

# Contemporary gene flow and the spatio-temporal genetic structure of subdivided newt populations (*Triturus cristatus*, *T. marmoratus*)

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## Abstract

Gene flow and drift shape the distribution of neutral genetic diversity in metapopulations, but their local rates are difficult to quantify. To identify gene flow between demes as distinct from individual migration, we present a modified Bayesian method to genetically test for descendants between an immigrant and a resident in a nonmigratory life stage. Applied to a metapopulation of pond-breeding European newts (*Triturus cristatus*, *T. marmoratus*) in western France, the evidence for gene flow was usually asymmetric and, for demes of known census size ( $N$ ), translated into maximally seven reproducing immigrants. Temporal sampling also enabled the joint estimation of the effective demic population size ( $N_e$ ) and the immigration rate  $m$  (including nonreproductive individuals).  $N_e$  ranged between 4.1 and 19.3 individuals,  $N_e/N$  ranged between 0.05 and 0.65 and always decreased with  $N$ ;  $m$  was estimated as 0.19–0.63, and was possibly biased upwards. We discuss how genotypic data can reveal fine-scale demographic processes with important microevolutionary implications.

## Introduction

The patchy occurrence of individuals in space and time forms the basis for the metapopulation concept, which has been widely applied to free-living organisms (Hanski & Gaggiotti, 2004). Metapopulation properties are important for determining the standing amount of neutral genetic variation, and thus have wide consequences for microevolutionary processes (reviewed in Pannell & Charlesworth, 2000). Early population genetic models for subdivided populations assumed stable demographic structures, and predicted that fixation rates and the amount of genetic drift will be lower under subdivision than for a single panmictic deme (Wright, 1969). A high variance in demic reproductive output resulting from source-sink and extinction-recolonization metapopulation dynamics, on the other hand, leads to a potentially drastic loss of genetic diversity (Whitlock & Barton, 1997).

The evolutionary importance of metapopulation processes has been further underlined by empirical investigations, which have demonstrated direct consequences for demic extinction rates and individual fitness-associated traits (e.g. Saccheri *et al.*, 1998; Haag *et al.*, 2002).

Seminal metapopulation studies have confirmed that the distribution of genetic variation is related to demographic extinctions and colonization events mostly as predicted by theory (Whitlock, 1992; Giles & Goudet, 1997; McCauley *et al.*, 2001). However, when looking at persistent demes without explicit turnover (a situation increasingly incorporated in the metapopulation framework, Hanski & Gaggiotti, 2004), differences in their allele frequencies are of limited value towards disentangling historic events, such as colonization history, from current dispersal (Rousset, 2001). Direct approaches, such as capture–mark–recapture (CMR) techniques, can reveal important information on population census size ( $N$ ) and migration rates (Bennetts *et al.*, 2001), but are spatially restricted and extraordinarily labour-intensive. Moreover, they are usually unable to distinguish between (i) individual movement and gene flow, or (ii) census and effective population size ( $N_e$ ). Only gene

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flow and effective population size have direct consequences for evolutionary processes.

Highly polymorphic genetic markers now allow the straightforward generation of individual multilocus genotypes, and maximum likelihood and Bayesian approaches provide a more flexible framework than traditional frequentist statistics (Beaumont & Rannala, 2004). As a result, genetic equilibrium assumptions associated with methods such as *F*-statistics can be relaxed to draw more direct inferences, and several parameters of interest can be measured simultaneously. Coalescent-based approaches usually consider events at least several generations back in a pedigree (e.g. Beerli & Felsenstein, 2001), whereas assignment methods use current genotypic disequilibria to delineate clusters of individuals, and to identify migrants and admixed genotypes (reviewed in Manel *et al.*, 2003). Assignment methods can focus on recent phenomena, and are therefore particularly promising for studying fine-scale metapopulation processes (Charbonnel *et al.*, 2002; Gaggiotti *et al.*, 2002; Gaggiotti, 2004).

Temperate pond-breeding amphibians congregate for several days or weeks to reproduce in confined aquatic sites, spending the remainder of the year on land which results in the dispersal of some individuals. As ponds are effectively islands to which most young return to breed, amphibians are particularly deme-structured organisms (e.g. Jehle *et al.*, in press). Their spatial genetic organization at the landscape scale is largely described by isolation by distance and, to some degree, by habitat properties (Hitchings & Beebee, 1998; Rowe *et al.*, 2000; Scribner *et al.*, 2001; Vos *et al.*, 2001). In western France, the two closely-related urodele species *Triturus cristatus* (the crested newt) and *T. marmoratus* (the marbled newt) locally occupy a particularly dense network of ponds, which enabled a rapid local range expansion of *T. cristatus* in the recent past (<50 years, Arntzen & Wallis, 1991). Moreover, given that the effective number of breeders has been estimated as usually below 100 individuals per pond (Jehle *et al.*, 2001), the amount of standing genetic variation in persisting demes can only have been maintained by occasional gene flow.

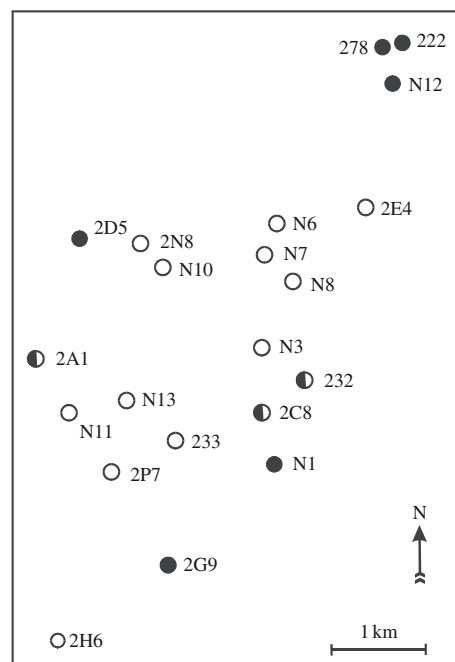
In the present paper, we focus on 21 ponds (demes) spaced at distances that could be covered by individual migrations (roughly 1 km, Arntzen & Wallis, 1991; Jehle *et al.*, in press), containing a total of 24 populations for the two species. The aims of our study are to employ microsatellite DNA markers to (i) quantify recent gene flow between demes with known *N*, and (ii) determine *N<sub>e</sub>* and *N<sub>e</sub>/N* in potentially open populations by jointly estimating drift and immigration using temporally successive genetic samples. To achieve (i), we sample a non-migratory life stage and modify the Bayesian approach presented in Wilson & Rannala (2003) to test for the presence of offspring from matings between immigrants and residents. The joint consideration of two syntopic species with subtly differing life-histories (Arntzen &

Hedlund, 1990; Jehle & Arntzen, 2000) should provide a deeper understanding of underlying demographic processes than focusing on one species alone (Amarasekare & Nisbet, 2001).

## Materials and methods

### Field work and census size estimates

The 21 study ponds are located in an area of approximately 7.5 × 3.5 km in the Département Mayenne, western France, bordered by the forests Forêt du Bourgon (west) and Bois d'Hermet (east), the village of Jublains (north), and an area with rather few ponds in the south. Most ponds are man-made cattle ponds. The analysis is based on 15 *T. cristatus* and nine *T. marmoratus* populations (Fig. 1). Between-pond geographic distances were measured to the nearest mm on topographical maps (1 : 25000), and ranged between 400 and 5875 m (average: 2270 m) in *T. cristatus*, and between 175 and 6425 m (average: 3442 m) in *T. marmoratus*. Nearest-neighbour distances did not exceed 2575 and 2000 m in *T. cristatus* and *T. marmoratus*, respectively. Tissue samples for microsatellite genotyping were obtained in 2001 and 2002, from embryos raised in water-filled containers until late egg stages or hatching, before sacrifice and storage in 96% ethanol. For six populations, additional temporal samples were collected between 1986 and



**Fig. 1** Distribution of study ponds. Open circles: *Triturus cristatus* populations; filled circles: *T. marmoratus* populations. See Table 1 for sample and demic census sizes.

1998, obtained from sacrificed or gill-clipped larvae, or toe clips from adults (Jehle *et al.*, 2001, Table 1). In Pond 2A1, several *T. cristatus* individuals from outside the present study area were introduced in 1994 (J.W. Arntzen, unpublished data), and consequently the data for this population were not fully interpretable.

For seven *T. cristatus* and four *T. marmoratus* populations, it was possible to obtain adult census sizes ( $N$ ) using a CMR approach (Begon's weighted mean, Begon, 1979), with group marks through clipping a single toe. We obtained all estimates in 2003, except for Pond 232 for which an estimate from 1998 was available (Jehle *et al.*, 2001). When only a small number of adults were present, CMR proved impractical. In such cases, we reconstructed the most likely number of parents from offspring genotypes using a Markov Chain Monte Carlo (MCMC)-based Bayesian method (Emery *et al.*, 2001). We chose the Ewens sampling formula (Ewens, 1972),

giving the joint distribution of the number of males and females contributing to the parentage share based on the parameter  $\alpha$  (for more detail see Emery *et al.*, 2001). The priors of  $\alpha$ , a gamma distribution with scale and shape parameters of 1 and 20, respectively (as paternal and maternal genotypes were indiscernible we chose equivalent priors for both sexes), were specified for the MCMC outcome to match CMR estimates from ponds 2P7 and N8, where enough individuals could be captured despite small census sizes. We approximated the number of parents by sampling 500 values taken every 400 iterations after  $2 \times 10^6$  burn-ins. The estimated numbers of fathers and mothers were summed to obtain  $N$ , and 2.5 and 97.5% quantiles were calculated. For large populations (also assessed by the large numbers of eggs found), this approach produced inconsistent results and/or results inconsistent with CMR estimates (data not shown). Therefore, no census size estimates

**Table 1** Details of study ponds and populations.

Pond	Species	Samples (2001, 2002)	Samples before 1999	$N$	$H_e$	$H_o$	HW disequilibria
2A1	<i>c</i>	17	12 (1997)	40.2 ± 10.1	0.61	0.59	0
2C8	<i>c</i>	11	–	5 (4–7)†	0.57	0.53	0
2E4	<i>c</i>	35	–	6 (5–8)†	0.60	0.58	0
2H6	<i>c</i>	60	–	–	0.63	0.67	0
2N8	<i>c</i>	78	–	28.2 ± 10.7	0.60	0.54	0
2P7	<i>c</i>	45	–	3.6 ± 1.6, 6 (4–9)†	0.62	0.64	0
232	<i>c</i>	48	8 (1989); 35*, 40 (1998)	72.5 ± 18.8	0.60	0.58	0
233	<i>c</i>	27	–	4 (3–6)†	0.49	0.49	0
N3	<i>c</i>	60	–	8 (6–9)†	0.55	0.52	0
N6	<i>c</i>	78	–	46.3 ± 19.0	0.62	0.55	0
N7	<i>c</i>	117	–	94.6 ± 19.7	0.59	0.57	0
N8	<i>c</i>	48	–	6.4 ± 3.7, 5 (4–7)†	0.55	0.50	0
N10	<i>c</i>	45	–	11 (8–14)†	0.62	0.51	<i>Tcri35</i>
N11	<i>c</i>	66	–	–	0.65	0.53	<i>Tcri13, Tcri29, Tcri35, Tcri43</i>
N13	<i>c</i>	36	–	–	0.56	0.52	0
2A1	<i>m</i>	18	–	3 (2–5)†	0.46	0.46	0
2C8	<i>m</i>	10	11 (1998)	8 (6–9)†	0.50	0.47	0
2D5	<i>m</i>	78	–	145.8 ± 25.4	0.42	0.37	<i>Tcri43</i>
2G9	<i>m</i>	30	42 (1997)	–	0.53	0.39	0
222	<i>m</i>	89	–	194.7 ± 54.1	0.55	0.38	<i>Tcri43</i>
232	<i>m</i>	50	23 (1989); 66*, 79 (1998)	147.4 ± 28.4	0.36	0.36	<i>Tcri27</i>
278	<i>m</i>	36	90 (1986); 43* (1998)	220.7 ± 35.4	0.39	0.37	0
N1	<i>m</i>	29	–	–	0.50	0.34	<i>Tcri27</i>
N12	<i>m</i>	8	–	2 (1–3)†	0.20	0.15	0

*c*, *Triturus cristatus*, *m*, *T. marmoratus*.  $N$ , population census size;  $H_e$ , expected heterozygosity;  $H_o$ , observed heterozygosity; HW disequilibria: loci out of Hardy–Weinberg equilibrium, excluding locus *Tcri42* which showed a consistent deficit of heterozygotes.

$H_e$ ,  $H_o$ , and disequilibria are reported for the pooled 2001 and 2002 samples only.

\*Tissue samples obtained from adults, all others were obtained from embryos and larvae.

†Most likely posterior number of parental genotypes producing the sampled offspring, assessed using the approach of Emery *et al.* (2001). Values in brackets denote 2.5 and 97.5% quantiles. All other: capture–mark–recapture estimates ± SE.

For five demes no size data were available.

are available for four medium-sized or large populations (Table 1).

### Laboratory procedures

Microsatellite genotypes were largely obtained using the PCR primers and protocols described in Krupa *et al.* (2002). Eight loci (*Tcri13*, *Tcri27*, *Tcri29*, *Tcri32*, *Tcri35*, *Tcri36*, *Tcri42*, *Tcri43*) were used in *T. cristatus*, five of which (*Tcri27*, *Tcri32*, *Tcri35*, *Tcri42*, *Tcri43*) were also used in *T. marmoratus*. For two loci, primers differing from Krupa *et al.* (2002) were used [*Tcri32*: f: GGCTCCCACACTGAGAAACT, r: TCAGTGAGTCTGACATCGGC, yielding PCR products of around 490 bp in *T. cristatus* and around 175 bp in *T. marmoratus*; *Tcri43* (corrected from Krupa *et al.*, 2002: see <http://www.shef.ac.uk/misc/groups/molecol/newts.html>): f: GAAGTAACTGAAAGATAACATGTAG, r: GTTTCTATTCAATTTTGTACGCAC]. The performance of locus *Tcri29* was significantly improved by adding 1% Bovine Serum Albumin to the PCR reaction. Primers were labelled with fluorochromes TET, HEX, and FAM; PCR products were run on an ABI 377 sequencer, and analysed using the softwares ABI GeneScan and ABI Genotyper (Applied Biosystems, Foster City, CA, USA). Individuals for which more than two (*T. cristatus*) or more than one (*T. marmoratus*) loci failed to amplify were discarded. The two study species are morphologically indiscernible at the embryo stage, and instead were distinguished using loci *Tcri32* (see above) and *Tcri36* (polymorphic bands around 275 bp in *T. cristatus*, a locally monomorphic band at 242 bp in *T. marmoratus*), as well as *Tcri14* scored on agarose gels (EMBL accession number AJ292504, the primer pair f: GGTGGACTGTATCAACCAGT, r: TCTTAGCTCGATAAGT GTTGAAG produces an approximately 320-bp PCR product diagnostic for *T. cristatus* and an approximately 180-bp band diagnostic for *T. marmoratus*). Putative hybrids (about 4% of individuals, Arntzen & Wallis, 1991) and individuals with putatively introgressed species-diagnostic alleles bias the calculations and were excluded from the analysis. As in a previous study (Jehle *et al.*, 2001), locus *Tcri42* showed a consistent excess of apparent homozygotes, probably because of null alleles, and was, therefore, omitted from all analyses requiring Hardy–Weinberg equilibrium, and for the gene flow analysis (see below).

### Basic genetic analysis

Observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, and departures from Hardy–Weinberg equilibrium at each locus and population were computed with Genepop 3.1d, using the implemented Markov Chain method ( $10^6$  runs) to obtain unbiased estimates of Fisher's exact tests (Raymond & Rousset, 1995). Spatial genetic differentiation between ponds was described using pair-wise  $F_{st}$ , tested for significance as implemented in Fstat (Goudet,

1995) with Bonferroni corrections to give table-wide significance levels of  $P = 0.05$ . Isolation-by-distance scenarios were tested using Mantel tests to correlate  $F_{st}$  and log-transformed geographic distances, based on the test statistics  $Z$  for matrix correlations as implemented in the software IBD (Bohonak, 2002).

We addressed the question of whether samples combined from two consecutive years can be pooled using a Bayesian clustering approach described in Pritchard *et al.* (2000). The approach assigns individual genotypes to a predefined number of clusters ( $K$ ) in a given sample ( $X$ ), in order to achieve Hardy–Weinberg and linkage equilibrium. We estimated the  $\ln$  posterior probabilities for  $K = 1$  (implying one gene pool) or  $K = 2$  (implying that the sampling years 2001 and 2002 represent different gene pools), followed by calculating  $P(K|X)$  using Bayes' rule. Results were obtained from  $10^6$  runs after  $10^5$  burn-ins, using the correlated frequency model as implemented in the software Structure 2.0. As in our gene flow analysis (see below), this approach uses individual genotypes as analysis units, whereas for example an estimation of  $F_{st}$  values between sampling years would be based on allele frequencies pooled for each sample.

### Recent gene flow

To measure recent gene flow between demes without considering nonreproductive immigrants, we modified a Bayesian method presented in Wilson & Rannala (2003). The original method assumes that first-generation migrants can be sampled, which will not occur with the sampling scheme described herein. Therefore, the method was modified to specifically test for the presence of offspring between a migrant and a resident, and to estimate migration rates from these estimates. Details are presented in Appendix 1, and the executing C++ program BayesAssNM is available at <http://www.rannala.org>.

For the analysis of the present paper, we ran a total of  $3 \times 10^6$  MCMC iterations, discarding the first  $10^6$  iterations as burn-in. For demes for which admixture proportions were above 0.05, we estimated the number of reproducing immigrants by dividing the admixture proportion by their demic census size (when available).

### Joint estimation of immigration rate and effective population size

We also simultaneously calculated effective demic sizes ( $N_e$ ) and per-generation immigration rates ( $m$ ) for the six demes for which temporal samples were available, using a maximum likelihood approach described in Wang & Whitlock (2003). This approach assumes that the allele frequencies of a focal population change temporally at random when isolated from a potential source of immigration, whereas allele frequencies converge to the source when immigration occurs. We defined the source by pooling samples from all ponds collected in 2001 and

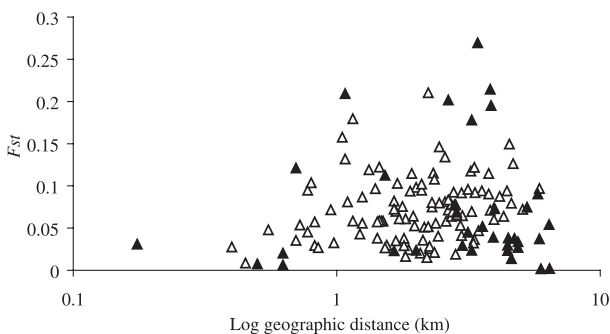
2002, except the population whose  $N_e$  was to be estimated, applying the large source model (Wang & Whitlock, 2003). Generation times were assumed to be 4.3 years for *T. cristatus* and 6.0 years for *T. marmoratus* (Jehle *et al.*, 2001). As suggested, sampling intervals ( $T$ ) were rounded to the nearest integer ( $t$ ) for the maximum likelihood calculations, and obtained  $N_e'$  was subsequently converted by  $N_e = (T/t) \times N_e'$ , and  $m'$  by  $m = 1 - e^{-(t/T) \log(1-m')}$ .

## Results

Adult demic census sizes ranged over two orders of magnitude, from one breeding pair to about 220 individuals (Table 1). In total, 771 *T. cristatus* and 702 *T. marmoratus* genotypes were analysed. The Bayesian reconstruction of parental genotypes suggested a most likely  $N$  of up to 11 individuals in ponds where this method was applied. Observed heterozygosity was usually slightly lower than expected, however significant deficiencies across more than one locus were found only in Pond N11 (*T. cristatus*, Table 1).  $P$  (1 $X$ ) ranged between 0.89 and 1.00 across all demes and species, providing no evidence for temporal within-pond substructure due to the two sampling years; several runs for each  $K$  confirmed that the Markov chains converged (data not shown). Overall  $F_{st}$  values across all populations were 0.07 and 0.11 in *T. cristatus* and *T. marmoratus*, respectively. Pairwise  $F_{st}$  values ranged from 0.012 to 0.183 in *T. cristatus* (71% of comparisons being significant), and from 0.007 to 0.303 in *T. marmoratus* (38% of comparisons being significant). Significant isolation by distance was observed across *T. cristatus* populations ( $Z = 14.60$ ,  $P < 0.05$ ), and not in *T. marmoratus* ( $Z = 10201.50$ , n.s., Fig. 2).

### Recent gene flow

In *T. cristatus*, seven (47%) demes appear to be isolated over the last generation. The proportion of individuals



**Fig. 2** Relationship between log-transformed geographic and genetic distances across *Triturus cristatus* (open symbols) and *T. marmoratus* (filled symbols) populations. Mantel tests reveal a significant correlation in *T. cristatus* only (for details see text).

considered to originate from the focal population was  $>0.9$ , and mean admixture proportions with other demes were between 0.00 and 0.02 (Table 2). Five (33%) demes had admixture rates of  $>0.05$  with one other deme, translating into an estimated recent immigration of less than one reproducing individual from Pond 2N8 to Pond 2P7 (2975 m distance) and Pond N10 (400 m), and from Pond N8 to Pond 2E4 (1325 m). Seven reproducing individuals dispersed from Pond N7 to the nearby Pond N6 (450 m), and six individuals moved from Pond N8 to the neighbouring Pond N7 (550 m). Three demes (2C8, 2A1 and N13) had admixture rates between 0.01 and 0.04 distributed across all other ponds (Table 2). For ponds 2A1 and 2C8, this is probably a consequence of small sample size, making more precise estimates of dispersal rates difficult.

In *T. marmoratus*, the analysis proved less informative (Table 3). Nonmigration rates were rather uniformly distributed between 0.74 and 0.94, and admixture rates between ponds revealed a flat distribution around 0.02. The strongest evidence for a recent migrant (0.40 individuals) was achieved from Pond 2C8 to the nearby Pond 232 (700 m).

### Effective population size and immigration rates

The effective size of single demes was always a subset of its census size (Table 4). Values ranged between 5.25 and 13.80 individuals assuming isolation, and between 4.11 and 19.03 individuals (78–151% of the value assuming isolation) when allowing for immigration. Estimated immigration rates ranged between 0.19 and 0.63. For the five populations for which census sizes were available,  $N_e/N$  ratios ranged from 0.07 to 0.51 (assuming isolation), or 0.05 to 0.65 (allowing for immigration). Regardless of assuming close or open populations,  $N_e/N$  always increased with decreasing  $N$  (Fig. 3).

## Discussion

A knowledge of migration rates is vital to the understanding of demographic processes in metapopulations, but the voluminous theory is far from being matched with empirical data, even for the most intensively studied taxa (Bowne & Bowers, 2004). Moreover, CMR studies can, for example, correlate inter-demic migration rates with population census sizes (Kuussaari *et al.*, 1996; Doncaster *et al.*, 1997), but, when the reproductive success of migrants is unknown, such demographic estimates only approximate the evolutionary consequences of connectivity. In this study, we present a Bayesian genetic approach to test for the presence of offspring between migrants and residents. The method is applicable whenever certain age classes can be sampled that cannot be migrants. When adult census sizes are available, this method also allows an approximation of the absolute number of immigrants from a most likely

**Table 2** Posterior distributions of admixture rates among *T. cristatus* populations.

	233	232	2A1	2C8	2E4	2H6	2N8	2P7	N10	N11	N13	N3	N6	N7	N8
233	<b>0.98</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
232	0.00	<b>0.93</b>	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.02	0.00	0.00	0.00
2A1	0.04	0.04	<b>0.70</b>	0.01	0.01	0.01	0.04	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01
2C8	0.02	0.02	0.02	<b>0.71</b>	0.01	0.02	0.03	0.01	0.02	0.02	0.02	0.02	0.03	0.04	0.02
2E4	0.02	0.01	0.01	0.01	<b>0.70</b>	0.01	0.04	0.01	0.03	0.01	0.01	0.02	0.03	0.04	<b>0.06/0.36</b>
2H6	0.00	0.00	0.00	0.00	0.00	<b>0.97</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2N8	0.00	0.00	0.00	0.00	0.00	0.00	<b>0.98</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2P7	0.00	0.01	0.00	0.01	0.00	0.00	<b>0.22/0.79</b>	<b>0.68</b>	0.00	0.01	0.00	0.00	0.01	0.01	0.01
N10	0.01	0.02	0.01	0.00	0.01	0.01	<b>0.07/0.77</b>	0.00	<b>0.78</b>	0.01	0.01	0.01	0.01	0.02	0.04
N11	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	<b>0.94</b>	0.00	0.00	0.00	0.00	0.00
N13	0.02	0.03	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.02	<b>0.80</b>	0.02	0.01	0.02	0.01
N3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	<b>0.97</b>	0.00	0.00	0.00
N6	0.01	0.01	0.00	0.00	0.00	0.01	0.03	0.00	0.01	0.01	0.00	0.02	<b>0.73</b>	<b>0.15/6.95</b>	0.02
N7	0.01	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.02	<b>0.86</b>	<b>0.06/5.68</b>
N8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	<b>0.97</b>

Admixture rates were estimated using modified prior parameter specifications for the Bayesian approach presented in Wilson & Rannala (2003).

The focal populations are listed in the rows, and the source populations are listed in the columns. Values with an SD >0.05 are in italics, and migration rates >0.05 are in bold, with the absolute number of migrants as calculated on the basis of census size added after the solidus.

source. We applied the approach to metapopulations of European newts, and found that gene flow is frequently unidirectional and largely influenced by a combination of

geographic distance and demographic properties. Using additional temporal samples, we also present findings from a joint estimation of demic effective population sizes and immigration rates. The implications of the results are discussed in detail below.

**Table 3** Posterior distributions of admixture rates among *T. marmoratus* populations.

b.	222	278	232	2A1	2C8	2D5	2G9	N1	N12
222	<b>0.94</b>	0.02	0.00	0.01	0.00	0.01	0.01	0.01	0.01
278	0.02	<b>0.88</b>	0.02	0.01	0.01	<i>0.04</i>	0.01	0.01	0.01
232	0.01	0.01	<b>0.93</b>	0.01	0.00	0.02	0.01	0.01	0.01
2A1	0.02	<b>0.05/0.15</b>	0.01	<b>0.83</b>	0.01	0.02	0.02	0.02	0.01
2C8	0.03	0.03	<b>0.05/0.40</b>	0.03	<b>0.74</b>	0.03	0.03	0.03	0.02
2D5	0.01	0.03	0.02	0.01	0.01	<b>0.89</b>	0.01	0.01	0.01
2G9	0.03	0.03	0.02	0.02	0.01	0.04	<b>0.82</b>	0.02	0.02
N1	0.04	<b>0.05/-</b>	0.02	0.03	0.02	0.03	0.02	<b>0.79</b>	0.01
N12	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	<b>0.82</b>

Values with an SD >0.05 are in italics, and migration rates >0.05 are in bold, with the absolute number of migrants as calculated on the basis of census size added after the solidus.

**Gene flow**

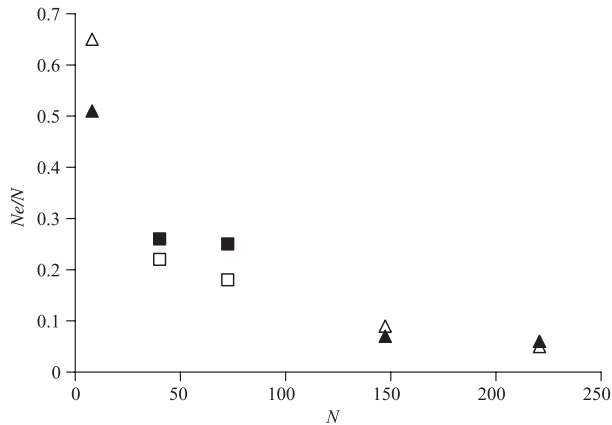
The developed methodology allows for deviations from Hardy–Weinberg equilibrium which arise from such factors as the absence of random mating (see also Wilson & Rannala, 2003). However, locus *Tcri42*, which exhibited a homozygote excess probably due to the presence of null alleles, was excluded from the analysis as no information about the alleles that were not visualized was available. Deviations from equilibrium for other loci in a few of the study ponds (see Table 1) could be due to low-frequency null alleles (such as in *T. marmoratus*, a species for which the primers were not originally designed), or due to demographic causes (such as for

Pond	Species	Samples	Generations	$N_{eo}$ (95% CI)	$m$ (95% CI)	$N_{ei}$ (95% CI)
232	<i>c</i>	3	3.02	13.41 (10.20–17.47)	0.37 (0.25–0.56)	19.03 (13.57–28.99)
232	<i>m</i>	3	2.17	13.80 (11.27–16.99)	0.19 (0.13–0.25)	13.38 (11.09–16.34)
278	<i>m</i>	3	2.50	10.43 (8.71–13.11)	0.23 (0.15–0.32)	13.42 (10.74–16.46)
2G9	<i>m</i>	2	0.83	11.55 (7.56–22.53)	0.58 (0.36–0.84)	17.47 (10.53–44.24)
2A1	<i>c</i>	2	1.63	9.03 (6.53–13.40)	0.63 (0.31–1.00)	10.61 (7.63–16.62)
2C8	<i>m</i>	2	0.67	5.25 (2.57–36.40)	0.59 (0.17–1.00)	4.11 (2.54–9.83)

*c*, *Triturus cristatus*, *m*, *T. marmoratus*;  $N_{eo}$ , effective population size assuming open populations;  $N_{ei}$ , effective population size assuming isolated populations.

Samples: number of temporal samples taken; generations: number of generations between first and last temporal sample.

**Table 4** Sampling specifications, effective population sizes ( $N_e$ ) and migration rates ( $m$ ) for five populations estimated after Wang & Whitlock (2003).



**Fig. 3** Relationship between  $N$  and  $N_e/N$ . Filled symbols denote  $N_e$  estimates assuming closed populations, open symbols denote estimates allowing for immigration (following Wang & Whitlock, 2003). Squares: *Triturus cristatus*; triangles: *T. marmoratus*.

*T. cristatus* in Pond N11: the population remained undetected in earlier surveys and might be the result of a recent colonisation). That the analysis was less informative in *T. marmoratus* is likely due to the lower number of loci, as average per-pond sample sizes and measures of between-population differentiation were higher than in *T. cristatus*. For guidelines to obtain informative outcomes for gene flow measures based on our method see the examples provided in Wilson & Rannala (2003). The non-migration rates of the *T. cristatus* population in Pond 2P7 was close to the constrained minimum of 0.67, implying that the actual value could be lower than that estimated here. The deme consists of only around five animals and, for example, a single immigrant could have accounted for a large proportion of offspring. If so, the most likely sampled source is 2N8, despite the distance separating these ponds. The probability that eggs could be passively transported between ponds, e.g. via waterfowl, can be ruled out as they were collected when attached to sedentary plants.

As in other measures of gene flow, our method of estimating the number of reproductively successful immigrants only provides an indication of the true figure. Some confidence intervals of admixture rates were high (particularly in *T. marmoratus*); moreover, individuals do not contribute equally to the next generation. Calculating the number of reproducing immigrants also requires equal reproductive success between immigrants and residents. Given the lack of marked differences in heterozygosities and allele frequencies, it seems unlikely that immigrants possess a higher fitness than residents through heterosis (as observed in Jones *et al.*, 1995; Haag *et al.*, 2002). However, no information is available on other factors that might influence reproductive success, such as familiarity with the local habitat.

This study confirms that dispersal usually takes place between neighbouring demes. However, over the contemporary timescale we looked at, not all nearby ponds exchanged individuals. The reason for this could be that migration is temporally irregular, related to pond density and quality, or that terrestrial habitats are of differential quality for migration. Among the most interesting results is the frequent occurrence of asymmetric gene flow. In our case, populations with high immigration proportions were also usually those which are rather small, and where the addition of a few animals can have a large effect on the observed genotypes (such as Ponds 2P7, 2E4). Using experimental populations with diagnostic loci, net gene flow from large to small demes has been reported previously, but only for certain interdemetic distances (in the plant *Silene*: Richards *et al.*, 1999), or only in males (small mammals: Aars & Ims, 2000). However, our approach also resulted in a large estimate of the number of migrants from a small to a large deme on the basis of only a modest amount of gene flow, such as the transfer from Pond N8 to Pond N7. Given the wide confidence limits associated with our method, such estimates might easily become too large. Source populations associated with better quality habitats can supply their demographic excess to sink populations, where mortality exceeds natality, and which may not persist without immigration (Pulliam, 1988; Dias, 1996). With the current data, we are however unable to specify whether populations receiving immigrants represent sinks or pseudosinks (cf. Watkinson & Sutherland, 1995). The frequent observation of asymmetric migration gives rise to the conclusion that, at least for our narrow spatio-temporal study window, demographic properties play a more important role in shaping patterns of gene flow than terrestrial habitat characteristics (un)favourable for migration (as studied in Hitchings & Beebe, 1998; Rowe *et al.*, 2000; Scribner *et al.*, 2001; Vos *et al.*, 2001).

Isolation by distance may occur among equilibrium populations with limited gene flow, or during range expansions following colonization in a stepwise fashion (Castric & Bernatchez, 2003). The isolation-by-distance scenario in *T. cristatus* is probably connected with the colonization of the study area over the post-war period (Arntzen & Wallis, 1991). In *T. marmoratus*, the invasion of *T. cristatus* has led to increased demographic fluctuations (J.W. Arntzen, unpublished data). Associated bottlenecks and non-equilibrium dynamics might explain the lack of significant isolation by distance. Alternatively, the statistical power could have been insufficient because of the small number of populations and loci.

### Census and effective population size

The reconstruction of parental genotypes offers an alternative to CMR-based census size estimates when  $N$  is small. As the parentage model only considers reproducing adults, biologically unrealistic priors are required

to compensate for reproductive failures. However, population-wide data on reproductive success do not exist for the genus *Triturus*, or any other aquatically breeding urodele. Also, this study revealed that  $N_e/N$  ratios were high when  $N$  was low, suggesting that most individuals reproduce under such circumstances.

Due to a longer study period, the availability of more than two temporal samples and the different analytical method, the  $N_e$  values obtained herein are not fully comparable with the results from Jehle *et al.* (2001). However, remarkably similar figures were obtained for the two reconsidered Ponds 232 and 278 (identical with Ponds 1 and 2 in Jehle *et al.*, 2001), for which  $N_e$  values were previously estimated as 12.2 (*T. cristatus*) and 13.4 (*T. marmoratus*), as well as 9.6 (*T. marmoratus*), respectively. Making the assumption that populations were closed or open biased the inferred  $N_e$  both upwards and downwards, but the differences were not large. Episodic immigration from a source differing in allele frequencies results in  $N_e$  estimates which are too low, whereas constant immigration results in the opposite effect; strongly biased estimates are probably only expected when sampling intervals are large (Wang & Whitlock, 2003). Incorporating nonreproducing dispersers, the inferred immigration rates were expected to be higher than the rates obtained from the analysis focusing on gene flow. Values of  $m$  indeed averaged around the 35–37% of 'transients' estimated from a demographic model for another congeneric species (*T. alpestris*, Perret *et al.*, 2003). However, given the average migration rates of individual *T. cristatus* and *T. marmoratus* are far below 1 km per year (Arntzen & Wallis, 1991; Jehle & Arntzen, 2000), we argue that these values are probably too high. The assumed source gene pool showed little divergence from the last sample for all but one focal population ( $F_{st} = 0.009–0.021$ ), a situation for which the performance of this approach has not much power (J. Wang, personal communication). The exception was the *T. marmoratus* population of Pond 232 ( $F_{st}$ : 0.178), and  $m$  was indeed lowest in this case. In several instances the number of genotypes obtained in the initial temporal sample was also rather low and therefore prone to stochasticity. This might have resulted in some allele frequencies approaching the source rather than diverging from it. Pooling all other demes except the focal population as a source pool also might have introduced a bias, as close by ponds are more likely to supply dispersers than the most distant ponds. However, in light of the episodic and unidirectional occurrence of migration (see above), we refrained from including groups of ponds close to the focal deme for example based on a neighbourhood model. Finally, we noted that  $m$  was very sensitive to the assumed number of generations between temporal samples (results not shown), a value we can only approximate for our study species. Whether the tendency of a rather large  $m$  at low  $N_e$  is a significant phenomenon can only be studied by sampling more populations.

The  $N$  of populations for which  $N_e$  could be calculated varied almost 30-fold, whereas  $N_e$  varied less than fivefold. More importantly,  $N_e/N$  was inversely related to  $N$ , an effect termed 'genetic compensation' in Arden & Kapuscinski (2003). When few individuals were present, a higher proportion of newts apparently reproduced successfully. In evolutionary terms, such a mechanism could decrease the loss of genetic diversity during population bottlenecks, as would immigration and selection in favour of heterozygotes (Keller *et al.*, 2001). Future research could address whether temporarily adverse environmental conditions have a negative impact on  $N_e/N$  at constant  $N$ . If this were the case, increased genetic erosion could remain undetected in suboptimal habitats when only census data are available.

A calculation method for the effective size of a metapopulation with varying deme  $N_e$  and between-deme gene flow is currently not available (Wang & Caballero, 1999). In our case, it is probably best approximated by summing up the  $N_e$  values of all demes. Extrapolating from typical  $N_e/N$  values across demes for which no temporal samples are available,  $N_e$  in our system is probably in the order of 100–200 individuals. Variance in productivity among demes has been shown in particular to reduce effective metapopulation size (Whitlock & Barton, 1997). The limited evidence from our system suggests that, at least for our narrow temporal study window, metapopulation processes do not markedly reduce the retention of neutral genetic diversity. It would be worthwhile resampling all study populations after at least one generation, in order to determine whether current rates and directions of gene flow vary over time, and to obtain a better picture of the fine-scale variation of genetic drift across all demes.

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## Appendix 1

Consider  $R$  populations of a diploid species with migration rates between populations of  $\mathbf{m} = \{m_{bq}\}$ , where  $m_{bq}$  is the fraction of individuals in population  $q$  that have

migrated from population  $b$ . If random mating is assumed, the expected proportion of offspring resulting from an immigrant-resident mating is  $2m_{bq}$ , and the expected proportion of residents in population  $q$  is then:

$$1 - \sum_{b \neq q}^R m_{bq} - \sum_{b \neq q}^R 2m_{bq} = 1 - \sum_{b \neq q}^R 3m_{bq}$$

This method assumes that migration rates are relatively low, such that the expected total proportion of migrant individuals is  $<0.3$  (Wilson & Rannala, 2003).

As the sampling strategy of this study eliminated the possibility of obtaining genotypes from migrant individuals, modifications to the previous method were required. The expected proportion of migrants obtained in the sample becomes 0, and the expected proportion of individuals in population  $q$  with one parent from population  $b$  is then:

$$\frac{2m_{bq}}{1 - m_{bq}}$$

The expected proportion of nonmigrants becomes:

$$1 - \sum_{q \neq b}^R \left( \frac{2m_{bq}}{1 - m_{bq}} \right)$$

As shown in Wilson & Rannala (2003), the joint posterior probability density of the model parameters, derived from applying Bayes' theorem, is:

$$f(\mathbf{m}, \mathbf{M}, \mathbf{t}, \mathbf{F}, \mathbf{p} | \mathbf{X}, \mathbf{S}) = \frac{\Pr(\mathbf{X} | \mathbf{S}; \mathbf{M}, \mathbf{t}, \mathbf{F}, \mathbf{p}) \times \Pr(\mathbf{M}, \mathbf{t} | \mathbf{m}) f_p(\mathbf{p}) f_m(\mathbf{m}) f_F(\mathbf{F})}{\Pr(\mathbf{X} | \mathbf{S})}$$

where  $\mathbf{M} = \{M_h\}$  is the source of migrant ancestry for individual  $h$ ,  $\mathbf{t} = \{t_h\}$  is the generation at which the migrant ancestor arrived,  $\mathbf{F} = \{F_b\}$  is the inbreeding coefficient for population  $b$ ,  $\mathbf{p} = \{p_{ijb}\}$  is the frequency of allele  $i$  at locus  $j$  in population  $b$ ,  $\mathbf{X} = \{X_{hj}\}$  is the genotype for individual  $h$  at locus  $j$ , and  $\mathbf{S} = \{S_h\}$  is the population individual  $h$  was sampled from.  $\Pr(\mathbf{X} | \mathbf{S}; \mathbf{M}, \mathbf{t}, \mathbf{F}, \mathbf{p})$  is also known as the likelihood of the data, or the probability of the observed genotypes given the model parameters. The other terms in the numerator represent the prior distribution of  $\mathbf{p}$ ,  $\mathbf{m}$ ,  $\mathbf{F}$ , and  $(\mathbf{M}, \mathbf{t} | \mathbf{m})$ . As the denominator involves high-dimensional sums and integrals, MCMC methods are used to estimate the joint posterior probability density. Due to the modifications of the proportions of individuals of each type of migrant ancestry described above, the prior probability distribution of  $\mathbf{M}$  and  $\mathbf{t}$ , given  $\mathbf{m}$ , now becomes:

$$\Pr(\mathbf{M}, \mathbf{t} | \mathbf{m}) = \prod_{b=1}^R n_b! \prod_{q \neq b}^R \left( \frac{[2m_{bq}/1 - m_{bq}]^{n_{bqt}}}{n_{bqt}!} \right) \times \prod_{b=1}^R \left( \frac{m_{bb}^{n_{bb0}}}{n_{bb0}!} \right)$$

where

$$m_{bb} = 1 - \sum_{q \neq b} \left( \frac{2m_{bq}}{1 - m_{bq}} \right)$$

and

$$n_{bqt} = \sum_{h=1}^n \mathfrak{S}(M_h, t_h, S_h)$$

where

$$\mathfrak{S}(M_h, t_h, S_h) = \begin{cases} 1 & \text{if } M_h = 1, S_h = 1, t_h = 1 \\ 0 & \text{otherwise} \end{cases}$$

All other probabilities are as described in Wilson & Rannala (2003).

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