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Combining landscape genetics, radio-tracking and long-term monitoring to derive management implications for Natterjack toads (*Epidalea calamita*) in agricultural landscapes

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ABSTRACT

Understanding the influence of landscape elements on movement and connectivity of potentially isolated populations is essential for successful conservation management, especially in agricultural landscapes. Here, we assessed the movement and genetic structure of the endangered Natterjack toad (*Epidalea calamita*) in an intensively managed agricultural landscape of the Central Plateau in Switzerland. Movement was analysed on the basis of molecular markers and radio-tracking, supplemented by long-term monitoring data describing population trends. A total of 19 migration events were detected by genetic first-generation assignment tests (11), by unintentional repeated genetic sampling of individuals (6) and by radio-tracking between predefined populations (2). None of the breeding sites was genetically isolated, although there was a trend that, in addition to geographic distances < 2.5 km, built-up areas enhanced genetic differentiation. Analysing the toad populations as a spatial network supported the inference that the two largest populations were sources, suggesting population size as a major driver of the movement pattern. We conclude that the Natterjack toad is well established in the intensely managed agricultural landscape, given that (i) large populations are maintained which may act as sources to spatially distributed occurrences, and (ii) the intensively managed landscape is sufficiently interspersed with suitable breeding habitats.

1. Introduction

Through the continuous modification of landscapes by humans and associated increase of traffic infrastructure (Jaeger, Bertiller, Schwick, & Kienast, 2009) and the intensification of agriculture (Billeter et al., 2008), natural habitats have been degraded, fragmented or even lost (Lindenmayer & Fischer, 2006). The decrease of suitable habitat is clearly one of the main causes of biodiversity losses (Curado, Hartel, & Arntzen, 2011). In particular, pioneer habitats in floodplains or wetlands have become scarce through canalisation, regulation of the flood dynamics and drainage in many parts of densely inhabited and cultivated areas (Collins & Storfer, 2003). Pond-breeding amphibians are therefore especially affected by the on-going landscape intensification, as—in addition to requiring breeding ponds — they are often philopatric and particularly vulnerable due to their seasonal migration between their aquatic breeding habitats and terrestrial foraging sites (Hamer & McDonnell, 2008). In addition, species such as the Natterjack toad (*Epidalea calamita*), which substituted the loss of their natural habitats with secondary habitats (*e.g.* gravel pits), are now increasingly losing their substitute habitats due to the intensification of gravel mining or the abandonment of mining activities and subsequent restoration of the original landscape (Sinsch, 1998; Schmidt & Zumbach, 2005). Not surprisingly, many amphibian species have strongly declined at a global scale during the past 30 years (Stuart et al., 2004), also in Switzerland (Schmidt & Zumbach, 2005).

A broad range of conservation initiatives has been set up to counteract this negative trend for amphibians (Semlitsch, 2003; Cushman, 2006; Tanadini & Schmidt, 2011; Smith & Sutherland, 2014). Increasing the availability of breeding ponds is among

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Table 1
Coordinates (Swiss national grid) of the weighted population centres, number of individuals and samples (in parentheses), allelic richness (A_r), expected (H_E) and observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), p values from Chi-square test for goodness-of-fit to Hardy–Weinberg equilibrium (pHWE), and the number of private alleles of the natterjack-toad populations in the Suhre valley, Switzerland. Additionally, means and trends of population sizes are listed, and it is stated in which population unit individuals were radio-tracked.

Population units	E	N	Genetic data							Population size		Radio-tracking data available
			Number of individuals (samples)	A_R	H_E	H_O	F_{IS}	pHWE	Private alleles	Mean[3]	Trend[4]	
PopA[1]	645′532	240′996	3 (3)*	–	–	–	–	–	–	2	↘	-
PopB[1]	646′598	238′811	–	–	–	–	–	–	–	3	↘	–
PopC	645′589	237′750	–	–	–	–	–	–	–	7	→	-
PopD[1]	646′421	237′169	68 (68)	2.51	0.58	0.59	−0.02	**0.0000**	6	149	→	x
PopE	646′556	235′902	24 (29)	2.37	0.54	0.55	−0.02	0.0693	0	–	–	x
PopF	646′143	235′182	36 (42)	2.45	0.57	0.56	**0.02**	**0.0002**	0	20	→	x
PopG	646′800	234′835	16 (21)	2.46	0.58	0.56	0.03	0.6742	1	–	–	x
PopH[2]	646′535	234′742	41 (41)	2.40	0.55	0.55	0.00	0.1054	0	32	↗	x
PopI[1]	649′820	234′310	9 (9)*	2.39	0.54	0.56	−0.04	0.8845	0	–	–	–
PopJ[2]	646′874	233′497	21 (22)	2.43	0.55	0.61	−0.11	0.8494	0	14	↗	x
PopK[1]	647′402	233′093	48 (48)	2.45	0.56	0.58	−0.04	0.3355	0	–	–	–

Statistically significant values ($p < 0.05$) are marked in bold.
* Sampled in 2014.
[1] Located in gravel pits.
[2] Artificial ponds built in 2009.
[3] Mean of 2009–2013.
[4] Trends of population size (past 15 years): ↗ increasing, → constant, ↘ decreasing (amphibian monitoring).

the most preferred actions in amphibian management (Le Lay, Angelone, Flory, Holderegger, & Bolliger, 2015). However, if ponds are built in environments such as intensely managed landscapes, the success of the measures is unclear as individuals and offspring may die prior to reproduction due to consequences of an unsuitable environment. In such cases, building ponds as well-meant conservation measure could easily result in an "ecological trap" (Griffin & Case, 2001), which might even weaken the overall reproductive capacity of the population network. Conservation management should be able to preclude such negative effects on populations. Therefore, spatially explicit analyses with particular focus on a detailed understanding of movement in a complex environment are most important as a basis of informed management decisions (Ferraro & Pattanayak, 2006; Wang & Bradburd, 2014; Keller, Holderegger, Van Strien, & Bolliger, 2015).

Movement patterns are traditionally assessed by direct monitoring through telemetry or GPS tracking (Jaquiery, Broquet, Hirzel, Yearsley, & Perrin, 2011). These methods provide highly resolved data on individual movement trajectories in space (*e.g.* Riley et al., 2006). Capture-mark-recapture studies are another possibility to estimate movement (*e.g.* Rouquette & Thompson, 2007). All these methods are costly, primarily because of high labour efforts (Bowne & Bowers, 2004), and often suffer from low sample sizes. Favoured by the fast advancement of genetic analyses in the past years, the upcoming use of landscape genetics in conservation applications provides promising tools to assess movement and connectivity in a landscape context (Manel & Holderegger, 2013; Segelbacher et al., 2010; Bolliger, Lander, & Balkenhol, 2014). Landscape-genetic approaches provide, among others, information on the degree of genetic differentiation among populations and individuals. This metric is taken as a surrogate for functional connectivity and can be used to identify barriers or corridors of gene flow in the landscape (Sork & Waits, 2010; Storfer, 2013) and to gain insights into population dynamics (Storfer, 2013). A disadvantage of genetic methods in contrast to telemetric approaches is, however, that contemporary movement paths and habitat preferences of individuals cannot be tracked in spatially explicit detail.

This study evaluates the movement of Natterjack toads as assessed by both genetic and radio-telemetric methods combined with long-term monitoring of population sizes in an intensively managed landscape in Switzerland. Natterjack toads (*E. calamita*, formerly *Bufo calamita*) are medium-sized anurans distributed across Western Europe. The toads breed in ephemeral ponds surrounded by bare ground or open vegetation (Sinsch, 1998). The loss of floodplains through channelling of watercourses, drainage and intensification of agriculture increasingly threatens the species. Although Natterjack-toad populations decline in central and Northern Europe, this species is of least concern (LC) on the IUCN Red List due to a strong abundance in western and Southern Europe (Beja et al., 2009). In Switzerland, however, more than 60 % of the known occurrences of Natterjack toads have disappeared in the past 30 years and the species is currently listed as endangered (EN) on the Swiss Red List (Schmidt & Zumbach, 2005). The goals of this conservation study were (i) to quantify the movement of Natterjack toads in a human-dominated landscape using a combination of genetic analyses and radio-tracking, (ii) to analyse the population dynamics using a spatially explicit network analysis, (iii) to study the influence of land use on the size and movement of natterjack-toad populations.

2. Material and methods

2.1. Study area

The study area covered around 25 km^2 of the upper Suhre valley, cantons of Argovia and Lucerne, on the Central Plateau of Switzerland (Fig. 1). The valley is located at 480 m a.s.l., encompassing a moraine with an elevation difference of approximately 50 m. Whereas the bottom of the valley is dominated by intensive agriculture, the valley slopes are mostly covered by forest (Fig. 1). Several settlements are located along the valley slopes. The canalised Suhre river, which is about 8 m wide, divides the study area into a western and an eastern part, and three main roads (not fenced) cross the study area (Fig. 1).

Twenty breeding ponds of the Natterjack toad are known in the upper Suhre valley. The majority was found at the bottom of the valley, and five were located in gravel pits (Fig. 1; Table 1). Because assessments on movement between ponds have shown that spatial units considerably larger than single ponds are necessary to maintain amphibian populations (Petranka, Smith, & Scott, 2004; Petranka, 2007), and because pond-breeding amphibians exhibit high site fidelity suggesting a (meta)population organisation, we considered a population-level analysis of the Natterjack toads. The toads found at breeding ponds and other temporary wet areas were subdivided into eleven *a priori* delineated population units (metapopulations, sensu Levins, 1969). The delineation of the

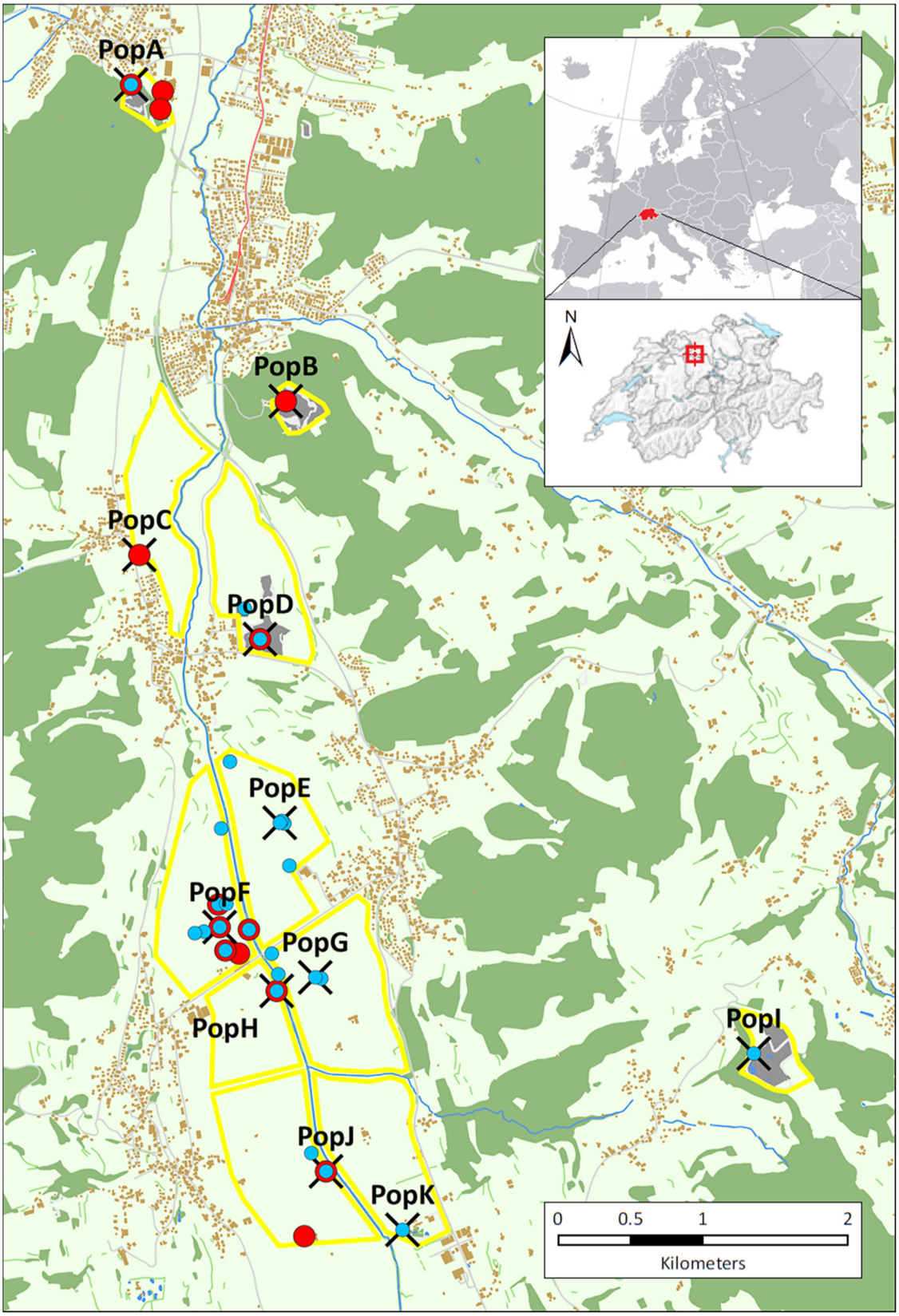

Fig. 1. Locations of genetic sampling (blue points) and regularly visited breeding ponds within the long-term amphibian monitoring (red points) in the upper Suhre valley, Switzerland. Yellow outlines show the borders of *a priori* delineated natterjack-toad populations. Black crosses show the weighted centres of each population which were used to measure distances between populations (coordinates in Table 1). Dark green areas mark forests and hedges, light green areas show open lands. Water bodies are blue, roads are grey and railway lines are red. Dark grey areas indicate gravel pits, light grey areas show slopes, and buildings are coloured in brown.

populations was such that distinct barriers to movement (main roads, rivers, moraine) were located between the populations (populations A-K; Fig. 1; Table 1) to test whether these landscape elements might affect the toad's gene flow. For each *a priori* delineated population, we determined the largest breeding pond (with most sampled toads) and the most central one (compared to the others) to be the population centre (Fig. 1). These population centres were used to measure distances between populations.

2.2. Data

Three types of data were available for the Natterjack toad in the Suhre valley: (i) genetic data derived from microsatellites, (ii) radio-tracking data (Schweizer, 2014), and (iii) census data of population sizes collected within the long-term amphibian monitoring program of the canton of Argovia.

2.2.1. Genetic data

We ensured that all breeding sites in the study area were genetically sampled, also checking potential non-permanent breeding ponds in the area. As we were interested in the movement and gene flow between the breeding ponds, we divided sampling sites into eleven discrete population units (as described above; Fig. 1, Table 1). These *a priori* populations were used for all analyses unless stated otherwise.

In 2013, we sampled 272 adult Natterjack toads, supplemented with twelve toads sampled in 2014 (Table 1). At each sampling site, we first collected as many adult individuals as possible and put them into a bucket. We then took non-invasive tissue samples using buccal swabs and photos of the dorsum of the collected toads. Buccal swabbing is a widely used method that has been shown to be an efficient approach to gain amphibian DNA (Pidancier, Miquel, & Miaud, 2003; Broquet, Berset-Braendli, Emaresi, & Fumagalli, 2007; Angelone & Holderegger, 2009), whereas the photos served as a reference if genetic analysis should be unresolved. After sampling, we immediately released the collected toads.

All 284 samples were successfully genotyped at 13 nuclear microsatellite (nSSR) loci (Buca1, Buca2, Buca5, Buca6: Rowe, Beebee, & Burke, 1997; Bcalμ10: Rowe, Beebee, & Burke, 2000; Bcalμ1–Bcalμ8: Rogell, Gyllenstrand, & Hoglund, 2005) as described below. Because one sampling site (PopA) comprised only three individuals (Table 1), we excluded this site from genetic analyses.

We extracted genomic DNA using the QIAmp 96 Blood Kit (Qiagen). Buccal swabs were first transferred to 96-well collection tube plates, and we added 320 μl PBS buffer (phosphate buffered saline, pH 7.4), 16 μl protease and 320 μl AL buffer per sample. Samples were then placed in a shaking incubator (Heidolph) for 3 h at 56 °C. We then added 320 μl of ethanol (puriss., SIGMA), transferred the samples to the QIAmp 96 plate and followed the manufacturer's protocol. DNA was eluted with 120 μl AE buffer.

We amplified the 13 nSSRs in two multiplex mixes. Because some primers amplified more effectively, primer concentrations were adjusted to obtain even peak heights. Reactions of 10 μl contained 4 μl (0.8× end concentration) of Type-it Multiplex Master Mix PCR (Qiagen) and 50–70 ng of DNA (in 2 μl undiluted DNA extract). Multiplex 1 contained 0.1 μM of primer pairs Bcalμ1(FAM), Bcalμ2(FAM), Bcalμ3(ATTO532), Bcalμ6(ATTO565), Bcalμ7(ATTO550) and Bcalμ8(ATTO532), 0.25 μM of Bcalμ4(ATTO550) and 0.2 μM of Bcalμ5(FAM). Multiplex 2 contained 0.075 μM of Bcalμ10(ATTO532), 0.3 μM of Buca1(ATTO532), 0.2 μM of Buca2(FAM) and Buca6(FAM) and 0.1 μM of Buca5(ATTO565). Each forward primer was labelled with the fluorescent dye indicated in brackets, and all primers were synthesized at Microsynth (Balgach). Polymerase chain reaction (PCR) was performed on a Veriti 96 well Thermal Cycler (Applied Biosystems) with the following thermal profile: multiplex 1: 5 min at 95 °C, 30 cycles of 30 s at 95 °C, 90 s at 58 °C, 30 s at 72 °C and a final extension step of 30 min at 60 °C; multiplex 2: 5 min at 95 °C, 32 cycles of 30 s at 95 °C, 90 s at 55 °C, 30 s at 72 °C and a final extension step of 30 min at 60 °C. PCR fragments were sized on an ABI 3130 sequencer (Life Technologies) with LIZ500 (Life Technologies) as internal size standard. Alleles were scored using GeneMapper 5.0 (Life Technologies).

2.2.2. Radio-tracking

Fifty males of the Natterjack toad were tracked in a radiotelemetry study between April and November 2013 (Schweizer, 2014). Similar approaches were used by Indermaur, Schmidt, and Tockner (2008) and Indermaur, Gehrig, Wehrle, Trockner, and Naef-Daenzer (2009). To track the toads, transmitters of the type BD-2 (1.95 g, Holohil System, Carp Ontario, Canada) were fixed with aluminium chains and connectors (Ball Chain Manufacturing, Mount Vernon, NY, USA) on the toads' bodies (Schweizer, 2014). Black Plasti-Dip was used to make the aluminium chains eudermic and as invisible for predators as possible (Indermaur et al., 2009). Because the weight of a transmitter should not exceed 10 % of the toad's body weight, only toads with a body weight >25 g were selected (Schweizer, 2014). The signals of the transmitters were localised with a foldable Yagi antenna (Yagi Mhz, three element antenna) and a receiver (R-1000 telemetry receiver, Titley Electronics, Ballina, Australia), and the coordinates of the toads were registered with a mobile GPS (Trimble GeoXT™ GPS CE handheld; Schweizer, 2014). The time span of observation was different for each male and varied from two days up to four months, depending on the time of the transmitter loss. On average, toad positions were measured about once a day (Schweizer, 2014). Although no sex bias was observed in movement distances of Natterjack toads (Sinsch, Oromi, Miaud, Denton, & Sanuy, 2012), it should be noted that only males were radio-tracked in the present study. All radio-tracked individuals were also genotyped.

2.2.3. Amphibian monitoring

Since 1999, population sizes of amphibian breeding sites have been recorded in the context of a long-term amphibian monitoring program conducted by the regional government of the canton of Argovia (Supplementary material—Table A1). Within this program, the Suhre valley is one out of ten core areas of which two or three are selected each year for a comprehensive survey of amphibian breeding sites. Four of these surveys were conducted in the Suhre valley in 1999, 2001, 2005 and 2009. In addition, a random sample of amphibian breeding sites is monitored every year. Trained volunteers visit each selected site three times per season and count all individuals heard or observed. The largest number of individuals counted during these three visits is taken to estimate the population size (Tanadini & Schmidt, 2011).

2.2.4. Landscape data

The landscape data characterising the Suhre valley were derived from the SwissTLM3D and SwissBUILDINGS^{3D} 1.0 datasets (swissTLM ^{3D} 1.0, 2013). As linear landscape elements are represented by lines in the SwissTLM^{3D}, we buffered roads according to their actual width between 2 and 5 m, hedges with 3 m, the Suhre river with 4 m and other smaller rivers with 3 m on both sides of respective elements using ArcGIS 10.2 (ESRI, USA). Finally, we merged all landscape features into a single raster map with a spatial resolution of 0.5 m. For further analysis, infrequent, highly correlated ($|r| > 0.7$) and thematically similar landscape elements were re-categorised into six instead of the originally ten landscape elements (Fig. 1): built-up area (buildings, roads, railways), ponds, streams, gravel pits, open land (agricultural fields, hedges) and forests.

2.3. Genetic diversity and structure

The genetic data was tested for Hardy–Weinberg and linkage equilibrium using GENEPOP (Rousset 2008). Genetic diversity was calculated as allelic richness (A_r), expected heterozygosity (H_E), observed heterozygosity (H_O) and an inbreeding coefficient (F_{IS}). These parameters were estimated with the diveRsity package in R 3.1.1 by performing 1000 replications of bootstrap resampling (Keenan, McGinnity, Cross, Crozier, & Prodohl, 2013; R Development Core Team, 2014). Genetic differentiation between pairs of populations was determined through fixation indices (F_{ST}) according to Weir and Cockerham, (1984), excluding PopA owing to low sample size. Statistical significance of pairwise F_{ST} was assessed in GENEPOP (Rousset, 2008). We estimated the effect of geographic distance on the genetic structure by isolation by distance (IBD) tests (Wright, 1943) using log-transformed geographic distance and standardised genetic differentiation between populations ($F_{ST}/1\text{-}F_{ST}$). Statistical significance of IBD was inferred using a Mantel test in R 3.1.1, calculated in the package ecodist (Goslee & Urban, 2007).

To describe the spatial genetic structure of the study populations (without PopA), we used STRUCTURE 2.3.2.1, a Bayesian partitioning approach (Pritchard, Stephens, & Donnelly, 2000; Falush, Stephens, & Pritchard, 2003). Because of a shallow genetic structure at the given small spatial scale, sample group information of the individuals was taken into account by the LocPrior model (Hubisz, Falush, Stephens, & Pritchard, 2009). Ten independent runs were calculated for each predefined cluster number of $K = 1\text{-}10$ performed with a burn-in length of 100,000 and a Markov Chain Monte Carlo (MCMC) of 1,000,000 repeats. We evaluated the cluster numbers (K) by following the STRUCTURE 2.3.2.1 guidelines (Pritchard et al., 2000) and the approach by Evanno, Regnaut, and Goudet, (2005) on the basis of result visualisation by STRUCTURE-HARVESTER 0.6.94 (Earl & Vonholdt, 2012). The STRUCTURE 2.3.2.1 output was edited in CLUMPP 2.1.1 to correct discrepancies between the ten runs of each K (Jakobsson & Rosenberg, 2007). The results were visualised with DISTRUCT 1.1 (Rosenberg, 2004).

2.4. Movement

The historical gene flow as measured by F_{ST} was supplemented with contemporary gene flow (first-generation migrants) using two Bayesian assignment tests on the basis of multilocus genotypes. A first-generation migration test was conducted with GENECLASS 2 (Piry et al., 2004). This assignment test identifies individuals sampled in a different population than the one where an individual was born (Piry et al., 2004). Because we sampled all known breeding ponds in the Suhre valley, the likelihood ratio $L = L_{home}/L_{max}$ was computed as the statistical criterion for assignment likelihood (Paetkau, Slade, Burden, & Estoup, 2004). For assignment, the partial Bayesian method of Rannala and Mountain (1997) together with the MCMC resampling algorithm of Paetkau et al. (2004) was used, and 1000 individuals were simulated at a threshold value of 0.01 (p value). Such a stringent setting usually identifies only a few first-generation migrants (Kraaijeveld-Smit, Beebee, Griffiths, Moore, & Schley, 2005) because of low genetic differentiation at small study extents and, thus, often low assignment resolutions (Piry et al., 2004).

Second, we calculated an assignment test as implemented in BIMR (Faubet, Waples, & Gaggiotti, 2007; Faubet & Gaggiotti, 2008), which defines posterior estimates of first-generation migration rates using MCMC and Reversible Jump MCMC methods. We tested several burn-ins between 10′000 and several millions, but it had no effect on the general pattern of migration rates. A burn-in period of 100,000 and a sample size of 10,000 runs with ten replicates per run were chosen. Mean and standard deviations for posterior regression model probabilities of the ten replicates per run were calculated separately for all ten landscape variables. Migration rates were related to landscape variables to test to what degree the landscape determines natterjack-toad movement. BIMR relies on a permuted generalized linear model (GLM). The correlation between landscape variables entering the GLM was maximally |0.7| to avoid multicollinearity. All six landscape variables (built-up area, ponds, streams, forests, gravel pits, open land) including geographic distance (Euclidean distance, *ED*) were considered.

2.5. Landscape effects on movement, gene flow and population characteristics

We tested the effects of landscape elements on the movement and on the genetic characteristics of Natterjack toads between the defined populations (corridors) and at each single population (sites). To analyse gene flow between populations, we followed an approach by Angelone, Kienast, and Holderegger, (2011) and Emaresi, Pellet, Dubey, Hirzel, and Fumagalli (2011). We buffered the geographic distances between all populations with 200 m on both sides in ArcGIS 10.2. Similarly, the individual toad sites were buffered with a 200 m radius. This buffer size was chosen to minimise spatial overlaps between buffers. The proportion of all landscape elements within each buffered corridor or buffered site was calculated. These proportions and geographic distance were then used to explain (i) inbreeding coefficient (F_{IS}) and demographic parameters (mean of population sizes of the last five years, trends of population size and the number of neighbouring populations within 3 km) at the toad sites, and (ii) gene flow as inferred from F_{ST} and the number of migrants between populations.

A principal component analysis (PCA) was used to assess the relationships between the genetic data on the Natterjack toad and the landscape elements. PCAs were conducted in R 3.1.1 (R Development Core Team, 2014) using the Lattice package (Sarkar, 2008). To test for statistical significance of the individual landscape elements to toad movement in addition to geographic distance, we applied a partial Mantel test in R 3.1.1 as available in the package ecodist (Goslee & Urban, 2007).

), which were, however, statistically significant in most cases.

Geographic distances (*ED*) between populations ranged between 281 and 8121 m (Table 2; Fig. 1). The relationship between F_{ST} and geographic distance (IBD) was statistically significant ($p < 0.05$; with a Mantel r of 0.89), but geographic distance only becomes effective for distances greater than ca. 2.5 km (Fig. 3

2.6. Functional toad network

We assessed the importance of each *a priori* delineated population (node) in the network of Natterjack toads using CONEFOR SENSINODE 2.6 (Pascual-Hortal & Saura, 2006; Saura & Pascual-Hortal, 2007; Saura & Torné, 2009). The node importance was measured by the graph-based index probability of connectivity *PC* (Saura & Rubio, 2010; Saura & Torné, 2009). *PC* requires a threshold value for the distance corresponding to the probability that the species is able to cover between two nodes. Based on studies assessing the terrestrial movement range of Natterjack toads, we set the threshold value at 2200 m with a probability of 0.5 (Sinsch, 1998; Miaud, Sanuy, & Avrillier, 2000; Jehle & Sinsch, 2007; Leskovar & Sinsch, 2005; Sinsch et al., 2012; Smith & Green, 2005). Nodes of the network were characterised by population sizes or allelic richness (A_r), and links either by *ED* or F_{ST} values. All four possible combinations (nodes x links) were calculated. Because recent historical data of population sizes were available from the amphibian monitoring program, we also assessed the node importance of the past by using

Table 2
Euclidean distances (*ED*, in metres) between the natterjack-toad populations in the Suhre valley are listed below and pairwise F_{ST} values above the diagonal. Statistically significant F_{ST} ($p < 0.05$) are marked in bold. PopA was excluded from genetic analysis due to low sample size.

	PopA	PopB	PopC	PopD	PopE	PopF	PopG	PopH	PopI	PopJ	PopK
PopA		–	–	–	–	–	–	–	–	–	–
PopB	2431		–	–	–	–	–	–	–	–	–
PopC	3247	1464		–	–	–	–	–	–	–	–
PopD	3929	1652	1015		**0.0103**	**0.0122**	0.0000	**0.0132**	**0.0339**	**0.0131**	**0.0139**
PopE	5196	2909	2086	1274		0.0038	0.0111	0.0031	**0.0627**	**0.0163**	**0.0159**
PopF	5846	3657	2627	2006	830		**0.0154**	0.0060	**0.0484**	0.0027	**0.0086**
PopG	6290	3981	3157	2365	1095	743		0.0041	**0.0279**	0.0000	**0.0125**
PopH	6334	4069	3153	2430	1160	589	281		**0.0421**	0.0006	**0.0099**
PopI	7943	5535	5453	4442	3632	3779	3065	3313		**0.0461**	**0.0270**
PopJ	7618	5321	4443	3700	2426	1837	1340	1290	3056		**0.0065**
PopK	8121	5774	4997	4192	2934	2439	1843	1863	2707	665	

five-year means, three-year means and single years of population sizes as nodes and *ED* as link measure.

3. Results

3.1. Genetic diversity and structure

The genetic analysis of the 284 toad samples showed that some individuals were able to move from one sampling site to another between two nights of sampling, so that 16 individuals were unintentionally sampled twice, and one individual even three times. This finding was confirmed by the photos of the toads. So, in total 266 individuals were sampled (Table 1). For genetic analysis, we only considered the first sampling of an individual.

Linkage disequilibrium was not statistically significant at the population level (result not shown). Only PopF and PopD showed a statistically significant deviation from Hardy–Weinberg equilibrium (Table 1). The measures for genetic diversity – allelic richness (A_r), expected heterozygosity (H_E) and observed heterozygosity (H_O) – differed only slightly between the *a priori* delineated populations (Table 1). Inbreeding coefficients (F_{IS}) were low in all populations (<0.03); only for PopF the positive F_{IS} value significantly deviated from zero (Table 1). Private alleles could only be found in PopD and PopG (Table 1).

The outcome of STRUCTURE-HARVESTER suggested a genetic substructure with five clusters according to ΔK (Evanno et al., 2005) and LnPr(X|*K*) graphs (Supplementary material—Figs. A1 and A2). However, when we evaluated graphs representing different numbers of *K*, we realised that all individuals were admixed to different degrees. The main information from the analysis was that a gradient of genetic structure could be observed between the North and South of the study area, with PopD and PopK consistently belonging to different clusters, independent of the number of *K*. Thus, most of the populations as defined in this study showed variable degrees of admixture, particularly so in the central part of the study area (Fig. 2). This shallow genetic structure is supported by an overall high level of gene flow between the defined populations as suggested by the low values of genetic differentiation (pairwise $F_{ST} < 0.06$; Table 2).

Overall, the genetic structure represented a gradient between North and South with admixed populations, high gene flow ($F_{ST} < 0.06$), and an IBD effect for populations separated by distances greater than 2.5 km.

3.2. Movement

Altogether, 19 migration events and migration probabilities >0.1 were revealed by assignment tests, unintentional repeated genetic sampling and radio-tracking (Fig. 4). The assessment of first-generation migrants from assignment tests in GENECLASS uncovered five migrants out of the 266 sampled toads with a type I error probability of $p < 0.01$ (Fig. 4). Assignment tests in BIMR detected six migration probabilities between 0.11 and 0.18 (Fig. 4).

Six migrants could be detected through the unintentional repeated genetic sampling of 17 individuals in 2013 (Fig. 4). In contrast to the assignment tests, these migrants were observed by chance and give an incomplete impression of migration events. As for the 50 radio-tracked males, only two toads were observed to move between populations (Fig. 4). All other movement assessed by radio-tracking occurred within the delineated populations.

The mean distance covered by the 19 migration events and probabilities was 1500 m with a minimum of 280 m and a maximum of 4200 m (two toads). Six toads covered between 280 and 1000 m and eleven toads moved between 1000 and 2300 m. At least five migrants crossed the canalised Suhre river, and seven migration events or probabilities were observed to cross main roads (Fig. 4).

In addition to the number of migrants, BIMR revealed details on the directionality of the first-generation migrants. Whereas populations D, H and K exhibited migration probabilities > 0.1, populations F and J showed first-generation migrants with probabilities between 0.01 and 0.1 (Fig. 5). In contrast, populations E, G, and I did not show any migration at all (migration rate = 0; Fig. 5). Although the probability of movement was low in all cases, populations D, F, H, J and K may be considered as potential sources for migrants, whereas populations E, G and I may act as sinks (Figs. 4 and 5). This source-sink pattern was independent of any distance or landscape features, as none of these explained the migration rates assessed by BIMR (results not shown).

3.3. Landscape effects on movement, gene flow and population characteristics

Six landscape elements were used for the PCA analysis (open lands, forests, streams, ponds, gravel pits, built-up areas; Supplementary material—Table A2). Remarkably, the first (x-axis) and second principal component (y-axis) of the PCA with these six landscape elements explained more than 75 % of the total variation, both for the site and corridor analysis (Fig. 6).

As expected due to the landscape characteristics of the Suhre valley, the proportions of forest and open land were negatively correlated (<−0.8) for both measures (sites and corridors, Supplementary material—Table A2). At the site measure, gravel pits were negatively correlated with open lands, and forests were negatively correlated with the trends of population size (Supplementary material—Table A2). In line with the results of IBD, positive correlations between geographic distance (*ED*) and F_{ST} were observed for the corridor analysis. The amount of built-up areas was also positively correlated with F_{ST} and *ED* (Supplementary material—Table A2).

The results of the PCA illustrate that built-up and forested areas were negatively correlated with all demographic and genetic site measures (F_{IS}, mean population size 2009–2013, trend of

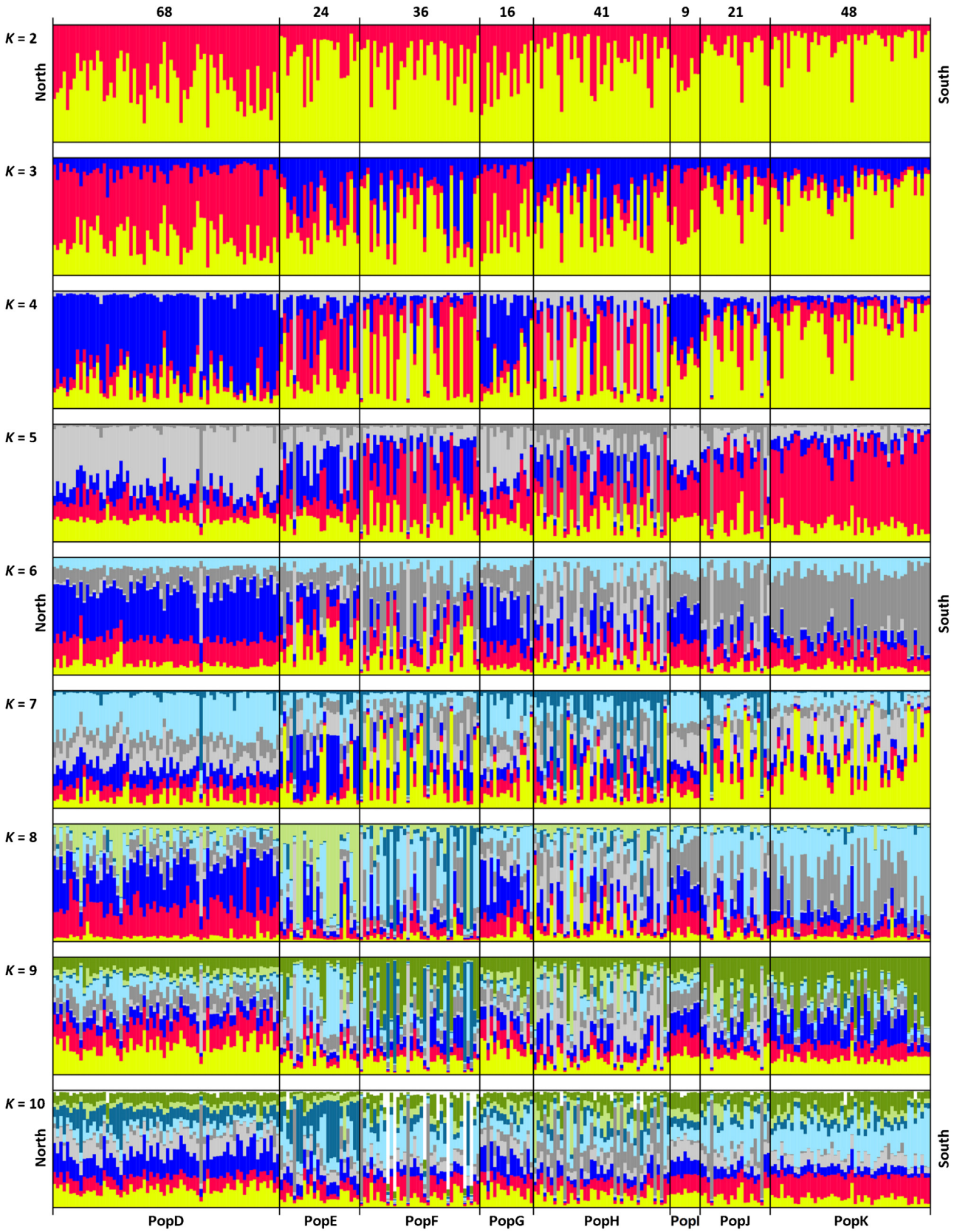

Fig. 2. Bar plots of STRUCTURE (Pritchard et al., 2000) analysis for clusters $K = 2$ to $K = 10$ of the natterjack-toad populations in the Suhre valley, Switzerland. PopA was excluded from this analysis due to low sample size. Each vertical bar represents one individual, and the colour composition displays the probability to belong to one of the K clusters defined by STRUCTURE. Black vertical lines delineate pre-defined populations, and the numbers of sampled individuals per population are shown above the illustration.

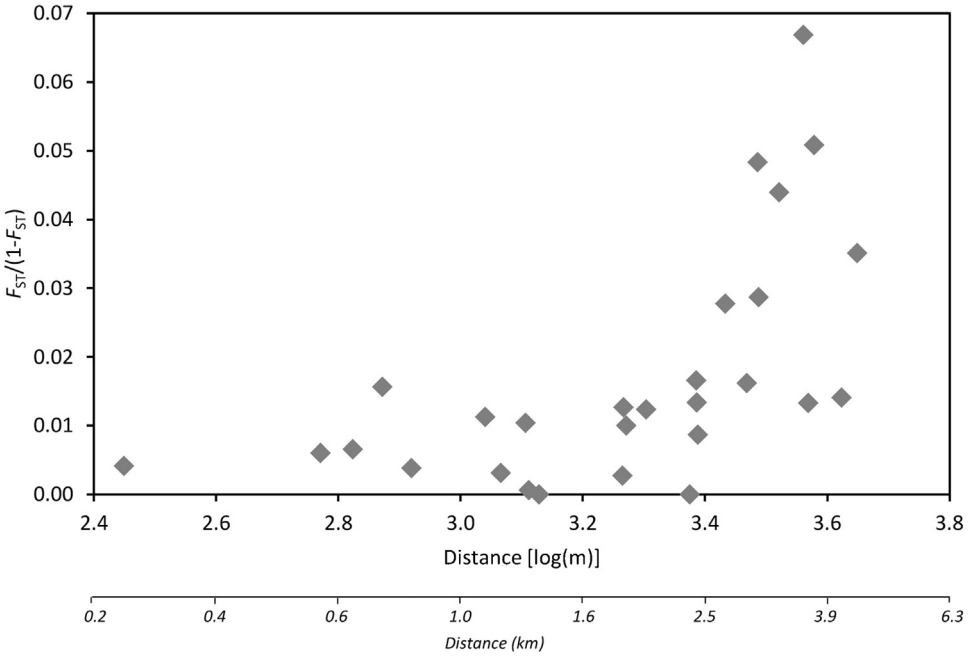

Fig. 3. Patterns of isolation by distance (IBD) of the natterjack-toad populations in the study area of the Suhre valley, measured between all populations except PopA. IBD is statistically significant at $p < 0.05$ with a Mantel r of 0.89.

population size, number of neighbours <3 km; Fig. 6a). The PCA also highlights positive correlations of F_{ST}, *ED* and built-up area (Fig. 6b), indicating more genetic differentiation with increasing geographic distance and higher proportions of impervious areas. This impeding effect of geographic distance and built-up area on movement is also supported by negative correlations between the number of migrants, *ED* and built-up area (Fig. 6b, Supplementary material—Table A2). A partial Mantel test revealed that both, *ED* and built-up area were statistically significant when related to F_{ST} ($p < 0.05$), but the correlation was highest for *ED* alone ($r = 0.89$). Relating both *ED* and built-up area to F_{ST} achieved a Mantel r of 0.74, whereas built-up area alone amounted to a Mantel r of 0.83; Supplementary material—Table A2. This shows that geographic distance is likely the most important environmental predictor for genetic differentiation in the study area.

3.4. Functional toad network

Overall, the importance of processes within a population (*i.e.* population size) was higher in larger populations, while connectivity was more important for smaller populations. However, in most cases the importance of internal processes was much lower compared to the importance of connectivity. Because of this, only the total node importance (*dPC*) is shown in Table 3, without a division in population internal processes and connectivity.

When characterising the population network for nodes by mean population size (data from amphibian monitoring), PopD was the most important in sustaining the overall natterjack-toad population network, irrespective of the characterisation of the link (connectivity) measure. Both, structural (*ED*) and functional measures (F_{ST}) indicated that PopD was by far the most important in the network (Table 3). The agreement between a network of structurally (*ED*) and functionally characterised links (F_{ST}) indicates that the landscape is very permeable for the toads as structure equals function. Looking at the toad network using allelic richness (A_r) to characterise nodes, populations F, G and H were the most important ones when characterising links with *ED* (Table 3). In contrast, when using F_{ST} to characterise links, none of the populations stuck out as particularly relevant (Table 3). This indicates that the role of structural connectivity (*ED*) in shaping A_r is overestimated because functional connectivity showed no differences in the populations for A_r.

Table 3
Node importance (*dPC*) of the natterjack-toad populations in the Suhre valley for all four combinations (node × links) measured by CONEFOR SENSINODE (Saura & Torné 2009). Links are measured as Euclidean distance (*ED*) or genetic differentiation (F_{ST}). The higher the value, the more important is a population for the population network. PopA was excluded from genetic analysis due to low sample size.

Population units	Node:	Population size[1]		Allelic richness[2]	
	Link:	*ED*	F_{ST}	*ED*	F_{ST}
PopA		0.68	–	–	–
PopB		1.51	–	–	–
PopC		5.40	–	–	–
PopD		88.80	90.08	18.92	22.56
PopE		–	–	22.82	21.37
PopF		13.65	17.55	24.58	22.05
PopG		–	–	25.79	22.14
PopH		19.62	27.26	25.17	21.63
PopI		–	–	14.63	21.54
PopJ		6.72	12.67	22.18	21.88
PopK		–	–	20.41	22.05

[1] Mean 2009–2013 (source: amphibian monitoring, Supplementary material—Table A1).
[2] 2013 (genetic data).

4. Discussion

According to our results, the investigated network of natterjack-toad breeding sites is genetically well connected and likely driven by two large populations in the North and South of the study area. Also, the landscape was rather permeable for movement as assessed by different methods (radio-tracking, genetic structure and assignment tests): none of the toad breeding sites was genetically isolated, although geographic distance was an important environmental determinant of the genetic pattern (for distances < 2.5 km) with a trend that also built-up areas may enhance genetic differentiation. Overall, it is unlikely that the inves-

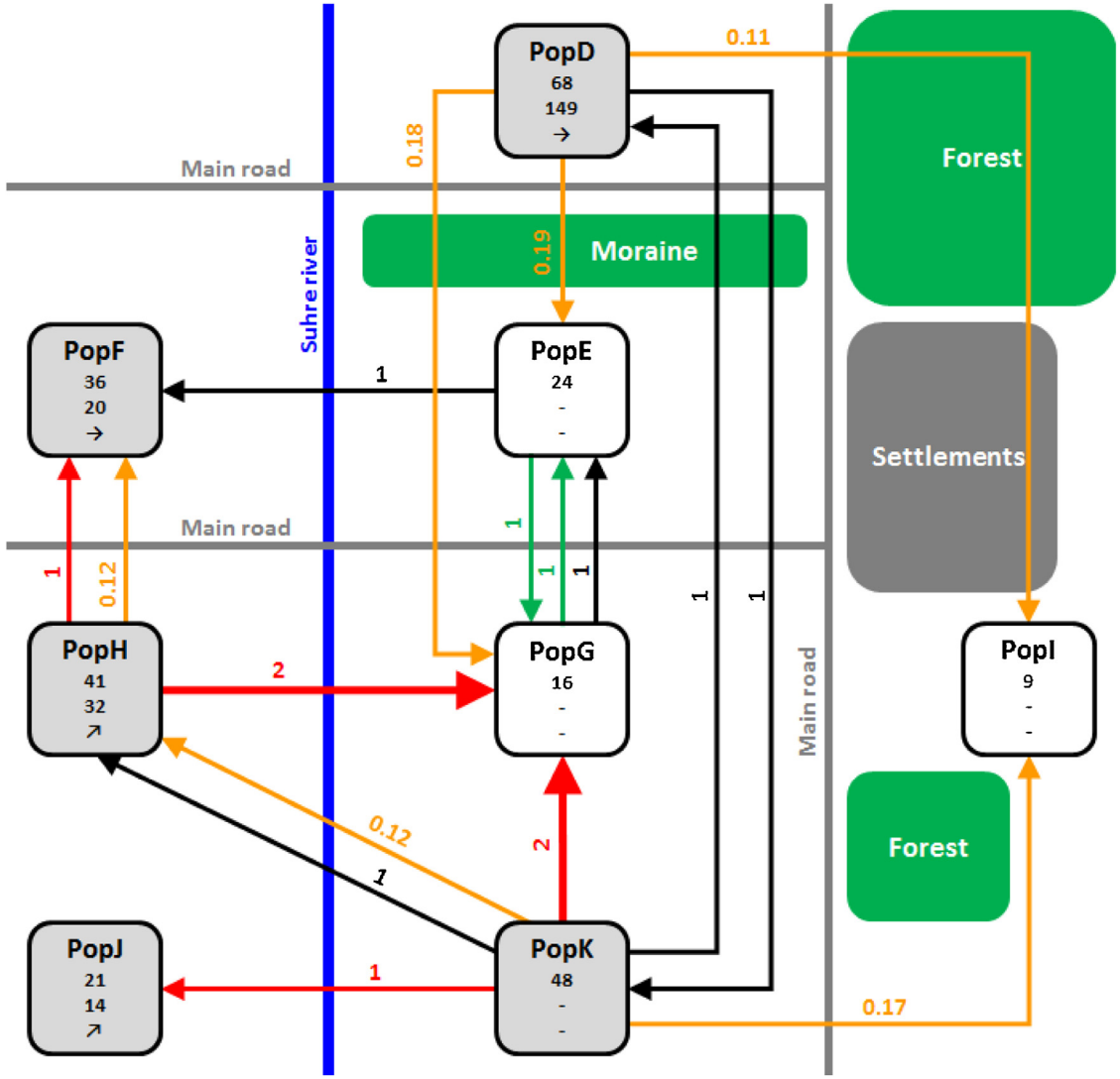

Fig. 4. Schematic illustration of the natterjack-toad populations and potential movement barriers in the Suhre valley, Switzerland. Arrows denote directly observed and indirectly detected migration events between populations, and the numbers of migrants are shown on each arrow. Green arrows show movement observed by radio-tracking, red arrows show movement by unintentional repeated genetic sampling of the same individuals, and black arrows show first-generation migrants. Orange arrows display migration probabilities detected by BIMR (only probabilities >0.1 are shown). Sources detected by BIMR are highlighted through grey background. The first number below the population name is the sample size, the second is the mean population size of the years 2009–2013, and arrows indicate the trend of population size.

tigated agricultural landscape represents an ecological trap, at least not in a situation where ephemeral breeding ponds are abundant.

4.1. Genetic diversity and structure

We observed a gradient of genetic assignment from North to South with none of the Natterjack toads unambiguously assigned to one of the up to ten clusters as calculated by STRUCTURE (Fig. 2). In line with this, gene flow was high (F_{ST} < 0.06) and IBD only became apparent (*i.e.* substantially increased genetic differentiation, indicative of reduced gene flow) for populations located at distances greater than 2.5 km (Fig. 3). The admixture was stronger in the central part of the study area compared to PopD and PopK, located in the North and South, respectively (Fig. 2). An explanation for this pattern might be that the two largest populations D and K (68 and 48 genetically sampled individuals, respectively; Table 1) may act as sources with individuals migrating into the centre of the valley. Genetically heterogeneous toad populations are particularly likely, because Natterjack toads have been recognised to be highly mobile (*e.g.* Jehle & Sinsch, 2007; Miaud et al., 2000; Sinsch et al., 2012). In line with the STRUCTURE results, our measured genetic parameters allelic richness (A_r), inbreeding coefficient (F_{IS}) and recent migrants also showed no genetic isolation or an indication of non-random mating, hence potential inbreeding, in our *a priori* delimited populations (Table 1; F_{IS} values around zero). Overall, genetic variability (heterozygosity) was at the same level as in well-connected populations in Belgium (Stevens, Polus, Wesselingh, Schtickzelle, & Baguette, 2006) and Luxembourg (Frantz, Proess, Burke, & Schley, 2009), but higher than in Denmark (Allentoft, Siegismund, Briggs, & Andersen, 2009) or Britain (Rowe et al., 1998, 1999), where populations were considered to be isolated.

4.2. Movement

Understanding movement is critical to many aspects of amphibian conservation (Cushman, 2006; Semlitsch, 2008). We used two complementary methods to assess or infer movement: radio-tracking and genetic assignment tests. Overall, two migration events could be observed out of the 50 radio-tracked individuals, eleven migration events/probabilities were detected by assignment

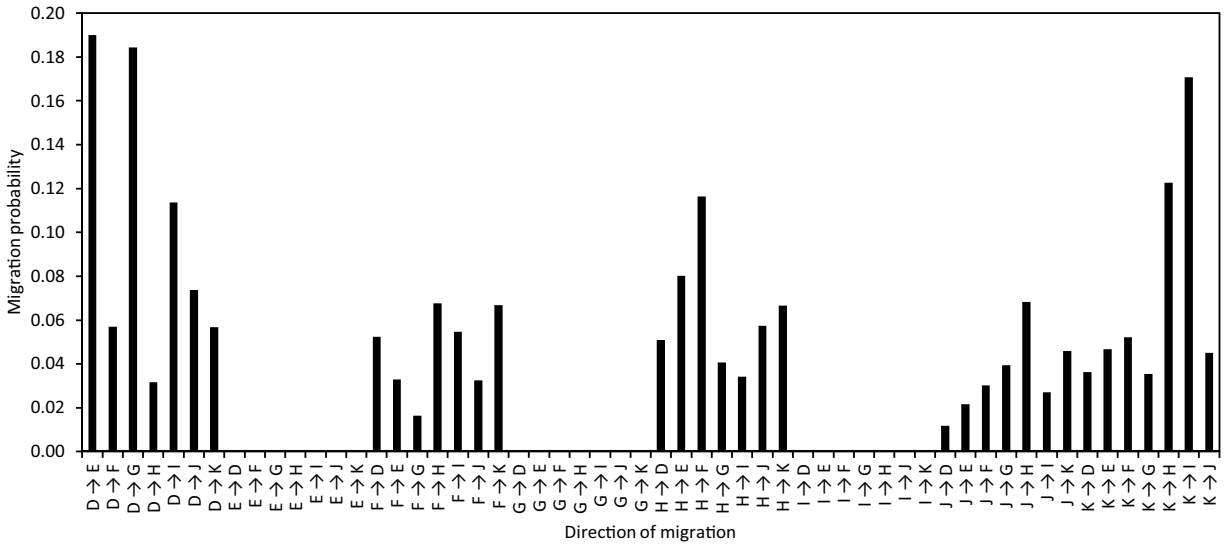

Fig. 5. Movement directionality of first-generation migrants as assessed by BIMR (Faubet & Gaggiotti 2008; Faubet et al., 2007) of the Natterjack toad in the Suhre valley, Switzerland. Populations D, F, H, J and K were identified as sources for migrants (migration probabilities > 0), whereas populations E, G and I were identified as sinks.

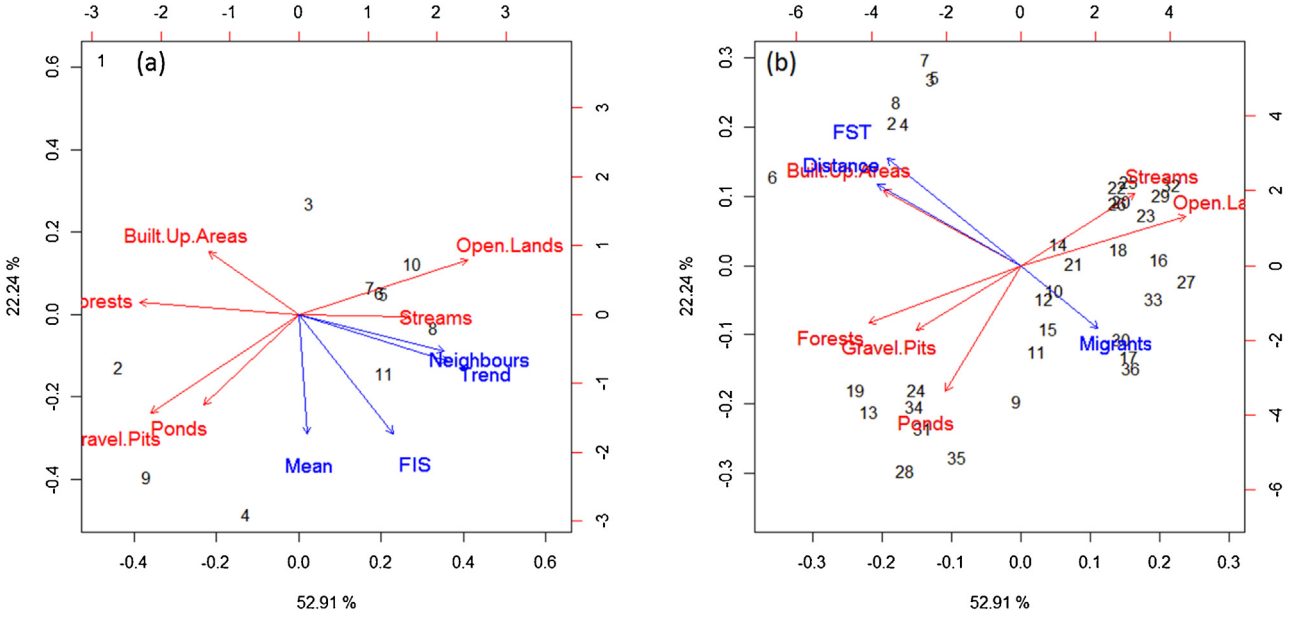

Fig. 6. Biplots of the principal component analyses (PCA) of the Natterjack toad in the Suhre valley, Switzerland. (a) Shows the results of the PCA for the site measures, (b) for the corridor measures. Landscape variables are red, demographic and genetic parameters are blue. The percentages on the axes show the amount of variation that is explained by the first (x-axis) and second principal component (y-axis).

tests and six migration events through unintentional repeated genetic sampling (and indirectly also by genetic analyses; Fig. 4).

Radio-tracking of individuals is a widely used method to observe movement (Jaquiery et al., 2011; Storfer, 2013), also of Natterjack toads (*e.g.* Husté et al., 2006; Miaud et al., 2000; Sinsch, 1988). The method reveals contemporary short-term movement which likely refers to smaller scale home-range assessments rather than movement between populations. Radio-tracking therefore allows for a spatially precise measurement of the movement and habitat preferences (Storfer, 2013).

In contrast to radio-tracking, genetic assignment tests allow for the detection of both, contemporary but also long-term movement between populations (gene flow) as genetic differentiation provides a proxy for a broader time horizon to assess movement than instant measures such as radio-tracking. In the Suhre valley, radio-tracked toads preferred to stay in the agricultural land with low ambition to move between our predefined populations (Fig. 4). Also, no radio-tracked individual was killed during the observation period (Schweizer, 2014). Contrarily, movement as assessed from genetic assignment tests indicated that migration has occurred both recently but also in the past. Radio-tracked toads in the middle of agricultural fields did not immediately escape, but, as shown by genetic assignment tests and by genetic structure, they can and do move if needed. So, there is evidence for both — remaining in intensively managed agricultural areas, but also moving through such a landscape. This led us to conclude that agricultural areas are not ecological traps for Natterjack toads in the Suhre valley, although the management of agricultural fields remains intensive.

4.3. Landscape effects on movement, gene flow and population characteristics

Many studies identified transportation infrastructures such as roads and railway tracks as strong barriers for amphibian movement (*e.g.* Mazerolle, Huot, & Gravel, 2005; Elzanowski, Ciesiolkiewicz, Kaczor, Radwanska, & Urban, 2009). In addition to anthropogenic barriers, also natural landscape elements such as forests, large or fast-flowing rivers likely represent challenges to the movement of many amphibians (Arntzen & Espregueira Themudo, 2008; Angelone & Holderegger, 2009).

In spite of the generally high level of gene flow between our *a priori* defined populations ($F_{ST} < 0.06$; Table 2) and a genetic gradient rather than discrete clusters (Fig. 2), roads and settlements may affect movement as indicated by the negative correlation between the number of migrants and built-up areas as well as the positive correlation between built-up areas and F_{ST} (Fig. 6b). Indeed, a partial Mantel test revealed that built-up area, in addition to geographic distance, was statistically significant when related to F_{ST} ($p < 0.05$). However, the correlation was highest for distance alone ($r = 0.89$), indicating that geographic distance is likely the most important determinant for genetic differentiation across the entire study area. Thus, similar to Le Lay et al. (2015) for European tree frogs, neither built-up area nor rivers or agricultural fields could be identified as true barriers to natterjack-toad migrants between our *a priori* defined populations (Figs. 4 and 5; Supplementary material—Table A2).

Natterjack toads have been reported to remain (Miaud et al., 2000) and also forage in intensively managed agricultural areas (Miaud & Sanuy, 2005). Similarly, Schweizer (2014) found that adult Natterjack toads used intensively managed agricultural areas as summer habitats. However, the effects of landscapes on Natterjack toads may differ depending on the life stage of the toads: quite in contrast to adults which were the focus of our study, toadlets preferred forested areas and bare grounds, whereas agricultural environments were largely avoided (Stevens, Polus, Wesselingh, Schtickzelle, & Baguette, 2004; Stevens et al., 2006). Our results confirmed these findings for adults, as forests surrounding breeding ponds were negatively correlated with trends in population size (Fig. 6a; Supplementary material—Table A2), indicating that tree encroachment at breeding ponds could be a reason for decreasing populations (Table 1; Fig. 1). The reason why we cannot see a negative effect of forests on gene flow could reflect the fact that toadlets move through forests as found by Stevens et al. (2004, 2006).

4.4. Functional toad network

The analysis of node importance, *i.e.* the relevance of single populations for the entire toad network, confirmed that larger populations tend to be more important for the persistence of a population network than small ones: PopD was the most important node if long-term population sizes were taken as node characterisation (Table 3). This stresses that PopD (and very likely also PopK) acts as a source population, as also indicated by the genetic structure (Fig. 2) and by the assignment test with BIMR (Fig. 5). In contrast, a toad network characterised with genetic parameters (A_r for node and F_{ST} for link characterisation) shows that, from a genetic point of view, all nodes contributed equally to the network (Table 3). This result confirms that the Natterjack toads are genetically well admixed. Our finding also underlines that functional assessments of landscape connectivity – such as network analyses – are indispensable supplements to fully understand structural connectivity assessments (Descout, Manel, Miaud, & Luque, 2012; Le Lay et al., 2015).

4.5. Natterjack toads in the Suhre valley—population, metapopulation or simply patchily distributed toad occurrences?

It is generally known that persistence of amphibian populations in fragmented landscapes depends on various environmental characteristics such as the distribution of roads, landscape types, breeding ponds, the population sizes in those ponds as well as the amphibian-specific dispersal potential (Cushman, 2006). The degree to which such environmental features and life-history traits shape the spatial distribution of amphibians is an issue particularly relevant to managers as they develop monitoring programs or management plans to assess whether local populations are represented as a single population or as several metapopulations consisting of population units (Petranka & Holbrook, 2006; Petranka et al., 2004). Clues from the genetic structures of wildlife have helped to delineate populations (Keller et al., 2015; Scribner et al., 2005). Although pond-breeding amphibians have shown a high site fidelity hinting towards a (meta)population organisation, previous studies on terrestrial movements of amphibians among ponds have concluded that spatial units larger than single ponds are necessary for the persistence of amphibian populations (Marsh & Trenham, 2001; Semlitsch, 2003). This is in line with findings that pond populations closer than some hundred meters are not demographically independent, which is why they should be treated as the same monitoring unit (Petranka et al., 2004; Petranka, 2007). Here, we subdivided the toad breeding ponds into *a priori* delineated population units using presumed landscape barriers as a criterion to investigate whether and to which degree landscape elements influence the genetic pattern and movement of the Natterjack toad. Results showed, however, that the toads exhibited only a weak genetic structure and low variation in the genetic diversity parameters with no major influence of landscape on gene flow or migration. Thus, in the case of this study for which all investigated ponds were well confined within 10 km (Fig. 1; Table 2), maybe even a metapopulation perspective is exaggerated, and managers may refer to the spatial structure of Natterjack toads as found in the Suhre valley as patchily distributed as suggested by Petranka et al. (2004).

4.6. Conclusions

This work was initiated by conservation management and subsequently tackled in a scientific context. Therefore, this project is an example to counteract the often found science–practice implementation gap (Arlettaz et al., 2010; Braunisch et al., 2012; Knight et al., 2008) by bundling competences between practitioners and scientists to bridge commitment, communication and collaboration gaps to mitigate conservation-relevant issues. The following conclusions for management are drawn:

1. Our analysis confirmed that the Natterjack toad is able to build viable populations in an agricultural landscape (Miaud et al., 2000). None of the predefined populations were genetically isolated, although there was a trend that built-up areas and roads fostered genetic differentiation in addition to geographic distance. Therefore, it is not likely that the investigated agricultural landscape is an ecological trap for Natterjack toads, at least not in situations with abundant ephemeral ponds.
2. The investigated toad network is presumably driven by two large source populations in the North and South of the study area (PopD, PopK). Apart from providing a dense network of breeding ponds, conservation priority should therefore also be given to the maintenance of currently large and well-established source populations.
3. Understanding movement in amphibians is a key to amphibian conservation (Semlitsch, 2008) and genetic methods to assess gene flow are important. However, to complete the long-term

picture of movement, we suggest to supplement gene flow, as assessed by *e.g.* F_{ST}, with assignment tests for contemporary gene flow and spatially exact movements, as provided by *e.g.* spatially and temporally highly resolved GPS tracking.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jnc.2016.04.002.

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