

Dispersal of *Triturus alpestris* and *T. vulgaris* in agricultural landscapes – comparing estimates from allozyme markers and capture-mark-recapture analysis

Peter Schmidt¹, Klaus Weddeling¹, Meike Thomas², Ruth Rottscheidt², David N. Tarkhishvili³, Monika Hachtel¹

Abstract. Demes of the two newt species *Triturus alpestris* and *T. vulgaris* occupying five water bodies in an agricultural landscape in western Germany were investigated with respect to their dispersal behaviour and population structure. We applied two different approaches to investigate dispersal and population structure: (1) mark-recapture methods for breeding adults and (2) F-statistics estimated from the outcome of allozyme electrophoresis. Both approaches yield similar results for the two species indicating low differentiation between the demes, suggesting either high level of gene flow caused by migration or a short time passed after deme separation. Mark-recapture-methods revealed a considerable number of migrants, indicating that low genetic differentiation probably is due to recent gene flow. The problems of data interpretation in cases of demes founded only a few years ago are discussed.

Introduction

Dispersal is a key factor for the survival of amphibian populations in agricultural landscapes (e.g. Cushman 2006). It strongly affects two crucial aspects of population ecology. First, dispersing animals usually contribute to gene flow between demes, preventing them from potential negative effects of inbreeding in isolated populations (overview in Whitlock, 2001). Second, migrating individuals may significantly affect the demography of populations and prevent the extinction of small demes (rescue effect, e.g. Stacey et al., 1997; Hanski, 2001; Trenham et al., 2001). Thus, estimates of the dispersal potential of organisms are of special interest for conservation biology because dispersal strongly affects survival probability of populations on different spatial scales.

Methodically, estimates of animal dispersal in a spatially limited study area can be obtained with two different approaches: So called direct methods use capture-mark-recapture-techniques (CMR) in the field to measure the portion of animals that disperse at a certain distance from the marking location (Gill, 1978, Perret et al., 2003). The biggest problem of this technique is, that recaptures are limited to a predefined study area, which may lead to biased results. Despite this approach is time and

cost expensive, it usually cannot distinguish between mortality and the proportion of animals that leave the study area (Fletcher et al., 2002, Koenig et al, 1997). Thus, this procedure systematically underestimates the proportion of dispersing animals, although recent developments of CMR-Statistics partly overcome such problems (Perret et al., 2003). On the other hand, not all dispersing animals contribute to gene flow, due to failure in successful breeding in the colonized demes (Jehle et al., 2001), which would lead to an overestimation of gene flow when using CMR data. One advantage of direct approaches is the possibility to identify the relevant stages in a life cycle, that contribute to dispersal (MacDonald and Smith, 1990). Nowadays, so called indirect methods are becoming more important for dispersal estimates. These approaches use genetic markers to estimate genetic differentiation between subpopulations (F_{st} and related measures) and are usually not as time and cost expensive as the direct methods. On the one hand the estimates usually are not biased by spatial limitations of study areas since they are not affected by non detected migrants or deme specific mortality (Fletcher et al., 2002). On the other hand these methods often cannot distinguish between the effects of recent and historical gene flow due to the low drift effects over short separation time between demes. Moreover, they usually cannot identify the dispersal relevant stage in the life cycle of an animal.

During a long-term monitoring of amphibian populations in an agricultural landscape in western Germany we compared the direct and indirect estimates of newt dispersal to get insights into the population structure and dispersal of two common pond breeding newt

1 Zoologisches Forschungsmuseum Alexander Koenig,
Adenauerallee 160, 53113 Bonn

2 Institute for Genetics, University of Cologne, Zùlpicher Straße 47, D-50674 Cologne

3 Georgian Center for Conservation of Wildlife, PO Box 56,
Tbilisi 0160, Georgia;
Email: peter_e_schmidt@yahoo.de

Table 1. Number of migrants, number of marked and recaptured newts differentiated by species and sex, sum of all ponds and years of survey.

species	sex	marked individuals	recaptures	migrants	Dispersal rate
<i>T. vulgaris</i>	male	5775	909	48	0.053
	female	7022	1229	56	0.046
<i>T. alpestris</i>	male	4868	3115	124	0.040
	female	4546	3457	169	0.049

species, *Triturus vulgaris* and *T. alpestris*. The study area comprised five ponds, two natural waters and three of artificial origin. The three artificial ponds have been created 12 years before genetic samples were taken. Newts represent a suitable model for comparing these approaches, because their local demes are spatially well defined in pond breeding communities.

Study area and methods

The study was carried out at five ponds situated in a typical agricultural landscape near Bonn in the western part of Germany (Fig. 1) situated at a edge of a forest. Ponds 3 and 5 are natural waterbodies, whereas ponds 1, 2 and 4 were artificially created in 1988. According to skeletochronological data, the average age of adult newts from the study area varies between 3–4 years (Rottscheidt 2002, Thomas 2002). The natural ponds represent ancestral breeding habitats, whereas in artificial ponds not more than four generations could pass after the first colonization events. Newts were captured in pitfalls at permanent drift fences encircling the breeding ponds from 2001 until 2003. Estimated census population sizes within study time in the ponds ranged from 126 to 1,874 individuals in alpine newt and 132 to 4,342 in smooth newt (Weddeling *et al.* 2004). Ponds were surrounded mainly by acres,

pastures and woodland (Fig. 1). The distances between the ponds ranged from 270 m to 1,800 m.

Adult newts were marked using toe-clipping with year specific group marking. We calculated the proportion of dispersing adult newts based on the total number of recaptures made as requested in Bowne and Bowers (2004) to prevent bias due to different capture effort and mortality. Dispersal of juveniles could not be tracked since it is not possible to mark them permanently due to tissue regeneration (Glandt, 1980).

In 2001, the tissue samples from toes and tail were taken in order to analyze allele frequencies in polymorphic allozyme loci as genetic marker. We sampled 250 adults and 182 juveniles of *T. vulgaris* as well as 300 adults and 184 juveniles of *T. alpestris*.

Tissues were kept in test tubes on ice during field work and afterwards stored at -80°C . We used vertical starch gel electrophoresis (STAGE) with Tris-Citrate TC 2 or Aminomorpholine-Citrat buffer (Hillis *et al.*, 1996). The staining protocols followed Hillis *et al.* (1996) with PMS and MLB as electron transmitter and MTT as dye. In both species, 18 allozyme loci were tested for polymorphism. 4 out of 18 tested allozyme loci (MDH, MPI, 6-PGDH, PGM) showed polymorphisms between the individuals in *T. vulgaris* whereas six loci in *T. alpestris* exhibited considerable variability (LDH, MDH, ME, MPI, 6-PGD, PGM). For both approaches, F_{st} was calculated and compared with the software ARLEQUIN3 (Excoffier *et al.*, 2005) and GENEPOP (Raymond and Rousset, 1995). We tested for isolation by distance using Mantel test software ISOLDE in GENEPOP.

Results

Direct approach

The analysis of deme specific marking showed an averaged dispersal rate of adult newts of 4.6 % for

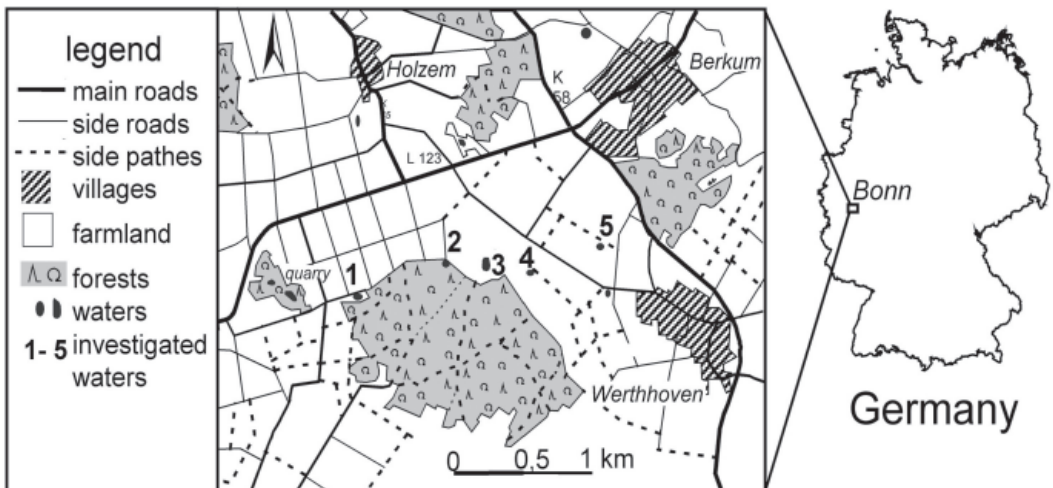
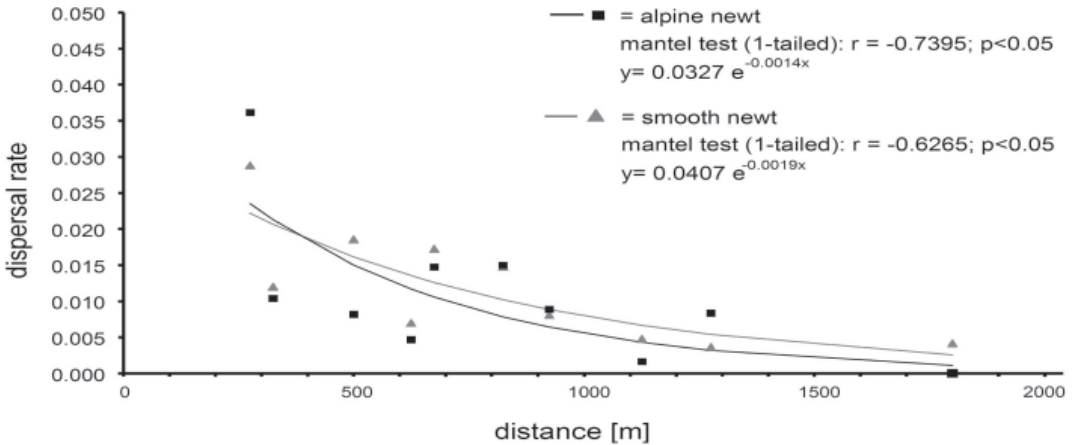


Figure 1. Map of the study area south of Bonn. The numbers mark the five investigated breeding ponds 1 to 5. Pond 3 and pond 5 are natural waterbodies, whereas the three remaining ones are artificially created in 1988.

Figure 2. Isolation by distance effect of dispersal rate in alpine newt and smooth newt within the study area (exponential function fitted), obtained from CMR data. Dispersal rate calculated as follows: no° of recaptures at different pond / no° of all recaptures with pond specific marking; sum of recaptures for both directions of migration (pond a towards b + pond b towards a)



all recaptures (Table 1), with no apparent differences between the species. Estimated pairwise adult migration rates strongly differ between ponds (Table 2). The local population size and geographical distance influences the number of migrants between demes. Mantel tests showed significant isolation by distance effects for adult newt dispersal (Fig. 2) with declining dispersal rates for increasing distances between the demes.

Wright's F_{st} was calculated from CMR data for the whole pond system (Berven and Grudzien, 1990) to correlate field data with results from the indirect approach. F_{st} values were calculated using Wright's equation $F_{st} \approx 1/(4Nm+1)$ and the harmonic mean of deme sizes (taken from Weddelling et al. 2004). Overall F_{st} estimates are low (*T. vulgaris*: $F_{st}=0.013$, *T. alpestris*: $F_{st}=0.016$), indicating low genetic differentiation between demes.

Indirect approach

Overall F_{st} values calculated from allozymes for the whole pond system were low (alpine newt $F_{st}=0.02$, smooth newt $F_{st}=0.014$). Pairwise estimates among the demes varied between 0.0006 and 0.0463 in *T. alpestris* and 0.0003 and 0.0613 in *T. vulgaris*. Most pairwise comparisons did not yield in F_{st} estimates significantly different from zero (Table 3), correspondingly the Mantel tests for isolation by distance were not performed. Remarkably, for both species the comparisons between the ponds 1 and 5 on the one hand and between ponds 3 and 5 on the other hand showed F_{st} values significantly different from zero, which represent the most distant

positioned respectively the natural ponds. Additionally, *T. alpestris* showed pairwise F_{st} values significantly different from zero between pond 2 and 4 on the one hand and between pond 4 and 5 on the other hand.

Discussion

The simultaneous application of the two approaches allows some conservative conclusions about recent and historical processes in the observed populations. The genetic analysis of the two newt species showed low differentiation between the five demes (overall F_{st} values for alpine newt $F_{st}=0.02$ and for smooth newt $F_{st}=0.014$). This result is consistent with our expectation given the small geographical distances between the demes and the recent origin of the artificial ponds. They have most likely been colonized by individuals from two natural ponds (pond 3 and 5) (Schaefer 1993). Via allele frequency based F_{st} values we are not able to discriminate between the influence of the recent colonization and actual gene flow between demes. As the separation time between the ponds is not longer than three to four generations we would not expect the subpopulations to show strong genetical differentiation even if no gene flow would have existed in the last 12 years. Thus, low differentiation between demes could be either a result of the short-time colonization history of the ponds or of recent gene flow. Whitlock and McCauley (1999) point out, that precondition for the usage of F_{st} estimates as a measure of dispersal is an equilibrium state of dispersal and drift.

Table 2. Migration rate of adult alpine newts and smooth newts (sum of migrating males and females from 2000-2003 calculated as proportion of all recaptures at the outer sides of the drift fences).

Species	destination pond	source pond				
		1	2	3	4	5
alpine newt	1	3395*	0.0199	0.0253	0.0243	0.0944
	2	0.0157	1990*	0.0572	0.0219	0.0708
	3	0.0046	0.0190	692*	0.0195	0.0708
	4	0.0014	0.0042	0.0080	378*	0.0708
	5	0.0011	0.0009	0.0053	0.0024	117*
smooth newt	1	344*	0.0069	0.0016	0.0043	0.0000
	2	0.0267	531*	0.0281	0.0028	0.0368
	3	0.0214	0.0450	589*	0.0128	0.2944
	4	0.0160	0.0069	0.0078	649*	0.1472
	5	0.0000	0.0000	0.0031	0.0028	25*

* sum of all recaptures at the outer sides of the drift fences of the source pond

The capture-mark-recapture data of adult newts detected migrants between all of the demes. A significant isolation by distance effect for these migrants could be shown, indicating that migration of adult newts in agricultural areas is possible, but even on small landscape scales (up to 2000 m between ponds) dispersal is limited due to landscape characteristics. But, since we were not able to measure natal dispersal of first breeding newts, we assume that this number might underestimate overall dispersal ability of the species (Gill 1978, Berven and Grudzien 1990).

The considerable number of migrants observed in the field supports the idea of gene flow, although since

nothing is known about breeding success of these individuals, the final proof remains open.

To our knowledge, there are only very few studies that use direct and indirect estimates of dispersal simultaneously for a single study area (e.g. Sumner *et al.*, 2001) and no such measures for amphibians. In situations where deme separation time lasts long enough, combination of genetic analysis and capture-mark-recapture investigations might be an interesting approach for further understanding of processes in amphibian population structures and dynamics since dispersal ability of these animals often might be underestimated (Smith and Green, 2005). Care should

	species	pond 1	pond 2	pond 3	pond 4
pond 2	<i>T.a.</i>	0.0037			
	<i>T.v.</i>	0.0042			
pond 3	<i>T.a.</i>	0.0006	- 0.0075		
	<i>T.v.</i>	0.0003	0.0028		
pond 4	<i>T.a.</i>	- 0.0022	0.0090*	0.0044	
	<i>T.v.</i>	0.0061	0.0047	0.0015	
pond 5	<i>T.a.</i>	0.0210*	0.0463	0.0427*	0.0284*
	<i>T.v.</i>	0.0613*	0.0201	0.0360*	0.0374

* sign. different from 0

Table 2. Pairwise F_{st} values for alpine newt (*T.a.*) and smooth newt (*T.v.*) between the five investigated ponds based on allele frequencies of investigated polymorphic allozyme loci.

be taken in cases of demes founded only a short time ago, since in case of low genetic differentiation it might be difficult, to draw conclusions on gene flow, even in cases where migrants can be directly tracked (Whitlock and McCauley 1999).

References

- Berven, K.A., Grudzien, T. (1990): Dispersal in the wood frog (*Rana sylvatica*): implication for genetic population structure. *Evolution* **44**(8): 2047-2056.
- Bowne, D.R., Bowers, M.R. (2004): Interpatch movements in spatially structured populations: a literature review. *Landscape Ecology* **19**: 1-20.
- Cushman, S.A. (2006): Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation* **128**(2): 231-240.
- Excoffier, L., Laval, G., Schneider, S. (2005): Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*.
- Fletcher D., Dawson S., Slooten E. (2002): Designing a mark-recapture study to allow for local emigration. *Journal of Agricultural, Biological and Environmental Statistics*, **7**(4): 586-593.
- Gill, D.E. (1978): Effective population size and interdemographic migration rates in a metapopulation of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). *Evolution*, **32**(4): 839-849.
- Glandt, D. (1980): Populationsökologische Untersuchungen an einheimischen Molchen, Gattung *Triturus* (Amphibia, Urodela) [= Herpetoskriptum **2**]. PhD Thesis, University of Münster, CD edition 2003, 191 p.
- Hanski, I. (2001): Population dynamic consequences of dispersal in local populations and in metapopulations. In: *Dispersal*, p. 283-298. Clobert, J., E. Danchin, A. Dhondt, Nichols, J., Oxford Univ. Press.
- Hillis, D.M., Moritz, C.M., Mable, B.K. (1996). *Molecular Systematics*. Sinauer Associates, 655 p.
- Jehle R., Arntzen J.W., Burke T., Krupa A.P., Hödl W. (2001): The annual number of breeding adults and the effective population size of syntopic newts (*Triturus cristatus*, *T. marmoratus*). *Molecular Ecology*, **10**(4): 839-850
- Koenig, W.D., Vuren, D. Van, Hooge, P.N. (1996): Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology and Evolution* **11**(12): 514-517.
- Macdonald, D. W., Smith, H. (1990): Dispersal, dispersion and conservation in the agricultural ecosystem. In: *Species dispersal in agricultural habitats*, p.18-64. Bunce, R.G.H., Howard, D.C., Behaven Press, London.
- Perret, N., Pradel, R., Miaud, C., Grolet, O., Joly, P. (2003): Transience, dispersal and survival rates in newt patchy populations. *Journal of Animal Ecology* **72**: 567-575.
- Raymond M., Rousset F. (1995): GENEPop (version 1.2): population genetics software for exact tests and ecumenism. *Journal of Heredity* **86**: 248-249
- Rottscheidt, R. (2002): Zur Populationsökologie des Bergmolches *Triturus alpestris* (LAURENTI, 1768) in der Agrarlandschaft des Drachenfelder Ländchens bei Bonn. Diploma thesis (unpubl.), University of Bonn, Germany, 117 pp.
- Schäfer, H.-J. (1993): Ausbreitung und Entwicklung von Amphibien-Populationen in der Agrarlandschaft. PhD Thesis, University of Bonn, 294 p.
- Smith M.A., Green D.A. (2005): Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**: 110-128.
- Stacey, P.B., Johnson, V.A., M.L. Taper (1997): Migration within Metapopulations – the impact upon local population dynamics. In: *Metapopulation Biology – Ecology, Genetics, and Evolution*, p. 267-291. Hanski, I., Gilpin, M.E., Acad. Press, London.
- Sumner J, Rousset F, Estoup A, Moritz C. (2001): “Neighbourhood” size, dispersal and density estimates in the prickly forest skink (*Gnypetoscincus queenslandiae*) using individual genetic and demographic methods. *Molecular Ecology*, **10**(8):1917-1927.
- Thomas, M. (2002): Populationsstruktur und -dynamik von *Triturus vulgaris* (LINNAEUS 1758) Fekundität – Demographie – Genetik. Diploma thesis (unpubl.), University of Bonn, Germany, 182 pp.
- Trenham, P.C., Koenig, W., Shaffer, H.B. (2001): Spatially autocorrelated demography and interpond dispersal in the salamander *Ambystoma californiense*. *Ecology* **82**(12): 3519-3530.
- Weddelling, K., Hachtel, M., Sander, U., Tarkhnishvili, D.N. (2004): Bias in estimation of newt population size: a field study at five ponds using drift fences, pitfalls and funnel traps. *Herpetological Journal* **14** (1): 17-23.
- Whitlock, M.C. (2001): Dispersal and the genetic properties of metapopulations. In: *Dispersal*, p. 273-282. Clobert, J., Danchin, E., Dhondt, A, Nichols, J., Oxford Univ. Press.
- Whitlock, M.C., McCauley, D.E. (1999): Indirect measures of gene flow and migration: $F_{st} \neq 1/(4Nm+1)$. *Heredity* **82**: 117-125.