

Scale-dependent effects of terrestrial habitat on genetic variation in the great crested newt (*Triturus cristatus*)

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Abstract

Context

Terrestrial landscapes surrounding aquatic habitat influence the persistence of amphibian spatially structured populations (SSPs) via their crucial role in providing estivation and overwintering sites, facilitating or hampering dispersal and colonisation, and consequently the maintenance or loss of genetic diversity.

Objectives

To highlight the landscape drivers of genetic variation, we investigated the relationship between the level of genetic variation measured within ponds of the great crested newt (*Triturus cristatus*), and the composition of the surrounding landscape at various spatial scales.

The present pdf is the author version of the manuscript.

The published version with the layout of the publisher is available in the publisher website (Springer):

<https://doi.org/10.1007/s10980-021-01297-5>

Methods

Based on the sampling of 40 ponds in thirteen SSPs, the influence of landscape features on several estimators of genetic variation was investigated via linear mixed models, with effects within and between SSPs incorporated.

Results

The best models depended on the spatial scale, with more significant associations within radii of 50 and 100 m of core ponds, particularly for allelic richness. Responses within and between SSPs were mostly similar. The availability of aquatic habitat in the landscape had a positive effect, while woodland, arable land and pasture had different effects depending on scale and response variable. Total length of roads within a 250 m radius influenced effective population size negatively.

Conclusions

Our results stress the need to investigate the influence of environmental predictors at multiple spatial scales for an adequate understanding of ongoing processes. Generally, the landscape affected genetic variation similarly within and between SSPs. This allowed us to provide general guidelines for the persistence of great crested newt populations, with an emphasis on the importance of the aquatic habitat.

Keywords

amphibians, genetic diversity, habitat fragmentation, spatial scales, *Triturus cristatus*

Introduction

Habitat loss and fragmentation due to human activities are the main causes of population declines and extinctions (Fischer 2000; Pimm and Raven 2000). They can lead to reduced gene flow among populations and decreasing population sizes with potentially negative consequences, such as an accelerated loss of genetic variation by genetic drift, inbreeding and an increasing risk of population extinction (Allendorf et al. 2013). As a population's adaptive and evolutionary potential is reflected in its level of genetic diversity (Frankham et al. 2017), it becomes important to learn how environmental factors might influence genetic variation.

Given their semi-aquatic life cycle, many amphibians occur in spatially structured populations. Spatially structured populations are typically described as a network of local populations that occupy discrete habitat patches connected by dispersing individuals (Thomas and Kunin 1999; Revilla and Wiegand 2008). The persistence of the spatially structured amphibian population does not only depend on the quality of the habitat sustaining the aquatic phase of the species, but also on the quality of the terrestrial habitat enabling estivating, overwintering, migration and dispersal. Terrestrial dispersal events include natal dispersal (from the natal site to the breeding site) and breeding dispersal (between breeding sites) (Semlitsch 2008). The configuration of the landscape matrix can therefore fundamentally affect connectivity of local populations (Van Buskirk 2005; Cayuela et al. 2020a). As the surrounding landscape plays an important role in dispersal, it is also involved in the maintenance or

loss of genetic diversity (Hamer and McDonnell 2008). Gene flow (i.e. dispersal followed by reproduction, also termed effective dispersal) decreases genetic divergence among local populations and counters genetic drift (i.e. random loss due to a finite population size) and inbreeding in local populations (Whitlock et al. 2000; Keller and Waller 2002). The loss of genetic diversity caused by genetic drift is inversely proportional to the effective population size (N_e). Consequently, already small populations will increasingly experience negative genetic effects under environmental pressures such as habitat destruction and pollution (Ralls et al. 2018).

So far, few studies directly investigated the relationship between habitat features and genetic variation within spatially structured amphibian populations (but see Curtis and Taylor 2004; Cosentino et al. 2012; Homola et al. 2019; Haugen et al. 2020). This should not be neglected as the habitat matrix might impose preconditions on genetic responses that are different from demographic observations. In addition, the spatial extent studied is also considered to play an important role (Cushman and McGarigal 2004; Galpern et al. 2012). Choosing a particular scale to identify landscape characteristics that influence populations can provide misleading predictions and leave landscape effects undetected (Jackson and Fahrig 2014). Although the number of studies using multiple geographical scales is steadily increasing, most of them have focussed on either abundance or presence/absence data (Gustafson et al. 2009; Hartel et al. 2010a; Denoël et al. 2013; Chambers et al. 2016; Macdonald et al. 2018; Boissinot et al. 2019), or proxies for gene flow such as genetic distances or data on migration (Johnson et al. 2002; Galpern et al. 2012; Coster et al. 2015; Cushman et al. 2016; Krishnamurthy et al. 2016; Zeller et al. 2017; Burgess and Garrick 2020; Winiarski et al. 2020).

Here, we studied thirteen spatially structured populations of the great (or northern) crested newt, *Triturus cristatus* (Laurenti, 1768). This amphibian species occurs throughout Europe (except southern Europe, where it is replaced by members of the same genus), and in parts of Western Siberia (Speybroeck et al. 2016). It receives EU-wide protection, being listed in Annexes II and IV of the European Habitats Directive (92/43/EEC 1992). As a result of significant population declines observed in monitoring programs and regional assessments, the species is red-listed in the majority of countries and regions within its range (Dufresnes and Perrin 2015). Denoël (2012) found similar decline patterns in different countries and therefore suggested the species to be more susceptible than other newt species to environmental disturbance. Several studies exist on the effects of pond and terrestrial habitat characteristics on the abundance and occurrence of great crested newt, on its reproductive success, and on its dispersal behaviour (see e.g. Jehle and Arntzen 2000; Joly et al. 2001; Sztatecsny et al. 2004; Hartel et al. 2010b; Denoël et al. 2013; Vuorio et al. 2015; Miró et al. 2017; Denoël et al. 2018). In this study, we investigated the relationship between the level of genetic variation of great crested newts within breeding ponds, and the composition of the surrounding terrestrial landscape at various spatial scales using a hierarchical sampling design across multiple spatially structured populations. In addition to the multi-scale approach, several metrics estimating genetic variation, including effective population size, were used to provide overall and complementary information on the terrestrial landscape drivers of genetic variation. The specific aims were to investigate (1) which land cover types and features affect current genetic variation and effective population size of great crested newts at the pond-level, (2) if landscape components may have a different effect within and between spatially structured populations, (3) if scale affects these relationships and (4) at what scale genetic variation and effective population are most likely to be influenced by the terrestrial landscape.

Material and methods

Study sites, sampling and genotyping methods

The study area comprises thirteen spatially structured populations (SSP) of great crested newt located in different landscapes in Belgium. The majority of the sampled SSPs are situated in rural to peri-urban areas with agricultural fields, mainly consisting of pastures and arable land, and with urban elements surrounding the breeding ponds and SSPs. Human population density in Belgium is very high (377 people/km² in 2018; <https://data.worldbank.org/indicator/EN.POP.DNST>), especially in northern Belgium where the land surface is dominated by arable land and urban areas (Fig. 1, Fig. S1). One to six ponds per SSP were sampled, delivering samples of 1179 adults, 58 metamorphosed juveniles and 299 larvae collected in 53 ponds in total (Fig. S1, Table S1).

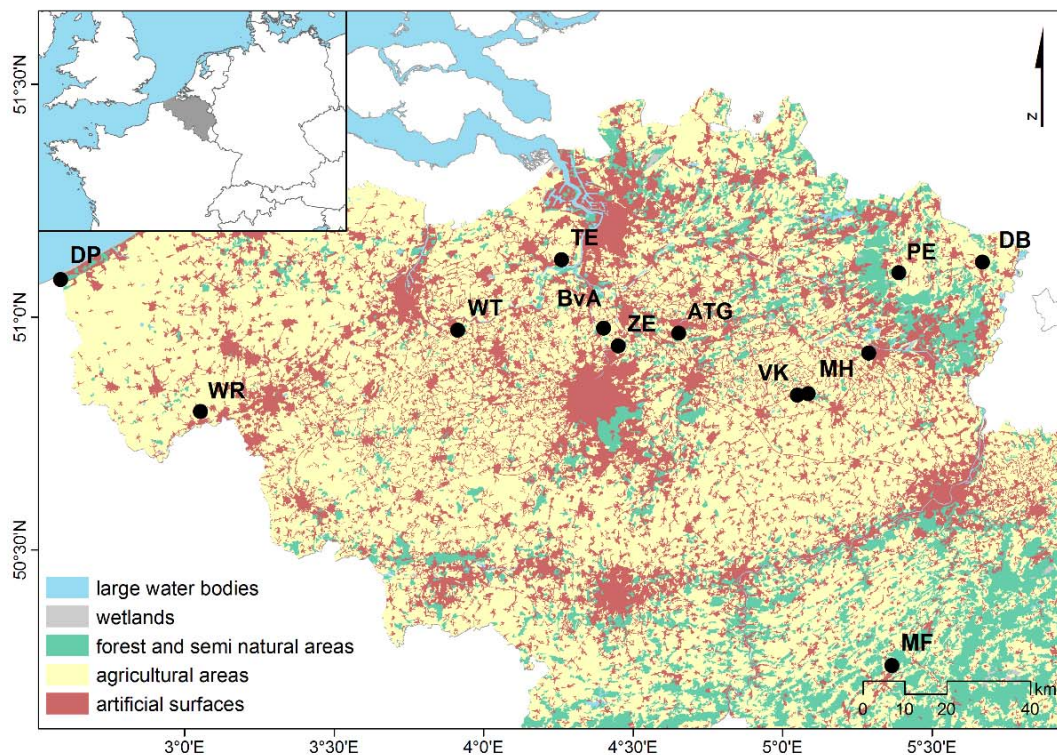


Fig. 1 Sampled spatially structured populations of great crested newt in Belgium indicated with black dots. The inset map shows part of Europe with Belgium indicated in grey. The background for Belgium is built from the Corine land cover data of 2018 (<http://dataservice.eea.europa.eu>) with the five major classes depicted here

DNA was extracted and genotyped at 31 microsatellite markers. Recaptured individuals were identified on the basis of the pattern on the newts' bellies and on their genotypes. Further potential bias in estimates of genetic variation can come from sampling close relatives due to the sampling design, such as sampling newt larvae in ponds (Goldberg and Waits 2010; O'Connell et al. 2019). Recaptures and all but one member per full-sib family were removed from the dataset for further analysis. We checked the remaining genotypes for the presence of null alleles, for possible deviations from Hardy-Weinberg

equilibrium (HWE), and for linkage disequilibrium (LD) between pairs of loci. A more detailed description of the methodology can be found in Supplementary Material (Text S1, Tables S1 and S2).

After excluding low quality genotypes, recaptures and full sibs among larvae, genotypes of 1009 adults, 55 metamorphosed juveniles and 268 larvae remained, divided over 52 ponds in thirteen SSPs (Table S1). In the models to study the landscape effects on genetic variation we only included ponds with a minimum sample size of six individuals (see further) which reduces the number of studied ponds to 40 (Table S1 and S3).

Population genetics

In order to identify genetic clusters among SSPs and among breeding ponds within SSPs, we used the Bayesian program BAPS v. 6.0 (Corander et al. 2008). We performed 10 runs for each $K = 1-40$. An admixture analysis (Corander and Marttinen 2006) was performed using 100 iterations, a minimum of four individuals per population, 200 reference individuals for each population, and 20 iterations of reference individuals.

A second approach to assess population structure was used, Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010) implemented in the R package *adegenet* 2.1.3 (Jombart 2008). Unlike BAPS, DAPC is independent from Hardy-Weinberg assumptions or linkage disequilibrium. We searched for clusters *de novo* by using the K-means procedure, with $K = 1 - 60$ with ten replicates per K . The best clustering model was selected based on the Bayesian Information Criterion (BIC). After an initial DAPC considering 150 principal components (PCs), the optimal number of PCs was chosen as the number PCs maximising the α -score. The α -score is the difference between the reassignment probability of the analysis and the reassignment probability obtained using random clusters.

Pairwise genetic differentiation F_{ST} (Weir and Cockerham 1984) among ponds, with a minimal sample size of six individuals, with corrected 95 % confidence intervals using 1000 bootstraps were calculated with the R package *diveRsity* 1.9.90 (Keenan et al. 2013). We further tested for differences in relatedness within and between ponds for each SSP with at least two sampled ponds with a minimum of six genotypes each using the DyadML coefficient (Milligan 2003) implemented in COANCESTRY 1.0.1.9 (Wang 2011). By randomly drawing dyads 1000 times while keeping the properties of the original groups, the average relatedness within ponds and difference in relatedness between ponds was then calculated each time, delivering a distribution with which the observed difference was compared. For each SSP and pond, observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (A_R) and the inbreeding coefficient (F_{IS}) were estimated using the R package *hierfstat* 0.04-22 (Goudet 2005).

Estimates of effective population size (N_e) were calculated using the linkage disequilibrium method LDNe with a bias correction (Waples and Do 2008) implemented in *NeEstimator* 2.01 (Do et al. 2014) assuming random mating. The jackknife method was used to estimate 95 % confidence intervals across loci and a threshold for minimum allele frequency was set at 0.02 to further reduce bias (Waples and Do 2010). Since the great crested newt is an iteroparous species and mostly mixed-age samples were collected, the number of effective breeders (N_b) refers to a mix of overlapping sets of parents in different years (Waples 2010). Waples et al. (2014) found that when a sample contains as many cohorts

as there are in a generation, the estimated N_b approaches N_e . We also used a sibship assignment (SA) method implemented in the software COLONY 2.0.6.5 (Wang 2009; Jones and Wang 2010) to estimate N_e . Estimates from the software COLONY were based on the assumption of polygamous and random mating, and using the same settings as for the identification of full sibs among larvae were (Text S1). We did not include the estimates using only larval samples, which equal N_b for one reproductive cycle, because the majority of samples per pond contain mostly or exclusively samples of adults. To obtain values for N_e that are better comparable among ponds, we used only the genotypes of adults and metamorphosed juveniles for the calculations. For the SA method, we divided the samples into candidate mothers and fathers. Given the mixed ages of the adults, we included both adults and juveniles as potential offspring (Wang 2009; Wang and Santure 2009).

Landscape effects on genetic variation within and between SSPs

To assess the influence of the landscape surrounding the investigated ponds on the genetic variation, linear mixed models were built with estimates of A_R or F_{IS} as the response variable. H_E was highly correlated with A_R (Pearson's $r = 0.96$, $p < 0.0001$). We therefore chose not to use H_E as a response variable. We used estimates of N_e calculated with the LD- and SA-method as response variables as well. We used only estimates of N_e with finite 95% confidence limits as a response variable (log transformed).

Mixed effects models allowed us to analyse nested data (breeding ponds within SSPs). Based on the genetic structure results, no higher level of hierarchy had to be added to the models. Ponds with less than six samples were discarded.

As explanatory variables we derived land cover data from the map layer 'Bodembedekkingskaart' (BBK; Agentschap Informatie Vlaanderen (AIV)) created for Flanders with a resolution of 1 m. We used the editions for the situation in 2012 and 2015, as close to the two sampling periods as possible (2011-2012 and 2018, respectively). Additional landscape features were extracted from the map layer 'Grootschalig Referentie Bestand' (GRB) from the editions for 2014 and 2018 (AIV). Furthermore, a map layer with all known lentic features, 'Waternlakken version 1.0' (WV) (Packet et al. 2018), was used. Since these layers were developed for the region of Flanders, border locations such as De Panne (DP; close to the French border), Wervik (WR; close to the border with the Walloon region) and Marche-en-Famenne (MF; in the Walloon region) were not or not completely covered. We, therefore, used orthophotographs (summer images, 2012 edn., AIV) to complete the missing information for location WR, for which only a small percentage of the area was not covered by the Flemish maps, and for MF (2009-2010 edn., Service Public de Wallonie), while CES-OSO (Centre d'Expertise Scientifique sur l'occupation des sols) land cover map for France 2018 (Inglada et al. 2017) with a resolution of 10 m was used to derive land cover data for the missing part in location DP. To translate the land cover types in this map to those used in the layers BBK, GRB and WV, we assessed the overlapping parts together with the information obtained from orthophotos. Specifically for the location in question, on the North Sea Coast within the dunes, this resulted in transforming coniferous stands, according to CES-OSO, to grassland and shrubs, as defined by the BBK. Roads and railways in the coastal population were further identified on orthophotos and transformed to shapefiles in ArcGIS 10.4.1 (Esri 2016).

The effect of particular land cover types and features on genetic variation can be scale-dependent. For that reason, we derived landscape data within buffers of different sizes around each of the ponds, with a radius of 50, 100, 250 and 500 m. These distances were chosen according to the diversity of landscape components in the studied areas and to encompass distances commonly crossed by great crested newts during migratory movements and dispersal to other breeding patches as well as during other terrestrial activities such as feeding, estivation, and overwintering (Miaud et al. 1993; Jehle 2000; Jehle and Arntzen 2000; Kupfer and Kneitz 2000). We did not include larger buffer sizes in the study due to the limited distances between the sampled ponds within the majority of the SSPs, ranging from 19 to 1702 m (mean = 327 m; Table S3). The following land cover classes were selected and calculated as the proportion of the investigated buffer area: natural grassland and shrubs, pastures, arable land, trees (small woodlands and forests) and surface water. Surface water included both lotic and lentic waters, since great crested newt was found in both ponds and some small canals, including the canal in ATG. However, this does not encompass larger lentic and lotic systems as none of these elements were present within the buffers used. The length of roads (all paved roads) was calculated as length (m) within a buffer. Distance to the nearest pond (m) was also included, which does not change with buffer size. The number of lentic systems within a buffer was also considered. The land cover types and landscape features are listed in Table S4. All operations to create buffers and to extract geospatial data from the maps within buffers were performed in the R environment (R Core Team 2019) using the packages raster 3.0-7 (Hijmans 2019), rgdal 1.4-8 (Bivand et al. 2019), rgeos 0.5-2 (Bivand and Rundel 2019), sp 1.3-1 (Pebesma and Bivand 2005; Bivand et al. 2013) and sf 0.8-0 (Pebesma 2018).

Adding a landscape component (which varies at the level of ponds) as explanatory variable may mask that the landscape component may have a different effect (in sign and/or strength) within versus between SSPs. Therefore, we used within-population centering to separate the within-SSP (W) effects from the between-SSP (B) effects (van de Pol and Wright 2009). The standard equation for a linear mixed model is given in Eq. 1, where x_{ij} is the pond-level covariate, u_{0j} the random intercept for SSP j and ϵ_{ij} the pond-level residual.

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + u_{0j} + \epsilon_{ij} \quad (\text{Eq. 1})$$

with $u_{0j} \sim N(0, \sigma_{u_{0j}}^2)$ and $\epsilon_{ij} \sim N(0, \sigma_{\epsilon_{ij}}^2)$

This can be changed to an equation with population-mean centered pond-level covariates, which expresses variation within SSP (β_W), and with population-mean (\bar{x}_j) added as a new covariate, which expresses the variation between SSPs (β_B) (Eq. 2).

$$y_{ij} = \beta_0 + \beta_W(x_{ij} - \bar{x}_j) + \beta_B(\bar{x}_j) + u_{0j} + \epsilon_{ij} \quad (\text{Eq. 2})$$

This adjustment to a standard mixed model allows us to test whether within-SSP and between-SSP effects are significantly different, by rearranging Eq. 2 (Eq. 3).

$$y_{ij} = \beta_0 + \beta_W x_{ij} + (\beta_B - \beta_W) \bar{x}_j + u_{0j} + \epsilon_{ij} \quad (\text{Eq. 3})$$

If the within-SSP and between-SSP effects for a certain variable are the same, then the population-mean is not included in the model (formulated according to Eq. 2).

We used the variance inflation factor (VIF) to evaluate the presence of multicollinearity, with a threshold of 3 to remove variables (Zuur and Ieno 2016). Since observations change with buffer size, the analysis was repeated for every buffer radius. Also, roads were only considered in the larger buffer sizes, starting at a radius of 250 m, as they were often absent in the smaller buffer sizes. The initial models did, therefore, not contain entirely the same set of land cover types as predictor variables. Still, we tried to create similar models with largely the same fixed predictor variables. Next, we used the *dredge* function of the R package MuMIn 1.43.17 (Bartón 2020) to find the best models with a $\Delta AICc$ of 2 or less than the top model. For this set of models, full model averaged coefficient estimates were calculated with their standard error, 95% confidence intervals, Z-values and *p*-values.

Results

Genetic diversity parameters for each microsatellite locus, averaged over ponds with at least six genotypes, are given in Table S5. Loci that were not in HWE after Bonferroni correction were locus TRCR427 in ponds 12s (BvA) and ZE6 (ZE), locus Tcri27 in pond PE (VK), loci Tcri29 and TRCR406 in pond P15 (ATG), and locus Tc50 in pond 12s (BvA). Proportions of null alleles were higher than 20% for several loci mostly in one to three ponds within a single SSP (common results from GENEPOP and ML-NULLFREQ). Locus TRCR427 appeared to have null alleles in ZE and pond 12s (BvA).

Several pairs of loci appeared to be in LD (6.7 % of all pairs), of which seven pairs tested positive for LD at multiple ponds (between two and four ponds) with Bonferroni correction. After excluding larvae and reducing the number of adults to limit the chance of including families or multiple cohorts (Text S1), the remaining pairs of loci still exhibiting significant LD (3.2 % of all pairs) were limited to one pond.

Signals of Hardy-Weinberg disequilibria and null alleles seemed mostly SSP dependent. However, loci TRCR427 and TRCR406 came up multiple times in these tests, including those of LD detection. We therefore calculated F_{ST} values with and without these loci. As F_{ST} values hardly changed, we included both loci for further analysis.

Population genetics

The analysis using BAPS produced an optimal K of 15. This generally means that each SSP forms a separate cluster, but with a substructure in two SSPs (Fig. 2). Pond WR2 clustered separately from WR3 in SSP WR, and the majority of the samples in pond TE2 formed a separate cluster from the remaining samples of population TE. One individual of TE4 and one of TE5 were assigned to that cluster of TE2 and a few other samples showed some admixture between both genetic groups. The K-means results delivered the lowest mean BIC for K = 15 (mean BIC = 2862.8). However, similar BIC values were obtained for K between 12 and 17 groups (maximum mean BIC= 2865.2; Fig. S2). The DAPC results for K = 15 also clustered most SSPs separately, except for PE, with individuals assigned to the cluster containing TO and to the cluster containing DB (Fig. 2). Individuals of ponds TE2, WR2 and WR3 were not assigned to separate clusters, while SSPs WT and BvA were subdivided in two groups each, though not according to pond membership. Higher K values did also not always deliver the same SSPs to be subdivided, while other SSPs were assigned to more than one cluster, again not according to their breeding patch (results not shown). The DAPC results for K =9 to 13 delivered no multiple clusters

within populations, although for $K = 14$ the majority of the individuals of TE2 was assigned to a separate cluster, comparable to the BAPS results.

The SSPs were highly differentiated from each other (Fig. S3), even SSPs that are relatively near each other such as ZE and BvA ($F_{ST} = 0.27$, CI = 0.26 – 0.29). ATG is less genetically differentiated from VK than is expected based on the distance between them ($F_{ST} = 0.07$, CI = 0.06 – 0.08). Genetic differentiation estimates among ponds within SSPs were mostly small, though frequently significant (Fig. S3). In agreement with the clustering results, samples from pond WR2 were moderately differentiated from the samples from WR3 ($F_{ST} = 0.09$, CI = 0.07 – 0.12). Pond TE2 showed, however, a maximum among pond F_{ST} value of only 0.05 within TE.

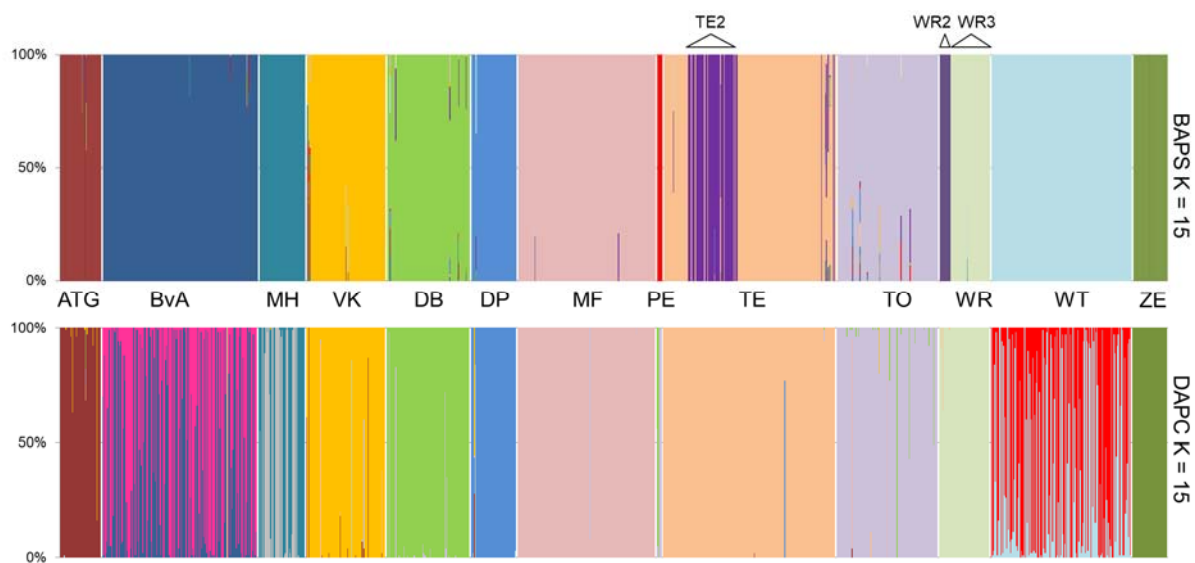


Fig. 2 Bar plot of the Bayesian Analysis of Population Structure (BAPS) results (upper) and of the Discriminant Analysis of Principal Components (DAPC) results (lower) of great crested newt genotypes. The different colours represent different genetic clusters (15) with population codes given on the x-axis. Ponds that clustered separately according to the BAPS results are indicated above the plot

Small but significant differences in relatedness within and among ponds were found in all of the SSPs tested, except in WT (Table S6). Relatedness within ponds was higher than among ponds, with differences ranging from 0.002 in WT to 0.05 in MH.

H_E values in ponds range from 0.43 to 0.71, A_R from 2.28 to 4.36 and F_{IS} from -0.15 to 0.11 (Table 1). Estimates of N_e using the LD-method and based on the genotypes of adult newts ranged from 6 to 502 (Table 1). This method often delivered confidence intervals with an infinite upper limit and in certain cases not even a finite estimate of N_e (in 47% of ponds with at least 6 adults). In contrast, 17% of the estimates of N_e calculated with the SA-method (ranging from 20 to 180) were not accompanied with finite confidence limits (Table 1). Both methods produced estimates (with finite confidence limits) that were comparable for those ponds exhibiting small N_e . Though, when LD-based N_e increased, SA-based estimates were often smaller.

Table 1 Genetic diversity and N_e estimates of the great crested newt spatially structured populations and ponds when the sample size exceeds five. The standard error for A_R , H_E , H_O and F_{IS} , and 95% confidence intervals for N_e estimates are given in parentheses

SPP-pond	N	Na	H_O	H_E	F_{IS}	A_R	LD- N_e	SA- N_e
ATG	52	52	0.56 (0.04)	0.62 (0.04)	0.09 (0.05)	4.52 (0.38)		
ATG-P15	32	32	0.54 (0.05)	0.61 (0.04)	0.12 (0.05)	3.59 (0.25)	30 (25–38)	34 (22–59)
BvA	190	190	0.47 (0.05)	0.45 (0.04)	-0.04 (0.06)	2.61 (0.38)		
BvA-12l	37	37	0.49 (0.06)	0.43 (0.04)	-0.13 (0.06)	2.28 (0.15)	168 (49–∞)	30 (17–52)
BvA-12s	84	84	0.46 (0.05)	0.45 (0.04)	-0.02 (0.07)	2.37 (0.15)	69 (37–181)	40 (26–63)
BvA-4A	66	66	0.48 (0.05)	0.46 (0.04)	-0.03 (0.05)	2.46 (0.16)	74 (43–184)	31 (19–53)
DB	101	96	0.71 (0.04)	0.69 (0.03)	-0.02 (0.04)	5.52 (0.39)		
DB-DB15	10	10	0.72 (0.04)	0.71 (0.03)	-0.01 (0.04)	4.36 (0.27)	∞ (273–∞)	180 (50–∞)
DB-DB2	14	13	0.71 (0.04)	0.68 (0.04)	-0.06 (0.05)	4.18 (0.29)	439 (72–∞)	62 (28–∞)
DB-DB3	54	52	0.71 (0.04)	0.69 (0.03)	-0.03 (0.03)	4.15 (0.23)	81 (68–98)	71 (50–108)
DB-DB5	23	21	0.69 (0.05)	0.64 (0.04)	-0.07 (0.04)	3.89 (0.24)	19 (16–22)	32 (18–62)
DP	55	31	0.57 (0.05)	0.55 (0.04)	-0.06 (0.05)	3.75 (0.33)		
DP-DP1	6	6	0.49 (0.06)	0.52 (0.05)	0.06 (0.08)	3.68 (0.54)	6 (3–16)	30 (10–∞)
DP-DP3	36	12	0.58 (0.05)	0.53 (0.04)	-0.11 (0.05)	3.00 (0.22)	∞ (57–∞)	66 (25–∞)
MF	168	168	0.63 (0.04)	0.60 (0.04)	-0.06 (0.04)	4.23 (0.34)		
MF-MFH4	29	29	0.62 (0.05)	0.59 (0.04)	-0.04 (0.06)	3.37 (0.23)	364 (119–∞)	62 (36–111)
MF-MFH7	30	30	0.65 (0.04)	0.60 (0.04)	-0.10 (0.05)	3.42 (0.23)	148 (85–467)	64 (40–114)
MF-MFH9	20	20	0.62 (0.05)	0.59 (0.04)	-0.03 (0.06)	3.49 (0.25)	∞ (299–∞)	63 (35–157)
MF-MFJ22	29	29	0.65 (0.04)	0.61 (0.04)	-0.07 (0.05)	3.48 (0.23)	481 (143–∞)	71 (43–133)
MF-MFJ33	30	30	0.62 (0.04)	0.59 (0.04)	-0.07 (0.05)	3.42 (0.24)	454 (144–∞)	60 (38–102)
MF-MFK2	30	30	0.61 (0.05)	0.60 (0.04)	-0.01 (0.06)	3.50 (0.26)	502 (94–∞)	54 (34–94)
MH	56	56	0.51 (0.05)	0.48 (0.04)	-0.06 (0.05)	3.04 (0.23)		
MH-Stad1	38	38	0.51 (0.05)	0.46 (0.04)	-0.10 (0.06)	2.55 (0.18)	26 (20–35)	24 (14–45)
MH-Stad2	11	11	0.49 (0.05)	0.46 (0.04)	-0.07 (0.06)	2.59 (0.18)	58 (20–∞)	20 (9–59)
PE-PE0	6	0	0.65 (0.05)	0.58 (0.04)	-0.15 (0.06)	3.06 (0.22)		
TE	210	113	0.58 (0.05)	0.56 (0.05)	-0.03 (0.05)	4.38 (0.45)		
TE-TE1	29	12	0.56 (0.05)	0.56 (0.05)	-0.01 (0.05)	3.43 (0.29)	∞ (∞–∞)	33 (16–141)
TE-TE2	61	40	0.54 (0.06)	0.52 (0.05)	-0.05 (0.05)	3.11 (0.28)	26 (22–33)	47 (30–76)
TE-TE4	106	59	0.60 (0.05)	0.56 (0.05)	-0.07 (0.05)	3.43 (0.29)	188 (132–317)	83 (59–117)
TE-TE5	14	2	0.61 (0.06)	0.56 (0.05)	-0.09 (0.06)	3.43 (0.32)		
TO	123	40	0.68 (0.04)	0.65 (0.03)	-0.04 (0.04)	5.12 (0.44)		
TO-TO0	9	9	0.71 (0.04)	0.65 (0.03)	-0.11 (0.05)	3.96 (0.33)	∞ (246–∞)	∞ (1–∞)
TO-TO1	14	5	0.65 (0.05)	0.64 (0.04)	-0.01 (0.06)	3.84 (0.28)		
TO-TO2	11	6	0.69 (0.04)	0.64 (0.03)	-0.09 (0.05)	3.84 (0.27)	∞ (∞–∞)	∞ (1–∞)
TO-TO3	17	8	0.71 (0.05)	0.63 (0.04)	-0.10 (0.04)	3.92 (0.29)	∞ (∞–∞)	31 (14–158)
TO-TO4	34	11	0.69 (0.04)	0.66 (0.03)	-0.04 (0.04)	3.94 (0.26)	∞ (107–∞)	55 (24–1551)
TO-TO5	38	1	0.65 (0.04)	0.63 (0.03)	-0.03 (0.04)	3.80 (0.29)		
VK	96	96	0.61 (0.04)	0.65 (0.04)	0.07 (0.04)	5.19 (0.39)		
VK-PA	28	28	0.60 (0.05)	0.67 (0.04)	0.11 (0.04)	4.17 (0.28)	134 (79–390)	52 (31–97)
VK-PB	26	26	0.63 (0.04)	0.65 (0.04)	0.02 (0.04)	4.07 (0.28)	445 (121–∞)	50 (31–98)
VK-PE	18	18	0.58 (0.05)	0.61 (0.04)	0.03 (0.05)	3.65 (0.24)	25 (18–38)	36 (19–73)
VK-PF	19	19	0.59 (0.05)	0.60 (0.04)	0.00 (0.05)	3.52 (0.22)	20 (16–26)	25 (13–52)
WR	61	35	0.60 (0.05)	0.58 (0.04)	-0.04 (0.05)	4.11 (0.38)		

WR-WR2	13	13	0.57 (0.05)	0.56 (0.04)	-0.04 (0.06)	3.10 (0.24)	44 (22—352)	31 (16—82)
WR-WR3	48	22	0.61 (0.05)	0.57 (0.04)	-0.07 (0.05)	3.24 (0.24)	56 (39—92)	42 (23—89)
WT	172	145	0.53 (0.05)	0.50 (0.05)	-0.07 (0.05)	3.34 (0.32)		
WT-WT1	41	41	0.52 (0.06)	0.49 (0.05)	-0.05 (0.06)	2.81 (0.24)	117 (72—275)	61 (36—128)
WT-WT2	19	19	0.52 (0.05)	0.50 (0.05)	-0.05 (0.06)	2.83 (0.23)	∞ (165—∞)	57 (30—147)
WT-WT4	81	60	0.55 (0.05)	0.51 (0.05)	-0.09 (0.05)	2.91 (0.24)	84 (63—120)	59 (40—89)
WT-WT6	30	24	0.54 (0.06)	0.50 (0.05)	-0.08 (0.06)	2.82 (0.23)	77 (43—263)	32 (19—61)
ZE	43	43	0.46 (0.05)	0.44 (0.04)	-0.02 (0.08)	2.72 (0.18)		
ZE-ZE6	38	38	0.46 (0.05)	0.43 (0.04)	-0.03 (0.08)	2.41 (0.15)	45 (30—78)	28 (17—49)

SSP: spatially structured population, N: total sample size, Na: number of adults and metamorphosed juveniles, A_R : allelic richness, H_E : expected heterozygosity, H_O : observed heterozygosity, F_{IS} : inbreeding coefficient, N_e : effective population size calculated for adult samples using the linkage disequilibrium method (LD- N_e) and the sibship assignment method (SA- N_e)

Landscape effects on genetic variation within and between SSPs

The results of the top models ($\Delta AIC_c \leq 2$) for each response variable and each buffer size are presented in Table S7. The model-averaged parameters from these top models are shown in Table 2 and with more detailed information in Table S8. The predictors included in the models with significant parameter estimates after model averaging were mostly their within-SSP effects. This implies that the trends in the response variables explained by the landscape within SSPs did not differ from the trends among SSPs. Because we only included a random intercept, the slope of the relationship is the same in all SSPs, only the intercept differs. In general, the models using landscape variables within a radius of 50 and 100 m held the most significant covariates, although sometimes partly different ones depending on the buffer radius (Table 2 and S8).

Table 2 Model averaged results of the top models with $\Delta AIC_c \leq 2$ for each of the response values and buffer sizes. Coefficients are given for each covariate still included in the model

Response	Buffer radius (m)	Within-SSP-distance to the nearest pond	Within-SSP-number of lentic systems	Within SSP-surface water	Within-SSP-arable land	Within-SSP-pasture	Between-SSP-pasture	Within-SSP-trees	Between-SSP-trees	Within-SSP-grassland and shrubs	Within-SSP-roads
A_R	50	-0.001***	-0.017*	1.765***	0.225						
	100	-0.001***	-0.014***	3.196***	0.749**	0.521*					
	250	-0.001**				0.126			-0.093		
	500	-0.001***		-0.672	-1.576*						
F_{IS}	50	-0.0002*				-0.222**	0.013	-0.048			
	100	-0.00002						-0.169**	0.025		
	250	-0.00001	0.0002		-0.031			-0.352*	0.010		-0.000004
	500	-0.0001			-0.103	-0.016		0.020			
LD- N_e	50		0.658		0.557				3.756*		
	100			10.192					2.297		
	250	0.0005				0.237		2.718			
	500			-26.854					1.392		
SA- N_e	50	-0.0002	-0.021	0.398	0.0856						
	100	-0.0028*		0.344				-0.907	0.144		
	250	-0.002									-0.0002*
	500	-0.002						0.543	0.213		

SSP: spatially structured population, N_e : effective population size calculated for adult samples using the linkage disequilibrium method (LD- N_e) and the sibship assignment method (SA- N_e), significant p -value indicated as < 0.05 (*), < 0.01 (**), < 0.001 (***)

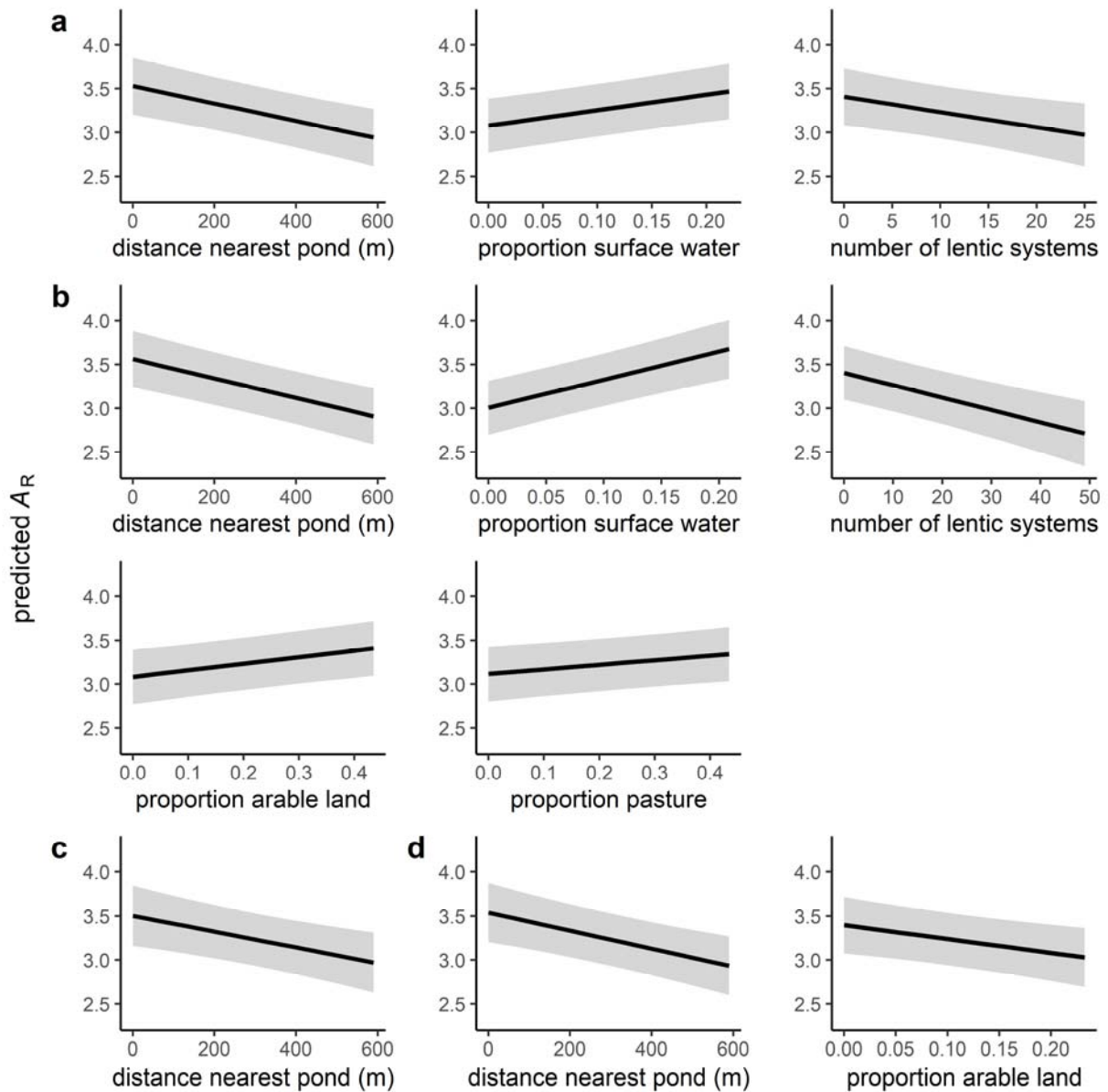


Fig. 3 Relationship between allelic richness (A_R) and each significantly contributing landscape variable at the different buffer sizes. Full model averaged results for within-SSP effects are shown for buffer radius 50 m (a), 100 m (b), 250 m (c) and 500 m (d) with fitted regression lines and 95% confidence interval (grey shaded area)

The distance to the nearest pond showed a significant relationship with A_R on all scales, with a negative effect of increasing distances to the nearest pond (Fig. 3, Table 2). The estimate of the effect of this variable was similar for all buffer sizes. The proportion of surface water in a buffer showed a positive relationship with A_R , while an increase in the number of ponds and other lentic systems had a negative effect within SSPs on the same estimates within a radius of 50 or 100 m (Fig. 3a and b). As the number of lentic systems within these small buffer sizes was often limited to one to three ponds (Table S4), the number of populations showing a negative trend was small. Within a 100 m radius, the land covered by arable land had a positive within-SSP effect (Fig. 3b). The sign of this relationship changed when the buffer radius was 500 m (Fig. 3d). The influence of pastures on A_R was positive within a radius of 100 m (Fig. 3b), though only slightly significant ($p = 0.03$; Table 2 and S8).

With F_{IS} as response variable, the distance to the nearest pond was again included in the final model on all levels with negative estimates, although only significant on the smallest scale ($p = 0.02$; Table S8 and Fig. 4a). Within a radius of 50 m, the within-SSP effect of pastures was significantly negative (Fig. 4a), as was the within-SSP effect of land covered by trees within radii of 100 and 250 m (Fig. 4b and c).

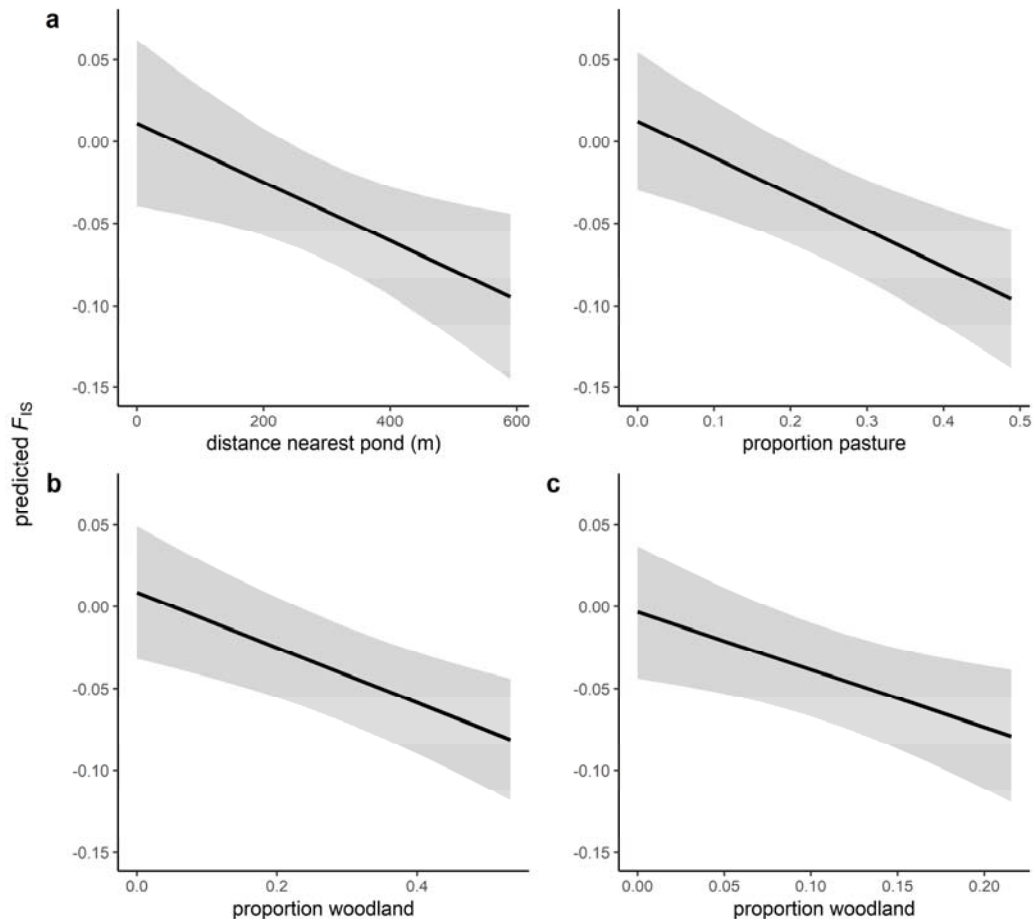


Fig. 4 Relationship between inbreeding coefficient (F_{IS}) and each significantly contributing landscape variable at the different buffer sizes. Full model averaged results for within-SSP effects are shown for buffer radius 50 m (a), 100 m (b) and 250 m (c) with fitted regression lines and 95% confidence interval (grey shaded area)

Sample size was reduced from 40 ponds in 13 SSPs to 30 ponds in 11 SSPs when N_e estimated with the SA-method was used as the response variable. On a scale of 100 m, the top models included a negative estimate for the within-SSP effect of the distance to the nearest pond and was significant after model averaging ($p = 0.02$; Table 2 and S8, Fig. 5a). The length of roads within SSPs calculated within a radius of 250 m was a significant negative predictor ($p = 0.02$; Table 2 and S8, Fig. 5b). Average models obtained by using the LD-method N_e estimates as response variable showed no significant estimates, except for the proportion of land covered by trees within a buffer radius of 50 m ($p = 0.01$; Fig. 6, Table 2 and S8). Sample size was, however, reduced to 19 ponds within 11 populations.

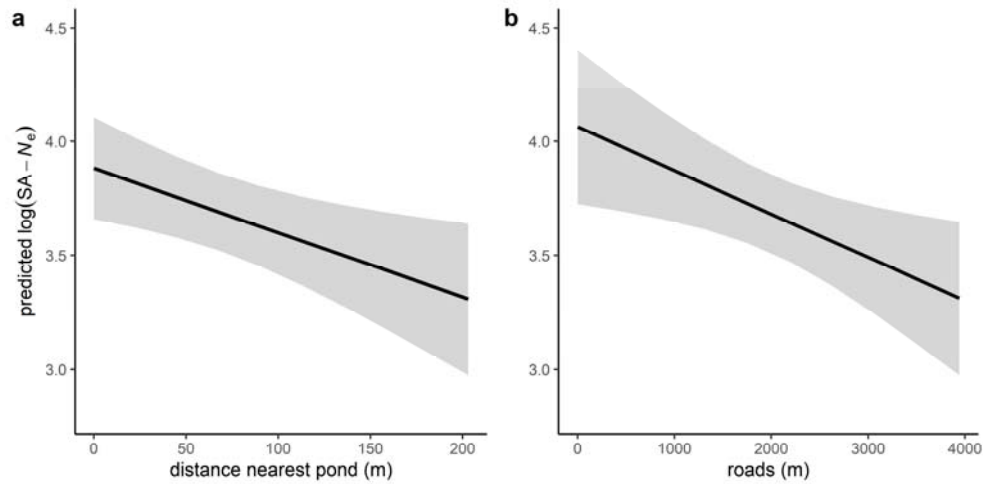


Fig. 5 Relationship between log transformed estimates of effective population size based on the sibship assignment approach ($SA-N_e$) and each significantly contributing landscape variable at the different buffer sizes. Full model averaged results for within-SSP effects are shown for buffer radius 100 m (a) and 250 m (b) with fitted regression lines and 95% confidence interval (grey shaded area)

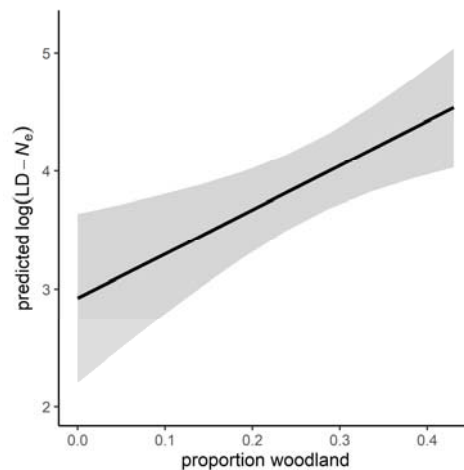


Fig. 6 Relationship between log transformed estimates of effective population size based on the linkage disequilibrium approach ($LD-N_e$) and between-SSP effect of proportion of woodland within a buffer radius of 50 m with fitted regression line and 95% confidence interval (grey shaded area)

Discussion

Using a multi-scale approach, we assessed the influence of the landscape surrounding breeding ponds on genetic diversity of the great crested newt. Our findings confirm the relevance and need to investigate the influence of environmental predictors on multiple spatial scales, as has been demonstrated in a growing number of ecological studies (e.g. Pellet et al. 2004a, 2004b; Hartel et al. 2010a; Angelone et al. 2011; Hamer and Parris 2011; Moraga et al. 2019). Such strategy is particularly meaningful, in terms of conservation, to highlight at which scale landscape features can affect patterns

of distribution and genetic diversity. Moreover, the results offer new insights on how the terrestrial landscape may affect the genetic variation and effective population size. The land cover types studied here appeared to be mostly relevant on smaller spatial scales, within a radius of up to 100 m around breeding ponds. While the importance of the aquatic breeding habitat to sustain the populations' genetic variation and thus persistence was confirmed (Jehle et al. 2005; Karlsson et al. 2007; Rannap et al. 2009; Schön et al. 2011; Arntzen et al. 2017), the proportion of the buffer area covered by trees, arable land, pasture and the presence of roads appeared to affect genetic measures variably, depending on scale and/or response variable.

Spatial scales and within- versus between-population effects

A population's history is known to significantly influence inferences on its current ecological processes (Anderson et al. 2010), especially past local extinction and (re)colonisation events (Wade and McCauley 1988). The landscape matrix of each population has its own history of change in habitat quality, amount and connectivity caused by environmental drivers such as land use change and invasive species (Wade and McCauley 1988; Kuussaari et al. 2009). Consequently, differences in landscape history and current composition as well as differences in past evolutionary processes within SSPs can lead to finding dissimilar relationships between genetic variation and current landscape features. Replicated empirical studies on the landscape scale are therefore warranted (Anderson et al. 2010). Instead of studying each population and landscape separately to obtain general conclusions (i.e. metareplication) (Johnson 2002), we addressed these issues by replacing the predictors by their within- and between-SSP effects. The difference in between- and within-SSP slope estimates was, however, in most models not significant, suggesting that the responses within and between SSPs were similar. Between-SSP effects still included in average models were often not significant, with the exception of wooded land cover within a radius of 50 m explaining the LD-based estimate of N_e . Here, the heterogeneous group of populations explained the between-SSP effect of the area covered by trees on the effective population size, which could not be clarified by the non-significant within-SSP predictor.

Complementarity of genetic responses

In general, the average models harbouring the most significant landscape variables were those for A_R . This response variable is considered to be particularly indicative of a population's resilience to change and its persistence (Caballero and García-Dorado 2013). It is therefore of high importance in conservation and management practices. H_E and A_R respond differently to population changes. For instance, in case of a short genetic bottleneck, H_E may not change severely while the loss of alleles could be substantial (Allendorf 1986). Then, H_E favours common alleles while A_R takes rare alleles into account which tend to get lost more quickly. Particularly when considering heterozygosity measures, the current landscape might not entirely explain the observed levels of genetic response, due to genetic time lags (Epps and Keyghobadi 2015). Nevertheless, A_R and H_E were highly correlated ($r = 0.96$) in our study. We therefore chose to use solely A_R as response variable to evaluate the effect of the current landscape on genetic variation.

As N_e is inversely proportional to the loss of genetic diversity caused by genetic drift, it can help predict or explain population genetic processes such as a reduction in genetic diversity, inbreeding and

population differentiation (Weckworth et al. 2013). Although the LD approach has been proven to be robust (Gilbert and Whitlock 2015) and wide confidence intervals occur frequently for single-sample estimates (Luikart et al. 2010), many of our LD-based estimates of N_e were not accompanied with limited confidence intervals. Large confidence intervals are likely to occur when sample sizes are too small, especially when true N_e is large (Waples and Do 2010). The number of N_e estimates with finite confidence bounds were limited, reducing the sample size of the model considerably. Consequently, only one significant relationship between N_e and the between population effect of wooded land cover was found within a buffer radius of 50 m. This relationship was, however, not present in the models with SA-based N_e as the response variable. For both the LD- and SA-based estimates we used mixed-age samples. According to Waples et al. (2014), sampling as many cohorts as there are in a generation should deliver an LD-based estimate of N_e close to true N_e . Based on the data provided in recent studies performed by Cayuela et al. (2020b) and Orchard et al. (2019), mean generation length in the great crested newt ranged from 2.4 to 10 years and depended on both sex and population. Since the age of the sampled adults in our study is unknown, there is uncertainty if we achieved to sample the necessary number of different cohorts in every pond. Increased bias is also expected when estimating N_e using a mixed-age sample with the SA-approach (Wang 2009; Wang and Santure 2009), although the number of markers in our study and their information content should be adequate to reduce bias (Wang 2009). Yet some bias is still expected due to low sample sizes in certain ponds. To obtain accurate SA-based N_e , sample sizes need to be near to or greater than true N_e (Ackerman et al. 2017). This condition was probably not met in those ponds with presumably larger true N_e .

F_{IS} is usually considered to express the level of inbreeding in a population. However, the values of F_{IS} were here mostly negative. This slight excess of heterozygotes could be caused by sampling an excessive proportion of relatives (Sánchez-Montes et al. 2017; Wang 2018). Although full sibs among larvae were excluded, close relatives among adults within a breeding pond could have been sampled, especially when the effective population size is small. Relatedness between individuals within ponds was significantly higher than among ponds within most of the studied SSPs, even in SSPs where ponds are very close to each other and F_{ST} values among ponds were low (e.g. TO). Because the majority of breeding patches are not in Hardy–Weinberg equilibrium, immigrants from other ponds will cause negative F_{IS} values to increase towards zero, rather than to a positive value. Instead of perceiving an increase in F_{IS} as an increase in inbreeding, we should therefore recognise it as an increase in immigration in our study. Still, not many significant explanatory variables were included in the models for F_{IS} , potentially because of its dependence on the composition of the samples making it difficult to standardise among ponds and SSPs.

Impact of the landscape

Across all buffer sizes, the distance to the nearest pond within-SSP was highly significant in the models explaining A_R . The negative relationship suggests that the closer the neighbouring pond the higher the chance of gene flow resulting in higher allelic diversity. In addition, a negative relationship with distance to the nearest pond appeared significant in the resulting model for F_{IS} within a radius of 50 m and for SA-based N_e within a radius of 100 m. Distance to the nearest pond appeared often to be an important connectivity metric to explain newt occurrence (Baker and Halliday 1999; Hartel et al. 2010b; Rannap et al. 2012; Miró et al. 2018). Denoël et al. (2018) further discovered a high rate of pond-infidelity among ponds within a few hundred meters in the great crested newt population of

Marche-en-Famenne, also included in this study (MF). Consequently, a positive relationship between genetic diversity and the density of ponds was expected, since the number of ponds within 100 m also had a positive effect on great crested newt abundance (Denoël et al. 2013). However, the within-SSP effect of number of lentic systems was negative with A_R as response variable within buffer radii of 50 and 100 m. At these spatial scales, pond density was very low (one to three ponds) in many of the studied populations. Hence, this negative relationship appeared to be confined to a few populations and mainly steered by the situation in populations TO and MF where a variable number of ponds could be counted within such small buffer sizes. Moreover, the between-SSP effect of this landscape variable was not included in the initial models due to collinearity, which potentially could have delivered a different perspective. Moreover, pond characteristics and local hetero- and conspecific density were not taken into account, which are known to influence occupancy, breeding and immigration probability (Griffiths and Wijer 1994; Denoël and Ficetola 2008; Gustafson et al. 2009; Cayuela et al. 2018; Cayuela et al. 2019). Nevertheless, the proportion of area covered by surface water within populations affected A_R positively within the 50 m and 100 m radii. Surface water is in this study restricted to lentic and at some sites small lotic systems which include breeding sites of great crested newts. Lotic systems such as large rivers, which can act as barriers to gene flow (Maletzky et al. 2010), were not present within the buffers.

Great crested newts tend to migrate towards trees and forest edges (Jehle and Arntzen 2000; Malmgren 2002; Vuorio et al. 2015), where they may use forested areas as refuge and hibernation sites (Joly et al. 2001). However, we found a negative within-SSP effect of the proportion of the area covered by trees on F_{15} within radii of 100 and 250 m. At close distances from ponds, an increasing amount of wooded area could mean higher proportions of shoreline shading which is not preferred by great crested newts (Oldham et al. 2000; Maletzky et al. 2007). Shading can limit growth of macrophytes, which deliver many services to newts, and causes lower levels of dissolved oxygen due to reduced photosynthesis, and lower water temperature, which in turn slows down larval development. Sztatecsny et al. (2004) also found shading to be negative for larval abundance in great crested newts. Gustafson et al. (2011) used two scales in their study, a radius of 100 m and of 500 m, to examine the relationship of the terrestrial landscape and the presence of the species. Although they did not find a clear difference between scales in land use variables, they concluded that among other land uses, the abundance of old deciduous forest was important at the larger, landscape scale. This is supported by the positive effect across SSPs (i.e. between-SSP effect) of the proportion of land covered with trees on LD-based N_e , although within 50 m of the ponds.

Positive estimates for pasture area and arable land cover (within-SSP effects) were included in the top models explaining A_R at the 100 m scale. At this small scale, the benefits of an open landscape could outweigh potential negative effects. In addition, arable land is often scattered and infrequent within this buffer size. Hedgerows and ditches associated to pastures and arable fields can provide additional habitat and putative dispersal corridors. For instance, Jehle and Arntzen (2000) found post-breeding migration activity in newts associated with hedgerows whereas adjacent pastures were avoided. Within the 500 m radius, A_R appeared to be affected negatively by the within-SSP proportion of arable land. In addition, F_{15} was negatively affected by the within-SSP pasture area within a 50 m radius. Arable land, pastures and rotation grasslands potentially deliver higher input of nutrients in nearby ponds. While adult great crested newts are able to thrive in aquatic habitats with a high nutrient content (Gustafson et al. 2009), eutrophication can speed up pond succession and potentially lead to toxicity

(Egea-Serrano et al. 2011). Moreover, high levels of nutrients can negatively affect occurrence and reproduction of great crested newts (Gustafson et al. 2009; Denoël et al. 2013). Amphibians are also known to be sensitive to pesticides and fertilizer (Quaranta et al. 2009; Brühl et al. 2011), especially when exposed directly during migration (Berger et al. 2012; Lenhardt et al. 2015). Further research at the studied sites is, however, needed to ascertain the presence and importance of potential ecotoxicological risks related to arable land use.

In general, roads appear to influence amphibian populations negatively due to fragmentation or depletion effects (Fahrig et al. 1995; Gibbs and Shriver 2005; Jackson and Fahrig 2011). In our study, roads appeared with a significant and negative estimate in the 250 m radius model explaining SA-based N_e . According to Gustafson et al. (2011), great crested newts probably do not actively avoid roads, yet they are affected by road mortality (Matos et al. 2019). Some SSPs in our study are enclosed by roads, such as TO, which means that no roads need to be crossed to reach other breeding ponds within the SSP. However, some populations are intersected by minor roads, such as VK, TE and MF. Despite the low traffic densities on these roads, mortality risk during seasonal migration and dispersal increases with the number of roads within the habitat matrix (Matos et al. 2012; Matos et al. 2017).

Although a reasonable number of SSPs and ponds were sampled, the low number of ponds in several SSPs could be a potentially limiting factor to reliably identify landscape features that affect genetic variation. Particularly increasing the number of ponds within SSPs would produce a stronger within-SSP signal and improve statistical power to distinguish between potentially different effects within and between SSPs. Although more ponds were sampled, they could not be included in the models as a result of the low capture success or of low local abundance. Due to the elusive and rare character of the species in our study region, a larger number of ponds with a substantial number of individuals caught, could, however, only be achieved in a small number of SSPs. We would recommend extending the sampling coverage in the future by adding new data from other SSPs occurring in landscapes with similar components as included in this study. This would allow the sample size to increase without the loss of previously acquired information. In addition, future studies could benefit from the use of a high number of genome-wide markers such as Single Nucleotide Polymorphisms (SNPs) to increase marker resolution and to investigate landscape influence on both neutral and adaptive variation (Balkenhol et al. 2019).

Conclusions

Although the scale of effect of the landscape can vary with the response variable (Moraga et al. 2019), the genetic response variables used here delivered models with significant predictors on mostly similar spatial scales. For great crested newts, genetic variation and effective population size as measured in individual ponds or demes, appear to be mainly influenced by the surrounding landscape on the smallest scales studied here (i.e. 50 – 100 m radius).

Since SSPs potentially went through different evolutionary processes and the surrounding terrestrial habitat is variable in quality and composition among SSPs, we expected different responses to the landscape within and among SSPs. Our approach eliminates the risk of mistakenly extrapolating from between-SSP effects to within-SSP effects or vice versa (van de Pol and Wright 2009). Results showed that, in general, the landscape variables studied here appeared to affect genetic variation within SSPs

in a similar fashion as across SSPs. This allowed us to provide more general guidelines that would benefit the persistence of each great crested newt SSP.

Acknowledgements

We would like to thank Peter Breyne for his help and expertise at the start of the project and Nancy Van Liefveringe for her help in extracting DNA and Sabrina Neyrinck for the lab work in the development of the microsatellites. We are grateful to two anonymous reviewers for their insightful and helpful comments. We would further like to thank Michael Timmermans, Mylène Hanoy, Sander Devisscher, Johan Auwerx, Eddy De Smedt, Wiske Teugels, Richard Soille, Pieter-Jan Alles, Arne Solheid, Kevin Lambeets, Koen Vertommen, Nicole Smeyers, Chantal Deschepper, Elke Leys en Maud Segal for their support during sample collection. We are also grateful to J.-L. Stilmant, M. Vanherck, Y. Goessens, P.-J. Henrottin, D. Cravatte and P. Bily for giving us logistic support and access to the military base (Arrête 111.08847), to H. Pirard and S. Vanderlinden for coordinating our stay in the field, to S. Liegeois, P. Goffart, and J.-Y. Grenson (Département Nature et Forêt) for their support. Capture permits were granted by the authorities of the Flemish and Walloon Region. All manipulations followed the appropriate regulations and legislations. Part of this study, conducted in Marche-en-Famenne, was funded by Service Public de Wallonie (33.01.11/GF/2009-64). M. Denoël is a Research Director at Fonds de la Recherche Scientifique – FNRS.

Declarations

Funding

Part of the field work, conducted in Marche-en-Famenne, was funded by Service Public de Wallonie (33.01.11/GF/2009-64).

Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

Microsatellite genotypes were deposited in the Dryad repository under the accession doi.org/10.5061/dryad.573n5tb7v.

Authors' contributions

KC, DA and GL conceived and designed the study. MD, DA, GL, SVdP and EP collected data and provided recourses. DH, LV and AVB performed the genetic analyses. KC performed the data analysis, with support from HVC, and wrote the first draft of the paper. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

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