Institut für Umweltwissenschaften, Universität Koblenz-Landau, Campus Landau

Are pesticides a factor for genetic differentiation of amphibian populations?

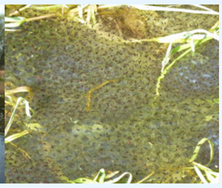
Patrick P. Lenhardt, Carsten A. Brühl, Kathrin Theissinger

Institute for Environmental Science, University of Koblenz Landau, Fortstr. 7, 76829 Landau, Germany

Introduction

In pond-rich agricultural landscapes breeding habitats are often completely surrounded by arable land. Therefore amphibians often have to cross agricultural land to migrate from terrestrial to aquatic habitats for reproduction and for genetic exchange between pond populations. During migrations over arable land an exposure to agro-chemicals, such as fertilizers and pesticides, is likely[1]. Due to toxic effects, arable land may act as sink habitat, inhibiting genetic exchange and entailing genetic differentiation of pond populations in agricultural areas.

The fragmentation of suitable habitat patches is mainly driven by the increase and intensification of agriculture as well as linear barriers (e.g. roads). It is necessary to understand how anthropogenic land use influences amphibian migration, in particular the genetic exchange between pond populations.



In the present study, we used ten of polymorphic microsatellites to analyze the population genetic structure of nine *Rana temporaria* pond populations in a vineyard monoculture and the adjacent forest (Fig. 1). We tested for significant relations of genetic population differentiation and landscape elements, including land use and linear barriers like streets and their associated traffic intensity, to explain the genetic structure in the study area.

Materials and Methods

Due to small pond populations in the agricultural area we used larvae sampling to obtain DNA (three eggs per clutch). To avoid inaccurate estimates of genetic differentiation (pairwise F_{ST}s) based on full-siblings in the data[2] we applied a repeated random selection approach (median over multiple F_{ST} estimates; MPF). We grouped MPFs based on their location into the groups forest (F; P7 to P9; N=3), vineyards (V; P1 to P6; N= 15) and vineyards/forest (V/F; N=18) and compared these groups for significant differences in the degree of population differentiation.

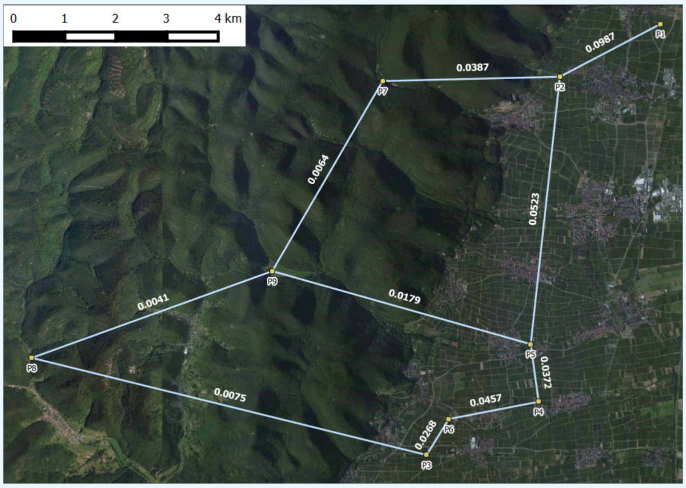


Fig. 1. - Overview of the study area and selection of MPFs between pond populations (P1 to P9).

We used Genepop's subprogram ISOLDE to test for relations of MPFs and linear geographic distance (LGD; isolation by distance), number of roads as well as accumulated traffic intensity between pond populations.

Finally, we identified all significant landscape elements (land uses and traffic related model factors) in a GLM (without interactions) by considering a 200 m wide strip between pond pairs. The best GLM was selected using the Akaike Information Criterion (AIC) as well as D^2 and adjusted D^2.

Results

Genetic differentiation

Pond populations within the Palatinate Forest showed significantly lower MPFs (< 0.017) than pond populations within vineyards in close proximity (e.g., LGD < 1 km: MPF = 0.0467; LGD < 2.5 km: MPF = 0.0987).

The highest MPF was estimated in vineyards between P1 and P2 at a LGD of 2.4 km. The lowest MPF was found between P3 (vineyards) and P7 (forest) at a LGD of 7.9 km. On average, genetic differentiation in vineyards (average MPF = 0.0523) was higher than both other groups (Fig. 2).

ISOLDE

Over all ponds, ISOLDE detected no statistically significant relation between MPFs and isolation by distance, number of roads and accumulated traffic intensity between population pairs ($p > 0.05$).

GLM

Out of all single factor models, land uses settlements and forests were the most relevant, followed by vineyards. The best model consisted of the model factors forest, settlements, vineyards, groves and the accumulated value of the product traffic intensity and length of respective road within the 200 m strip between ponds (all model factors statistically significant; AIC=-240.76; D^2=0.6; adjusted D^2=0.54).

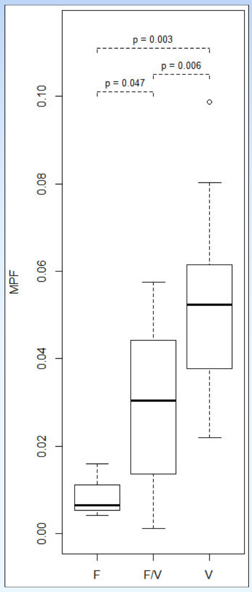


Fig. 2. - Boxplot of MPF groups (F = between forest pond populations (N=3), F/V = between forest and vineyards pond populations (N=18), V = between vineyards pond populations (N=15)) and their statistical significant differences (shown above bars) tested with Mann-Whitney-Wilcoxon Test.

Discussion

Our data exhibited higher genetic differentiation among agricultural pond populations than among forest pond populations and we demonstrated that the genetic differentiation of *R. temporaria* in agricultural areas can't be satisfactorily explained by visible barriers, i.e., LGD or traffic related variables on their own. However, in the best GLM viniculture was not the most significant model factor to explain the genetic differentiation within the study area.

As *R. temporaria* becomes sexually mature in the third (rarely second) year of life, about 25 to 40 overlapping generations have passed since the intensification of viniculture in the early 20th century started and habitats became progressively fragmented. Due to few passed generations, overall population differentiation is still low (MPF < 0.1) but may increase due to time-delay in genetic differentiation.

Yet, habitat fragmentation is not only caused by habitat loss but also by increased barrier effects of arable land due to pesticides use and mechanical soil cultivation[3]. Currently the magnitude of chemical fragmentation by pesticides can't be quantified but seems obvious since genetic differentiation outside of agricultural areas is remarkably lower, even over great distances (e. g. MPF < 0.041 at about 40 km).

Generally, the presence of groves between pond pairs decreased genetic differentiation (stepping stone habitats). Also, some MPFs between forest and vineyard pond populations were low (< 0.02), probably due to a one directional genetic exchange (e.g. drift of larvae within streams).

Conclusion

Based on our results we are concerned about the sustainability of amphibians in agricultural areas since we can clearly recognize negative trends on the genetic level.

- Agriculture has negative effects on amphibian population structure
- Pesticides are partially responsible
- Genetic differentiation may increase (time-delay)
- Chemical and physical fragmentation endanger survival of pond populations

We recommend more scientific effort to evaluate the potential toxicity of pesticides to ecologically relevant amphibian species. Also we ask for further research at the population level to improve our understanding how anthropogenic land use influences the amphibian life cycle and population dynamics.

References

1. Lenhardt, P. P., Brühl, C. A., & Berger, G. (2014). Temporal coincidence of amphibian migration and pesticide applications on arable fields in spring. *Basic and Applied Ecology*, *16*, 54–63.
2. Goldberg, C. S., & Waits, L. P. (2010). Quantification and reduction of bias from sampling larvae to infer population and landscape genetic structure. *Molecular ecology resources*, *10*, 304–13.
3. Lenhardt, P. P., Schäfer, R. B., Theissinger, K., & Brühl, C. a. (2013). An expert-based landscape permeability model for assessing the impact of agricultural management on amphibian migration. *Basic and Applied Ecology*, *14*, 442–451.

Contact details

Patrick P. Lenhardt, Email: lenhardt@uni-landau.de
Institute for Environmental Science , University of Koblenz Landau
Fortstr. 7, 76829 Landau, Germany