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Genetic diversity under the strain of habitat fragmentation: a population genetic analysis of two *Salamandra salamandra* populations from the Vienna Woods

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Abstract

The possession and maintenance of genetic diversity plays a crucial role in the survival of species as it enables populations to evolve in response to environmental change. In this context, habitat fragmentation has been identified as a major issue, as it creates small isolated populations that are exposed to high genetic drift, and thereby accelerates the loss of genetic variation. In this study I compared genetic parameters of two populations of *Salamandra salamandra* in the Vienna Woods that differ in their habitat structure. The location “Neuwaldegg” is an “open” habitat, whereas the habitat “Liesing” is constrained to a fragmented forest patch. Therefore I expected the salamander population from “Liesing” to be less genetically diverse in contrast to the population from “Neuwaldegg”. The results show a rather large degree of genetic subdivision between these two populations. The genetic diversity, however, hardly differed between the sites. I suggest the unexpected results to be attributable to a sufficiently large population size in “Liesing”, allowing the maintenance of the genetic variation at this location. The existence of remaining corridors around this area allowing genetic exchange

among neighbouring (sub-) populations, is a further possible reason for the lack of the originally expected genetic pauperisation in the “Liesing” population.

Introduction

It is of prominent interest in evolutionary biology to understand the coherences between the effects of habitat fragmentation and genetic diversity on the population genetic structures (Frankham, 1995; Debinski and Holt, 2000; Fahrig, 2003). This is getting more and more important in particular for the conservation of amphibians that are currently facing severe worldwide declines (Beebee and Griffiths, 2005; Allentoft and O’Brien, 2010). Today scientists are aware that the decline in amphibian populations is caused by the complex interaction of multiple stressors, but still habitat loss and fragmentation are indicated as major threats to the viability of populations (Cushman, 2006; Blaustein et al., 2011).

Genetic diversity plays a crucial role for the survival and adaptability of species (Soulé, 1986; Hedrick, 2000). It enables populations to evolve in response to environmental change (Frankham, 1996). Accordingly, the World Conservation Union stated, that genetic diversity is one of three levels of biodiversity requiring conservation (McNeely et al., 1990). The major source of genetic variability is mutation (Bürger, 1999) whereas genetic drift due to a finite small population size is the reason for the decrease of genetic variability. Habitat fragmentation is suspected to accelerate the loss of genetic variation (Andersen and Damgaard, 2004), because per definition it is a process of losing and breaking apart of habitat. Thus, it creates isolated patches of suitable habitat embedded in an adverse matrix (Fahrig, 2003). Consequently, residual

populations get reduced in size conditional on the size and quality of the remaining habitat island (Wiegand et al., 2005). A finite small population, in turn, increases the risk of inbreeding and accelerates the loss of genetic variability due to genetic drift (Lande and Barrowclough, 1996). Finally, the probability of (local) extinction increases (Andrén, 1994). To disrupt the chain of reaction started by habitat fragmentation, possibilities for genetic exchange and the maintenance of genetic variability are necessary. Depending on the capacity to disperse and associated with the distance between suitable habitat patches, corridors are needed to connect fragmented populations (Opdam, 1990; Rothermel and Semlitsch, 2002).

Owing to their complex life histories involving spatially separated stages, European amphibian populations may be especially vulnerable to local extinction because of human made habitat destruction, (Gibbs, 1997; Scribner et al., 2001). Their physiological requirement to remain near moist refuges, their tendency to site fidelity and their relatively low mobility compared to other vertebrates (Bowne and Bowers, 2004) make it difficult to cope with the effects of habitat fragmentation (Gibbs, 1998). Therefore, amphibian species provide proper models to investigate the impact on genetic diversity due to habitat fragmentation.

The present study compares the genetic diversity of two spatially separate *Salamandra salamandra* populations from the recreational area “Wienerwald”, Vienna Woods. The broadleaf forest habitat in “Neuwaldegg” (NEW) lies at the beginning of an extensive part of the Vienna Woods enabling individuals to disperse more freely, whereas “Liesing” (LIE) is restricted to a diminutive forest area. Interestingly, in LIE temporary ponds are used for larvae deposition, which

are an atypical larval habitat for *S. salamandra* in Central Europe (see Weitere et al., 2004; Steinfartz et al., 2007).

The objective was to determine if habitat fragmentation in the *Salamandra salamandra* population from LIE already led to genetic pauperization. Furthermore, I investigated whether this fragmentation even lead to a complete genetic isolation that impeded genetic exchange between the population of LIE and NEW. Utilizing microsatellite DNA markers, a comparison of the genetic structure of the two spatially separated populations with a different situation in forest patch connectivity was conducted.

Materials and methods

Study area and samples

Samples were taken from two spatially separated locations from the Vienna Woods differing in habitat composition (Fig. 1). Located in the north-western end of Vienna and continuing in Lower Austria the study site “Neuwaldegg” NEW (+48°14'51.49", +16°15'55.31", approximately 14 ha) is part of an extensive forest area of the Vienna Woods (Fig. 2). It is characterized by common beeches, hornbeam and oaks. The creeks are flanked by steep slopes with crevices and holes which offer hiding places for the salamanders. The sampling area “Liesing” LIE (+48°9'6.58", +16°14'46.62", about 1,5 km²) is a hilly oak and hornbeam forest habitat (Fig. 3). In this site the present water bodies are creeks, temporary ponds, tarns and seepage spring. In the north LIE is confined by the wildlife park “Lainzer Tiergarten”. Its stone wall, erected in 1782, displays a serious barrier for

animals like salamanders. To the east side LIE is bounded by urban area, to the south and west by large meadows. Hence, NEW offers a large and continuous habitat for fire salamanders, whereas LIE is a fragmented habitat patch.

From March 2010 until the end of June morning or evening visits were conducted every other day alternately to both locations (in total 23 times to LIE and 24 times to NEW). Individuals were recorded via GPS (mobile GIS software ArcPad 8.0™ ESRI on Magellan MobileMapper 6) and their position data was further handled in ArcGIS™ 9.3 (ESRI). Each individual was documented with digital photographs and identified by means of their dorsal pattern as well as sexed by their cloaca (male: swollen, female: not swollen). Body size (snout to base of the tail length) was calculated with imageJ 1.43u. To obtain the DNA-samples I took non-invasive buccal swabs with a common cotton bud and stored each in an Eppendorf tube in a freezer at -20°C prior the genetic analyses.

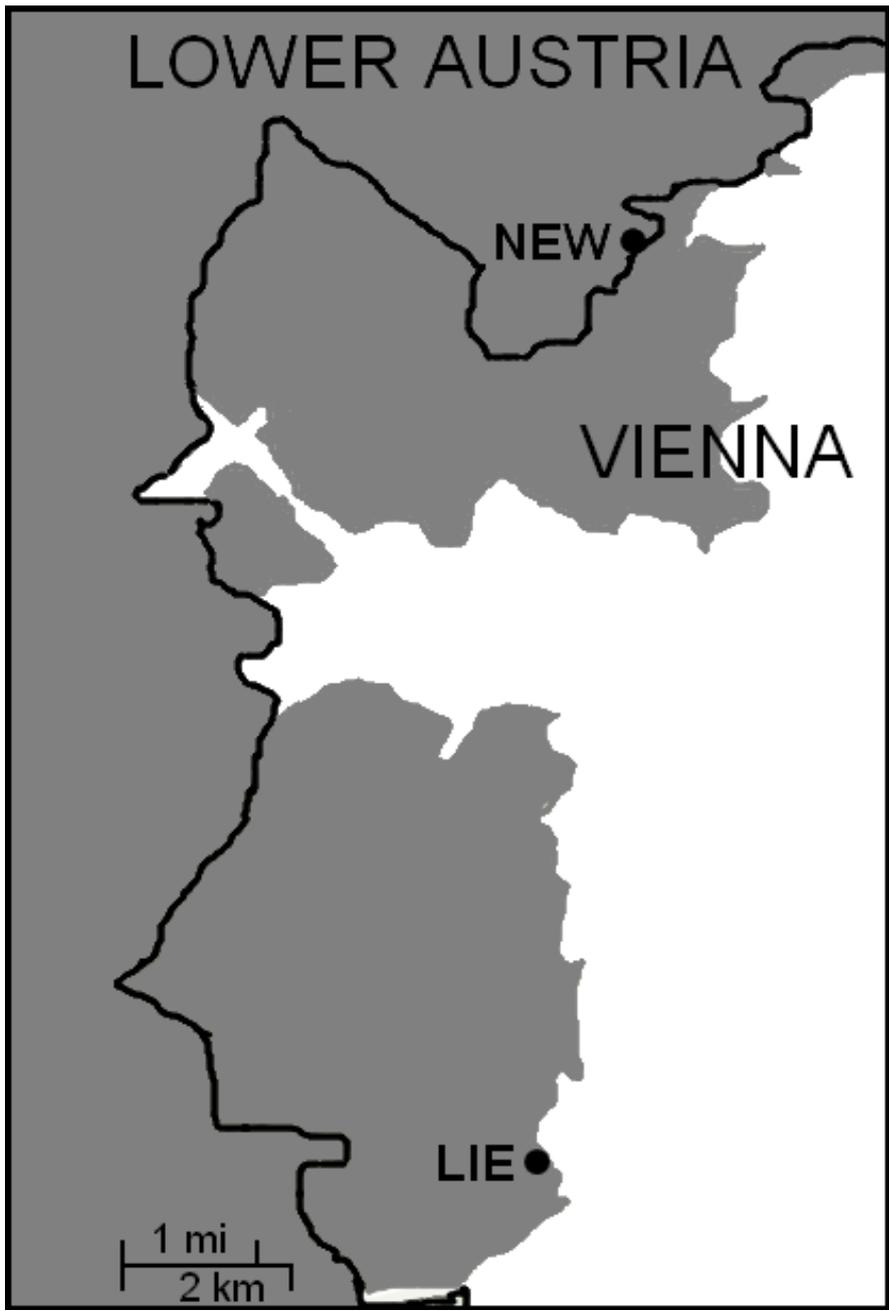


Fig. 1 Sampling locations of *Salamandra salamandra* in the study area Vienna Woods. The urban area of Vienna is shown in white, whereas grey represents a part of the Vienna Woods. Sample sites are marked by points: NEW “Neuwaldegg” in the northwest of Vienna at the border (black line) to Lower Austria and LIE “Liesing” in the southwest of Vienna.

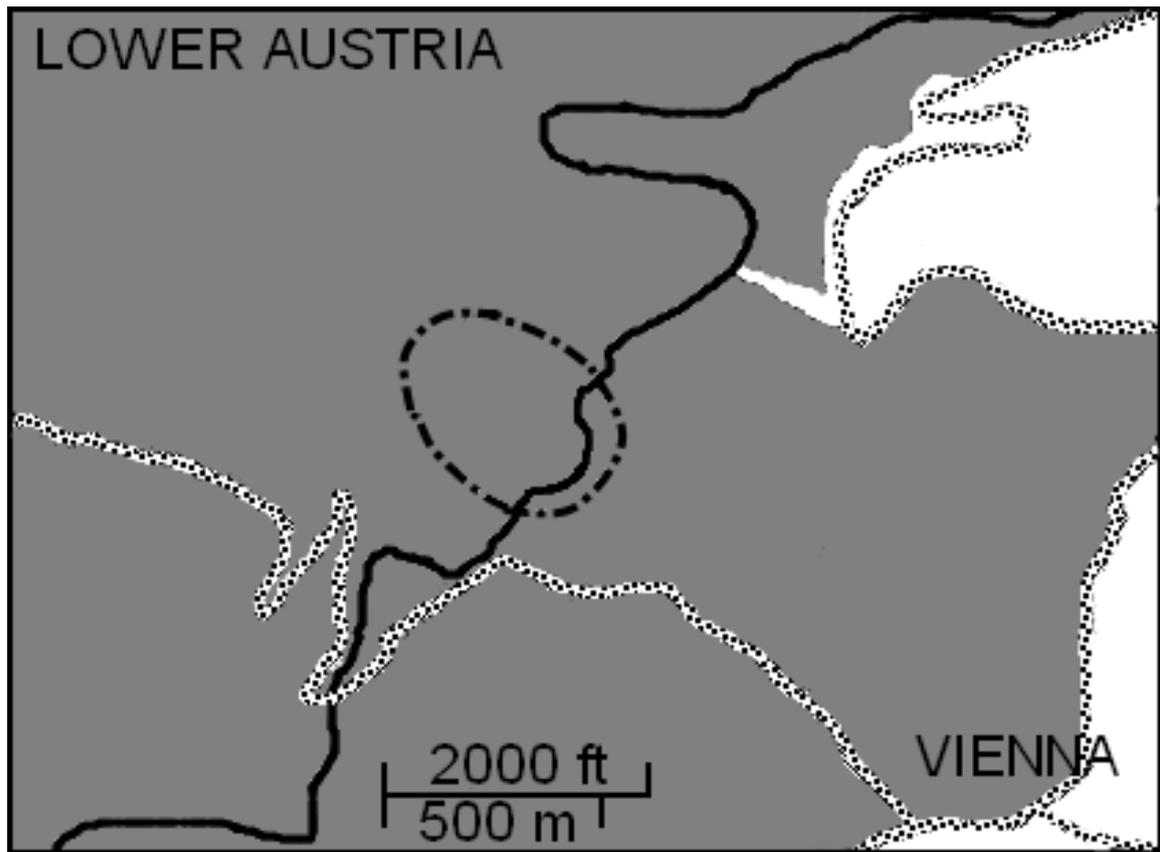


Fig. 2 *Salamandra salamandra* sampling site "Neuwaldegg". The dashed and dotted line indicates the collecting area NEW "Neuwaldegg" (enlarged section of Fig.1); grey: forest area; white: urban area of Vienna; solid black line: border of the city of Vienna; dotted line: road.

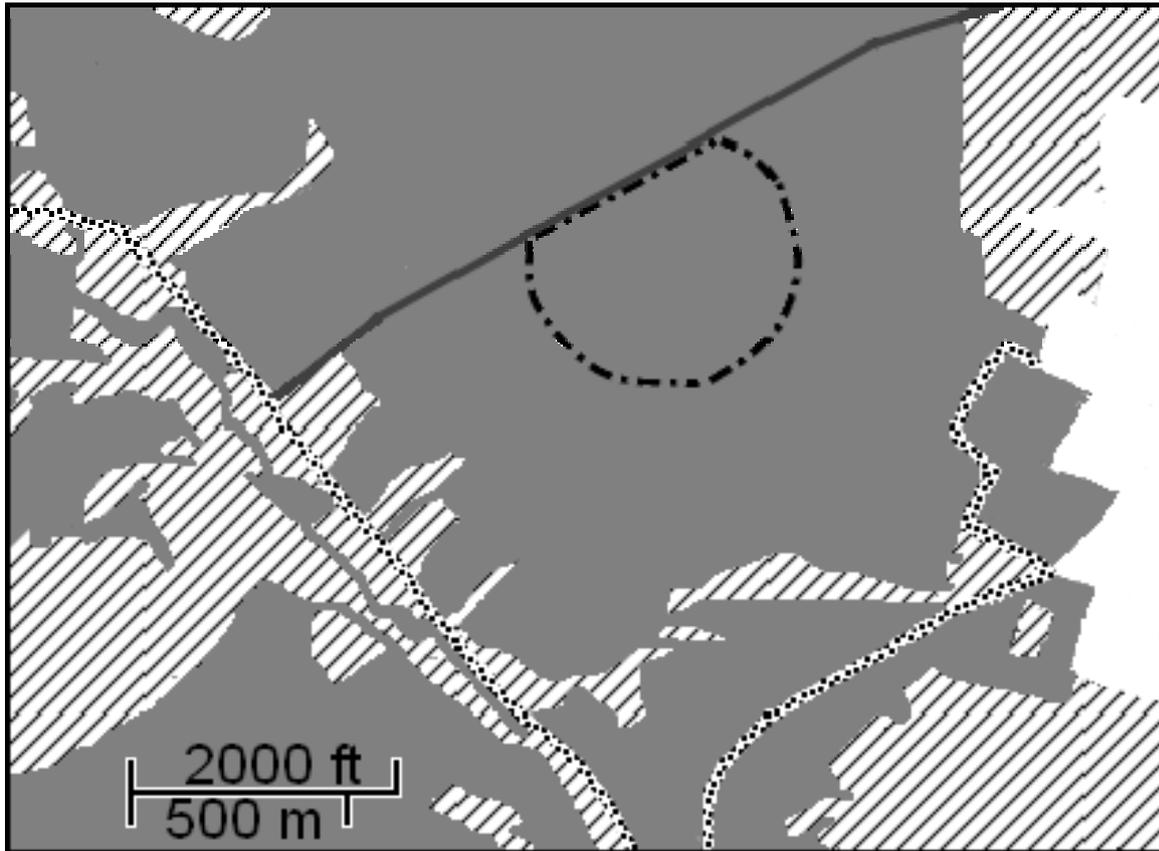


Fig. 3 *Salamandra salamandra* sampling site "Liesing". The circle marks the collecting area LIE "Liesing" (enlarged section of Fig.1). Note the enclosed forest patch situation. Grey: forest parts; white: urban area of Vienna; hachured: meadows; dark grey line: stone wall; dotted line: road.

Microsatellite genotyping

To extract the genomic DNA standard phenol-chloroform procedures (Sambrook et al., 1989) were performed. Primers were used for 10 tetranucleotide microsatellite loci (locus *Sal E2*, *Sal E6*, *Sal E7*, *Sal E8*, *Sal E11*, *Sal E12*, *Sal E14*, *Sal 3*, *Sal 23* and locus *Sal 29*), as published in Steinfartz et al. (2004). PCR protocols were modified to improve amplification rates (Table 1). Each 1,5 mM MgCl₂ amplification reaction contained 1,2 µL genomic DNA (diluted to 10 ng/µL); 1 µL 10x NH₄ reaction buffer (Axon); 4,35 µL deionised (DI) water; 0,6 µL MgCl₂; 1µL 2 mM of each dNTP; 0,05 µL *Taq* DNA polymerase and 1 µL of each primer (5 pmol/µL). The amplification conditions were: one cycle of 94°C for 4 min; 39 cycles of 94°C for 45 sec, primer specific temperature (Tab.1) for 45 sec, 72°C for 45 sec and a final extension step of 72°C for 5 min.

PCR products were diluted with water and mixed with internal size standard ROX500 to run on an ABI 3130xl sequencer. Further on, sequencing products were analyzed using PeakScanner 1.0 (Applied Biosystems). All loci were visually identified and the final allele sizes were determined using the binning software Tandem 1.01 (Matschiner and Salzburger, 2009). Samples for which more than three loci failed to amplify were not included in the analysis.

Statistical analysis

The program FSTAT v.2.9.3.2 (Goudet, 2002) was used to calculate probability tests for genotypic linkage disequilibrium and to test departures from Hardy–Weinberg equilibrium at each locus. Furthermore, I determined gene diversity within populations, frequencies of observed (H_o) and expected (H_E)

heterozygotes, the mean number of alleles per locus, allelic richness and Wright's (1965) inbreeding coefficient (F_{IS}).

The allelic richness (AR) of the population at a particular locus is the total number of allele types present in the population at that locus. It is a measure that accounts for variable sample sizes through rarefaction. A F_{IS} value of 0 indicates that there is no inbreeding ($F_{IS} = 1$ for complete inbreeding) or outbreeding (-1: all individuals are heterozygous) in the population.

Further, the degree of differentiation between populations was estimated with Wright's index of population subdivision (F_{ST}). F_{ST} values vary from 0 to 1, considering that values of 0-0.05 represent little genetic differentiation, 0.05-0.15 moderate, 0.15-0.25 great and values of >0.25 indicate pronounced levels of genetic differentiation (Wright, 1978).

To determine the effective population size (N_e) the single-sample estimates from the sibship assignment method of Colony 2.0.1.3 (Wang, 2009) was chosen. To compare values of genetic diversity indexes between both populations, the non-parametric Wilcoxon-W test was conducted with PASW Statistics 17.0.2.

Results

During spring and early summer of 2010 a total of 245 (NEW) and 90 (LIE) individuals were sampled. For this study 73 mouth swab samples from LIE and 65 samples from NEW were genetically analyzed (Table 2). Locus designation, primer sequences and repeat motif for the nine used microsatellite loci referring to Steinfartz et al. (2004) are summarized in Table 1. Moreover, the size range of alleles, allelic diversity per locus, expected (H_E) and observed (H_O) heterozygosity of both Vienna Woods populations combined are compiled. The microsatellite locus *Sal E11* was excluded from the statistical analysis due to a low PCR amplification success.

For Hardy–Weinberg equilibrium within samples FSTAT permuted alleles among individuals within samples based on 900 randomizations with an indicative adjusted nominal level of 5% = 0.00556. For the microsatellite locus *Sal E2* from LIE the proportion of randomizations that gave a *larger* F_{IS} than the observed deviated from Hardy–Weinberg equilibrium, indicating a homozygote excess. The population NEW showed deviation from Hardy-Weinberg equilibrium for locus *Sal E14* also due to homozygote excess. Concerning the proportion of randomizations that gave a *smaller* F_{IS} than the observed, none of the used loci, neither for LIE nor for NEW, revealed p-values below the adjusted nominal level.

No significant genotypic linkage disequilibrium between loci was observed (pairwise comparisons for each population, adjusted P-value for 5% nominal level based on 3600 permutations = 0.00139).

Table 1 Characterization of the Fire salamander (*Salamandra salamandra*) microsatellite loci of the two studied populations combined. H_o observed heterozygosity, H_E expected heterozygosity, N number of individuals typed.

Locus	Primer sequences (5'-3'), fluorescence labelling	Repeat motif	Annealing temp.	Size range (bp)	No. of alleles	H_o	H_E	N
Sal E2 AY612894	F: EXCACGACAAAATACAGAGAGTGGATA R: ATATTTGAAATTGCCCATTTGGTA	(GATA) ₆ (GACA) ₅ (GATA) ₁₂	53°C	217-293	11	0,565	0,765	115
Sal E6 AY612885	F: FAMGGACTCATGGTCACCCAGAGGTTCT R: ATGGATTGTGTCGAAATAAGGTATC	(GATA) ₂ GATG(GATA) ₁₅	59°C	264-304	7	0,55	0,691	111
Sal E7 AY612886	F: HEXTTTTCAGCACCAAGATACCTCTTTTG R: CTCCTCCATATCAAGGTCACAGAC	(GATA) ₆ (GACA) ₁₁ (GATA) (GACA)(GACA)(GATA) ₁₂	53°C	132-252	15	0,534	0,599	118
Sal E8 AY612887	F: FAMGCAAGTCCATGCTTTCCCTTTCTC R: GACATACCAAGACTCCAGAATGGG	(TATC) ₁₆	59°C	150-190	9	0,846	0,778	130
Sal E12 AY612889	F: TETCTCAGGAACAGGTGCCCCCAAATAC R: CTCATAATTTAGTCTACCTCCAC	(CTAT) ₁₅	59°C	103-287	22	0,781	0,864	114
Sal E14 AY612890	F: TETGCTGCCCTCTCTGCCTACTGACCCAT R: GCCAAGACATGGAACACCCCTCCCGC	(CTAT) ₁₆	65°C	154-282	10	0,505	0,603	109
Sal 3 AY612891	F: FAMCTCAGACAAGAAATCCTGCTTCTTC R: AATAATCTGCTCTGTTCTTAATCAG	(GAGT) ₁₅	59°C	179-223	9	0,661	0,543	127
Sal 23 AY612893	F: HEX-TCAGTGTATCTTTGTTCTTTTAT R: AATTATTTGTTGAGTCGATTTTCT	(GACA) ₈ (GATA) ₄	50°C	286-310	7	0,653	0,702	118
Sal 29 AY612892	F: TET-CTCTTTGACTGAACCAGAACCC R: GCCTGTCGGCTCTGTGTAACC	(GATA) ₁₄	58°C	159-231	13	0,541	0,602	109

Table 2 Characterization and comparison of the polymorphic microsatellite loci observed in the two Fire Salamander populations Liesing (LIE) and Neuwaldegg (NEW). Numbers of individuals genetically analyzed of each population are added in parentheses. N, number of individuals typed per locus; k , number of alleles; AR , allelic richness; H_E , expected heterozygosity; H_O , observed heterozygosity; F_{IS} , inbreeding coefficient; P , P-value for Hardy–Weinberg within samples with proportion of randomizations that gave a smaller (<) or larger (>) F_{IS} than the observed (indicative adjusted nominal level: 0.00556); \bar{k} , Mean number of alleles; \bar{H}_E , Mean expected heterozygosity; \bar{PIC} , mean polymorphic information content.

	Locus	Sal E2	Sal E6	Sal E7	Sal E8	Sal E12	Sal E14	Sal 3	Sal 23	Sal 29	\bar{k}
LIE (73)	N	65	60	60	69	60	60	67	67	57	
	k	11	5	11	8	12	9	2	6	9	8.11
	AR	10.356	4.950	10.794	7.478	11.750	8.798	2.000	5.829	9.000	
	H_O	0.554	0.550	0.483	0.855	0.733	0.583	0.627	0.657	0.544	0.621
	H_E	0.774	0.670	0.493	0.776	0.817	0.587	0.498	0.667	0.642	0.6582
	F_{IS}	0.286	0.181	0.019	-0.102	0.103	0.006	-0.261	0.015	0.154	
	$P < F_{IS}$	0.0011	0.0422	0.4756	0.9767	0.0444	0.5544	0.9889	0.4733	0.0378	
	$P > F_{IS}$	1	0.9767	0.6867	0.0611	0.9800	0.5822	0.0278	0.6522	0.9867	
PIC										0.6016	
NEW (65)	N	50	51	58	61	54	49	60	51	52	
	k	8	6	10	7	22	5	9	7	11	9.44
	AR	7.940	5.960	9.511	6.956	21.156	5.000	8.201	6.960	10.766	
	H_O	0.580	0.549	0.586	0.836	0.833	0.408	0.700	0.647	0.538	0.631
	H_E	0.724	0.714	0.686	0.776	0.904	0.624	0.595	0.738	0.546	0.7006
	F_{IS}	0.201	0.233	0.147	-0.079	0.079	0.348	-0.179	0.124	0.013	
	$P < F_{IS}$	0.0111	0.0089	0.0244	0.9089	0.0567	0.0022	0.9656	0.0844	0.5333	
	$P > F_{IS}$	0.9978	0.9967	0.9889	0.1622	0.9833	1	0.0589	0.9622	0.6522	
PIC										0.6516	

The population LIE showed a little less polymorphism with the average number of 8.11 alleles per locus (ranging from 2-11 alleles per locus) compared to NEW with an average of 9.44 (ranging from 5-22) alleles per locus (Table 2). For LIE the observed heterozygosities were between 0.48 and 0.86 (mean = 0.62) and for the population NEW H_O was between 0.41 and 0.84 (mean = 0.63). Whereas the average expected heterozygosity for LIE was 0.66 and for NEW 0.7. Applicable for both populations, H_O values lay above H_E for loci *Sal 3* and *Sal E8* but for the other seven loci the observed were below the expected heterozygosities.

For both populations the values of the *allelic richness* were equal to or minimally below the numbers of alleles (k) of each corresponding locus (Table 2). Further, no difference between the two populations concerning the effective population size could be found. With N_e at LIE = 28 (95% CI = 17.50) and N_e at NEW = 29 (95% CI = 18.50).

The highest F_{IS} value for LIE was found at locus *Sal E2* (0.286), whereas NEW showed the highest F_{IS} value at *Sal E14* (0.348). For loci *Sal E8* and *Sal 3* negative F_{IS} values for both populations were observed. Therefore, the F_{IS} calculations suggest little to no evidence of strong inbreeding, given that all values were close to zero (Table 2).

F-statistics for each locus over both populations are given in Table 3. The measure of differentiation among LIE and NEW (F_{ST} estimated pairwise) is 0.1749 suggesting a moderate level of genetic differentiation between populations.

None of the parameters *number of alleles*, *gene diversity* (or H_E), *allelic richness*, or F_{IS} showed significant differences between the populations of LIE and NEW (Wilcoxon W test, Table 4).

Table 3 Overall inbreeding coefficient (F_{IT}), fixation index (F_{ST}) and inbreeding coefficient (F_{IS}) values for each locus over both populations.

Locus	F_{IT}	F_{ST}	F_{IS}
Sal E2	0.320	0.092	0.251
Sal E6	0.265	0.075	0.205
Sal E7	0.410	0.350	0.093
Sal E8	-0.038	0.049	-0.091
Sal E12	0.207	0.128	0.091
Sal E14	0.473	0.369	0.165
Sal 3	0.144	0.297	-0.219
Sal 23	0.166	0.108	0.065
Sal 29	0.111	0.021	0.092
Over all loci	0.239	0.175	0.078

Table 4 Wilcoxon W test conducted with four parameters characterizing genetic diversity. k , number of alleles; H_E , gene diversity; AR , allelic richness; F_{IS} , inbreeding coefficient.

	k	H_E	AR	F_{IS}
W	-0,538	-1,186	-0,533	-0,889
p	0,591	0,236	0,594	0,374

Discussion

During the last decades an intense attention has been made to the effects of human made landscape modification. It has been shown that habitat fragmentation triggers several factors, such as the reduction in the census population size, migration rates, and in population density. Further, habitat fragmentation can lead to demographic instability and increase habitat edge effects (Saunders et al., 1991; Johansson et al., 2005). Particularly the reduction in effective population size will subsequently lead to a reduction in genetic diversity. In this study I compared the population genetic structure of two *Salamandra salamandra* populations in the Vienna Woods. The two populations differed significantly in their spatial setup. While NEW can be described as an “open” habitat, the location in LIE seemed to be constrained to a fragmented forest patch. Accordingly, I expected the population in LIE to be less genetically diverse compared to the population in NEW. Furthermore, I wanted to investigate whether this fragmentation even lead to a complete genetic isolation that impeded genetic exchange between these two populations.

The genetic analysis showed that although in LIE the habitat is fairly enclosed by human made landscape modifications (Fig. 3), the two populations from the Vienna Woods were equal in their genetic diversity, but not different in their effective population sizes. This can either be attributable to the fact that the population size in LIE is sufficiently large to maintain the genetic variation in this area, or that the effects of pauperization are not yet detectable. A computer simulation model (Lacy, 1987) that tried to estimate the time latency for a population to respond to changes associated with habitat fragmentation, showed that in small populations the reduction of genetic variability due mainly to genetic

drift was only noticeable after about ten generations. Given that our model organism, the fire salamander, has a very long lifespan of more than twenty years, reaching sexual maturity with two to four years (Nöllert and Nöllert, 1992), overlapping generations exist with an average generation time of 4 years (Steinfartz et al., 2007). In conjunction with the situation of LIE, the erection of the wall from the “Lainzer Tiergarten” was probably enough to significantly decrease gene flow with surrounding (meta-) populations. Considering this barrier as the beginning of fragmenting LIE (228 years ago), the LIE population may have been fragmented for over 50 generations. According to the simulation model, this should be a sufficient amount of time to be reflected in genetic data, however, LIE was genetically not inferior to NEW. This could indicate that the LIE population is not as fragmented as previously expected, or that its size is sufficiently large to maintain the genetic variation.

Additionally, the existence of corridors might explain the lack of differences in the genetic variability between the two Vienna Woods populations. Corridors to increase connectivity of fragmented landscapes (Soulé and Simberloff, 1986; Hudson, 1991; Simberloff et al., 1992; Rosenberg et al., 1997; Tewksbury et al., 2002) were discussed ever since habitat loss and fragmentation was viewed as the major threats for biological diversity (e.g. Pimm and Raven, 2000 animals in general; Cushman, 2006 for amphibians). Several researchers suggest habitat connectivity to be important for regional viability of fragmented populations (in small mammals: Bennett, 1990; Henein and Merriam, 1990; in insects: Sutcliffe and Thomas, 1996; in plants: Murphy and Lovett-Doust, 2004; in birds: Uezu et al., 2005; in amphibians: Olson et al., 2007). In some cases even small corridors between habitat patches may allow enough genetic exchange to sustain a

sufficient level of genetic diversity in a population (Mills and Allendorf, 1996; Mech and Hallett, 2001). Hereto it is important to emphasize that connectivity is depending on the distance between suitable habitat patches and on the dispersal capacity of individuals (Vos et al., 2001). In case of our model organism, contemporary research provides evidence to presume that the dispersal ability of *Salamandra* species is much greater than previously suggested (Bar-David et al., 2007; Schmidt et al., 2007). If only some individuals are able to complete long-distance movements it could increase connectivity and contribute to genetic exchange (Cushman, 2006). Therefore, the lack of genetic pauperization in the LIE population can also be on account of existing corridors allowing genetic exchange.

Surprisingly, the large degree of genetic subdivision (F_{ST}) between LIE and NEW suggest low connectivity among the two sites, indicating that these populations are genetically independent. Beside their differences in the spatial setup, the populations in LIE and NEW also differed in their reproductive strategy. In NEW *S. salamandra* exhibit the typical reproductive behaviour with larval deposition in small streams. Contrastingly, the salamanders in LIE mainly used standing water bodies for larval deposition. A similar situation was described by Steinfartz and his colleagues, where in some locations in western Germany *S. salamandra* larvae were found in small steep streams but also in temporary ponds and pond-like water bodies (Weitere et al., 2004; Steinfartz et al., 2007). The observed genetic differences between the populations were assumed to constitute an early stage of adaptive differentiation under sympatric conditions, as they could rule out geographical factors as the reason for habitat differentiation. However, since I compared two spatially separated populations, I cannot attribute

the observed differences in larval habitats to spatial (“isolation by distance”) or behavioural (“reproductive isolation”) effects.

In this study I found a rather large degree of genetic subdivision between two populations of the fire salamander in the Vienna Woods. The genetic diversity, however, hardly differed between the sites. According to this finding, future research should investigate the existence of remaining corridors that allow genetic exchange among neighbouring (sub-) populations. This information will particularly be of significant value for conservation management strategies. Furthermore, comparative studies at the LIE site will give insight in a possible plasticity of the reproductive behaviour in fire salamanders.

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References

- Allentoft, M., O'Brien, J. (2010): Global Amphibian Declines, Loss of Genetic Diversity and Fitness: A Review. *Diversity* **2**: 47-71.
- Andersen, L.W., Fog, K., Damgaard, C. (2004): Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). *Proceedings of the Royal Society B: Biological Sciences* **271**: 1293-1302.
- Andrén, H. (1994): Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos* **71**: 355-366.
- Bar-David, S., Segev, O., Peleg, N., Hill, N., Templeton, A.R., Schultz, C.B., Blaustein, L. (2007): Long-Distance Movements by Fire Salamanders (*Salamandra infraimmaculata*) and Implications for Habitat Fragmentation. *Israel Journal of Ecology and Evolution* **53**: 143-159
- Beebee, T.J.C., Griffiths, R.A. (2005): The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation* **125**: 271-285.
- Bennett, A.F. (1990): Habitat corridors and the conservation of small mammals in a fragmented forest environment. *Landscape Ecology* **4**: 109-122.
- Blaustein, A.R., Han, B.A., Relyea, R.A., Johnson, P.T.J., Buck, J.C., Gervasi, S.S., Kats, L.B. (2011): The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. *Annals of the New York Academy of Sciences* **1223**: 108-119.
- Bowne, D.R., Bowers M.A. (2004): Interpatch movements in spatially structured populations: a literature review. *Landscape Ecology* **19**: 1-20
- Bürger, R. (1999): Evolution of Genetic Variability and the Advantage of Sex and Recombination in Changing Environments. *Genetics* **153**:1055-1069
- Cushman, S.A. (2006): Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation* **128**: 231-240.

- Debinski, D.M., Holt, R.D. (2000): Review: A Survey and Overview of Habitat Fragmentation Experiments. *Conservation Biology* **14**: 342-355.
- Fahrig, L. (2003): Effects of Habitat Fragmentation on Biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **34**: 487-515.
- Frankham, R. (1995): Conservation genetics. *Annual review of genetics* **29**: 305-327.
- Frankham, R. (1996): Relationship of Genetic Variation to Population Size in Wildlife. *Conservation Biology* **10**: 1500-1508.
- Gibbs, J.P. (1997): Distribution of woodland amphibians along a forest fragmentation gradient. *Landscape Ecology* **13**: 263-268
- Gibbs, J.P. (1998): Amphibian Movements in Response to Forest Edges, Roads, and Streambeds in Southern New England. *The Journal of Wildlife Management* **62**: 584-589
- Goudet, J. (2002): FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3.2. Universite' de Lausanne, <http://www.unil.ch/izea/software/fstat.html>.
- Hedrick, P.W. (2000): *Genetics of populations*, 2nd Edition. Sudbury, Jones and Bartlett Publishers
- Henein, K., Merriam, G. (1990): The elements of connectivity where corridor quality is variable. *Landscape Ecology* **4**: 157-170.
- Hudson, W.E. (1991): *Landscape linkages and biodiversity*. Defenders of Wildlife. Washington, Island Press.
- Johansson, M., Primmer, C.R., Sahlsten, J., Merilä, J. (2005): The influence of landscape structure on occurrence, abundance and genetic diversity of the common frog, *Rana temporaria*. *Global Change Biology* **11**: 1664-1679.
- Lacy, R. (1987): Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology* **1**:143-158

- Lande, R., Barrowclough, G.F. (1996): Effective population size, genetic variation, and their use in population management. In: *Viable populations for Conservation*, p. 87-124. Soulé, M.E., Ed., Cambridge, University Press.
- Matschiner, M., Salzburger, W. (2009): TANDEM: integrating automated allele binning into genetics and genomics workflows. *Bioinformatics* **25**: 1982–1983.
- McNeely, J.A., Miller, K.R., Reid, W.V., Mittermeier, R.A., Werner, T.B. (1990): *Conserving the world's biological diversity*. Gland, Switzerland, IUCN
- Mech, S.G., Hallett, J.G. (2001): Evaluating the Effectiveness of Corridors: a Genetic Approach. *Conservation Biology* **15**: 467-474.
- Mills, L.S., Allendorf, F.W.(1996): The one-migrant-per-generation rule in conservation management. *Conservation Biology* **10**:1509-1518.
- Murphy, H.T., Lovett-Doust, J. (2004): Context and connectivity in plant metapopulations and landscape mosaics: does the matrix matter? *Oikos* **105**: 3-14.
- Nöllert, A., Nöllert, C. (1992): *Die Amphibien Europas: Bestimmung, Gefährdung, Schutz*. Stuttgart, Franckh-Kosmos.
- Olson, D.H., Anderson, P.D., Frissell, C.A., Welsh Jr, H.H., Bradford, D.F. (2007): Biodiversity management approaches for stream–riparian areas: Perspectives for Pacific Northwest headwater forests, microclimates, and amphibians. *Forest Ecology and Management* **246**: 81-107.
- Opdam, P. (1990): Dispersal in fragmented populations: the key to survival. In: *Species Dispersal in Agricultural Habitats*, p. 3-17. Bunce, R.G.H., Howard, D.C., Eds., London, Belhaven Press.
- Pimm, S.L., Raven, P. (2000): Biodiversity – extinction by numbers. *Nature* **403**: 843-845.
- Rosenberg, D.K., Noon, B.R., Meslow, E.C. (1997): Biological Corridors: Form, Function, and Efficacy. *BioScience* **47**: 677-687.

- Rothermel, B.B., Semlitsch, R.D. (2002): An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. *Conservation Biology* **16**:1324-133.
- Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): *Molecular Cloning: a Laboratory Manual*, 2nd Edition. New York, Cold Spring Harbour Laboratory Press.
- Saunders, D.A., Hobbs, R.J., Margules, C.R. (1991): Biological consequences of ecosystem fragmentation – a review. *Conservation Biology* **5**: 18-32.
- Schmidt, B.R., Schaub, M., Steinfartz, S. (2007): Apparent survival of the salamander *Salamandra salamandra* is low because of high migratory activity. *Frontiers in Zoology*, doi: 10.1186/1742-9994-4-19.
- Scribner, K.T., Arntzen, J.W., Cruddace, N., Oldham, R.S., Burke, T. (2001): Environmental correlates of toad abundance and population genetic diversity. *Biological Conservation* **98**: 201-210.
- Simberloff, D., Farr, J.A., Cox, J., Mehlman, D.W. (1992): Movement Corridors: Conservation Bargains or Poor Investments? *Conservation Biology* **6**: 493-504.
- Soulé, M.E. (1986): *Conservation biology: the science of scarcity and diversity*. Sunderland, Sinauer Associates.
- Soulé, M.E., Simberloff, D. (1986): What do genetics and ecology tell us about the design of nature reserves? *Biological Conservation* **35**: 19-40.
- Steinfartz, S., Küsters, D., Tautz, D. (2004): Isolation and characterization of polymorphic tetranucleotide microsatellite loci in the Fire salamander *Salamandra salamandra* (Amphibia: Caudata). *Molecular Ecology Notes* **4**: 626-628.
- Steinfartz, S., Weitere, M., Tautz, D. (2007): Tracing the first step to speciation: ecological and genetic differentiation of a salamander population in a small forest. *Molecular Ecology* **16**: 4550-4561.
- Sutcliffe, O.L., Thomas, C.D. (1996): Open Corridors Appear to Facilitate Dispersal by Ringlet Butterflies (*Aphantopus hyperantus*) between Woodland Clearings. *Conservation Biology* **10**: 1359-1365.

- Tewksbury, J.J., Levey, D.J., Haddad, N.M., Sargent, S., Orrock, J.L., Weldon, A., Danielson, B.J., Brinkerhoff, J., Damschen, E.I., Townsend, P. (2002): Corridors affect plants, animals, and their interactions in fragmented landscapes. *Proceedings of the National Academy of Sciences* **99**: 12923-12926.
- Uezu A., Metzger, J.P., Vielliard, J.M.E. (2005): Effects of structural and functional connectivity and patch size on the abundance of seven Atlantic Forest bird species. *Biological Conservation* **123**: 507-519.
- Vos, C.C., Antonisse-De Jong, A.G., Goedhart, P. W., Smulders, M.J.M. (2001): Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity* **86**: 598-608.
- Wang, J. (2009): A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology* **18**: 2148-2164.
- Weitere, M., Tautz, D., Neumann, D., Steinfartz, S. (2004): Adaptive divergence vs. environmental plasticity: tracing local genetic adaptation of metamorphosis traits in salamanders. *Molecular Ecology* **13**: 1665-1677.
- Wiegand, T., Revilla, E., Moloney, K. A. (2005): Effects of Habitat Loss and Fragmentation on Population Dynamics. *Conservation Biology* **19**: 108-121.
- Wright, S. (1965): The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**: 395-420.
- Wright, S. (1978): *Evolution and the Genetics of Populations: Variability Within and Among Natural Populations*, 4th Edition. Chicago, University of Chicago Press.

Zusammenfassung

Der Besitz und die Erhaltung der genetischen Vielfalt spielen eine entscheidende Rolle für das Überleben von Arten, denn genetische Variabilität ermöglicht den Populationen, sich auf Veränderungen in der Umwelt anzupassen. In diesem Zusammenhang ist die Zersplitterung / Fragmentierung von Lebensräumen zu einem wichtigen Thema geworden, denn es verursacht die Bildung kleiner, isolierter Populationen, die daraufhin verstärkt genetischer Drift ausgesetzt sind und beschleunigt so den Verlust genetischer Variation. In dieser Studie habe ich genetische Parameter zweier Populationen von *Salamandra salamandra* aus dem Wienerwald verglichen, die sich im Hinblick auf die Lebensraum-Struktur unterscheiden. Der Standort "Neuwaldegg" ist ein "offener" Lebensraum, während der Lebensraum "Liesing" ein begrenztes Waldfragment ist. Daher ging ich davon aus, dass die Salamander Population aus "Liesing" weniger genetisch divers, verglichen mit der Population aus "Neuwaldegg" wäre. Die Ergebnisse zeigen ein großes Maß an genetischer Differenzierung zwischen beiden Populationen. Das Ausmaß der genetischen Vielfalt unterscheidet sich jedoch kaum zwischen den Standorten. Das unerwartete Ergebnis führe ich auf eine ausreichend große Population in "Liesing", welche die Aufrechterhaltung der genetischen Variation an diesem Standort ermöglicht, zurück. Zudem können verbliebene, aber bisher unbekannte Korridore den genetischen Austausch zwischen benachbarten (Sub-) Populationen ermöglichen und somit das Fehlen der ursprünglich erwarteten genetischen Verarmung der „Liesing“ Population erklären.

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