Genetic assessment of translocations: a case study of two endangered amphibians

Master thesis

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Abstract

Conservation managers face complex decisions in the attempt to halt further population declines and reduce biodiversity losses. Instead of basing decisions on personal belief, scientifically validated conservation is needed. Such an evidence-based approach has been applied to various conservation strategies, including translocations. They have been proven to be an effective tool for restoring endangered populations to portions of their former range. Especially when natural recolonisation is absent and/or too patchy. Unfortunately, the assessment of the genetic consequences of translocations are still lacking for various projects.

In this research I examined the genetic consequences of translocations for two endangered amphibians occurring in Switzerland; midwife toad (*Alytes obstetricans*) and natterjack toad (*Epidalea calamita*). Each species was genotyped with 13 microsatellite markers in order to deduce the genetic consequences of the translocations. For both species translocated populations were not genetically impoverished in comparison to the natural populations for either species. The populations that were created with several hundred individuals from mixed donor sites were more genetically diverse and admixed than other translocated populations, which were established with a limited founder group size from a single donor population.

Natterjack toads were only released where recolonisation through natural migration was not expected. I tested whether there were signs of natural migration with gene flow, network and landscape analysis in the region. My gene flow results indicated strong evidence that the investigated *E. calamita* populations are even more connected than previously assumed and that the species can cover up to 25.4 km between potential habitats in the study region. Landscape and network investigations suggest that there are no substantial barriers to gene flow in the area and further support the gene flow calculations.

These findings indicate that future translocations can create genetically diverse and structurally admixed populations when they are started with several hundred individuals from multiple populations. My results also suggest that gene flow between seemingly isolated

natterjack toad populations is higher than previously thought. However, future conservation work, which increases natural gene flow, would be beneficial for these populations.

Zusammenfassung

Verantwortliche für Natur- und Artenschutz stehen aufgrund von Populationsrückgängen und dem Verlust der biologischen Vielfalt vor komplexen Entscheidungen. Anstatt diese nur auf persönliche Überzeugungen zu stützen, braucht es evidenzbasierte Ansätze. Nach wissenschaftlichen Massstäben haben sich Wiederansiedlungen als wirksames naturschützerisches Werkzeug zum Erhalt gefährdeter Populationen in ihrem ehemaligen Verbreitungsgebiet erwiesen. Insbesondere, wenn natürliche Wiederbesiedelung kaum und/oder zu lückenhaft stattfindet. Leider fehlt die anschliessende Beurteilung der genetischen Konsequenzen solcher Wiederansiedlungen oft.

In der vorliegenden Arbeit konzentriere ich mich auf die genetischen Konsequenzen der Wiederansiedlung zweier gefährdeter Amphibien in der Schweiz; der Geburtshelfer-kröte *(Alytes obstetricans)* und der Kreuzkröte *(Epidalea calamita)*. Beide Arten wurde mit 13 Mikrosatelliten-Markern genotypisiert, um Rückschlüsse auf die genetischen Folgen der Translokationen zu erhalten. Die wiederangesiedelten Populationen sind genetisch nicht weniger divers als natürliche Populationen. Wiederangesiedelte Populationen, für die mehrere hundert Individuen aus unterschiedlichen Populationen ausgesetzt wurden, waren genetisch vielfältiger als andere, welche mittels einer beschränkten Anzahl von Individuen von einer Spenderpopulation gegründet wurden.

Die Kreuzkröten wurden nur in Gebieten ausgesetzt in denen eine Rekolonialisierung durch natürliche Migration nicht erwartet wurde. Ob trotzdem eine natürliche Migration stattfand, untersuchte ich mittels Genfluss-, Netzwerk- und Landschaftsanalysen. Es fanden Genflüsse über Distanzen von 25.4 km statt. Dies zeigt, dass die Kreuzkröten-Standorte stärker mit einander verbunden sind, als bisher angenommen wurde. Auch die Landschafts- und Netzwerkuntersuchungen bestätigten den berechneten Genfluss in dieser Gegend.

Zusammengefasst sprechen die Ergebnisse dieser Arbeit dafür, dass Wiederansiedlungen zu genetisch diversen und gemischten Populationen führen können, wenn mehrere hundert Individuen aus verschiedenen Populationen ausgesetzt werden. Zudem konnte gezeigt werden, dass der Genfluss zwischen scheinbar isolierten Kreuzkrötenpopulationen höher ist

als zuerst angenommen wurde, trotzdem wären weitere Naturschutzmassnahmen, welche den natürlichen Genfluss weiter verbessern wünschenswert.

Introduction

Conservation managers face complex decisions in relation to the approaches they apply to halt biodiversity losses (Uusitalo et al. 2015; Laurila-Pant et al. 2015). Due to their complex nature, conservation decisions are often based on personal knowledge and experience (Pullin et al. 2004; Cook et al. 2010; Drolet et al. 2014). A move towards more systematic appraisal of evidence would benefit conservation efforts and provide vital information that can be used to improve the efficacy of future conservation work (Gusset et al. 2008; Schofield et al. 2013; Drolet et al. 2014).

Such an evidence-based approach could also benefit translocation projects (IUCN/SSC 2013). A conservation approach which have become an important and well acknowledged tool (Fischer and Lindenmayer 2000; Bouzat et al. 2009; Germano et al. 2015; Mowry et al. 2015; Reiter et al. 2016) and can be for example executed when natural recolonisation is very slow and/or too patchy (Watson and Watson 2015). Translocations are inherently complex (Germano et al. 2015; Heikkinen et al. 2015), requiring careful consideration of multiple factors including the selection of suitable founder populations, analysis of suitable habitats and potential threats. This makes it challenging to know when, where and how translocations are best applied (Wolf et al. 1998; IUCN/SSC 2013; Batson et al. 2015; Heikkinen et al. 2015). They do not only require information about ecological, environmental, disease and demographic aspects but also genetics (Smith et al. 2012). Projects often lack a comprehensive assessment of genetic indicators such as genetic diversity and gene flow (Latch and Rhodes 2005; Sigg 2006; Cardoso et al. 2009; Michaelides et al. 2015; Mowry et al. 2015), although the need for genetic analysis of translocated populations has long been known (Haig et al. 1990; Mowry et al. 2015). Such insights are indispensable in the context of translocation, particularly because small populations are very susceptible to loss of diversity through processes including genetic drift, inbreeding depression in newly founded populations, and possibly even extinction (Saccheri et al. 1998; Westemeier et al. 1998; Hedrick and Kalinowski 2000; Sigg 2006; Frankham et al. 2011).

Translocations involve a disproportionate number of mammal and bird species (Seddon et al.

2005) although other taxonomic groups are also clearly in need of effective conservation actions. Amphibians are the worlds most threatened animal class, with more than 41 % of the living amphibian species currently considered to be threatened (Pimm et al. 2014; Catenazzi 2015). Early studies of Dodd and Seigel (1991) suggested the approach as unsuitable for herpetofauna due to the potential for disadvantages (e.g. disease spread) and low possibilities of the establishment of self-sustaining populations. However, attitudes towards translocations as a viable intervention for amphibians have increased in recent years (Germano and Bishop 2009; Schröder et al. 2012; Taddey 2013; Zeisset and Beebee 2013).

For the establishment of viable, self-sustaining populations, the eventual aim of translocation (Dodd and Seigel 1991), a founder group size of over 1000 individuals for amphibians has been recommended (Germano and Bishop 2009; Zeisset and Beebee 2013). However, endangered amphibian populations are often small (Schmidt and Zumbach 2005). Additionally, for certain species, such as *A. obstetricans*, females produce approximately 50 eggs (Kordges 2003) per year. Hence the removal of such large quantities of founder individuals is often not possible without harming the existing populations. As a consequence, a small number of founder individuals is released and genetic erosion, loss of genetic diversity, has been reported in various studies (Griffith et al. 1989; Maudet et al. 2002; Sigg 2006; Cardoso et al. 2009; Miller et al. 2014; Michaelides et al. 2015).

To study genetic translocation outcome and to contribute additional knowledge to future evidence-based translocations to support the decision-making process, I assessed the population and landscape genetic compounds in natural and translocated populations of two endangered amphibian species (*Alytes obstetricans* and *Epidalea calamita*). Both species have exhibited strong population declines in Switzerland with approximately 50 % - 60 % of the populations lost over the last 30 years; thus both are listed in the Red list of Swiss amphibians (Schmidt and Zumbach 2005; Cruickshank et al. 2016). In response to these declines, local authorities began to restore fragmented populations and took measures aimed to increased population connectivity (Borgula and Zumbach 2003; Ryser et al. 2003; Meier and Hoffmann 2004a; Meier and Hoffmann 2004b; Lippuner 2013a). Amongst various conservation actions, such as the creation of new habitats, individuals were also translocated.

The first translocations started in 2000 for *A. obstetricans*. Since then habitats have been continuously created or enhanced in Lucerne and St. Gallen allowing translocations in these locations. For *E. calamita* translocations started in 2007 in Zurich following the recommendations of prior studies that argued for increases in habitat quality and actions to halt further isolation of remaining populations (Lippuner 2013b). Various new habitats were created and translocations were only pursued in case natural colonisation were not anticipated (Lippuner 2013a).

In 2015 I sampled and analysed various donor and translocated population to gain an understanding of the possible genetic erosion of translocations and whether translocation were needed in Zurich. As such, I defined the following research questions:

How do genetic diversity indicators of natural populations differ from translocated populations?

Are translocated populations more strongly admixed than natural populations?

What are the effects of the number of founder sites and number of translocated individuals upon genetic diversity indicators?

Are translocated populations equally important in sustaining the population network?

Is there recent gene flow between natural *E. calamita* populations in intensely managed landscapes?

What are the effects of landscape elements on genetic diversity and gene flow of *E. calamita*?

To answer these questions, I compared natural populations to the translocated populations of the two species in three study areas, located in St. Gallen, Lucerne and Zürich in Switzerland.

Material and Methods

Study species

My study focuses on two endangered Swiss amphibians; *Alytes obstetricans* (midwife toad) and *Epidalea* (*Bufo*) *calamita* (natterjack toad). The two species differ in various aspects of their biology. *A. obstetricans* mates on land. Uniquely, the male carries the clutch of approximately 50 eggs twined around their hind legs until the larvae are ready to hatch (Kordges 2003). Further larval development takes place in water. Some tadpoles might not metamorphose in the same year and hibernate in the pond (Thiesmeier 1992). One to two years after metamorphosis the toad reproduces (Böll et al. 2012). *A. obstetricans* is considered as a relatively sessile species (Borgula and Zumbach 2003) and colonisations of new habitat usually occur mostly only over short distances of less than 1.5 km (Ryser et al. 2003). In contrast, the hatching and larval development of *E. calamita* is entirely aquatic. Between 3000 and 4000 individuals are spawned directly into the ponds (Sinsch 1998). The larval development period is the shortest of all Swiss amphibian species and can be completed within three to six weeks and a total generation time of around three years until the first reproduction (Rowe et al. 2000). The species has been observed to colonise new habitats frequently (Banks and Beebee 1987) and over large distances. Annual movement distances up to four to five kilometres with telemetry and mark-recapture have been recorded (Jehle and Sinsch 2007). So far the largest detected connectivity distance was 12 km between neighbouring breeding ponds (Sinsch et al. 2012).

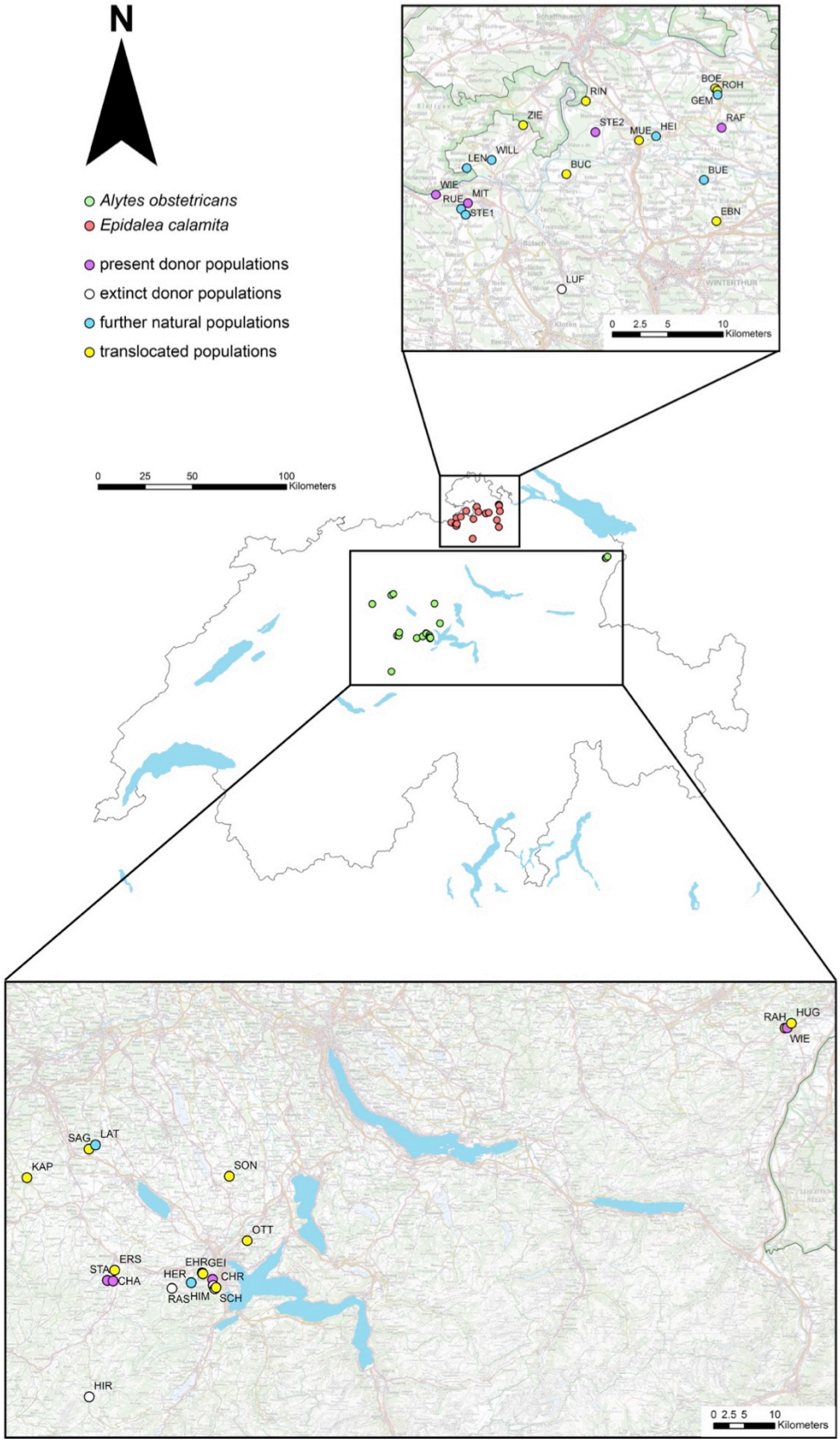
Like many amphibian species, *A. obstetricans* and *E. calamita* have been facing diverse threats during past decades (Catenazzi 2015; Ficetola et al. 2015). As a consequence, the species have suffered severe losses across their distribution range in Europe (Barrios et al. 2012; Buckley et al. 2014). In Switzerland around 50 % *A. obstetricans* and approximately 60 % *E. calamita* populations have gone locally extinct during the past 30 years, resulting in both species being listed as “endangered” at the national level (Schmidt and Zumbach 2005).

Study area

The study took place north of the Alps in Switzerland (Fig. 1; Appendix S1). 16 study sites for *A. obstetricans* are located in pre-alpine areas of the cantons Lucerne and St. Gallen. The mean elevation of the study sites was 621 m.a.s.l (range: 527-795) for Lucerne and 542 m.a.s.l (range: 539-548) for St. Gallen. The study area in Lucerne covered approximately 1400 km^2 and 0.3 km^2 in St. Gallen (Fig. 1). Lucerne has 38 known *A. obstetricans* populations (Röösli, pers. comm. 2016) whereas in Altstätten, St. Gallen, four populations are present (Moser, pers. comm. 2016).

Study sites for *E. calamita* were located in the canton Zurich (Swiss midlands). 18 *E. calamita* populations are known (Lippuner, pers. comm. 2016). The mean elevation was 400 m.a.s.l (range: 336 – 483 m.a.s.l). The river Thur, approximately 50m wide, divides the study area into a northern and a southern part and a highway, approximately 20m wide, into a western and eastern part. Approximately 6 % of the 300 km^2 study area consists of settlements, 15 % open land and 67 % forest.

Figure 1 Map of the donor, translocated and additional studied natural populations in the study area.



Translocation history

Due to the strong population declines of the two endangered amphibians, local authorities initiated translocation projects for the both species in the regions presented above. The translocation histories are presented in Figure 2.

The canton Lucerne initiated the first official Swiss amphibian translocation project in 2000 (Fig. 2A). *A. obstetricans* individuals were collected from 11 donor populations and officially released at nine translocation sites. Five additional populations originated from unofficial translocations. The numbers of founders released ranged from 150 to 385 from two to three sites (for detailed numbers per population, see Appendix S2). By 2015, four out of nine officially translocated had become locally extinct and one donor population, which was severely weakened due to larval removal, became extinct. Other donor sites were lost due to other anthropogenic or natural reasons (Röösli, pers. comm. 2016).

In Altstätten St. Gallen, *A. obstetricans* tadpoles were collected from one donor population and released at three close-by sites between 2003 and 2007. Founder group size ranged from 17 to 55. None of the populations became extinct up to 2015 (Moser, pers. comm. 2016).

In the canton of Zürich, *E. calamita* translocations were conducted between 2007 and 2011 (Fig. 2B). Five donor populations contributed tadpoles to eight translocation sites. The founder group size in the release years ranged from 700 to 8000 individuals collected from one to five sites. None of eight translocated populations has gone locally extinct. One donor population has become extinct but not because of translocation interventions. Two newly created ponds were colonised independently of translocations in 2015 (Lippuner, pers. comm. 2016).

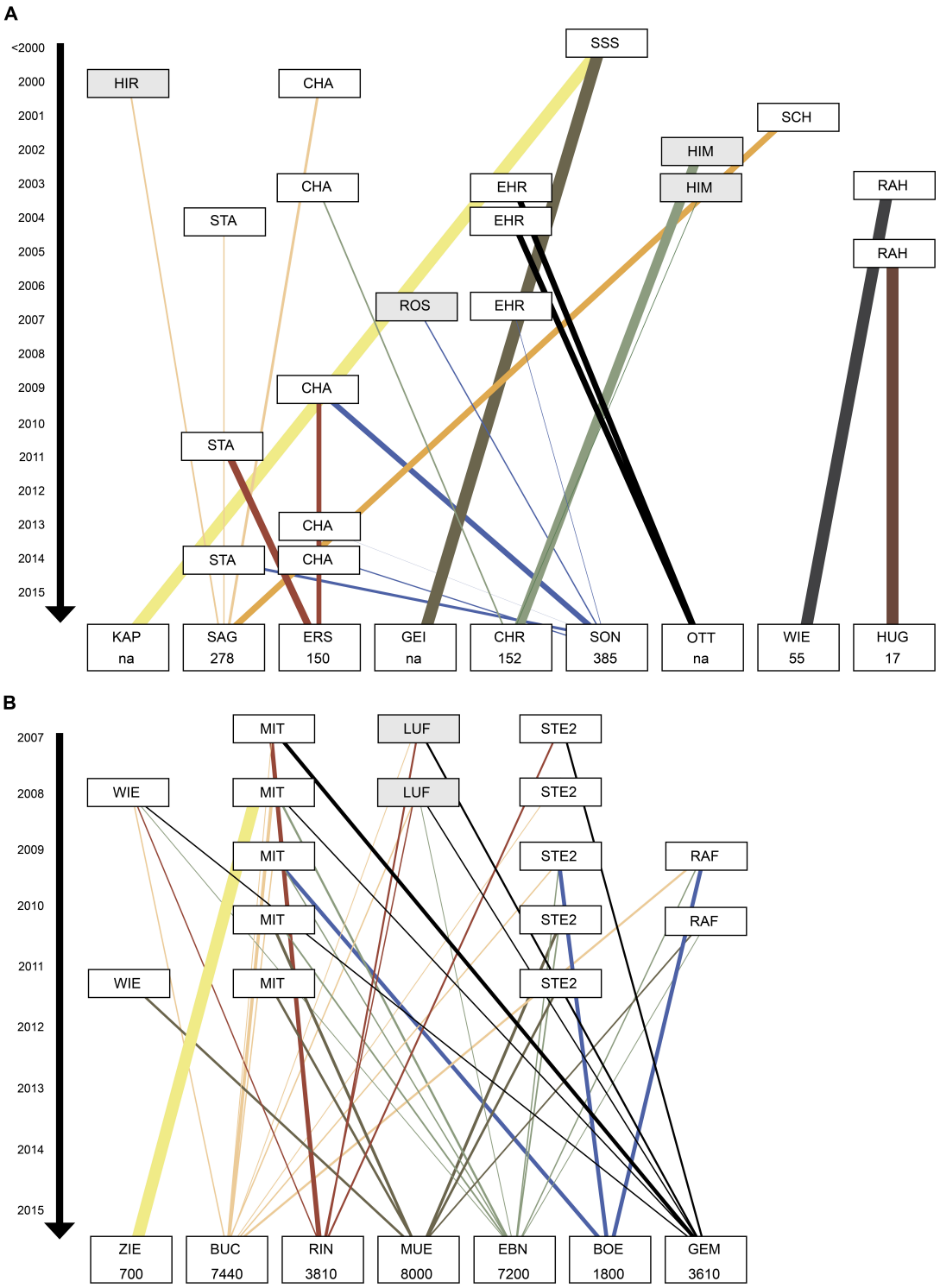


Figure 2 Translocation history of *A. obstetricans* (A) and *E. calamita* (B) since the first individual releases of the studied translocated populations. The names of the populations at the bottom represent the translocated populations from west to east with the number of founder individuals. The populations above represent the donor populations from which individuals were removed, also from west to east. The line thickness is proportional to the contribution (number of founder individuals) of a donor population to a translocated population. Populations in white boxes indicate present populations and populations in grey boxes locally extinct populations in 2015.

Population sampling

During spring and early summer 2015 *A. obstetricans* (April – May and July) and *E. calamita* (April – July) DNA was sampled. Standard hygiene procedures were applied to avoid spread of diseases during field work (Schmidt et al. 2009).

A. obstetricans tadpoles were sampled by dip-netting. For every population, a maximum of 32 tadpoles was caught. For *A. obstetricans* in Lucerne, the genetic data derived from six natural and seven translocated populations (Table 1). I sampled four official translocated and four donor populations. Additionally, two large natural populations (LAT, HER) that were never used for translocations and three populations that originated from unofficial translocations were also sampled (GEI, KAP, OTT). In St. Gallen, I sampled the only donor population and two translocated populations. With a scalpel blade tissue for DNA extraction was collected by removing approximately 3 mm from the tail tip. An exception was the population SAG in Lucerne, where we used sterile swabs (Copan Diagnostics, California, USA, code 155C) to collect DNA because of landowner concerns to harm the tadpoles. Tissue samples were put in 94 % ethanol and were stored in the freezer (-20.5 °C ± 0.8 °C) prior to laboratory analysis as were the swabs.

For *E. calamita,* all four existing donor populations, all seven translocated populations and all seven additional natural populations in the region were sampled. In order to avoid sampling full siblings, a different sampling approach was applied. As *E. calamita* females produces usually only one spawn string per year (Sinsch 1998) collecting eggs of different strings increased the probability that the samples originated from different females. For *A. obstetricans* this approach was not possible due to their different reproduction biology. I collected ten eggs per string and brought them to the laboratory. There, the eggs of a string were hatched in an individual 5 l tub and tadpoles were raised under greenhouse conditions until the tails reached 3 mm. Subsequently, the largest larvae per string was anaesthetized with Ethyl 3-aminobenzoate methanesulfonate (Sigma–Aldrich, Buchs, Switzerland, code MS-222), preserved in 94 % ethanol and stored in the freezer. The remaining tadpoles were released back into the population from which they were originally captured.

Table 1 The amount of studied natural and translocated population in the region with sample size (number of sampled tadpoles) in brackets.

Species	*A. obstetricans*		*E. calamita*
Canton	Lucerne	St.Gallen	Zurich
Number of natural populations (sample size)	6 (172)	1 (30)	11 (142)
Number of translocated populations	7 (180)	2 (46)	7 (96)

Genetic data

I analysed the samples genetically using microsatellite markers. For extraction, I used the BioSprint 96 DNA Blood Kit (Qiagen, Hombrechtikon, Switzerland, code 940054) and followed the kit's protocol for tissue extractions. *A. obstetricans* swabs were analysed using DNeasy Blood & Tissue Kit according to manufacturer's guidelines (Qiagen, Hombrechtikon, Switzerland, code 69504). Polymerase chain reaction (PCR) was performed with fluorescent-labelled primers.

All *A. obstetricans* samples were genotyped with 16 microsatellite markers. 12 markers, developed by Tobler et al. (2013), were supplemented with four new primers (Table 2). The new primers were designed by ecogenics GmbH (Zurich, Switzerland). The new primers exhibited clear and reliable amplification, polymorphism, and showed no evidence of null alleles in preliminary tests when generating population genetic data with MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004).

Table 2 Microsatellite primer information of the four newly developed *A. obstetricans* marker.

Muliplex	Locus		Primer sequences 5'-3'	Size bp	Modification 5'	Repeat type	Concentration per reaction
4	Alyobs_01107	F	GTCTCCCCACTCTACCATGC	133-145	Atto565	$(AGAT)_{19}$	0.2 µM each
		R	AGTTTAGCATAAAAAGGCCCAC				
4	Alyobs_04782	F	TGTCCCTATCACAAGAACCAAG	187-223	Atto532	$(TCTA)_{22}$	0.1 µM each
		R	ACAATACAATGTTGCAATCTGGAC				
4	Alyobs_06184	F	TGTAGCAATCCTAGTGGGTC	132-140	FAM	$(TCTA)_{10}$	0.1 µM each
		R	ACCTGGCAACTCATTGTCTC				
4	Alyobs_08127	F	TGAGGATCAACAGCCCTACAC	177-196	Atto550	$(ATCT)_{15}$	0.1 µM each
		R	CATCCTGACCAGGCATACAC				

The primers for *A. obstetricans* were assembled in four different multiplexes. The first 12 markers of Tobler et al. (2013) were multiplexed as followed: Multiplex 1 consisted of Alyobs3, Alyobs4, Alyobs7, and Alyobs28; multiplex 2 consisted of Alyobs 8, and Alyobs 16; multiplex 3 consisted of Alyobs 17, Alyobs 19, Alyobs 20, Alyobs 23, Alyobs 24, Alyobs 25.

The fourth multiplex contained the newly developed markers (Alyobs 01107, Alyobs 04782, Alyobs 06907, Alyobs 08127). Each well contained 4 µl BioSprint 96 DNA Blood Kit material (Qiagen, Hombrechtikon, Switzerland, code 940054), the primer concentrations from Tobler et al. (2013) respectively (see Table 2) and approximately 40 ng of template DNA. PCRs were performed on a Veriti 96 well Thermal Cycler (Thermo Fisher Scientific, Waltham, USA) with polymerase activation at 95 °C for 15 min, followed by 33 cycles (multiplex 1), 30 cycles (multiplexes 2 and 4) or 28 cycles (multiplex 3) of denaturing for 30 sec at 94 °C, annealing for 1.5 min at 52°C (multiplex 1) or 56 °C (multiplexes 2, 3, 4) and extension for 1 min at 72 °C, followed by a last extension for 30 min at 60 °C.

For *E. calamita* 13 polymorphic microsatellite markers were used: Buca1, Buca2, Buca5, Buca6 were designed by Rowe et al. (1997), Bcalµ10 by Rowe et al. (2000) and Bcalµ1, Bcalµ2, Bcalµ3, Bcalµ4, Bcalµ5, Bcalµ6, Bcalµ7, Bcalµ8 by Rogell et al. (2005). These markers have been successfully applied in various studies (Frantz et al. 2009; Allentoft et al. 2009; Oromi et al. 2012; Frei 2014). The microsatellites were analysed in two multiplexes. For each PCR a well consisted of 5 µl BioSprint 96 DNA Blood Kit material (Qiagen, Hombrechtikon, Switzerland, code 940054), the primer concentration from Frei (2014) and approximately 40 ng of template DNA. The PCR temperature and time periods were also based on the previous study of Frei (2014) following a thermal profile with 5 min at 95 °C, 30 (multiplex 1) and 32 cycles (multiplex 2) of 30 sec at 95 °C, 90 sec at 58 °C (multiplex 1) and 55 °C (multiplex 2), 30 sec at 72 °C as well as a final extension step at 30 min at 60 °C: PCR products of both species were sequenced on ABI 3730 Avant capillary sequencer (Applied Biosystems, Rotkreuz, Switzerland) with size standard Gene-Scan-500 LIZ. Subsequently, peaks from the sequencing were visually evaluated with GENEMAPPER 5 (Applied Biosystems, California USA).

Landscape data

I assessed the relationship between genetic indicators and landscape elements between all *E. calamita* populations in the study area. The landscape data were derived from the

SwissTLM3D 2013 and SwissBUILDINGS3D 1.0 datasets (Swisstopo, coordinate system: CH 1903 LV03), extracted using ARCGIS 10.3.1. In a first step, I identified 12 landscape elements hypothesised to either increase or decrease gene flow: wetlands, standing water, gravel pits, hedges, large roads (> 6 m broad), intermediate roads (3 m – 6 m broad), small roads (< 3 m), railways, large rivers (> 50m broad), small rivers (< 50 m broad), settlements, forests, open lands, slopes (> 25°). Information about each layer source is described in Appendix S3. In addition, elevational differences between populations was analysed with a 25 m resolution digital height model (DHM25; Swisstopo). Linear landscape elements from the SwissTLM3D were buffered on both sides of elements using ArcGIS 10.2 (ESRI, USA). Buffer width in meters was chosen to reflect real conditions. If buildings were closer than 100 m from each other, I aggregated them into settlements. Otherwise they were not considered to be a gene flow barrier. The subsequent landscape classes were then merged into a raster map with a spatial resolution of 0.5 m.

Genetic analysis

Genetic diversity

As it was not possible to exclude close siblings as part of the study design for *A. obstetricans* samples, siblings were identified using COLONY version 2.0.5.9 (Wang 2004) and excluded from the analysis. Furthermore, I grouped all *A. obstetricans* coming from two different regions (Luzern and St. Gallen) together for further analyses. All other analyses were applied equally to the *A. obstetricans* and the *E. calamita* datasets. First I carried out standard genetic analyses to test for deviations from Hardy-Weinberg equilibrium (HWE) as well as linkage disequilibrium (LD), both calculated with Fisher's exact test in GENEPOP 4.0 (Rousset 2008). The following settings were applied: number of dememorization was set to 1000, number of batches to 100 with 1000 iterations per batch. Significance of HWE and LD was evaluated after Bonferroni correction (Rice 1989). Linked loci might affect F-statistics while loci departing from HWE might be an indication for the presence of null alleles (Chapuis and Estoup 2006). For the latter, I used MICROCHECKER version 2.2.3 (Van Oosterhout et al.

2004) to determinate allelic amplification failure and probable genotyping errors ($p < 0.05$). The genotyping error rate was set to 0.001.

Genetic diversity indicators were assessed for each microsatellite marker to compare translocated and natural populations. I calculated the number of alleles (A), private alleles (P_A), fixed alleles (F_A), observed (H_O), and expected heterozygosity (H_E) with GENALEX version 6.5 (Peakall and Smouse 2012) whereas I used FSTAT version 2.9.3.2 for the calculation of allelic richness (A_R) and the inbreeding coefficient (F_{IS}) (Goudet 1995; Goudet 2002). I evaluated differences in genetic diversity measures between natural and translocated populations using Wilcoxon tests and Tukey's HSD test. The effective number of breeders (N_e) also affects the genetic diversity of breeding individuals (Frankham 1995). I calculated N_e with COLONY version 2.0.5.9 (Wang 2004) with a typing error rate of 0.001. Bottlenecks and the subsequent decrease in genetic diversity occur if population size is strongly reduced, e.g. in case of the establishment of translocated populations. As such, I decided to test for evidence of genetic bottlenecks from differences in genetic diversity between natural and translocated populations, using the program BOTTLENECK version 1.2.02 (Piry 1999). I performed bottleneck tests only on the largest populations where a minimum of 20 individuals was sampled. Three models were applied: the infinite alleles model (IAM), the stepwise mutation model (SMM) and the two-phase model (TPM). The latter was run as using recommended parameters set to 95 % SMM and variance to 12 % (Piry 1999). Significance was evaluated using a Wilcoxon signed-rank test, which is the most appropriate and powerful test for less than 20 loci (Piry 1999). An additional test calculated allele frequency distortion to determine shifts from the equilibrium L-shape (Luikart et al. 1998).

Genetic differentiation and structure

Genetic relationships and distances among natural or translocated populations were analysed by the calculation of pairwise genetic differentiation (F_{ST}) with ARLEQUIN (Excoffier et al. 2005). I used the exact test of population differentiation in ARLEQUIN to evaluate departures from zero (number of steps in Markov chain = 1000 and dememorization steps = 10'000). I tested for isolation by distance (IBD) using F_{ST} / (1-F_{ST})-transformed F_{ST} values and

log-transformed Euclidian distances (Excoffier et al. 2005). IBD was calculated with the statistical software R 3.2.2 (R Core Team 2015) and the package adegenet (Jombart 2008).
I used the programme STRUCTURE 2.3.4 (Pritchard et al. 2000) to investigate the spatial genetic structure using a Bayesian clustering approach. This approach assigns individuals to one of *K* clusters based on multilocus genotype data. Runs were conducted with the admixture model as I expected individuals in translocated populations to have mixed ancestry. Five independent runs were calculated to assess the most likely number of putative populations (*K* = 1 to K = the number of populations sampled), using a burn-in period of 500'000 and 1'000'000 Markov Chain Monte Carlo (MCMC) iterations. The optimal cluster number (K_{max}) was examined with STRUCTURE HARVESTER version 0.9.94 (Earl and von Holdt 2012) using the approach of Evanno et al. (2005). The optimal *K* outputs were rerun ten times with STRUCTURE and then merged in CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007) to avoid stochastic discrepancies between the 15 runs. The CLUMPP output was visualised with DISTRUCT 1.1 (Rosenberg 2003).
For all natural or translocated populations an analysis of molecular variance (AMOVA) was conducted to evaluate genetic variances within and among the groups, using GENALEX version 6.5.

Founder size and sites correlations

I determinate the consequences of the number of founder individuals and the number of contributing founder sites on genetic diversity indicators for the translocated populations. To do this, I calculated Pearson correlations between number of founder sites respectively amount of individuals translocated versus allelic richness, as well as both observed and expected heterozygosity. Coefficient significance was analysed with the test for association between paired samples, using Pearson's product moment correlation coefficient ($p < 0.05$). I carried out these analyses using the package "corrplot" (Wei 2013) implemented in R 3.2.2 (R Core Team 2015).

Connectivity in the network

I analysed the population networks in St. Gallen and in Zurich with CONEFOR SENSINODE version 2.6 (Pascual-Hortal and Saura 2006; Saura and Pascual-Hortal 2007; Saura and Torné 2009) to assess structural and the functional connectivity in toad networks. The programme evaluates populations (nodes) or links by comparing the distance between nodes and an assigned dispersal distance threshold. Node sizes were based on the latest census sizes estimation (*N*) by local experts or A_R, and links either by geographic (Euclidian distance, *ED*) or genetic (F_{ST}) distances. The species-specific threshold was set according to previous dispersal studies. Ryser et al. (2003) reported one out of 11 *A. obstetricans* toads migrated a distance of 2250 m. Therefore, I set the threshold to 2250 with a migration probability of 0.09. For *E. calamita* the threshold was set to 2200 m with a migration probability of 50 %, in line with the results from previous studies (Frei 2014). The effect of geographic distance on F_{ST} was assessed with linear regressions to determine genetic thresholds for the two species using Microsoft Excel 2010 (Appendix S4). Two graph-based connectivity indices; the integral index of connectivity and the probability of connectivity were used for the calculations because they have been successfully applied for the goal of prioritisation of habitats and links (Pascual-Hortal and Saura 2006; Saura and Torné 2009; Aavik et al. 2014).

Recent gene flow

For natural *E. calamita* populations I analysed whether there were signs of recent gene flow and if so over what distances. Gene flow was identified with two models: first-generation migrants and assignment-test with GENECLASS (Piry 2004). For first-generation migrants analysis I selected the L_{home} / L_{max} ratio. Probability values were determined using the partial Bayesian method of Rannala and Mountain (1997) and the Monte Carlo resampling method, simulating 1000 individuals and with a critical value of the test statistic at 0.01 (Paetkau et al. 2004) for the entire study area.

An additional assignment-test with GENECLASS (Piry 2004) complemented the FGM assessments. Again the methods of Rannala and Mountain (1997) with the Monte-Carlo

resampling algorithm of Paetkau et al. (2004) were applied (type I error, number of simulated individuals: 1000). All individuals that were assigned with a probability of ≥ 0.8 were characterized as migrants (Le Lay et al. 2015).

Effect of landscape elements on diversity and gene flow

I analysed the landscape data similar to the studies of Angelone et al. (2011) and Emaresi et al. (2011). Each population was buffered with a 140 m radius using ArcGIS 10.2 (ESRI, USA). The buffer size was chosen in order to avoid spatial overlap between populations (shortest distance between populations: 286 m). Within each buffer, the proportion of each landscape classification was calculated. Pearson correlations were calculated to assess the statistical correlations between landscape elements. Correlations were conducted in the software R 3.2.2 (R Core Team 2015) with package corrplot (Wei 2013). In a second step the effect of landscape elements on pairwise F_{ST} and natural gene flow was investigated with buffered straight-line corridors with a width of 140 m. Coefficient significance was elevated with the test for association between paired samples, using Pearson's product moment correlation coefficient ($p < 0.05$). To account for possible effects of multicollinearity, I considered only correlation coefficients above |0.6| as statistically important as in the study of Frei (2014).

Results

Genetic diversity

I removed 64 *A. obstetricans* full siblings after sibship analysis. Mean sample size per population was 23 (range: 11 – 32). Loci Alyobs19, 20, 25 were monomorphic in all studied populations and excluded while 13 microsatellite markers were polymorphic and used for further analysis. I did not find deviances in terms of null alleles, large allelic dropout (p > 0.05) or departure from HWE across the loci following Bonferroni correction for the species. The results from pairwise comparisons did not reveal any loci that showed consistent linkage patterns across populations.

The genetic diversity indicators; allelic richness (A_R), observed (H_O) and expected heterozygosity (H_E) did not differ significantly between natural and translocated *A. obstetricans* populations (Wilcoxon-test, p > 0.05). Overall, natural populations had a mean allelic richness value of 2.24 with a standard error (SE) of 0.07 and translocated populations of 2.25 (SE 0.13; Table 3). Also, differences among the F_{IS} of all populations were not significant (Tukey's HSD test, p > 0.05). Mean inbreeding values for natural populations were -0.017 (SE 0.025) and translocated populations -0.029 (SE 0.022).

For *E. calamita* 18 populations were analysed with a mean sample size of 13 (range: 4 – 26). All 13 markers were polymorphic in all studied populations. No evidence of allele dropout or genotyping errors was seen (p > 0.05), and no loci deviated significantly from HWE. I identified no evidence of any loci that were linked. A_R and H_O did not differ significantly between natural and translocated populations. Mean A_R for natural populations was 2.18 (SE 0.06) and for translocated populations 2.19 (SE 0.03). H_E was significantly higher in translocated populations (0.49, SE 0.01 vs. 0.42, SE 0.02; Wilcoxon-test; p = 0.035). Tukey's HSD test revealed that the differences among the F_{IS} of all populations were not significant (p > 0.05). Mean F_{IS} for natural populations were 0.013 (SE 0.051) and the mean F_{IS} of translocated populations was -0.053 (SE 0.029).

Table 3 Measures of genetic diversity for *A. obstetricans* and *E. calamita* conducted with 13 *A. obstetricans* and 13 *E. calamita* microsatellite markers: Number of alleles (*A*), number of fixed alleles (F_A), number of private alleles (P_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), mean pairwise differentiation of the populations (F_{ST}), population size according to Grossenbacher (1988; *N*) and number of effective breeding individuals (N_e) with 95 % confidence intervals.

	Type	population	sample size	*A*	F_A	P_A	A_R	H_o	H_e	F_{IS}	mean F_{ST}	*N*	N_e (95 % CI)
A. obstetricans	natural	LAT	15	35	1	4	2.48	0.41	0.39	0.007	0.36	1	19 (11 - 38)
		STA	32	39	3	2	2.45	0.35	0.34	-0.001	0.30	3	19 (11 - 38)
		CHA	25	31	4	0	2.04	0.33	0.28	-0.142	0.35	1	18 (10 - 38)
		HER	27	32	3	0	2.07	0.31	0.29	-0.033	0.29	3	12 (6 - 28)
		EHR	27	40	2	0	2.40	0.38	0.37	-0.041	0.24	2	17 (9 - 35)
		SSS	25	33	3	2	2.14	0.36	0.37	0.025	0.25	2	19 (11 - 38)
		RAH	26	31	2	2	2.08	0.38	0.37	0.066	0.40	3	18 (10 - 40)
	Mean		**25.29**	**34.43**	**2.57**	**1.43**	**2.24**	**0.36**	**0.35**	**-0.017**	**0.31**	**2.14**	**17.43**
	SE		**1.94**	**1.41**	**0.37**	**0.57**	**0.07**	**0.01**	**0.02**	**0.025**	**0.02**	**0.34**	**0.95**
	translocated	KAP	27	33	3	1	2.09	0.30	0.28	-0.031	0.30	3	16 (8 - 34)
		SAG	20	31	2	0	2.48	0.37	0.37	0.028	0.22	3	17 (9 - 36)
		ERS	26	39	3	2	2.57	0.46	0.42	-0.097	0.27	2	15 (8 - 34)
		GEI	20	30	4	3	2.11	0.32	0.32	0.045	0.35	2	17 (10 - 35)
		CHR	24	38	3	1	2.52	0.38	0.38	0.054	0.23	1	10 (5 - 25)
		SON	20	43	1	1	2.84	0.49	0.46	-0.040	0.21	2	17 (9 - 38)
		OTT	11	25	5	0	1.75	0.29	0.29	-0.012	0.34	1	16 (9 - 34)
		WIE	14	30	3	0	2.15	0.41	0.37	-0.062	0.37	1	15 (8 - 34)
		HUG	22	25	4	0	1.74	0.32	0.29	-0.146	0.46	2	18 (10 - 36)
	Mean		**20.44**	**32.67**	**3.11**	**0.89**	**2.25**	**0.37**	**0.35**	**-0.029**	**0.31**	**1.89**	**15.67**
	SE		**1.75**	**2.07**	**0.39**	**0.35**	**0.13**	**0.02**	**0.02**	**0.022**	**0.03**	**0.26**	**0.78**

	Type	population	sample size	*A*	F_A	P_A	A_R	H_o	H_e	F_{IS}	mean F_{ST}	*N*	N_e (95 % CI)
E. calamita	natural	WIE	12	35	2	1	2.14	0.47	0.43	-0.064	0.27	3	21 (12 - 47)
		RUE	22	39	0	0	2.29	0.50	0.47	-0.030	0.13	2	36 (20 - 59)
		STE1	3	32	1	0	2.46	0.44	0.47	0.269	0.09	3	6 (2 - inf)
		MIT	20	39	1	0	2.30	0.50	0.48	-0.011	0.12	4	32 (18 - 65)
		LEN	4	29	4	0	2.11	0.33	0.36	0.239	0.15	1	15 (3 - inf)
		WILL	24	37	1	0	2.02	0.37	0.37	0.017	0.24	2	26 (16 - 49)
		STE2	19	32	1	0	2.04	0.43	0.41	-0.033	0.18	3	28 (16 - 54)
		HEI	17	29	2	1	1.73	0.38	0.31	-0.202	0.42	2	24 (10-inf)
		BUE	4	33	1	0	2.36	0.52	0.47	0.030	0.15	2	6 (2 - inf)
		ROH	13	38	0	0	2.26	0.38	0.45	0.201	0.14	3	29 (19 -50)
		RAF	4	31	2	0	2.23	0.60	0.43	-0.274	0.22	3	7 (3 - 33)
	Mean		**12.91**	**34.00**	**1.36**	**0.18**	**2.18**	**0.45**	**0.42**	**0.013**	**0.19**	**2.55**	**20.91**
	SE		**2.43**	**1.14**	**0.34**	**0.12**	**0.06**	**0.02**	**0.02**	**0.051**	**0.03**	**0.25**	**3.27**
	translocated	ZIE	9	34	1	1	2.15	0.45	0.53	-0.131	0.20	3	16 (8 -44)
		BUC	12	38	1	1	2.13	0.43	0.42	0.048	0.17	3	15 (7 -44)
		RIN	19	39	1	0	2.18	0.45	0.51	-0.121	0.13	3	28 (16 -51)
		MUE	26	41	1	0	2.27	0.48	0.52	-0.077	0.11	4	33 (20 -59)
		EBN	6	31	3	0	2.14	0.42	0.50	-0.111	0.24	1	12 (5 -311)
		BOE	5	31	2	0	2.14	0.40	0.46	-0.034	0.15	1	10 (2 - inf)
		GEM	19	43	0	2	2.30	0.48	0.47	0.052	0.12	3	34 (17 -76)
	Mean		**13.71**	**36.71**	**1.29**	**0.57**	**2.19**	**0.44**	**0.49**	**-0.053**	**0.16**	**2.57**	**21.14**
	SE		**2.96**	**1.81**	**0.36**	**0.30**	**0.03**	**0.01**	**0.01**	**0.029**	**0.02**	**0.43**	**3.86**

Five *A. obstetricans* populations showed evidence of recent population reductions based on the IAM model (two natural populations and three translocated populations, Table 4) and two translocated populations experienced recent reductions according the mode-shift test. However, I found no consistent significance across all models. The highest consistency was detected for a translocated *A. obstetricans* population (SAG) where both tests indicated a recent bottleneck.

Two *E. calamita* populations showed a recent population reduction with the IAM test (one natural and one translocated population) and two natural populations were recently reduced according to the mode-shift model. One natural population (MIT16) had the strongest evidence for bottlenecks with two out of four models detecting a recent bottleneck.

Table 4 Evidence of recent bottleneck according to four models calculated for populations with sample size ≥ 20. Numbers indicate probability values, bold numbers indicate a significantly bottleneck sign ($p < 0.05$), - normal L-shaped and + shifted mode for the Mode-shift test.

Type	*A. obstericans*					*E. calamita*				
	Population	IAM	TPM	SMM	Mode-shift	Population	IAM	TPM	SMM	Mode-shift
natural	STA	0.060	0.470	0.472	-	RUER17	0.054	0.243	0.248	+
	CHA	0.096	0.633	0.614	-					
	HER	0.370	0.101	0.101	-	MIT16	**0.023**	0.136	0.151	+
	EHR	0.131	0.376	0.368	-					
	SSS	**0.009**	0.088	0.093	-	WILL14	0.069	0.283	0.127	-
	RAH	**0.003**	0.330	0.346	-					
translocated	KAP	0.364	0.098	0.105	-					
	SAG	**0.008**	0.240	0.243	+					
	ERS	**0.002**	0.309	0.441	-					
	GEI	0.094	0.630	0.611	-	MUELL04	**0.005**	0.380	0.388	-
	CHR	0.077	0.467	0.470	-					
	SON	0.237	0.582	0.578	+					
	HUG	**0.010**	0.105	0.104	-					

Genetic structure and population differentiation

Analysis of population structure and population history suggested that the 16 sampled ponds with *A. obstetricans* comprise seven (ΔK = 152) genetic clusters (Fig. 3A & 4A, Appendix S5). Further three high ΔK were found with two (ΔK = 1735), three (ΔK = 588), and 13 (ΔK = 61) genetic clusters. Official translocations with different founder sites (SAG, CHR, SON) in Lucerne showed a more mixed cluster assignment than unofficial translocations (KAP, GEI, OTT). The officially translocated populations tended to be more mixed than source populations across all simulations of *K*. The populations in St. Gallen (RAH, HUG, WIE) were always grouped in one single cluster.

The four most suitable *K* values for 18 *E. calamita* populations were three (ΔK = 37), six (ΔK = 24), seven (ΔK = 55) and 11 (ΔK = 15) (Fig. 3B). In contrast to the *A. obstetricans* populations, natural and translocated *E. calamita* populations were generally more admixed with the exception of HEI. This population forms a separate cluster across all suggested

K-values. Given that founder individuals derived from five populations, and the strong signal suggesting HEI as an independent cluster, I inferred that six clusters was the most reasonable (Fig. 4B).

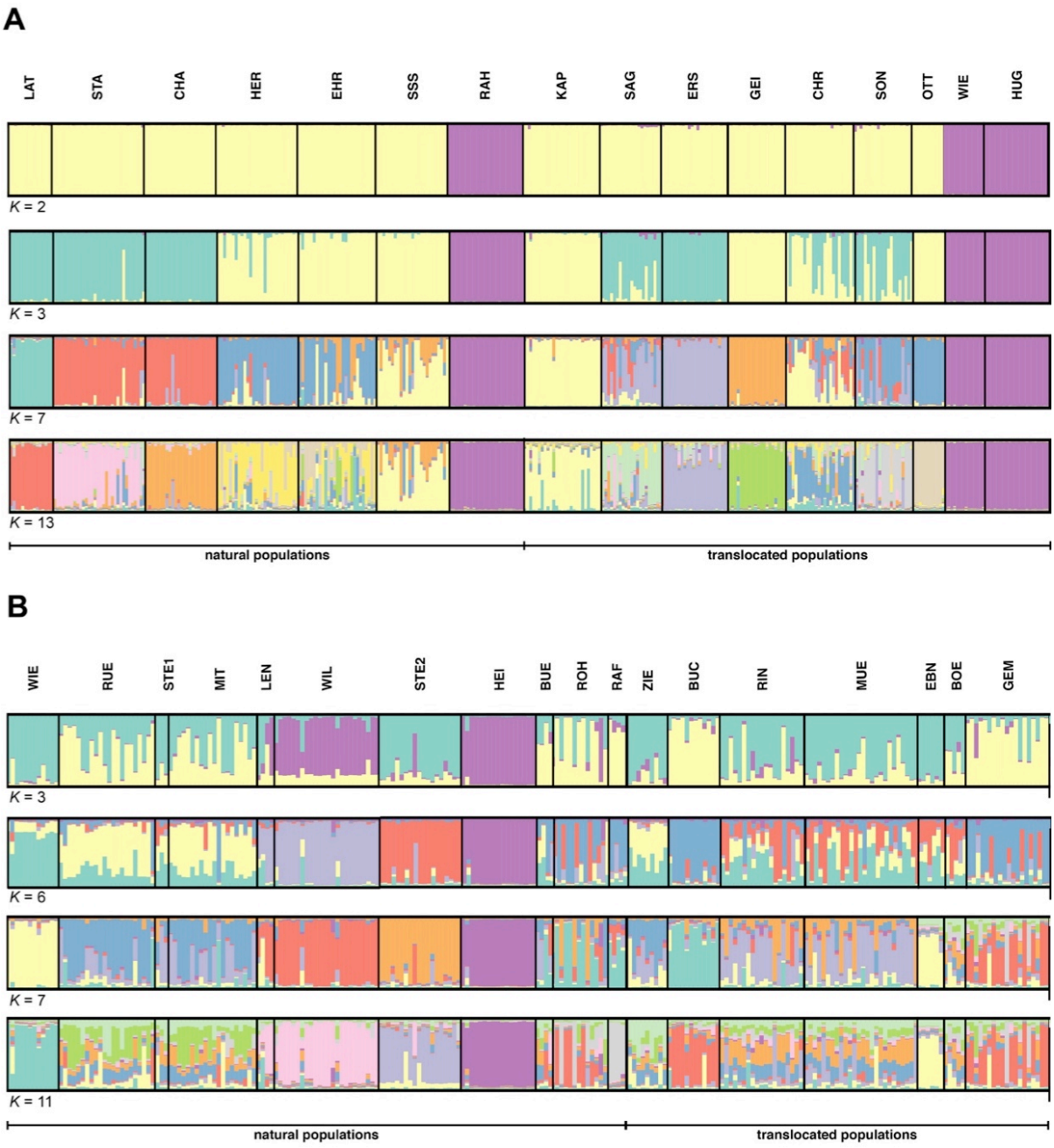


Figure 3 Bar plots obtained from the STRUCTURE analysis with **(A)** 364 *A. obstetricans* sampled in 16 populations and **(B)** 238 *E. calamita* from 18 populations. For *A. obstetricans* the most suitable *K* were two, three, seven and 13 whereas for *E. calamita* they were three, six, seven, and 11.

The analysis of molecular variance (AMOVA) showed that the neutral genetic variance is as equally distributed as among *A. obstetricans* populations (48 % vs. 52 %, Fig 5.; Table 5).

Similar findings were reported when the AMOVA was run with natural and translocated populations separately. For *E. calamita* populations, 26 % of genetic variance was found among the populations while 2.8 times more molecular variance was found within the populations (74 %). With 19 % variance among populations, the translocated populations of *E. calamita* marked the highest within population variance (81 %).

All *A. obstetricans* differentiation values were significantly different from zero ($p < 0.05$). Overall mean pairwise differentiation indices (F_{ST}) were 0.31 for *A. obstetricans*. Mean pairwise F_{ST} were not significantly different for the natural and translocated populations ($p > 0.05$). Pairwise F_{ST} values ranged for from 0.21 to 0.46. Isolation by distance (IBD) was highly significant for all *A. obstetricans* populations ($p = 0.001$, $R^2 = 0.427$) (Fig. 6). The significance did not differ when natural or translocated populations were tested separately (natural populations: $p = 0.002$, $R^2 = 0.885$ and translocated group: $p = 0.013$, $R^2 = 0.393$). Separate analyses for the two regional *A. obstetricans* groups (Lucerne and St. Gallen) resulted in a non-significant value for Lucerne ($p = 0.056$, $R^2 = 0.057$). For St. Gallen, the number of populations was not sufficient to calculate IBD.

Not all pairwise *E. calamita* F_{ST}s were significantly different from zero (Appendix S6). As a consequence of large amount of non-significant pairwise F_{ST} values, I removed STE1 from all genetic differentiation analyses. The remaining populations had a mean pairwise F_{ST} of 0.15. Pairwise F_{ST} values ranged from 0.02 to 0.39. There were no significant mean pairwise F_{ST} values between natural and translocated populations ($p > 0.05$). No statistically significant IBD values for the *E. calamita* populations were identified; neither when including all *E. calamita* populations ($p = 0.078$, $R^2 = 0.006$, Fig. 6) nor when analysing natural and translocated populations separately (natural: $p = 0.53$, $R^2 < 0.001$ and translocated: $p = 0.24$, $R^2 = 0.073$).

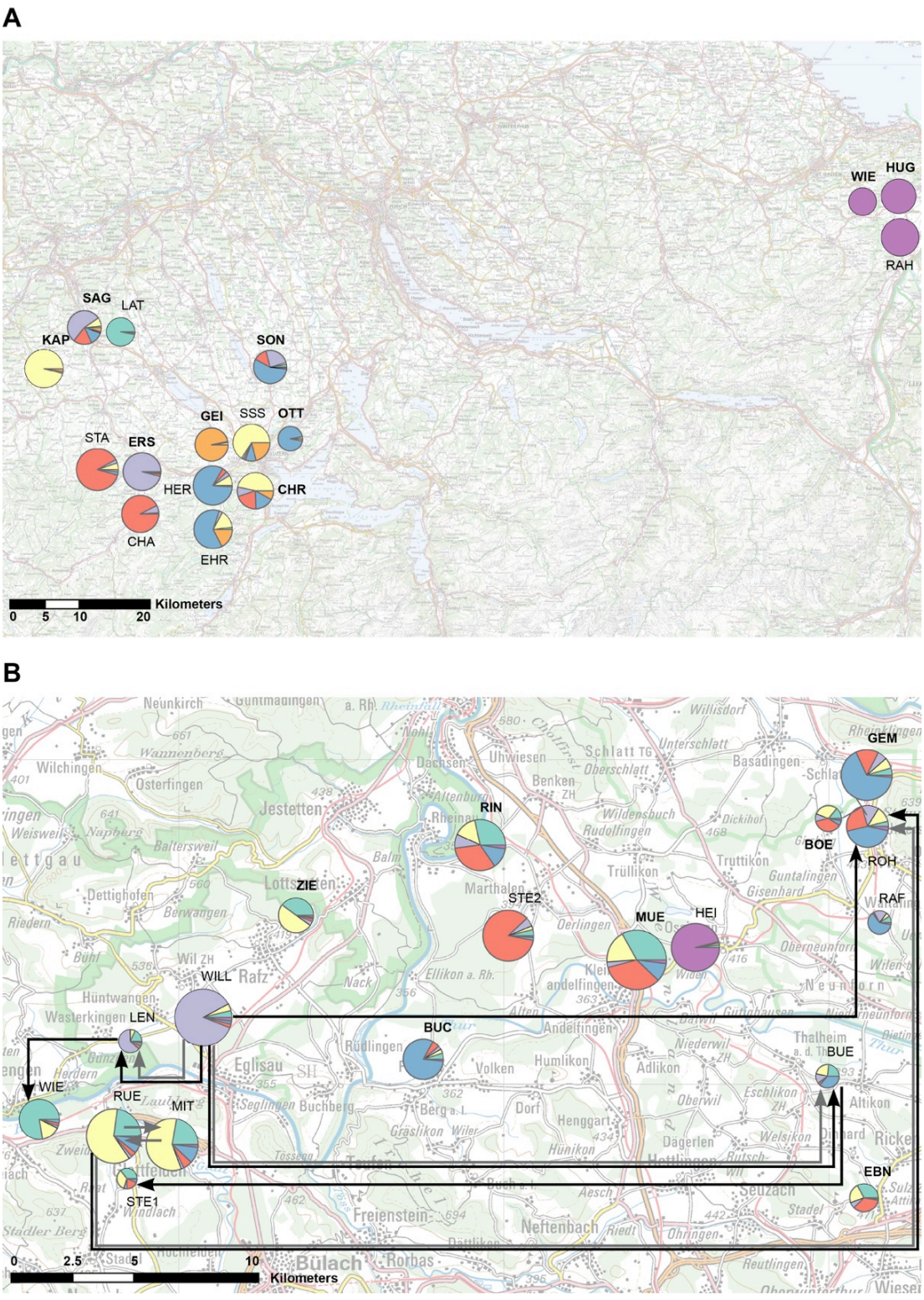


Figure 4 Population structure for **(A)** *A. obstetricans* and **(B)** *E. calamita*. For both species the most likely *K* due to statistical analysis and founder history was chosen for the populations structure (*K A. obstetricans* = 7 and *K E. calamita* = 6). Pie size represents sample size and bold labels indicate translocated populations. Additionally, gene flow detected with first-generation migrant analysis and assignment-tests between natural *E. calamita* populations is shown. The arrows indicate the directions of first-generation migrant toads (black) and assignment-tests (grey).

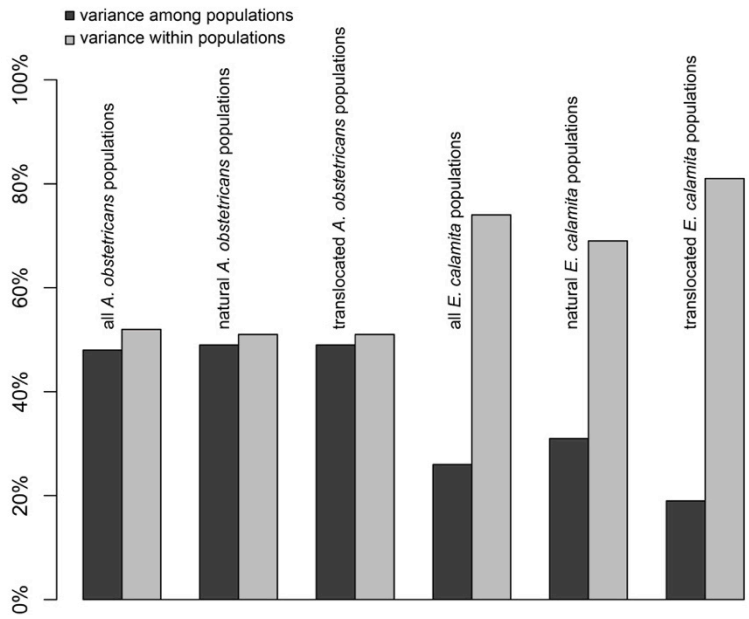


Figure 5 Genetic variance distributed among (dark grey) and within the populations (light grey) using AMOVA.

Table 5 AMOVA of the study populations.

Species	Type	Source of variation	df	Sums of squares	estimated variance	% of variance
A. obstetricians	all	Among Pops	15	1509.45	4.25	48%
		Within Pops	347	1613.59	4.65	52%
		Total	362	3123.04	8.90	100%
	natural	Among Pops	6	691.74	4.38	49%
		Within Pops	171	781.57	4.57	51%
		Total	177	1473.31	8.95	100%
	translocated	Among Pops	8	776.49	4.53	49%
		Within Pops	176	832.02	4.73	51%
		Total	184	1608.51	9.25	100%
E. calamita	all	Among Pops	17	536.26	1.99	26%
		Within Pops	220	1252.68	5.69	74%
		Total	237	1788.94	7.69	100%
	natural	Among Pops	10	368.47	2.51	31%
		Within Pops	131	734.08	5.60	69%
		Total	141	1102.54	8.11	100%
	translocated	Among Pops	6	140.47	1.34	19%
		Within Pops	89	518.60	5.83	81%
		Total	95	659.07	7.17	100%

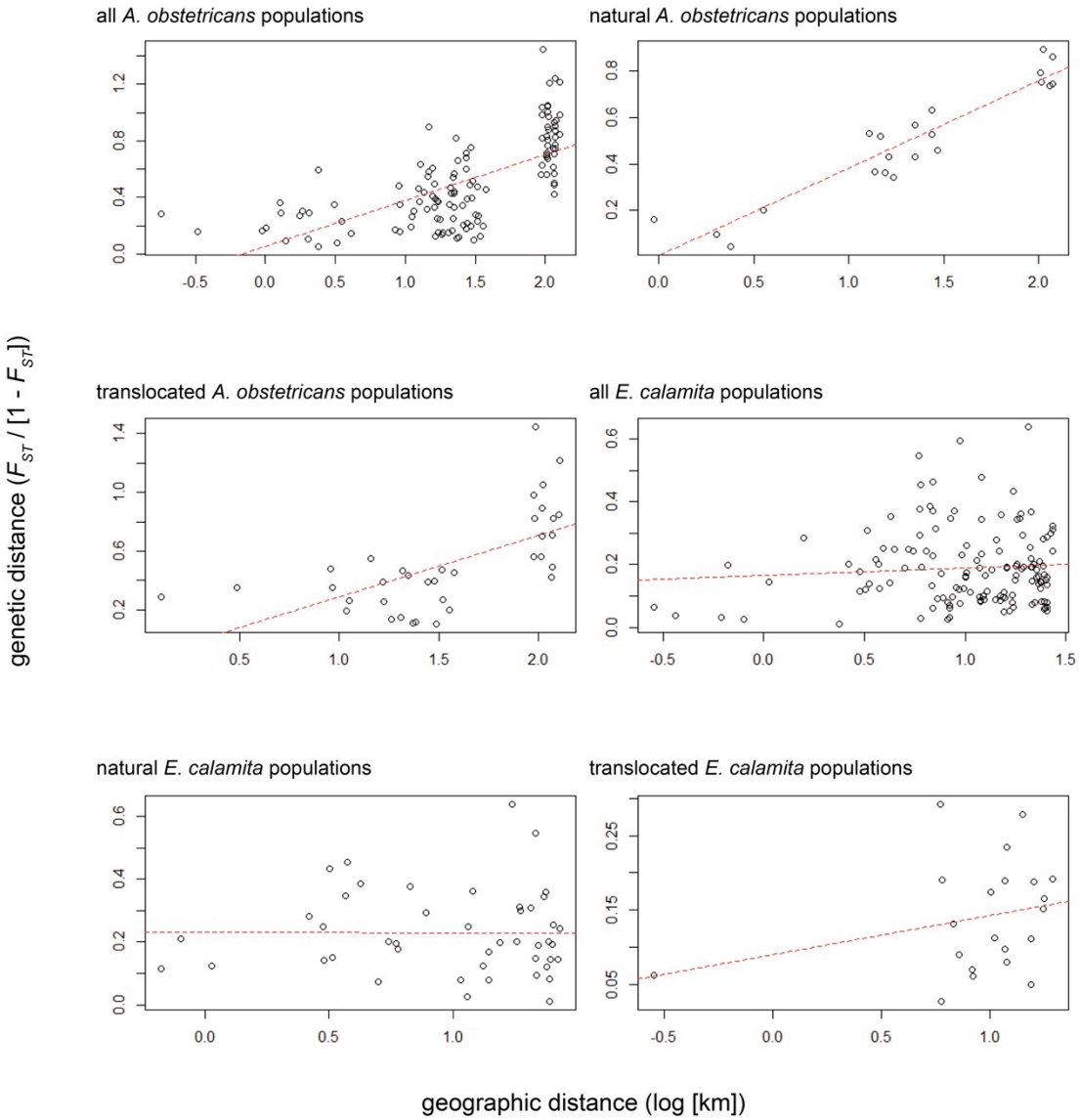


Figure 6 Genetic differentiation (F_{ST} / [1-F_{ST}]) plotted against geographic distance (log [km]) of both species.

Release characteristics

The number of founder sites and number of individuals released at a translocation site correlated significantly with diversity indicators in *A. obstetricans* (Appendix S7). I identified significant and mostly strong Pearson correlations for A_R (sites: r = 0.79 and individuals released: r = 0.86, p < 0.05), for H_O (r = 0.62 and 0.73, p < 0.05) and H_E (r = 0.56 and 0.74, p

< 0.05). For *E. calamita* I did not detect significant coefficients higher than 0.6 ($p < 0.05$) between diversity indicators and founder sites or the amount of founder individuals.

Recent gene flow

Gene flow analyses were conducted with all natural *E. calamita* populations. I detected six first-generation migrants out of 142 samples (Fig. 4B). Mean distance between the populations was 13.79 km (SE 3.93 km) with a minimum of 2.36 km and a maximum of 25.38 km. The conventional assignment-test assigned five individuals to another natural population than the population where I sampled them ($p \geq 0.8$). The mean geographic movement distance was 9.72 km (SE 5.24 km) with the lowest straight-line distance of 0.80 km and an uppermost of 25.38 km. If translocated populations were added to the gene flow analysis, I found similar gene flow distance findings.

Connectivity in the network

The network analysis for *A. obstetricans* in St. Gallen revealed that integral index of connectivity was significantly lower (Wilcoxon-test, $p > 0.05$) compared to the probability of connectivity and thus I decided to carry out all analysis only using probability of connectivity. Node importance of *A. obstetricans* populations is presented in Table 6. In St. Gallen, the natural *A. obstetricans* population was the most important, regardless of the connectivity measure (link). This was supported by both the structural (Euclidian Distance; *ED*) and the functional measures (F_{ST}). Structurally characterized networks (*N*, *ED*) showed that the node importance of the natural population was two to three times higher than the node importance for translocated populations (74 versus 27 and 39). In contrast, the node importance of the fully functionally characterized network (A_R, F_{ST}) of the natural population was 1.2 times higher than the importance of the translocated populations.

The node importance of natural structurally and functionally characterized networks of *E. calamita* populations were within the same range (mean: 10 and 11, Table 6). For translocated populations, however, the mean node importance of structurally characterized

networks ranged around eight and was underestimated compared to the functional network, where node importance amounted to a mean of 12.

Table 6 Node importance (*dPC*) of the *A. obstetricans* populations in St. Gallen and the *E. calamita* populations for two node types (N, A_R) and Euclidian distance (*ED*) and genetic differentiation (F_{ST}) as links.

	Type	Population	Population size		A_R	
			ED	F_{ST}	ED	F_{ST}
A. obstetricans	natural	RAH	74.18	71.69	57.78	51.04
	translocated	WIE	26.78	17.76	56.66	47.96
		HUG	38.96	39.90	32.61	34.30

	Type	Population	N		A_R	
			ED	F_{ST}	ED	F_{ST}
E. calamita	natural	WIE	11.33	9.61	9.54	8.09
		RUE	11.47	10.12	14.36	13.52
		MIT	21.04	19.76	14.38	13.57
		LEN	3.84	6.65	10.23	13.74
		WILL	6.70	8.64	8.69	10.49
		STE2	15.52	12.47	12.11	9.97
		HEI	7.36	4.19	6.86	4.23
		BUE	4.37	10.14	7.44	13.95
		ROH	13.60	14.50	13.58	13.04
		RAF	10.27	11.10	9.61	9.67
	Mean		**10.55**	**10.72**	**10.68**	**11.03**
	SE		**1.60**	**1.29**	**0.84**	**0.96**
	translocated	ZIE	7.62	12.36	6.24	10.43
		BUC	7.30	13.53	5.57	11.30
		RIN	6.97	14.63	5.34	12.48
		MUE	13.51	21.01	8.23	14.32
		EBN	1.17	3.49	4.57	8.94
		BOE	4.83	4.98	12.61	12.29
		GEM	13.43	15.78	13.87	14.49
	Mean		**7.83**	**12.25**	**8.06**	**12.03**
	SE		**1.68**	**2.32**	**1.41**	**0.76**
	Mean all		**9.43**	**11.35**	**9.60**	**11.44**
	SE all		**1.22**	**1.21**	**0.82**	**0.66**

Effect of landscape elements on diversity and gene flow

E. calamita site analysis showed that the genetic diversity had a negative relationship with open land (r = -0.31, p < 0.05; Fig. 8A), whereas higher inbreeding was seen in proximity to large roads (r = 0.47, p < 0.05) and proximity to standing water resulted

in lower inbreeding (r = -0.48, p < 0.05).

The corridor analysis revealed that large roads and hedgerows are significantly and negatively correlated with F_{ST} (r = -0.26 and -0.23, p < 0.05; Fig. 8B). In addition, small roads between natural populations were seen in proximity to lower gene flow (r = -0.35, p < 0.05) whereas gravel pits were significantly and positively correlated with gene flow (r = 0.59, p < 0.05).

Overall no site and corridor correlation coefficient was > |0.6| for the studied genetic and biological indicators.

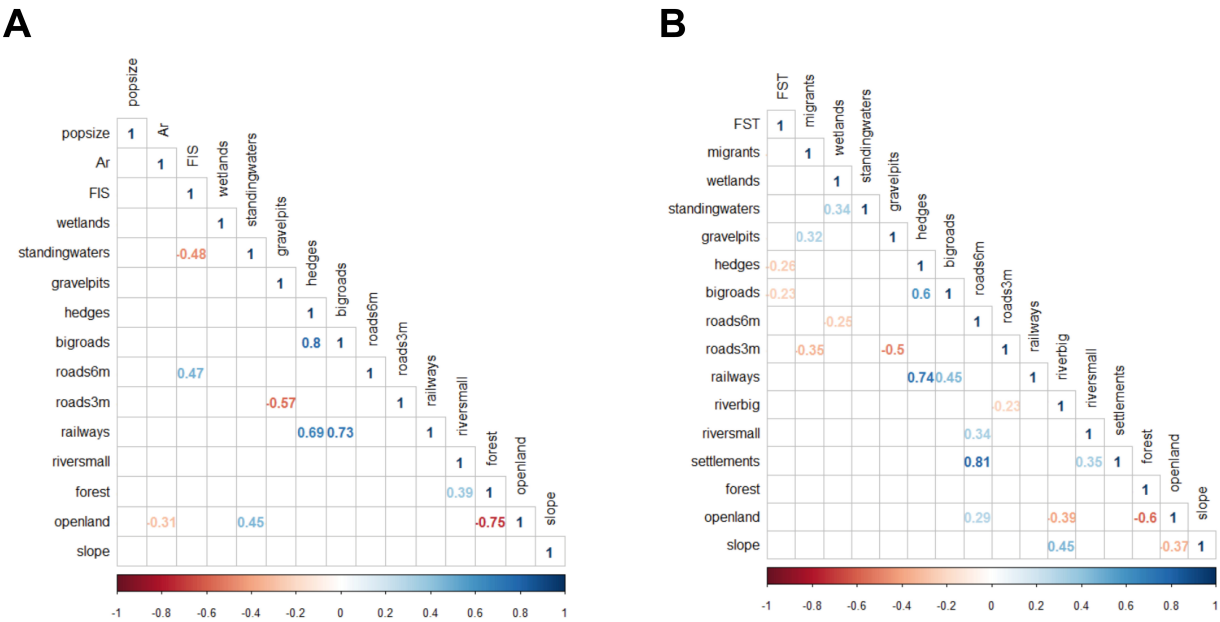


Figure 7 *E. calamita* correlation matrixes for sites (A) and corridor analyses (B). Shown are only significant values (p < 0.05).

Discussion

I compared the genetic structure of translocated and natural *A*. *obstetricans* and *E. calamita* populations in Switzerland. I found that translocated populations were no less genetically diverse than natural populations (Table 3) and that genetic admixture is higher in translocations that have large numbers of founder individuals from several source populations. As for *E*. *calamita* in Zurich, I also found evidence for natural recent gene flow over large distances (Fig. 4) and no apparent gene flow barriers (Fig. 8).

Genetic consequences of translocations

Loss of genetic variation is a major concern in translocation biology because genetic variability is known to be significantly correlated with population viability (Storfer 1999; Witzenberger and Hochkirch 2008; Schröder et al. 2012; Smith et al. 2012). I did not note overall genetic erosion and thus translocations have not led to negative effects on the genetic structure for natural and translocated populations.

Despite the fact that I did not note overall genetic differences between translocated and natural population (Table 3) the founder group size had an effect of genetic diversity indicators. I found a strong positive correlation of founder number and genetic diversity for *A. obstetricans*. I did not detect the same *E. calamita*. However, this is likely due to the fact that the majority of translocated populations in *E. calamita* derived from multiple donor populations and received at least 700 founding individuals compared to a maximum of 385 founding individuals in *A. obstetricans*. Other studies with *E. calamita* and *Hyla arborea*, which also did not find a reduction in genetic diversity in translocated populations, used similar founder numbers as in the *E. calamita* project in Zurich (Rowe et al. 1998; Taddey 2013). Since translocated *A. obstetricans* populations showed an overall reduction in genetic diversity with decreasing founder numbers, I suspected a higher presence of genetic bottlenecks in these populations. However, I did not discover signatures of a recent bottleneck for either species (Table 4). In some populations, a bottleneck was likely under

IAM but not SMM or the mode shift test. The SMM model is generally more appropriate when testing microsatellite loci (Luikart et al. 1998; Piry 1999). Despite a sufficient number of markers for an adequate model resolution, my bottleneck results should be interpreted with caution. I only tested populations with a sample size of ≥ 20. Normally, at least 30 individuals are recommended to ensure enough statistical power ($p > 0.80$) (Piry 1999). Nevertheless, the results of the bottleneck analysis match with the findings on genetic diversity and can be regarded as further evidence that translocated populations did not suffer from a reduction in genetic variation. This was also supported by the AMOVA, which noted similar genetic variation values between natural and translocated population of both species (Fig. 5 & Table 5).

Apart from the positive consequences of a larger founder group size on diversity, I also tested whether site admixture resulted in higher genetic diversity. And indeed I detected that the more sites were mixed, the higher the diversity was. However, there is an risk in some instances of using donors from different and distant locations (Huff et al. 2010). Outbreeding depression has often been discussed in the literature (Allentoft and O'Brien 2010; Weeks et al. 2011; Albert et al. 2015; Zenboudji et al. 2016), and occurs when the offspring of translocated individuals from diverse source populations have lower fitness than their parents (Edmands and Timmerman 2003; Tallmon et al. 2004). If the donor populations were only recently fragmented and the populations inhabit similar environments, then there may not be sufficient divergence to result in outbreeding depression (Frankham et al. 2011). This appears to be the case for both of my species; seeing that only since the mid-1980s approximately 50 % to 60 % of populations have been extirpated (Schmidt and Zumbach 2005) thus population fragmentation is mostly likely to have increased recently and translocations were conducted from population within a small scale region.

STRUCTURE analysis further detected that the combination of a large founder group size from different donor populations were more admixed (Fig. 3). This was also the reason why I found higher admixture levels for translocated *E. calamita* populations than for translocated *A. obstetricans* populations. Such admixture is regarded as a powerful process to increase genetic diversity in translocated populations (Biebach and Keller 2012). In contrast to

admixed official translocations, unofficial *A. obstetricans* translocations represented more unique clusters and higher genetic differentiation. If release numbers are small, and this is likely due to the unofficial nature, rapid genetic differentiation poses a risk for genetic diversity (Lacy 1987; Frankham 1996; Biebach and Keller 2009; Michaelides et al. 2015). Despite the fact that translocated and natural population did not differ according to genetic differentiation indices (F_{ST}, Table 3; IBD, Fig. 6), translocated population with decreased number of individuals released showed the highest differentiation and lowest diversity indicators.

Overall, my results suggest, that translocated populations did not differ under various genetic aspects (diversity, differentiation, variation, etc.) from natural populations. I regard these findings as positive genetic translocation outcome for both species. If I break certain aspects down on a population level, I did find differences. So I detected a tendency that the more founders from different donor populations were released, the lower the risks of genetic erosion was.

Demographic consequences of translocations

Apart part from genetic processes that can impose a risk to the success of translocations, demographic processes can have negative consequences for the translocated populations as well (Smith et al. 2012).

Out of the 20 translocated populations in my study, four became extinct again when translocations were stopped. All extinct populations were noted in Lucerne although there were not executed differently than other established translocated populations in the same area. Unfortunately, since these extinct populations do no longer exist, they cannot be screened for negative translocation consequences such as low genetic diversity anymore. As I was unable to find substantial genetic differences between successfully translocated and natural populations in this area, it seems unlikely that the failure was due to genetic reasons. Although my studies could not answer the question as to why these translocated populations disappeared, one commonly suggested reason for failure in literature is that poor habitat

quality or an absence of specific habitat characteristics having prevented the successful establishment of the species (Germano et al. 2015). Further investigations are needed to test this hypothesis. I recommend conducting habitat surveys comparable to other *A. obstetricans* studies (Kroepfli 2011; Vuichard 2016). They should be done to determine whether habitat characteristics such as the presence of heavy predation could have resulted in the extinctions. Another aspect that also remains unclear is to which extent diseases contributed to the population extinction. Studies showed that *Batrachochytrium dendrobatidis*, the pathogen causing amphibian chytridiomycosis, has led to severe host population declines (Laurance et al. 1996; Albert et al. 2015; Ackleh et al. 2016). The chytrid fungus was also detected in various *A. obstetricans* populations in Lucerne (Tobler et al. 2012) and could have well had an effect on translocation success, seeing that acclimation may result in the inability of released animals to deal with such a pest (Germano and Bishop 2009).

In addition to the loss of translocated populations, the harvesting of large amounts of founder individuals can cause the loss of donor populations. Thus the potential benefits of establishing translocated populations needs to be balanced against the negative impact on donor populations, especially if these populations are small (Armstrong and Seddon 2008; Sherley et al. 2010). It has been claimed that populations with an estimated N_e of less than 100 individuals are small and can negatively affect the fitness and viability of populations Lande 1998; Albert et al. 2015). Population estimates from all of my ponds were lower than this level and further harvesting is likely to lower population viability. If translocations were to continue, donor populations could be threatened. So far, one out 11 *A. obstetricans* donor populations in Lucerne has likely to become extinct due to overharvesting. To avoid future donor population loss, modelling techniques could be used to predict potential consequences upon donor populations. Although the question might be simple at first glance, predicting population dynamics, especially in response to perturbations (i.e. harvesting) can be very complicated (Dimond and Armstrong 2007; Earnhardt et al. 2014).

Overall, the reasons why various populations became extinct cannot be answered with this study. However, there is a clear need for investigation with habitat surveys, disease detection or population modelling to gain additional insights into the causes of these extirpations.

Population connectivity

In contrast to the conservation *A. obstetricans* projects, in Zurich translocation were only considered in case *E. calamita* was not suspected to colonise newly created habitats naturally (Lippuner 2013a). However, in various occasion it has been shown that amphibians can move over much longer distances than has long been anticipated and even populations commonly thought to be isolated are often connected to the a whole network (Smith and Green 2005). Indeed my first-generation migrants and assignment-tests results showed strong evidence of gene flow distances up to 25.4 km between natural populations (Fig. 4). In literature movement over five to 12 km for these species have been reported (Miaud et al. 2000; Jehle and Sinsch 2007; Sinsch et al. 2012; Lippuner 2013a) but such large gene flow distances for this species have not been mentioned to my knowledge. Other genetic studies with different amphibians, such as *Bufo boreas* and *Rana temporaria*, have already demonstrated that genetic analyses can suggest much greater dispersal distances than observed from tracking studies (Moore et al. 2011; Safner et al. 2011).

Network analysis showed that structural (*ED*) and functional connectivity measures (F_{ST}) did not differ between populations (Table 6). Thus the role of geographical distance in driving the population network is overestimated: connectivity as measured by gene flow appears to be higher than a structural network suggests, this is also true for *A. obstetricans*. This underlines the possibility of large *E. calamita* dispersal distances. Nevertheless, previous studies noted similar node and link pattern but overall higher functional and structural F_{ST} and *ED* values (Frei 2014). As the mean geographic and genetic distances between populations were higher in my work than in this previous study, it is not surprising that lower values were observed. According the gene flow and network it appears that intensely managed landscapes do not influence movement of *E. calamita* in Zurich. Due to these findings I did not expect that landscape elements had strong effect on genetic indicators. Indeed, I did not detect strong correlations between either genetic diversity, population size, genetic differentiation or recent gene flow and any of the landscape elements, albeit that the study area is located in highly managed landscapes with motorways, railway tracks and large proportions of settled land

(Fig. 8). These are all landscape elements which are normally regarded as barriers for amphibian movement (Carr and Fahrig 2001; Arntzen and Espregueira Themudo 2008; Emel and Storfer 2012). However, various amphibian studies showed that such barriers reduce but do not necessarily stop gene flow (Van Buskirk 2012; Frei 2014; Sinsch 2014; Furman et al. 2015; Le Lay et al. 2015). If gene flow between populations had been absent or very weak, I would have expected a differentiation and unique population structure. This was not the case according to STRUCTURE and IBD (Fig. 4 & 6).

Interestingly, one naturally colonised population after 2007 in the centre of the population network contrasted my conductivity findings and marked an exception. The population noted the highest mean F_{ST} as well as unique STRUCTURE cluster across all ΔK (Fig. 3 & 4). Two explanations seem possible for this apparent differentiation: either migrants from outside the study area colonised the newly created pond or a recent unofficial translocation took place with founder individuals being sourced from outside the study area. I suspect an unofficial translocation with various founder individuals from a distinct area seeing that the population reached already a large census size. Given the generation times of about three years (Rowe et al. 2000), I doubt that a few founder adults from a distant area could have already created a population in which at least 17 females reproduced in 2015.

Due to the potential of *E. calamita* to cover large geographical distances up to 25.4 km as well as the lack of apparent landscape barriers to gene flow, I conclude that all ponds created to support the toad breeding-pond network would have been colonised naturally over time without translocation efforts, as it was already observed for two newly built ponds. Thus this represents a promising management measure to further strengthen existing *E. calamita* populations without anthropogenic intervention.

Implications for future conservation management

In my study, the genetic structure of the amphibian network of natural and translocated populations of *A. obstetricans* and *E. calamita* was thoroughly investigated. I deduce the following conservation implications from my findings:

Future translocations should use large numbers of founder individuals from different populations.

I could show that a large number of founder individuals resulted in high genetic diversity, hence increasing the probability of some released individuals and next generations thriving under novel conditions. As for *E. calamita* females which produce 3000 and 4000 egg per string (Sinsch 1998), large founder group size do not pose a problem. However, for other species such as *A. obstetricans* for which females produce only 50 eggs a year (Kordges 2003) the removal of several hundred individuals pose a threat to present populations. Given the problems with harvesting large number of founders, and the beneficial effects of diverse donor populations, population mixture is an important technique to increase diversity for species such as *A. obstetricans.* Therefore I recommend for future translocations that for *E. calamita* similar procedures should be applied than in Zurich and that for *A. obstetricans* donor populations should be more mixed. Of course all founder individuals should be screened for diseases first before they were released.

Further environmental monitoring will be necessary to study the *A. obstetricans* populations in Lucerne.

In Lucerne certain populations failed to establish despite having the same donor background and translocation protocol as the successful populations. Therefore, I strongly recommend the assessment of habitat quality to try to understand potential environmental causes for their failure before further conservation methods are performed.

Actions should aim to increase population sizes and connectivity through provision of additional stepping stone habitats for both species.

Increasing population size has often been stated to be the most effective conservation strategy as large populations are less vulnerable towards genetic, environmental and demographical stochasticity and are more likely to immigrate and strengthen the population network (Tallmon et al. 2004; Weeks et al. 2011; Tobler et al. 2013). Considering the small number of N_e for both species there is a crucial need for population size increase as next

future conservation steps. For that matter it is pivotal that environmental, demographical and habitat knowledge about the species is present to enlarge existing and build new habitats. I regard the *E. calamita* conservation project in Zurich, where elaborate pre-translocation studies of such components were conducted (Lippuner 2013a; Lippuner 2013b), as a good model for future projects. If population size is increased, as a next step I recommend further conservation actions that aim to enlarge the population network through the creation of sustainable habitats within migration distance; so-called stepping stone ponds. Such stepping stone habitats facilitate species dispersal and range expansion through a sequence of adjacent stepping-stone movement steps (Saura et al. 2014) and can provide a new habitat for migrants.

Outlook

My results show that translocations, if executed properly, are suitable to establish populations that genetically resemble natural populations. However, certain studied translocated populations, which derived from single donor population and with small founder group size, were less diverse and structurally admixed than others.

My gene flow, network and landscape analyses results showed that *E. calamita* would have been likely to colonise new sites naturally over time. These findings again show that amphibians can move over much longer distances than have been expected.

These are two findings emphasise the importance of scientifically validated evidence and show how an evidence-base can reduces complex decision making process and improve future conservation projects.

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Appendix

Statement of Authorship

I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Zurich, 31.03.2016

Ramon Müller

Supporting information

S1) Study area

Table S1 Population abbreviations, canton, where the population is found (LU = Lucerne, ZH = Zurich, SG = St. Gallen), population name, coordinates (according to the Swiss national grid) and elevation of a population site (m.a.s.l).

abbreviation	canton	population name	X	Y	m.a.s.l.
KAP	LU	Kapelhugel	633640	225470	560
SAG	LU	Sagerhüsli	643800	230180	685
LAT	LU	Lätten Uffikon	644875	230850	757
FON	LU	Fontannen	646300	208175	621
STA	LU	Stägplatz	646810	208680	612
CHA	LU	Chalchloch	647750	208600	602
ERS	LU	Ersatzweiher Bahndamm Rossei	648010	210340	587
HER	LU	Hergiswald	660550	208300	795
EHR	LU	Ehrendingen (Iwert)	662340	209870	598
GEI	LU	GKW Geissenrain	662475	209750	554
SSS	LU	Schloss Schauensee	664050	208823	571
CHR	LU	Chräuel	664610	207520	625
SON	LU	Sonderschule Hohenrain	666775	225700	602
OTT	LU	Ottigenbüel	669725	215175	527
WIE	ZH	Kiesgrube Weiach	675710	268935	336
RUE	ZH	Rürifeld	678001	267643	389
STE1	ZH	Steinächer	678406	267115	404
LEN	ZH	Lengg	678509	271341	376
MIT	ZH	Mittlerboden	678612	268156	391
WILL	ZH	Kiesgrube Will	680751	272070	375
ZIE	ZH	Ziegeleigrube	683607	275216	483
BUC	ZH	Buchbrunnen	687521	270784	349
RIN	ZH	Rinauer Feld	689271	277376	382
STE2	ZH	Steinacker	690137	274590	355
MUE	ZH	Müllersbuck	694087	273845	397
HEI	ZH	Heidiboden	695622	274227	411
BUE	ZH	Büelhüsli	699957	270260	393
BOE	ZH	Bötschigrube	700974	278531	424
EBN	ZH	Ebnet	701080	266519	455
GEM	ZH	Gemeindegrube	701162	278316	433
ROH	ZH	Rohräcker	701187	277952	425
RAF	ZH	Raffolterseekiesgrube	701558	274992	431
WIE	SG	Wiedist	757841	249954	548
RAH	SG	Mächler, Rahnstrasse	758168	249995	539
HUG	SG	Hugentobler, Schwalbenweg 16	758856	250744	539

S2) Translocation history

Table S2 Release history for each translocated population with release date, type of founders (tadpoles, juvenile or adult toads), the amount released and from which site they derived. For population abbreviation see table S1.

species	translocated populations	release date	tadpoles	juveniles	adults	total	donor populations
A. obstetricans	CHR	22.06.02	-	-	5	5	HIM
	CHR	06.08.02	17	-	-	17	HIM
	CHR	11.09.02	101	-	-	101	HIM
	CHR	02.07.03	9	-	-	9	HIM
	CHR	24.07.03	20	-	-	20	CHA
	ERS	15.07.11	50	2	-	52	STA
	ERS	17.08.11	37	-	-	37	STA
	ERS	19.08.09	61	-	-	61	CHA
	KAP	< 2000	-	-	-	na	SSS
	GEI	< 2000	-	-	-	na	SSS
	OTT	2003	40	-	-	40	EHR
	OTT	2004	na	-	-	na	EHR
	SAG	27.09.01	147	-	-	147	SCH
	SAG	10.08.04	11	18	-	29	STA
	SAG	21.09.00	102	-	-	102	CHA / HIR
	SON	14.09.07	35	0	-	35	ROS/ EHR
	SON	27.06.07	14	9	-	23	ROS
	SON	27.07.14	43	45	-	88	STA
	SON	25.08.09	156	24	-	180	CHA
	SON	22.09.13	-	6	-	6	CHA
	SON	26.05.14	53	-	-	53	CHA
	HUG	21.03.03	17	-	-	17	RAH
	WIE	09.05.05	55	-	-	55	RAH
E. calamita	BOE	5.09	100	-	-	100	STE2
	BOE	5.09	100	-	-	100	RAF
	BOE	5.09	100	-	-	100	MIT
	BOE	6.09	500	-	-	500	STE2
	BOE	6.09	500	-	-	500	RAF
	BOE	6.09	500	-	-	500	MIT
	BUC	6.07	480	-	-	480	MIT
	BUC	6.07	480	-	-	480	LUF
	BUC	6.07	480	-	-	480	STE2
	BUC	5.08	800	-	-	800	MIT
	BUC	5.08	800	-	-	800	LUF
	BUC	5.08	800	-	-	800	WIE
	BUC	6.08	1200	-	-	1200	MIT
	BUC	5.09	800	-	-	800	STE2
	BUC	5.09	800	-	-	800	RAF
	BUC	5.09	800	-	-	800	MIT
	EBN	5.08	500	-	-	500	LUF
	EBN	5.08	500	-	-	500	WIE
	EBN	5.08	500	-	-	500	MIT
	EBN	6.08	800	-	-	800	MIT
	EBN	6.09	800	-	-	800	STE2
	EBN	6.09	800	-	-	800	RAF
	EBN	6.09	800	-	-	800	MIT
	EBN	6.10	500	-	-	500	STE2
	EBN	6.10	500	-	-	500	RAF
	EBN	6.10	500	-	-	500	MIT
	EBN	7.10	500	-	-	500	STE2
	EBN	7.10	500	-	-	500	MIT
	GEM	5.07	550	-	-	550	MIT
	GEM	6.07	620	-	-	620	MIT
	GEM	6.07	620	-	-	620	STE2
	GEM	6.07	620	-	-	620	LUF
	GEM	5.08	400	-	-	400	MIT
	GEM	5.08	400	-	-	400	WIE
	GEM	5.08	400	-	-	400	LUF
	MUE	6.10	1000	-	-	1000	STE2
	MUE	6.10	1000	-	-	1000	RAF
	MUE	6.10	1000	-	-	1000	MIT
	MUE	7.10	900	-	-	900	STE2
	MUE	7.10	900	-	-	900	MIT
	MUE	7.11	900	-	-	900	STE2
	MUE	7.11	900	-	-	900	MIT
	MUE	8.11	700	-	-	700	WIE
	MUE	8.11	700	-	-	700	MIT
	RIN	5.07	750	-	-	750	MIT
	RIN	6.07	620	-	-	620	LUF
	RIN	6.07	620	-	-	620	STE2
	RIN	6.07	620	-	-	620	MIT
	RIN	5.08	400	-	-	400	MIT
	RIN	5.08	400	-	-	400	LUF
	RIN	5.08	400	-	-	400	WIE
	ZIE	5.07	700	-	-	700	MIT

S3) Landscape data

Table S3 Landscape date information of the 13 landscape elements and their data source. If the element was buffer or if in the case of settlements buildings were aggregated, size and distanced is presented.

Object	Buffer size	Aggregation distance	Source
wetlands	-	-	GEOLIB.TLM_BODENBEDECKUNG_2013
standing water	-	-	GEOLIB.TLM_BODENBEDECKUNG_2013
gravel pits	-	-	GEOLIB.TLM_NUTZUNGSAREAL_2013
hedges	-	-	GEOLIB.TLM_BAUM_GEBUESCHREIHE
big roads (>6m broad)	20m	-	GEOLIB.TLM_STRASSE_2013
road (3m – 6m broad)	5m	-	GEOLIB.TLM_STRASSE_2013
small road (< 3m)	2m	-	GEOLIB.TLM_STRASSE_2013
railways	1m	-	GEOLIB.TLM_EISENBAHN_2013
river big	-	-	GEOLIB.TLM_FLIESSGEWAESSER_2013
riversmall (< 80m broad)	1m	-	GEOLIB.TLM_FLIESSGEWAESSER_2013
settlements	-	100m	GEOLIB.TLM_GEBAEUDE_FOOTPRINT_2013
forests	-	-	GEOLIB.TLM_BODENBEDECKUNG_2013
open lands	-	-	remaining land was categorized into open lands

S4) Genetic effect of landscape elements

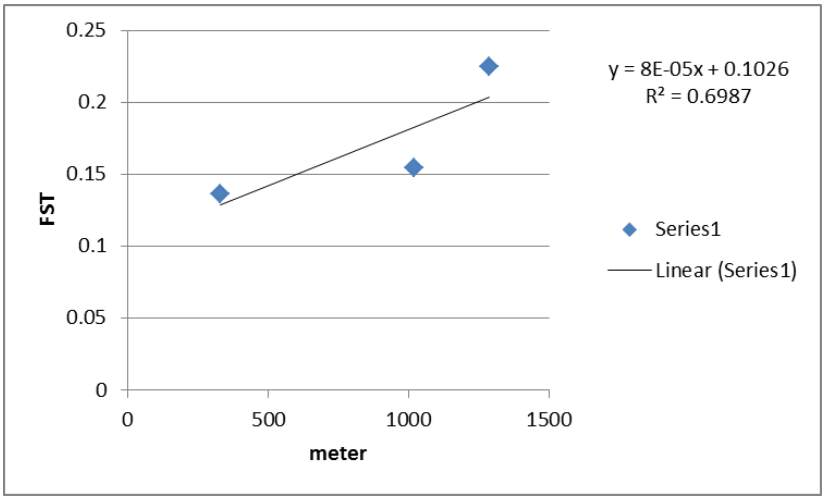

Figure S4$_1$ *A. obstetricans*-St. Gallen-Scatter plot for functional distance calculation with CONEFOR.

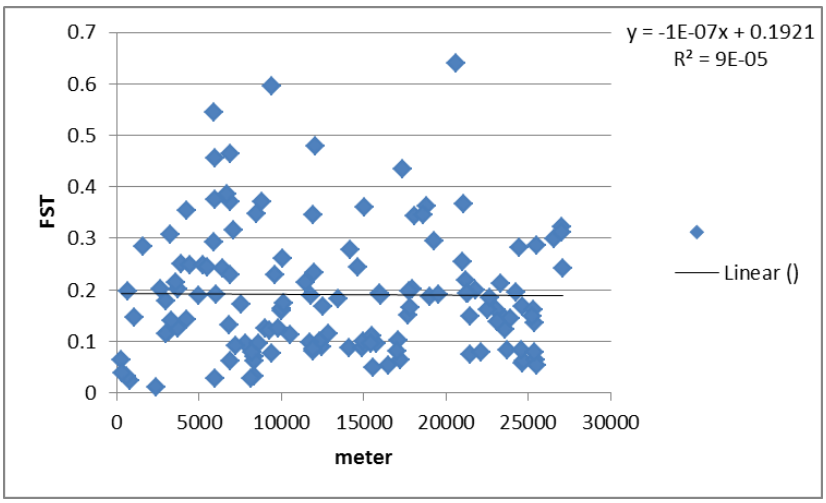

Figure S4$_2$ *E. calamita*-Zurich-Scatter plot for functional distance calculation with CONEFOR.

S5) Genetic structure

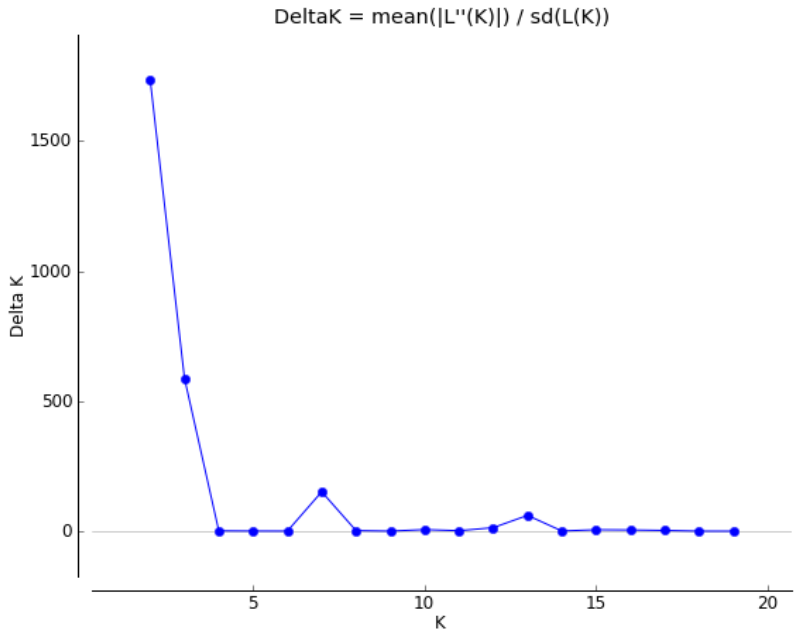

Figure S5$_1$ Estimation of clusters (*K*) by the *ΔK* method (A) by Evanno et al. (2005) for the *A. obstetricans* populations.

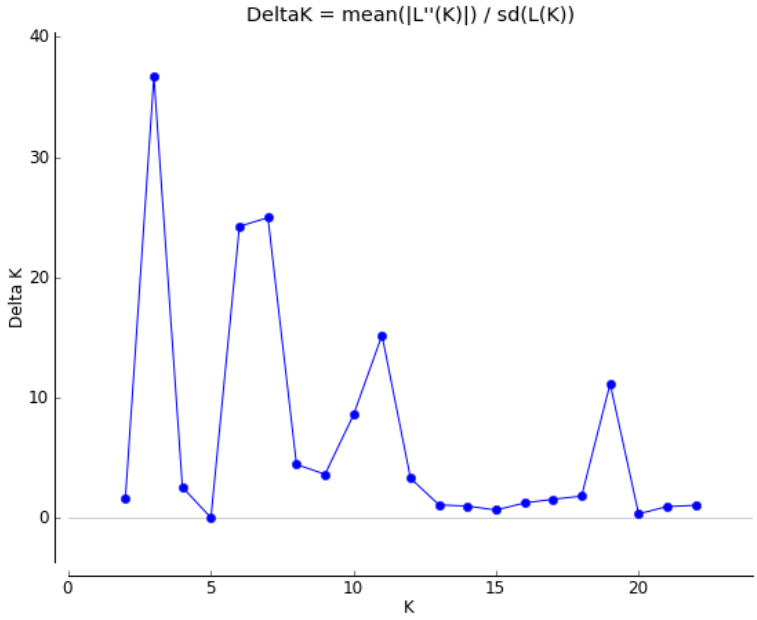

Figure S5$_2$ Estimation of clusters (*K*) by the *ΔK* method (A) by Evanno et al. (2005) for the *E. calamita* populations.

S6) Genetic differentiation and Euclidian distance

Table S6 Euclidean distances (m) between the *A. obstetricans* (upper table) resp. *E. calamita* populations (lower table) are presented above and pairwise F_{ST} values below the diagonal. Statistically significant F_{ST} from zero ($p < 0.05$) are marked in bold.

	KAP	SAG	LAT	STA	CHA	ERS	HER	EHR	GEI	SSS	CHR	SON	OTT	RAH	WIE	HUG
KAP	-	11198.65	12456.71	21339.00	21992.93	20866.57	31921.11	32665.73	32841.68	34671.99	35795.86	33135.80	37524.85	126920.05	126591.29	127741.23
SAG	**0.21**	-	1266.70	21709.68	21938.53	20281.76	27555.34	27499.59	27679.24	29439.14	30765.76	23407.71	29954.23	116071.85	115742.65	116879.26
LAT	**0.32**	**0.26**	-	22254.28	22434.98	20748.21	27462.85	27298.11	27476.72	29212.62	30557.47	22497.39	29380.74	114899.24	114569.98	115704.10
STA	**0.30**	**0.14**	**0.31**	-	943.40	2048.32	13745.25	15575.53	15701.50	17236.48	17837.76	26235.12	23817.69	118775.14	118454.32	119681.60
CHA	**0.35**	**0.20**	**0.36**	**0.14**	-	1759.32	12803.52	14645.17	14769.84	16297.33	16894.56	25580.47	22937.55	117922.35	117601.79	118830.38
ERS	**0.32**	**0.13**	**0.26**	**0.23**	**0.21**	-	12704.85	14337.71	14477.03	16109.01	16837.83	24249.84	22246.76	117078.20	116756.66	117980.16
HER	**0.21**	**0.18**	**0.42**	**0.30**	**0.39**	**0.27**	-	2380.97	2410.01	3532.72	4134.25	18480.01	11465.00	106149.64	105832.86	107077.37
EHR	**0.19**	**0.15**	**0.37**	**0.29**	**0.37**	**0.24**	**0.05**	-	180.62	2009.55	3267.32	16439.53	9092.92	103889.47	103572.04	104814.23
GEI	**0.32**	**0.28**	**0.40**	**0.38**	**0.47**	**0.35**	**0.37**	**0.22**	-	1831.78	3087.25	16519.46	9055.01	103811.42	103494.13	104736.84
SSS	**0.11**	**0.16**	**0.33**	**0.27**	**0.33**	**0.25**	**0.19**	**0.10**	**0.23**	-	1406.07	17111.02	8531.69	102739.09	102423.12	103670.46
CHR	**0.17**	**0.09**	**0.34**	**0.20**	**0.27**	**0.20**	**0.13**	**0.07**	**0.26**	**0.08**	-	18308.46	9206.64	102748.36	102433.70	103685.21
SON	**0.21**	**0.10**	**0.25**	**0.17**	**0.25**	**0.10**	**0.13**	**0.11**	**0.28**	**0.13**	**0.12**	-	10930.60	94567.05	94240.51	95425.95
OTT	**0.31**	**0.28**	**0.43**	**0.40**	**0.45**	**0.30**	**0.23**	**0.13**	**0.32**	**0.15**	**0.26**	**0.16**	-	95050.50	94731.24	95966.08
RAH	**0.50**	**0.33**	**0.43**	**0.44**	**0.47**	**0.36**	**0.49**	**0.45**	**0.43**	**0.45**	**0.42**	**0.39**	**0.51**	-	329.56	1017.03
WIE	**0.46**	**0.30**	**0.38**	**0.43**	**0.46**	**0.33**	**0.47**	**0.40**	**0.41**	**0.41**	**0.36**	**0.36**	**0.50**	**0.14**	-	1286.21
HUG	**0.55**	**0.41**	**0.48**	**0.49**	**0.55**	**0.45**	**0.55**	**0.50**	**0.51**	**0.51**	**0.47**	**0.45**	**0.59**	**0.15**	**0.22**	-

	BOET10	BUC03	BUEL11	EBN12	GEM09	HEI05	LEN15	MIT16	MUELL04	RAF06	RIN01	ROH08	RUER17	STE02	STE18	WIE19	WILL14	ZIE13
BOET10	-	15524.15	8333.29	12012.47	285.60	6867.92	23587.55	24651.57	8330.03	3586.86	11759.86	616.94	25422.57	25291.10	11531.35	27025.04	21230.03	17680.55
BUC03	**0.10**	-	12447.03	14213.96	15582.29	8802.30	9029.20	9288.52	7244.45	14654.17	6820.33	15431.78	10024.78	9825.72	4618.34	11954.85	6891.06	5912.87
BUEL11	0.03	**0.08**	-	3905.92	8145.62	5876.16	21475.22	21448.45	6878.16	4995.50	12838.54	7789.72	22111.41	21779.27	10732.26	24283.18	19291.10	17084.63
EBN12	**0.19**	**0.22**	**0.20**	-	11797.28	9444.74	23080.33	22527.56	10127.80	8486.47	16041.41	11433.50	23106.35	22681.83	13597.44	25484.78	21073.25	19517.77
GEM09	**0.06**	**0.05**	0.03	**0.16**	-	6885.60	23702.51	24733.14	8369.32	3347.51	11928.10	364.86	25501.86	25363.32	11637.60	27125.77	21345.29	17826.61
HEI05	**0.32**	**0.27**	**0.35**	**0.37**	**0.27**	-	17354.65	18060.93	1581.82	5985.09	7088.82	6696.63	18810.87	18627.16	5497.00	20603.23	15026.62	12055.64
LEN15	**0.13**	**0.11**	0.13	**0.14**	**0.08**	**0.30**	-	3186.67	15777.96	23336.37	12338.63	23621.96	3732.73	4227.26	12073.38	3690.97	2357.54	6403.53
MIT16	**0.05**	**0.11**	**0.07**	**0.14**	**0.06**	**0.26**	**0.11**	-	16487.58	23942.64	14093.36	24608.78	797.80	1061.19	13199.32	3004.74	4460.35	8648.33
MUELL04	**0.07**	**0.08**	**0.06**	**0.15**	**0.06**	**0.22**	**0.09**	**0.05**	-	7558.53	5971.75	8202.28	17240.19	17064.19	4019.64	19021.63	13453.61	10569.30
RAF06	**0.18**	**0.20**	**0.16**	**0.26**	**0.12**	**0.31**	**0.17**	**0.13**	**0.15**	-	12516.14	2983.16	24676.71	24455.31	11428.07	26548.19	21011.17	17952.40
RIN01	**0.09**	**0.12**	**0.10**	**0.16**	**0.07**	**0.24**	**0.09**	**0.08**	**0.03**	**0.14**	-	11929.91	14891.08	14944.44	2917.49	15973.45	10037.13	6061.89
ROH08	0.03	**0.09**	0.09	**0.18**	**0.04**	**0.28**	**0.11**	**0.08**	**0.07**	**0.10**	**0.08**	-	25374.52	25227.26	11550.13	27025.61	21265.65	17791.63
RUER17	**0.06**	**0.14**	**0.07**	**0.12**	**0.05**	**0.27**	**0.11**	**0.02**	**0.06**	**0.14**	**0.08**	**0.07**	-	665.44	13983.68	2630.20	5211.61	9422.18
STE02	**0.14**	**0.11**	**0.17**	**0.16**	**0.12**	**0.26**	**0.13**	**0.13**	**0.07**	**0.22**	**0.09**	**0.13**	**0.17**	-	13910.14	3252.82	5481.88	9626.87
STE18	0.05	0.08	0.02	**0.13**	0.05	**0.28**	0.06	-0.01	-0.01	**0.15**	0.04	0.06	**0.06**	**0.04**	-	15495.72	9718.41	6559.94
WIE19	**0.24**	**0.26**	**0.16**	**0.22**	**0.20**	**0.39**	**0.17**	**0.15**	**0.16**	**0.23**	**0.16**	**0.24**	**0.17**	**0.24**	**0.08**	-	5936.32	10090.27
WILL14	**0.18**	**0.19**	**0.23**	**0.27**	**0.16**	**0.26**	0.01	**0.20**	**0.15**	**0.20**	**0.14**	**0.16**	**0.20**	**0.20**	**0.16**	**0.27**	-	4249.01
ZIE13	**0.13**	**0.23**	**0.09**	**0.16**	**0.14**	**0.32**	**0.19**	**0.09**	**0.10**	**0.17**	**0.16**	**0.16**	**0.07**	**0.19**	**0.11**	**0.21**	**0.26**	-

S7) Correlation matrix for diversity indices

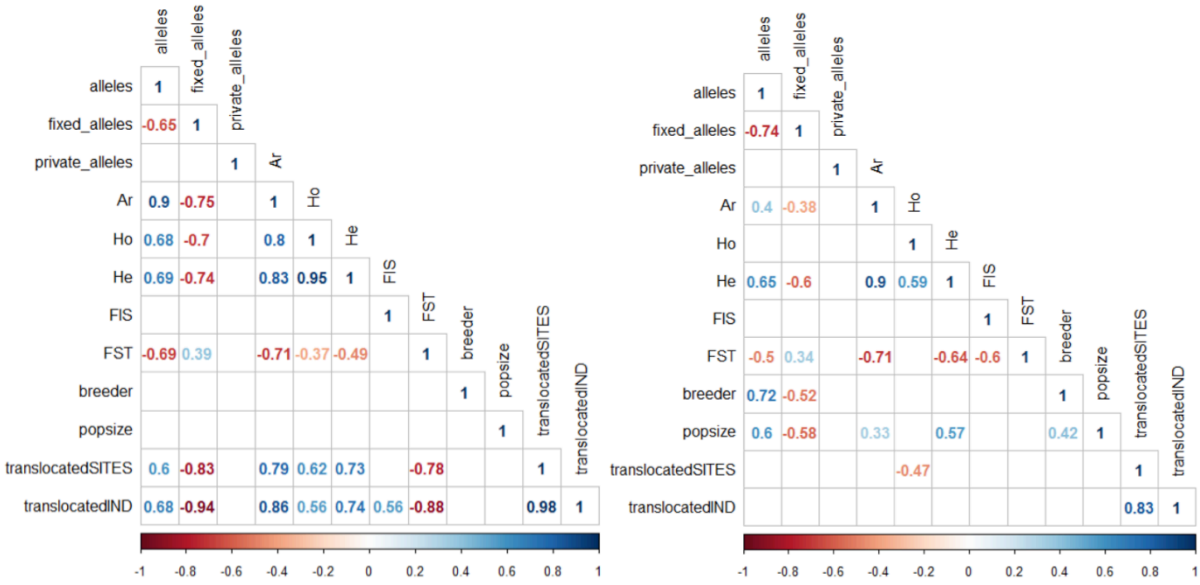

Figure S7 Correlation matrix for *A. obstetricans* (left) and *E. calamita* (right) diversity indices. Shown are only significant values ($p < 0.05$).

Raw data

Results of genotyping and landscape analysis are on additional files.