

# Microsatellite marker data suggest sex-biased dispersal in the common frog *Rana temporaria*

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## Abstract

Despite being important models in ecological, evolutionary and conservation biology research, very little is known about the dispersal in anuran amphibians, and juvenile dispersal in particular. Using microsatellite data, we assessed signatures of sex-biased migration in the common frog (*Rana temporaria*) in Scandinavia. Significant heterozygosity deficiency ( $F_{IS}$ ) and lower assignment value ( $mAIC$ ) among females suggest that dispersal in *R. temporaria* is female biased. Also variance of assignment ( $vAIC$ ), estimated separately for the two sexes, was consistent with this inference, although the difference was not statistically significant. Possible proximate and ultimate explanations for female-biased dispersal in amphibians are discussed.

**Keywords:** microsatellites, sex-biased dispersal, *Rana temporaria*

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## Introduction

Natal dispersal is defined as the movement of a juvenile individual from its place of birth to its first site of reproduction (Greenwood 1980). It is one of the central evolutionary forces affecting natural populations, being 'probably the most important life-history trait involved in both species persistence and evolution' (Clobert *et al.* 2001). From the population ecological point of view, dispersal rates determine to what degree different populations will function as independent demographic units. From the population genetic point of view, dispersal determines the rate of genetic differentiation of the semi-isolated populations. Effective dispersal delays increase of coancestry within and differentiation among populations significantly (e.g. Nei 1987). Therefore, information about dispersal patterns is vital for understanding population dynamics and structure.

Depending on a species' life history strategy, the cost/benefit asymmetries of dispersal are expected to affect the sexes differently (Perrin & Mazalov 2000; see also Vitalis 2002). This may lead to a sex-biased migration pattern, where individuals of the migrating sex disperse into a

non-natal area where they breed, while the philopatric sex remains in the vicinity of their natal site. Understanding why dispersal is sex-biased in many taxa is still a major issue in evolutionary ecology. A number of studies based on either direct (capture–recapture) or indirect (genetic markers) methods have shown that dispersal tends to be male biased in mammals (Greenwood 1980; Dobson 1982; Goudet *et al.* 2002; Vitalis 2002; Coltman *et al.* 2003) and female biased in birds (Piertney *et al.* 1998; Piertney *et al.* 1999; Piertney *et al.* 2000; Shorey *et al.* 2000; Shorey 2002), but counter-examples exist (see, e.g. Favre *et al.* 1997). In contrast, relatively little is known about possible sex-biased dispersal in other taxa.

A recent study by Austin *et al.* (2003) based on microsatellite data provided evidence for female-biased dispersal in the bullfrog *Rana catesbeiana*. Here, we assess sex-biased dispersal in another anuran, the common frog *Rana temporaria*, a medium-sized brown frog with a wide Palearctic distribution (Gasc *et al.* 1997). In contrast with the bullfrog, the common frog is a typical explosive breeder (*sensu* Wells 1977). During the breeding season the males aggregate to choruses which vary in size from a few to hundreds of males. The spawning period lasts about 20 days; however, the majority of the eggs are laid during the first few days (e.g. Haapanen 1982). As with most other amphibians, direct estimation methods have suggested that common frogs are largely philopatric, but move between different

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**Table 1** Genetic variation in the studied populations.  $N$ : average number of individuals sampled,  $N_G$ : average number of individuals genotyped successfully for each locus,  $A$ : average number of alleles/locus,  $H$ : gene diversity,  $F_{IS}$ : deviation from Hardy–Weinberg expectations

Population	Coordinates	$N$	$N_G$ (females:males)	$H$	$A$	$F_{IS}$
Kilpisjärvi	69°03' N, 20°47' E	46	44.7 (16.1:28.6)	0.589	5.7	−0.040
Kiruna	67°51' N, 21°02' E	46	45.0 (14.6:17.9)	0.644	5.7	0.033
Ammarnäs	65°54' N, 16°18' E	32	28.9 (10.0:18.9)	0.497	4.3	−0.086
Hamtjärn	63°52' N, 20°13' E	33	28.3 (11.0:17.3)	0.514	5.9	−0.016
Umeå	63°49' N, 20°14' E	49	48.3 (17.9:30.4)	0.559	6.1	0.021
Uppsala	59°51' N, 17°14' E	52	47.6 (12.3:35.3)	0.616	8.0	0.058
Lund	55°42' N, 13°26' E	57	55.1 (20.1:35.0)	0.643	10.0	0.190
Overall		315	297.9 (102.0:195.9)	0.591	15.0	0.048 <sup>NS</sup>

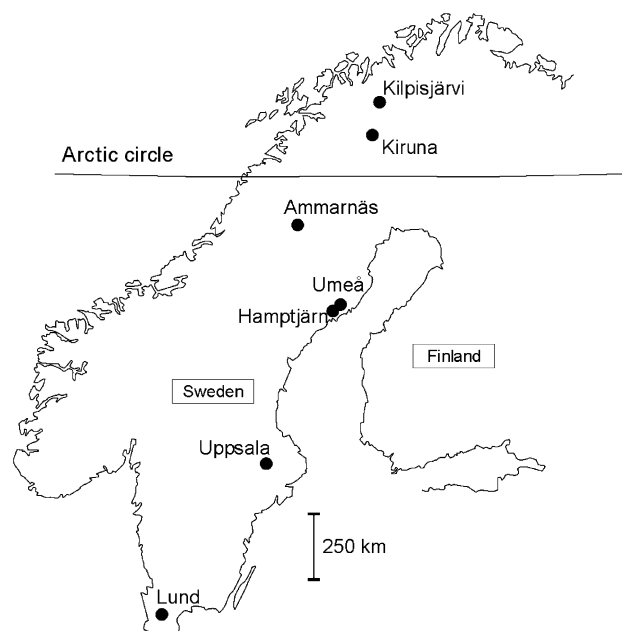
seasonal habitats (Savage 1961; Haapanen 1970; Loman 1994; Tramontano 1998) and are known to colonize new ponds at distances up to 950 m (e.g. Baker & Halliday 1999). Although many aspects of common frog ecology have been studied extensively, basically nothing is known about natal dispersal as it is very difficult to observe and measure.

At least part of the problems associated with direct dispersal studies can be circumvented by obtaining molecular marker data. Several basic population genetic indices are indicative of interpopulation dispersal rates; consequently, analysing and comparing these indices separately for the sexes can reveal differences in the migration rates between the sexes (Goudet *et al.* 2002; Vitalis 2002). Here, we have assessed signatures on (short-term) sex-biased dispersal at seven biparental microsatellite loci in seven north European common frog populations.

## Materials and methods

The 315 common frog samples included in the study were collected as point samples of adult common frogs from seven populations from different parts of Fennoscandia (Table 1, Fig. 1). The adults were collected from the choruses during early breeding season in spring 1998 (except Hamtjärn, which was sampled during spring 1999), i.e. after potential dispersal, before mating. The distance between different populations ranged from 3.5 km (Hamtjärn–Umeå) to 1535 km (Lund–Kilpisjärvi), the average distance between localities being 728 km.

DNA was extracted using standard SDS–proteinase K digestion treatment followed by NaCl purification and isopropanol precipitation (e.g. Bruford *et al.* 1992). Microsatellite variation at seven microsatellite loci: Rt2Ca2–22, Rt2Ca25 (Trent Garner unpublished), RRD590 (Vos *et al.* 2001), RtμH (Pidancier *et al.* 2002), Rtempμ4, Rtempμ5 and Rtempμ7 (Rowe & Beebe 2001) was assessed for 32–57 individuals from each of the populations. Polymerase chain reaction (PCR) amplifications were performed in a total volume of 10 μL, in conditions described in Palo *et al.*



**Fig. 1** Map showing the approximate locations of the sampled populations.

(2003). PCR products for each individual were resolved by denaturing PAGE (5% LongRanger gel, BMA, Rockland, USA) on a single lane using an ABI 377 DNA sequencer; data collection and allele scoring were performed using the GENESCAN 3.1 and GENOTYPER 2.5 software.

Allele frequency, observed heterozygosity and unbiased estimates of expected heterozygosity (Nei 1987) were calculated for each population using the software FSTAT version 2.9.3 (Goudet 2001). Deviations from Hardy–Weinberg equilibrium within populations at each locus were assessed by estimating  $F_{IS}$ . The amount of differentiation of allele frequencies between each pair of populations was quantified using Weir & Cockerham's (1984) standardized  $F_{ST}$ ; the statistical significance of the  $F$ -indices were determined by bootstrapping (10 000 replicates).

In order to detect potential differences in migration rates between females and males, deviation from Hardy–Weinberg equilibrium ( $F_{IS}$ ), mean assignment index ( $mAI_C$ ) and variance of the assignment index ( $vAI_C$ ) were quantified separately for both sexes over all populations. Statistical significance of differences in these within-population indices was determined using the randomization method implemented in *rSTAT* version 2.9.3 (10 000 permutations). Differences in the dispersal between the sexes are expected to result in significant dissimilarity in the population genetic parameters. As dispersal introduces genotypes unlikely to arise from the local allele frequencies,  $F_{IS}$  is expected to be positive in the dispersing sex due to the Wahlund effect. For the same reason, dispersal leads to lower assignment probabilities, higher variance of the assignment indices, as well as lower relatedness and interpopulation differentiation ( $F_{ST}$ ) in the dispersing sex. The relative power of these indices to detect a sex bias in the dispersal depends on the magnitude of the bias, dispersal rates, sampling scheme and markers examined (Goudet *et al.* 2002). Given the geographical distance between the populations studied here (> 300 km between most pairs), it is very unlikely that the populations exchange migrants (except perhaps Hamptjärn and Umeå). Therefore, (among-population) statistics such as  $F_{ST}$  bear little information on actual dispersal rates, and were excluded from the analysis. It is worth noting that the biparental markers, such as microsatellites utilized here, convey information on short-term dispersal (i.e. after dispersal, before mating); the sex-biased migration signal in biparental markers disappears in mating due to the Mendelian segregation (Goudet *et al.* 2002).

## Results

The average number of individuals genotyped successfully for each locus varied from 28.3 to 55.1 between populations, the mean being 42.6 (14.6 females, 28.0 males; Table 1). On average, 15 alleles per locus were observed in the total data set. Apart from Lund, the genotype frequencies within the populations were in the Hardy–Weinberg equilibrium (Table 1).

Differentiation between the populations was notable; the overall  $F_{ST}$  was 0.232 [95% confidence interval (CI) from 10 000 bootstrap replicates: 0.175–0.280], pairwise estimates ranging from 0.022 (Umeå–Hamptjärn) to 0.351 (Kilpisjärvi–Ammarnäs).

A total of 206 males and 109 females was included in the study for which the population genetic parameters were estimated separately. Between males and females, significant differences were observed in the overall  $F_{IS}$  and mean assignment values. The assignment index for females was negative ( $mAI_C = -0.764$ ), whereas it was well above zero for males ( $mAI_C = 0.404$ ,  $P = 0.002$ ). The most notable difference between the sexes was observed in the  $F_{IS}$ ,

**Table 2** Deviation from Hardy–Weinberg expectation ( $F_{IS}$ ), mean assignment ( $mAI_C$ ) and variance assignment ( $vAI_C$ ) values estimated separately for male and female common frogs. Significance ( $P$ ) was assessed using the randomization method of Goudet *et al.* (2002)

	$N$	$F_{IS}$	$mAI_C$	$vAI_C$
Females	109	0.089	-0.764	13.3
Males	206	-0.002	0.404	9.624
$P$		0.003	0.002	0.155

heterozygosity deficiency being significantly higher in females ( $F_{ISf} = 0.089$ ) than males ( $F_{ISm} = -0.002$ ;  $P = 0.003$ ), suggesting that dispersal is female biased in *R. temporaria*. This interpretation is supported further by the higher variance of the mean assignment index although this difference was not statistically significant. The results are summarized in Table 2.

## Discussion

All the indices estimated in this study suggest that in the common frog dispersal rates are higher among females than among males. The significant differences between the sexes were observed in the mean assignment index and in the  $F_{IS}$ , whereas differences in the assignment index variance was not significant.

Relatively few studies have examined sex-biased dispersal patterns in amphibians. Austin *et al.* (2003) concluded that the dispersal is female biased in the bullfrog; female bias has also been observed in the Columbia spotted frog (*R. luteiventris*) using direct methods (Pilliod *et al.* 2002). In contrast, dispersal appears male biased in the túngara frog (*Physalaemus pustulosus*; Lampert *et al.* 2003) whereas no sex bias was observed in the wood frog dispersal (*R. sylvatica*; Berven & Grudzien 1990). Austin *et al.* (2003) suggested that the difference in the dispersal pattern between the bullfrog and the wood frog is due to their different breeding strategies. In contrast to territorial bullfrogs showing prolonged breeding strategy, wood frogs are mainly monogamous explosive breeders and do not exhibit territoriality (Berven & Grudzien 1990). The Eurasian common frog appears ecologically similar to the wood frog, yet the current results suggest that the dispersal in this species is female biased. These two species differ in their maturation times: as with the bullfrogs, male common frogs mature 1–2 years earlier than females (Savage 1961), whereas in the wood frog both sexes mature at the age of 3–4 years. Even though adult common frogs appear as site-tenacious (see above), the longer maturation time — and hence also larger size — would allow broader dispersal for female juveniles and could serve as a proximate reason for differences in the dispersal patterns. However, it is perhaps relevant to note that we cannot deduce whether the genetic differences

observed between the sexes are due to higher emigration rates or longer dispersal distances in females, or both.

Several ultimate reasons for the observed male philopatry in *R. temporaria* may be speculated. First, the resource competition hypothesis (Greenwood 1980) predicts that the sex that benefits most from a familiarity with its birth site would be more philopatric. Male common frogs may gain significant advantage by occupying (familiar) high quality microhabitats early before the mating and may therefore be more site-tenacious compared to females. In *R. temporaria*, the males arrive at the breeding sites notably earlier than the females (Alho 2004), which would lend further support to this hypothesis.

Second, due to male–male competition involved in the reproduction of *R. temporaria* (explosive, dense aggregation, competition for females), males may best enhance their own (inclusive) fitness by joining choruses with related males. Furthermore, as vocalization has been found to be one of the main attraction factors for females (although weak in *R. temporaria*, see Wells 1977), formation of large calling choruses of related males is likely to increase the attractiveness of the breeding sites for females.

Third, demographic differences between sexes may also be invoked to explain the dispersal pattern observed. Indeed, male-biased predation has been observed in a closely related *R. dalmatina* (Lodé *et al.* 2004), potentially reducing the proportion of males that actually disperse. If males are less likely to survive from year to year, it may result in an observed female-biased dispersal even if both sexes tend to disperse equally. Nevertheless, capture–recapture data from the northernmost population do not support this hypothesis: male and female survival rates are equal in adulthood (Alho 2004).

Inbreeding avoidance is thought to be the primary cause of sex-biased dispersal in birds and mammals (Greenwood 1980; Perrin & Mazalov 2000); it is usually sufficient to minimize inbreeding (Greenwood 1980; Favre *et al.* 1997). In *R. temporaria*, mating with relatives does not necessarily imply a cost for males as individuals may mate repeatedly over a season. The opposite is expected for females as they typically reproduce once per season, even though a certain level of multiple paternity has been observed (Laurila & Seppä 1998). This asymmetry of costs between the sexes could favour the evolution of female-biased dispersal in *R. temporaria*.

Compared to male-biased dispersal in mammals and female-biased dispersal in birds, our results confirm a trend for female-biased dispersal in anurans. However, following other studies (Berven & Grudzien 1990; Lampert *et al.* 2003) our results illustrate that although the general breeding structure apparently promotes dispersal and outbreeding, the resulting genetic pattern may be very complex, resting on a trade-off between inbreeding avoidance and dispersal.

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This study is the result of a collaboration between researchers interested in utilizing molecular methods to study population history and microevolutionary processes in wild vertebrates.

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