer	VILL_Puer
PEGA_Larv	0	0.09	0.31	*0.23*	*0.16*	*0.54*
EUME_Larv	18.96	0	0.14	*0.28*	*0.20*	*0.50*
SGAL_Larv	13.84	17.22	0	*0.95*	*0.96*	*0.98*
INFA_Puer	*15.18*	*14.33*	*5.22*	0	0.93	0.98
BRAN_Puer	*16.56*	*15.83*	*4.82*	5.77	0	0.86
VILL_Puer	*10.86*	*11.28*	*4.05*	4.23	6.84	0

Appendix 14: Matrices of pairwise t^2 values (below diagonal) and respective *p* values (above diagonal) between males from different sampled localities

Matrices of pairwise t^2 values (below diagonal) and respective p-values (above diagonal) between males from different sampled localities. These analyses aimed at testing if males from populations exhibiting the same or different (italics) reproductive modes showed significant differences in fine-scale genetic structure at distance class level. Six distance classes were evaluated: 100 (0–100 m), 200 (101–200 m), 300 (201-300 m), 500 (301–500 m), 700 (501–700 m), and 1000 (701–1000 m). No pairwise comparison was significant ($p_{t2} < 0.01$; Banks and Peakall 2012).

Population	PEGA_Larv	EUME_Larv	SGAL_Larv	INFA_Puer	BRAN_Puer	VILL_Puer
100 m						
PEGA_Larv	0	0.02	0.76	*0.04*	*0.75*	*0.07*
EUME_Larv	5.66	0	0.01	*0.55*	*0.04*	*0.45*
SGAL_Larv	0.09	6.39	0	*0.04*	*0.55*	*0.07*
INFA_Puer	*4.17*	*0.36*	*4.29*	0	0.09	0.79
BRAN_Puer	*0.11*	*4.15*	*0.36*	2.86	0	0.15
VILL_Puer	*3.16*	*0.58*	*3.17*	0.07	2.05	0
200 m						
PEGA_Larv	0	0.83	0.84	*0.83*	*0.67*	*0.70*
EUME_Larv	0.04	0	1.00	*0.68*	*0.53*	*0.58*
SGAL_Larv	0.04	0.00	0	*0.70*	*0.57*	*0.60*
INFA_Puer	*0.05*	*0.17*	*0.15*	0	0.82	0.83
BRAN_Puer	*0.18*	*0.37*	*0.32*	0.05	0	0.97

Population	PEGA_Larv	EUME_Larv	SGAL_Larv	INFA_Puer	BRAN_Puer	VILL_Puer
VILL_Puer	*0.16*	*0.31*	*0.27*	0.04	0.00	0
300 m						
PEGA_Larv	0	0.83	0.84	*0.83*	*0.67*	*0.70*
EUME_Larv	0.04	0	1.00	*0.68*	*0.53*	*0.58*
SGAL_Larv	0.04	0.00	0	*0.70*	*0.57*	*0.60*
INFA_Puer	*0.05*	*0.17*	*0.15*	0	0.82	0.83
BRAN_Puer	*0.18*	*0.37*	*0.32*	0.05	0	0.97
VILL_Puer	*0.16*	*0.31*	*0.27*	0.04	0.00	0
500 m						
PEGA_Larv	0	0.69	0.59	*0.46*	*0.27*	*0.66*
EUME_Larv	0.16	0	0.85	*0.76*	*0.14*	*1.00*
SGAL_Larv	0.29	0.03	0	*0.93*	*0.12*	*0.86*
INFA_Puer	*0.57*	*0.10*	*0.01*	0	0.05	0.72
BRAN_Puer	*1.23*	*2.22*	*2.34*	3.90	0	0.10
VILL_Puer	*0.19*	*0.00*	*0.03*	0.13	2.76	0
700 m						
PEGA_Larv	0	0.11	0.83	*0.69*	*0.25*	*0.25*
EUME_Larv	2.56	0	0.19	*0.26*	*0.58*	*0.60*
SGAL_Larv	0.04	1.74	0	*0.87*	*0.42*	*0.46*
INFA_Puer	*0.16*	*1.27*	*0.02*	0	0.48	0.47
BRAN_Puer	*1.33*	*0.31*	*0.65*	0.48	0	0.99
VILL_Puer	*1.34*	*0.28*	*0.56*	0.51	0.00	0
1000 m						
PEGA_Larv	0	0.53	0.12	*0.66*	*0.96*	*0.52*
EUME_Larv	0.40	0	0.05	*0.77*	*0.54*	*0.26*
SGAL_Larv	2.41	3.85	0	*0.06*	*0.11*	*0.36*
INFA_Puer	*0.18*	*0.08*	*3.71*	0	0.70	0.27
BRAN_Puer	*0.00*	*0.37*	*2.62*	0.15	0	0.49
VILL_Puer	*0.40*	*1.24*	*0.87*	1.20	0.47	0

Appendix 15: Matrices of pairwise t^2 values (below diagonal) and respective *p* values (above diagonal) between females from different sampled localities

Matrices of pairwise t^2 values (below diagonal) and respective p-values (above diagonal) between females from different sampled localities. These analyses aimed at testing if females from populations exhibiting the same or different (italics) reproductive modes showed significant differences in fine-scale genetic structure at distance class level. Six distance classes were evaluated: 100 (0–100 m), 200 (101–200 m), 300 (201–300 m), 500 (301–500 m), 700 (501–700 m), and 1000 (701–1000 m). Significant t^2 tests are in bold and underlined ($p_{t2} < 0.01$; Banks and Peakall 2012).

Population	PEGA_Larv	EUME_Larv	SGAL_Larv	INFA_Puer	BRAN_Puer	VILL_Puer
100 m						
PEGA_Larv	0	0.05	0.92	*0.82*	*0.68*	*0.68*
EUME_Larv	3.95	0	0.03	*0.06*	*0.01*	*0.08*
SGAL_Larv	0.01	4.65	0	*0.71*	*0.74*	*0.55*
INFA_Puer	*0.05*	*3.68*	*0.14*	0	0.46	0.82
BRAN_Puer	*0.16*	*6.18*	*0.11*	0.52	0	0.32
VILL_Puer	*0.17*	*3.13*	*0.36*	0.05	1.00	0
200 m						
PEGA_Larv	0	**≤0.01**	0.06	*0.02*	*0.02*	*0.05*
EUME_Larv	**6.95**	0	0.42	*0.62*	*0.77*	*0.38*
SGAL_Larv	3.70	0.66	0	*0.71*	*0.56*	*0.98*
INFA_Puer	*5.37*	*0.25*	*0.14*	0	0.82	0.68
BRAN_Puer	*5.73*	*0.08*	*0.34*	0.05	0	0.51
VILL_Puer	*3.90*	*0.77*	*0.00*	0.17	0.43	0
300 m						
PEGA_Larv	0	0.59	0.18	*0.34*	*0.13*	*0.34*
EUME_Larv	0.29	0	0.33	*0.63*	*0.23*	*0.61*
SGAL_Larv	1.77	0.96	0	*0.61*	*0.87*	*0.60*
INFA_Puer	*0.89*	*0.24*	*0.26*	0	0.47	0.99
BRAN_Puer	*2.25*	*1.43*	*0.03*	0.51	0	0.45
VILL_Puer	*0.92*	*0.27*	*0.27*	0.00	0.57	0
500 m						
PEGA_Larv	0	0.94	0.96	*0.46*	*0.68*	*1.00*
EUME_Larv	0.01	0	0.98	*0.48*	*0.70*	*0.93*
SGAL_Larv	0.00	0.00	0	*0.43*	*0.65*	*0.95*
INFA_Puer	*0.57*	*0.51*	*0.63*	0	0.70	0.41
BRAN_Puer	*0.18*	*0.14*	*0.20*	0.14	0	0.62
VILL_Puer	*0.00*	*0.01*	*0.00*	0.68	0.25	0
700 m						
PEGA_Larv	0	0.38	0.96	*0.79*	*0.74*	*0.91*
EUME_Larv	0.80	0	0.29	*0.25*	*0.52*	*0.42*
SGAL_Larv	0.00	1.12	0	*0.80*	*0.64*	*0.85*
INFA_Puer	*0.07*	*1.36*	*0.06*	0	0.52	0.70
BRAN_Puer	*0.11*	*0.40*	*0.23*	0.43	0	0.81
VILL_Puer	*0.01*	*0.65*	*0.04*	0.16	0.06	0
1000 m						
PEGA_Larv	0	0.84	0.12	*0.24*	*0.32*	*0.41*
EUME_Larv	0.04	0	0.15	*0.30*	*0.40*	*0.51*
SGAL_Larv	2.45	2.06	0	*0.70*	*0.59*	*0.50*
INFA_Puer	*1.38*	*1.04*	*0.15*	0	0.87	0.77
BRAN_Puer	*1.00*	*0.73*	*0.30*	0.03	0	0.88
VILL_Puer	*0.67*	*0.43*	*0.45*	0.09	0.02	0

Appendix 16: Results of heterogeneity t^2 tests, as well as respective p values, between males and females in each sampled locality

Results of heterogeneity t^2 tests, as well as respective p-values, between males and females in each sampled locality. These tests aimed at examining differences in fine-scale genetic structure between sexes in each sampled locality at global and distance class levels. A total of six distance classes were evaluated: 100 (0–100 m), 200 (101–200 m), 300 (201–300 m), 500 (301–500 m), 700 (501–700 m), and 1000 (701–1000 m). No significant differences in genetic structure were found ($p_{t2} < 0.01$; Banks and Peakall 2012).

Pop (males vs. females)	100	200	300	500	700	1000
PEGA_Larv						
t^2 (p_{t2})	3.48 (0.06)	1.56 (0.21)	2.12 (0.15)	0.80 (0.37)	0.05 (0.81)	2.47 (0.12)
EUME_Larv						
t^2 (p_{t2})	1.44 (0.23)	1.84 (0.17)	0.20 (0.65)	0.42 (0.52)	1.99 (0.16)	1.94 (0.17)
SGAL_Larv						
t^2 (p)	3.99 (0.04)	1.22 (0.27)	0.35 (0.55)	0.33 (0.56)	2.17 (0.14)	1.46 (0.23)
INFA_Puer						
t^2 (p_{t2})	0.11 (0.75)	1.60 (0.21)	0.08 (0.77)	0.14 (0.71)	0.09 (0.77)	0.08 (0.77)
BRAN_Puer						
t^2 (p_{t2})	1.30 (0.25)	1.26 (0.26)	0.88 (0.35)	0.47 (0.50)	0.46 (0.49)	0.48 (0.49)
VILL_Puer						
t^2 (p)	0.84 (0.36)	0.57 (0.46)	0.01 (0.93)	0.05 (0.81)	0.05 (0.83)	0.56 (0.45)

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