MOLECULAR ECOLOGY

Female philopatry and male-biased dispersal in a direct-developing salamander, *Plethodon cinereus*

ERIC B. LIEBGOLD,* EDMUND D. BRODIE III* and PAUL R. CABE†

Department of Biology, The University of Virginia, Charlottesville, VA 22904-4328, USA, †Department of Biology, Washington and Lee University, Lexington, VA 24450-2116, USA

Abstract

The local resource competition hypothesis and the local mate competition hypothesis were developed based on avian and mammalian systems to explain sex-biased dispersal. Most avian species show a female bias in dispersal, ostensibly due to resource defence, and most mammals show a male bias, ostensibly due to male–male competition. These findings confound phylogeny with mating strategy; little is known about sex-biased dispersal in other taxa. Resource defence and male–male competition are both intense in *Plethodon cinereus*, a direct-developing salamander, so we tested whether sex-biased dispersal in this amphibian is consistent with the local resource competition hypothesis (female-biased) or the local mate competition hypothesis (male-biased). Using fine-scale genetic spatial autocorrelation analyses, we found that females were philopatric, showing significant positive genetic structure in the shortest distance classes, with stronger patterns apparent when only territorial females were tested. Males showed no spatial genetic structure over the shortest distances. Mark–recapture observations of *P. cinereus* over 5 years were consistent with the genetic data: males dispersed farther than females during natal dispersal and 44% of females were recaptured within 1 m of their juvenile locations. We conclude that, in this population of a direct-developing amphibian, females are philopatric and dispersal is male-biased, consistent with the local mate competition hypothesis.

Keywords: genetic spatial autocorrelation, microsatellite, *Plethodon cinereus*, sex-biased dispersal

Received 24 August 2010; revision received 1 November 2010; accepted 2 November 2010

Introduction

Sex-biased dispersal, when one sex remains mostly philopatric to its natal site and the other sex disperses further, is common in at least some vertebrate taxa (Greenwood 1980). Birds typically have female-biased dispersal and mammal species usually have male-biased dispersal (Greenwood 1980; Pusey 1987; Lawson Handley & Perrin 2007). Inbreeding avoidance is sometimes considered a cause of sex-biased dispersal, although it does not predict which sex disperses (Pusey 1987; Perrin & Mazalov 2000; Lebigre *et al.* 2010). There are a number of hypotheses for why a particular sex disperses, all of which have been generated exclusively from observations of sex-biased dispersal in birds and mammals (Pusey 1987; Goudet *et al.* 2002).

According to the local resource competition hypothesis, resource-defence monogamy, as found in birds, may be important in causing philopatry by males and dispersal by females (Greenwood 1980). Philopatry of one sex results from the asymmetry in the costs of dispersal as well as greater benefits of familiarity with a natal area by males resulting in greater acquisition of resources (Perrin & Mazalov 1999). In the local mate competition hypothesis, female choice and high male–male competition can cause male dispersal (Dobson 1982). Males usually compete strongly for mates, not resources, in many mammals. Models have shown that dispersal occurs in the sex with high competition for mates through the avoidance of kin competition, potentially outweighing the effects of resource-defence (Perrin & Mazalov 2000).

Correspondence: Eric B. Liebgold, Fax: +1 434 982 5626;
E-mail: caecilian@virginia.edu

Based on these models, we hypothesized that the local mate competition affects the direction of sex-biased dispersal more than local resource competition. Although this idea has been supported by examining exceptions to the normal sex-dispersing patterns in birds and mammals (Lecomte *et al.* 2009), true a priori hypothesis testing of sex-biased dispersal hypotheses requires data from other taxa (Pusey 1987; Goudet *et al.* 2002) because (i) the hypotheses were generated from observations of birds and mammals (Pusey 1987) and (ii) the dispersing sex may be derived feature from an ancestor (Perrin & Mazalov 1999).

Some data on sex-biased dispersal in fish (Bekkevold *et al.* 2004), amphibians (Palo *et al.* 2004), and reptiles (Dubey *et al.* 2008) is available and the diversity in mating strategies across these taxa is extensive, yet the number of studies is too small and the results too varied to reveal any general trends (Goudet *et al.* 2002). Direct-developing amphibians (i.e. amphibians without an aquatic larval stage) may be especially useful models for testing the importance of local resource competition and local mate competition in sex-biased dispersal for two main reasons. First, in comparison with most amphibians, juveniles of direct-developers occupy the same terrestrial habitat as adults, allowing familiarity with resources to play a role in resource competition. As a result, the mating systems and territorial behaviours of direct-developing amphibians share many similarities with both mammals and birds in terms of competition for mates and resources (Mathis *et al.* 1995) as well as parental care (Pough *et al.* 2004). Second, knowledge of sex-biased dispersal in birds and mammals may confound phylogeny with direction of sex-biased dispersal (Perrin & Mazalov 1999; Lawson Handley & Perrin 2007). Mating systems of most amphibians involves explosive breeding, lekking in large numbers in ephemeral or permanent bodies of water, and a life cycle that includes a free-living aquatic larval stage (Pough *et al.* 2004). Direct-development is a derived feature of some amphibian species in each of the three major amphibian clades (salamanders, frogs, and caecilians) with at least five independent evolutionary origins (Chippindale *et al.* 2004). Sex-biased dispersal in 'explosive breeders' tested to date includes both male- and female-biased dispersal as well as an absence of sex bias (Joly & Grolet 1996; Austin *et al.* 2003; Lampert *et al.* 2003; Palo *et al.* 2004; Smith & Green 2006), hinting at potential phylogenetic independence to sex-biased dispersal in the direct-developing clades. To date, the only test of sex-biased dispersal in any direct-developing amphibian was inconclusive (Cabe *et al.* 2007).

Specifically, we hypothesized that male-biased dispersal occurs in the red-backed salamander, *Plethodon cinereus*, a direct-developing salamander, consistent with

the predictions of the local mate competition hypothesis despite a mating system with intense resource defence. As in many birds, resource-defence monogamy occurs in *P. cinereus* with males defending territories based on territory quality (Gabor 1995). Females of *P. cinereus* are more likely choose a male if he occupies a high-quality territory (Mathis 1991a; Gillette *et al.* 2000). If resource defence monogamy is important in determining which sex disperses (Greenwood 1980), then females should disperse in *P. cinereus* because philopatric males would have an advantage by being familiar with local resources (*sensu* Perrin & Mazalov 1999). Alternatively, the local mate competition hypothesis posits that female choice and high male–male competition for mates can cause male dispersal (Dobson 1982) through avoidance of kin competition (Perrin & Mazalov 2000). Females of most populations of *P. cinereus* breed biennially (Petranka 1998) leading males to use elaborate courtship rituals and male–male combat (biting) to secure mates (Gergits & Jaeger 1990). These factors result in high levels of multiple paternity despite social monogamy after the courtship (Liebgold *et al.* 2006). Thus, male–male competition for mates is potentially strong (Mathis 1991a), which may lead to male-biased dispersal.

Plethodon cinereus is not a species conducive to typical measures for detecting sex-biased dispersal. Dispersal ability is limited (Marsh *et al.* 2004), with most dispersal on the scale of a 0–5 m in unfragmented habitat (Ousterhout & Liebgold 2010) and an average intergenerational dispersal distance of 3.5 m (Cabe *et al.* 2007). Assignment indices failed to identify any sex-biased dispersal in *P. cinereus* (Cabe *et al.* 2007). However, differences in dispersal distances may be more powerful than assignment indices in detecting sex-biased dispersal for species with predominantly short-distance dispersal (Lawson Handley & Perrin 2007).

Genetic spatial autocorrelation (Smouse & Peakall 1999) is a method for determining differences in dispersal distances between males and females by testing for the presence of within-population spatial genetic structure on a very local scale (Peakall *et al.* 2003). Genetic spatial autocorrelation uses pairwise relatedness (r) between individuals to test for differences in relatedness between binned distance classes. Mean pairwise relatedness is always zero for all sampled individuals (Hardy 2003). Positive values of r indicate that a pair of individuals shares more alleles than the average pair of individuals in the sample, while negative values of r indicate less sharing of alleles than average. Therefore, it is the relative differences expressed by r, not the absolute values of relatedness (i.e. not ~0.5 for parent–offspring), that are used to discern patterns of spatial genetic structure (Pardini & Hamrick 2008). Higher than average pairwise relatedness at near, but not far distance classes, is

indicative of restricted dispersal (Peakall *et al.* 2003; Vignieri 2007). The advent of microsatellite loci has made these tests robust for discerning patterns of dispersal (Peakall *et al.* 2003). Of the small number of animal studies that have tested for sex-biased dispersal using genetic spatial autocorrelation, most have detected it (Temple *et al.* 2006; Vignieri 2007; Dubey *et al.* 2008).

We used these local tests of genetic spatial autocorrelation as well as global tests (Mantel tests) with highly polymorphic microsatellite loci to discern sex differences in spatial genetic structure in a population of *P. cinereus*. If females are philopatric, they should be more related to proximate females than distant females. The opposite pattern should occur if males are philopatric. We also analysed spatial genetic structure between males and females to test for greater than average relatedness at short distances or a lack thereof. If males are more related to proximate females, then inbreeding may occur (Lebigre *et al.* 2010). In *P. cinereus*, male and female breeding pairs have overlapping territories (Mathis 1991b), so we predict that positive spatial genetic structure between males and females will not be found at short distances.

The interpretation of spatial genetic structure data is difficult without incorporating observational data (Temple *et al.* 2006; Vignieri 2007; Beck *et al.* 2008). Combining mark–recapture data with genetic relatedness data can confirm dispersal as a cause of spatial genetic structure (Vignieri 2007), so we also used data from a 5-year mark–recapture study to test for sex differences in natal dispersal distances in *P. cinereus*. Many animal species disperse during the juvenile phase (Dufty & Belthoff 2001), so natal dispersal can be an important component of sex-biased dispersal (Lawson Handley & Perrin 2007). Juvenile *P. cinereus* move larger distances between years than adults, while adult movements are limited (Ousterhout & Liebgold 2010) with males and females both occupying small home ranges that do not differ in size between the sexes (Mathis 1991b; Liebgold & Jaeger 2007). As in our spatial genetic structure analyses, we predicted that females would be philopatric and males recaptured as adults would have moved further distances from their natal areas if local mate competition influences sex-biased dispersal. We predicted the opposite pattern to occur if resource-defence competition affects which sex disperses.

Materials and methods

Study area

This study took place at the University of Virginia's Mountain Lake Biological Station (MLBS) in Giles County, VA, USA. MLBS is in the Appalachian Mountains and has an elevation of approximately 1200 m.

The forest is mixed deciduous, predominantly red oak (*Quercus rubra*), white oak (*Q. alba*), red maple (*Acer rubrum*), and Canadian hemlock (*Tsuga canadensis*).

Study species

Plethodon cinereus, the red-backed salamander, is a small, direct-developing salamander in the family Plethodontidae that is distributed in forests across most of eastern North America (Petranka 1998). During the day, *P. cinereus* inhabits moist retreats under rocks and logs or underground (Mathis 1991b). At night, *P. cinereus* emerge from retreats to forage or mate on the open forest floor (Liebgold & Jaeger 2007). Mating seasons are prolonged and occur during the spring and autumn, although male *P. cinereus* maintain foraging territories throughout the summer months (Mathis 1991b).

Field surveys

We delineated a 12 m × 12 m plot in an undisturbed section of the forest near the station. We collected data on individuals of *P. cinereus* captured during the day under rocks and logs and on the surface on rainy nights. The data were collected from May to August in 2005 (35 surveys) and 2006 (25 surveys). We individually marked salamanders with unique combinations of five fluorescent elastomers (Northwest Marine Technologies Inc., Shaw Island, WA, USA). Marking salamanders with elastomers does not appear to influence behavior or survivorship of *Plethodon* salamanders (Davis & Ovaska 2001). We removed tail-tips for DNA analysis by applying pressure with forceps approximately 0.5 cm from the tips of tails of adults (*sensu* Adams *et al.* 2005), whereby the tails subsequently autotomized. Snout-vent lengths (SVL) of individuals were measured from the tip of the snout to the posterior end of the vent using digital calipers (±0.01 mm; Mitutoyo America Corporation). We assigned salamanders to age-classes using Sayler's (1966) criteria based on SVL: juvenile (<34 mm) or adult (>36 mm). We determined the sexes of adults using Gillette & Peterson's (2001) candling method to look for the presence (male) or absence (female) of testes. Testes are transparent in smaller (<34 mm) males so sex of juveniles was unknown. Points of capture were flagged upon capture and spatial locations recorded (±1.0 cm). After processing, salamanders were released at their point of capture.

Microsatellite genotyping

DNA was extracted from tail clippings using a Wizard Genomic DNA Purification Kit (Promega Corporation).

We genotyped male and female adult salamanders at seven dinucleotide repeat microsatellite loci. The loci were amplified following the PCR protocols in Connors & Cabe (2003). PCR amplicons of the loci (PcFX08, PcI16, PcJX06, PcLX16, PcLX23, PcI14, and PcDX23) were then resolved with an ABI 3130xl Automated Sequencer (Applied Biosystems). We sized alleles for these loci using GeneMapper 4.0 software (Applied Biosystems).

Genotypes of the males and females collected were used to calculate the number of alleles per locus, allele frequencies, expected and observed heterozygosities, and we tested for Hardy–Weinberg equilibrium at each of the loci (GENALEX 6.0; Peakall & Smouse 2006). We also tested each locus for Hardy–Weinberg equilibrium and each pair of loci for linkage disequilibrium (GENE POP 3.4, Raymond & Roussett 1995).

Statistical analysis of spatial genetic structure

We analysed spatial genetic structure for all adult males, all adult females, territorial males only, and territorial females only (captured ≥ 3 times: Mathis 1991b) with the spatial autocorrelational methods in the program GENALEX 6.0 (Peakall & Smouse 2006). This analysis bins the distances into classes. We chose 2 m as a biologically relevant distance class based on the home ranges of *P. cinereus* (median$_{females}$ = 2.07 m^2; median$_{males}$ = 1.85 m^2, unpublished data). The distance classes in the analysis were binned as ≤ 2 m, ≤ 4 m, etc. up to 14 m.

Because individuals of *P. cinereus* within populations often share similar genotypes (Cabe *et al.* 2007), and pairwise *r* values in any group of sampled individuals are, on average, zero (Hardy 2003), pairwise *r* was calculated after including *regional* genotypes (from salamanders captured 3–5 km away) in addition to the *local* genotypes from our site of interest (Pardini & Hamrick 2008). Inclusion of regional genotypes in calculations of *r* prevents some Type II errors preventing detection of spatial genetic structure between highly related individuals (Pardini & Hamrick 2008). Ten or 20 regional genotypes were included for territorial or all individuals, respectively.

In GENALEX 6.0, *P* values for each distance class were calculated by 999 permutations of expected *r* values, based on random pairwise *r* values, tested against the actual *r* values within that distance class (Peakall *et al.* 2003). In addition, we used Mantel tests to test for a linear correlation between pairwise genetic distance and geographic distance (Guillot *et al.* 2009) among males and among females even though they are not as powerful as genetic spatial autocorrelation in detecting fine scale spatial genetic structure (GENALEX 6.0).

We analysed spatial genetic structure between territorial males and territorial females (>3 captures: *sensu* Mathis 1991b) using the same protocols. However, in order to test exclusively for male–female spatial genetic structure, we manually excluded all male–male and female–female pairwise values from both the genetic and geographic distance matrices calculated by GENALEX 6.0 before spatial autocorrelational analysis and Mantel test.

Goudet *et al.* (2002) used simulations to examine the power of four types of F_{ST} and assignment tests to detect sex-biased dispersal while varying a number of parameters. These simulations may not be directly comparable to this study because F_{ST} and assignment tests are used to test for inter-population biases in dispersal (Goudet *et al.* 2002) and genetic spatial autocorrelation is used to test for within-population sex biases in dispersal (e.g. Temple *et al.* 2006), but there are some relevant insights into the power of this study. Goudet *et al.* (2002) concluded five parameters increased power to detect sex-biases in dispersal: polymorphism of loci, number of loci, proportion of individuals sampled, dispersal rate, and intensity of sex-bias in dispersal. The last two parameters are beyond the control of methodology. With regards to this study, the markers we used were highly polymorphic, though few in number (seven). The only other available loci for *P. cinereus* (PcCC04, PcJX05 and PcJX24) have low polymorphism (Connors & Cabe 2003; Noël *et al.* 2007). However, when genetic patterns are strong, a small number of highly polymorphic loci can detect patterns; increasing the number of loci does not necessarily increase power (Goudet *et al.* 2002; Ryman *et al.* 2006; Björklund & Bergek 2009). Goudet *et al.* (2002) suggest investing in the proportion of individuals in a population rather than the number of loci, so we intensively sampled salamanders in the field during 60 thorough surveys and were able to genotype 94% of the males and 89% of the females that we captured.

Natal dispersal

Searching for salamanders marked in 2005 and 2006, we surveyed the plot and 12 m surrounding the plot seven times from June to August 2007. Because recapture of salamanders more than 2 m outside the plot was very low compared with the search effort (Ousterhout & Liebgold 2010), we surveyed the plot and at least 2 m surrounding the plot 28 times from May to August 2008 and 27 times from May to August 2009. All recaptured salamanders were measured, assigned to age-classes, sexes were determined, and spatial locations recorded (±1.0 cm).

When salamanders that were originally captured as juveniles in 2005 and/or 2006 were recaptured as sexually mature adults, we calculated the Euclidean distance from the original grid coordinate location (±1.0 cm) to the recaptured location (as a male or female adult) to determine dispersal distance from their natal area. When salamanders were captured in 2 years as juveniles (Sayler 1966), we only used the earliest year for natal location. Similarly, when salamanders were captured in multiple years as adults, we only used the latest year's spatial location as the most accurate adult location (some young sexually mature salamanders are non-territorial: Mathis 1991b). For individuals captured multiple times in one year, we used the centroid of their home range for that year as their location (Vignieri 2007).

The natal dispersal data were not normal and no transformations allowed the data to meet the assumptions of normality. We used a non-parametric Wilcoxon two-sample test (SAS Institute Inc. 2004) to compare natal dispersal distances of males and females. Wilcoxon two-sample tests are non-parametric equivalents of *t*-tests.

Results

Statistical analysis of spatial genetic structure

We collected DNA from 76 females and 63 males captured in 2005 and 2006; 41 of these females and 34 males were captured ≥3 times. Most of the microsatellite loci had high heterozygosity and were not found to deviate significantly from Hardy–Weinberg equilibrium (Table 1). PcI16 deviated significantly from Hardy–Weinberg equilibrium ($\chi^2_{10} = 76.31$; $P < 0.001$) so it was excluded from the spatial autocorrelational analyses (Björklund & Bergek 2009). No pairs of loci exhibited statistically significant linkage disequilibrium.

Table 1 Variation in seven *Plethodon cinereus* microsatellite DNA loci

Locus	Size range (bp)	No. of alleles	H_O	H_E
PcLX16	183–211	7	0.724	0.739
PcLX23	166–200	12	0.675	0.672
PcI14	109–137	7	0.758	0.677
PcI16	142–150	5	0.500	0.575
PcFX08	179–211	14	0.776	0.831
PcDX23	194–222	12	0.628	0.672
PcJX06	101–133	11	0.571	0.579

Shown is size range of alleles, number of alleles, observed heterozygosity (H_O) and expected heterozygosity (H_E) for each locus.

Females ($n = 76$) were significantly more related when they were separated by less than 4 m apart (0–2 m: $P = 0.030$; 2–4 m: $P = 0.025$; Fig. 1b). Males ($n = 63$) were not significantly more related when they were less than 2 m apart ($P = 0.680$) but were in some larger distance classes (2–4 m: $P = 0.020$; 6–8 m: $P = 0.001$; Fig. 1a). Mantel tests were not significant for males ($P = 0.194$, $R_{xy} = 0.036$, $y = 0.056x + 6.20$, $R^2 = 0.001$) or females ($P = 0.105$, $R_{xy} = 0.040$, $y = 0.066x + 6.14$, $R^2 = 0.002$) implying no overall linear correlation of genetic and geographic distances.

Territorial adults showed similar patterns of spatial genetic structure. Territorial females ($n = 41$) were significantly more related in the shortest distance classes (0–2 m; $P = 0.006$; 2–4 m; $P = 0.010$; Fig. 2b) and there was a trend for females 8–10 m apart to be less related ($P = 0.061$; Fig. 2b). Territorial males ($n = 34$) were
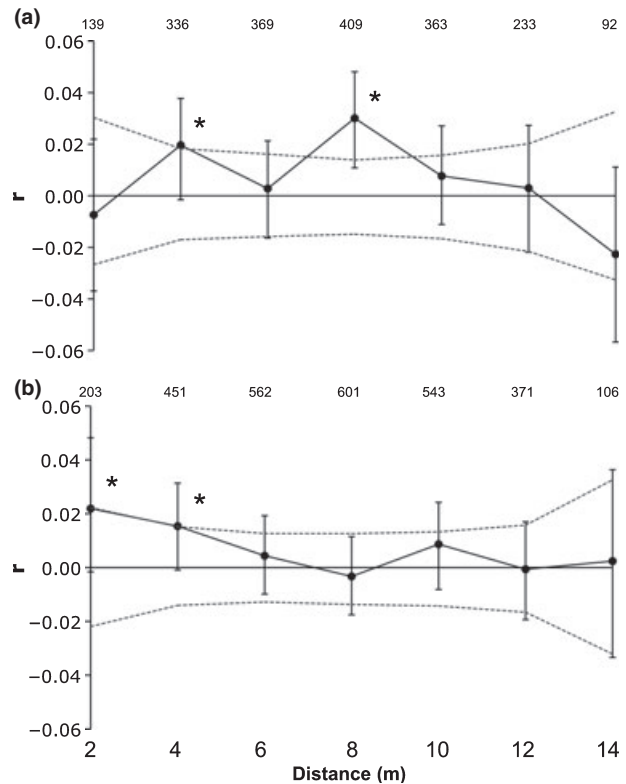

Fig. 1 Spatial genetic structure of (a) all males ($n = 63$) and (b) all females ($n = 76$) of *Plethodon cinereus*. The genetic correlation, *r*, denotes pairwise relatedness (±95% confidence error bars). The numbers above the points are the sample sizes for the number of pairwise relatedness comparisons at each distance class. The dashed lines represent the permuted 95% confidence limits for expected relatedness based on random subsampling within the entire pool of pairwise relatedness estimates and the sample size for each distance class. *Relatedness that was significantly greater than expected by chance ($P < 0.05$).
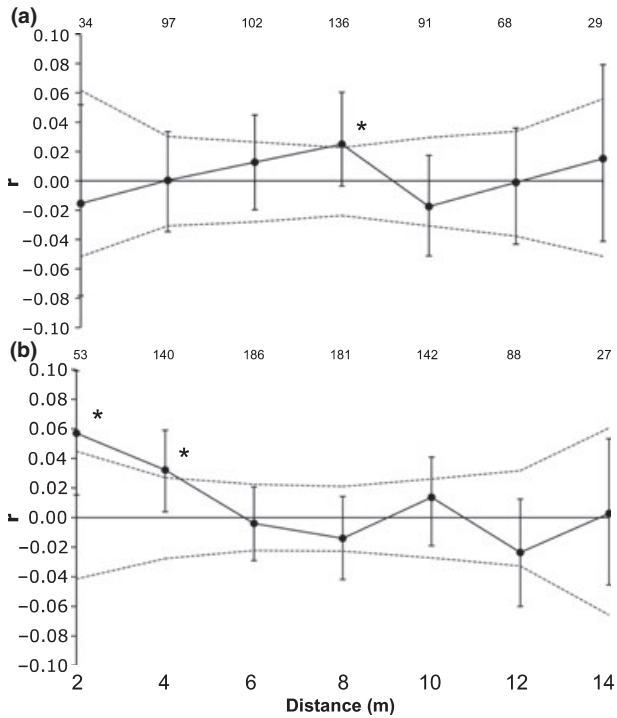
Fig. 2 Spatial genetic structure of (a) territorial males ($n = 34$) and (b) territorial females ($n = 41$) of *Plethodon cinereus*. See Fig. 1 text for more information.

Fig. 3 Male–female spatial genetic structure of territorial males ($n = 34$) and territorial females ($n = 41$) of *Plethodon cinereus*. See Fig. 1 text for more information.

Fig. 4 Natal dispersal distances of males and females of *Plethodon cinereus*. Distances were binned into 1 m distance classes.

significantly more related only when separated by 6–8 m ($P = 0.023$; Fig. 2a). Mantel tests showed a linear correlation between genetic and geographic distances for territorial females ($P = 0.013$, $R_{xy} = 0.114$, $y = 0.204x + 4.82$, $R^2 = 0.013$) but not territorial males ($P = 0.335$, $R_{xy} = -0.028$, $y = -0.044x + 7.21$, $R^2 < 0.001$).

Male–female relatedness was significantly above average only at 4–6 m ($P = 0.035$; Fig. 3), not in the shortest distance classes (0–2 m and 2–4 m). In the Mantel test, there was no significant linear correlation between male–female genetic and geographic distances ($P = 0.361$, $R_{xy} = -0.009$, $y = -0.015x + 6.66$, $R^2 < 0.001$).

Natal dispersal

Fewer males ($n = 22$) were recaptured as adults than females ($n = 36$). Males dispersed more than twice as far as females (Fig. 4; $Z = 2.08$, $P = 0.038$; $\bar{x}_{Males} = 4.05 \pm 1.19$, $\bar{x}_{Females} = 1.74 \pm 0.28$). 44% of adult females were recaptured within 1 m of their natal location and 69% were recaptured within 2 m (Fig. 4).

Discussion

We found differences in fine-scale spatial genetic structure between males and females of the salamander, *P. cinereus*, with females, but not males, more related
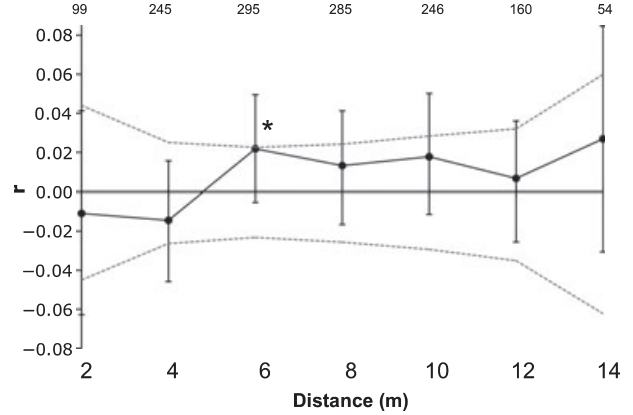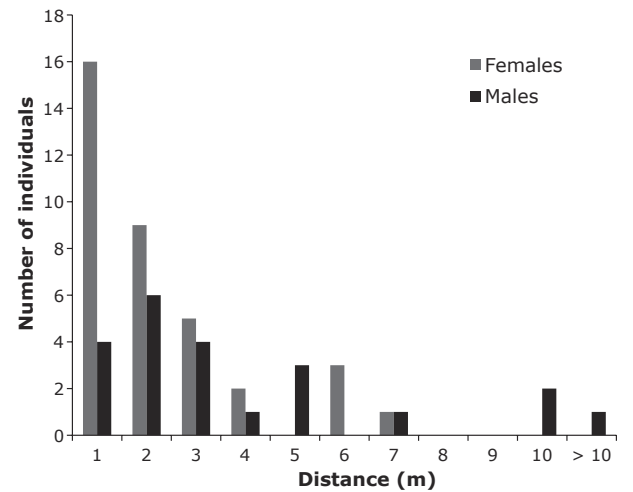
at short distance classes (Figs 1 and 2). Our mark–recapture data corroborates genetic inferences of male-biased dispersal, with males dispersing, on average, more than twice as far as females. Most females were recaptured adults within 2 m of their natal locations (Fig. 4), which explains why females were more related in the 0–2 m and 2–4 mm distance classes as many of them were likely siblings or half-siblings, although actual distances between siblings varies depending on the direction of each sibling's movements. Similarly, higher than expected relatedness was found for males located 6–8 m apart (Figs 1 and 2), a distance that would be expected by the mean male natal dispersal distance of just over 4 m (Fig. 4) as, on average, siblings would be up to ~8 m apart as adults. The congruence of our genetic measure of dispersal, genetic spatial autocorrelation, with a direct measure of natal dispersal

is encouraging, as it shows that genetic spatial autocorrelation may be useful in estimating as well as detecting short-distance dispersal.

Our data showing male-biased dispersal are consistent with predictions of the local mate competition hypothesis (Dobson 1982; Perrin & Mazalov 2000), potentially because male–male competition in *P. cinereus* is high (Gergits & Jaeger 1990; Mathis 1991a), even though resource defence occurs in males of this species (Mathis 1990; Gabor 1995). Models have shown that competition for mates causes dispersal by the sex with higher competition except in circumstances where resource competition is unusually intense, causing dispersal by the opposite sex (Perrin & Mazalov 2000). Males can also disperse in species where resource-defence occurs if male parental investment is small compared to females (male birds typically have large parental investment in offspring) and there are other benefits to male dispersal (Perrin & Mazalov 1999). Females of *P. cinereus* have a large parental investment in offspring in terms of brooding costs (Ng & Wilbur 1995; Yurewicz & Wilbur 2004) whereas males rarely attend eggs (Liebgold *et al.* 2006). This differential in parental investment might cause female salamanders to be philopatric as females, but not males, are limited in their rate of processing resources (Perrin & Mazalov 1999).

There are additional noteworthy differences between resource-defence monogamy in birds and in *P. cinereus* that may explain the taxonomic differences in sex-biased dispersal. First, both female and male *P. cinereus* are aggressive and defend moist substrates such as rocks and logs during the day (Mathis 1990) and small home ranges at night as feeding territories (Mathis 1991b; Liebgold & Jaeger 2007). Further, while extra-pair copulations occur frequently in over 75% of socially monogamous bird species, the rate of extra-pair copulations in birds are typically low (Griffith *et al.* 2002). In the socially monogamous *P. cinereus* (Gillette *et al.* 2000), male partners sire ≤50% of eggs in a clutch (Liebgold *et al.* 2006). These differences may explain why resource-defence monogamy may not affect sex-biased dispersal in *P. cinereus*.

Research on socially monogamous lizards of the genus *Egernia* has found similar patterns of female philopatry and male-biased dispersal (Gardner *et al.* 2001; Stow *et al.* 2001; Chapple & Keogh 2005). As in *P. cinereus*, while males disperse further than females, male movements in *Egernia* are also limited. Further tests of sex-biased dispersal in social monogamous amphibians, reptiles, and fish may be useful in understanding whether local mate competition is indeed important in sex-biased dispersal across taxa (Greenwood 1980; Dobson 1982) or whether other factors, such as parental investment, drives the determination of which sex disperses in species with resource-defence competition (Perrin & Mazalov 2000).

Genetic structure within populations can be a reflection of inbreeding avoidance (Schiegg *et al.* 2006; Lebigre *et al.* 2010), so inbreeding may be the ultimate cause of sex-biased dispersal, though it does not affect which sex disperses (Pusey 1987; Perrin & Mazalov 2000). Variation in r values across our set of samples was low (Figs 2 and 3), implying that all individuals of *P. cinereus* in our study were similar genetically (Pardini & Hamrick 2008). However, individuals should not move farther than needed to reduce inbreeding (Perrin & Mazalov 1999). We found that male-biased dispersal of males that averaged just four meters was concomitant with low male-female relatedness at proximate distances (<4 m; Fig. 3). The highest male–female relatedness was between 4 and 6 m, a distance that should be enough to prevent overlapping territories of closely related male–female pairs (see Mathis 1991b). In species where there are limitations to long-distance dispersal, such as *P. cinereus*, even small differences in sex-biased dispersal distances is likely sufficient to reduce inbreeding (Perrin & Goudet 2001).

In conclusion, we found differences in dispersal distances between the sexes in *P. cinereus* over short distances using genetic and observational tools, consistent with the local mate competition hypothesis. We stress, however, that it is difficult to infer the underlying causation of sex-biased dispersal from an observational studies of a single species (Perrin & Mazalov 2000). Meta-analyses or large experimental frameworks are needed to tease out the relative importance of local mate competition and inbreeding in sex-biased dispersal. This study adds to the little information on sex-biased dispersal in amphibians and other taxa with resource-defence monogamy, important taxa for testing sex-biased dispersal hypotheses. Fine-scale genetic spatial autocorrelation is a potentially significant and underused tool in molecular ecology for detecting sex-biased dispersal (Temple *et al.* 2006; Dubey *et al.* 2008), and its utility in future research is supported by the congruence of our spatial genetic structure analyses and our direct observations of natal dispersal.

Acknowledgements

References

Adams E, Jones AG, Arnold SJ (2005) Multiple paternity in a natural population of a salamander with long-term sperm storage. *Molecular Ecology*, **14**, 1803–1810.

Austin J, Davila J, Lougheed S, Boag PT (2003) Genetic evidence for female-biased dispersal in the bullfrog, *Rana catesbeiana* (Ranidae). *Molecular Ecology*, **12**, 3165–3172.

Beck N, Peakall R, Heinsohn R (2008) Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs. *Molecular Ecology*, **17**, 4346–4358.

Bekkevold D, Hansen M, Mensberg K (2004) Genetic detection of sex-specific dispersal in historical and contemporary populations of anadromous brown trout *Salmo trutta*. *Molecular Ecology*, **13**, 1707–1712.

Björklund M, Bergek S (2009) On the relationship between population differentiation and sampling effort: is more always better? *Oikos*, **118**, 1127–1129.

Cabe PR, Page RB, Hanlon TJ, Aldrich ME, Marsh DM (2007) Fine-scale population differentiation and gene flow in a terrestrial salamander (*Plethodon cinereus*) living in continuous habitat. *Heredity*, **98**, 53–60.

Chapple DG, Keogh JS (2005) Complex mating system and dispersal patterns in a social lizard, *Egernia whitii*. *Molecular Ecology*, **14**, 1215–1227.

Chippindale PT, Bonett RM, Baldwin AS, Wiens JJ (2004) Phylogenetic evidence for a major reversal of life-history evolution in plethodontid salamanders. *Evolution*, **58**, 2809–2822.

Connors LM, Cabe PR (2003) Isolation of dinucleotide microsatellite loci from the red-backed salamander (*Plethodon cinereus*). *Molecular Ecology Notes*, **2003**, 131–133.

Davis T, Ovaska K (2001) Individual recognition of amphibians: effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. *Journal of Herpetology*, **35**, 217–225.

Dobson F (1982) Competition for mates and predominant juvenile male dispersal in mammals. *Animal Behaviour*, **30**, 1183–1192.

Dubey S, Brown G, Madsen T, Shine R (2008) Male-biased dispersal in a tropical Australian snake (*Stegonotus cucullatus*, Colubridae). *Molecular Ecology*, **17**, 3506–3514.

Dufty AJ, Belthoff J (2001) Proximate mechanisms of natal dispersal: the role of body condition and hormones. In: *Dispersal* (eds Clobert J, Danchin E, Dhondt A, Nichols J), pp. 217–229. Oxford University Press, Oxford.

Gabor CR (1995) Correlational test of Mathis' hypothesis that bigger salamanders have better territories. *Copeia*, **1995**, 729–735.

Gardner M, Bull CM, Cooper SJB, Duffield G (2001) Genetic evidence for a family structure in stable social aggregations of the Australian lizard *Egernia stokesii*. *Molecular Ecology*, **10**, 175–183.

Gergits WF, Jaeger RG (1990) Field observations of the behavior of the red-backed salamander (*Plethodon cinereus*): courtship and agonistic interactions. *Journal of Herpetology*, **24**, 93–95.

Gillette JR, Peterson MG (2001) The benefits of transparency: candling as a simple method for determining sex in red-backed salamanders (*Plethodon cinereus*). *Herpetological Review*, **32**, 233–235.

Gillette JR, Jaeger RG, Peterson MG (2000) Social monogamy in a territorial salamander. *Animal Behaviour*, **59**, 1241–1250.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology*, **11**, 1103–1114.

Greenwood P (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, **11**, 2195–2212.

Guillot G, Leblois R, Coulon A, Frantz A (2009) Statistical methods in spatial genetics. *Molecular Ecology*, **18**, 4734–4756.

Hardy O (2003) Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. *Molecular Ecology*, **12**, 1577–1588.

Joly P, Grolet O (1996) Colonization dynamics of new ponds, and age structure of colonizing Alpine newts, *Triturus alpestris*. *Acta Oecologica*, **17**, 599–608.

Lampert K, Rand A, Mueller U, Ryan M (2003) Fine-scale genetic pattern and evidence for sex-biased dispersal in the tungara frog, *Physalaemus pustulosus*. *Molecular Ecology*, **12**, 3325–3334.

Lawson Handley LJ, Perrin N (2007) Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology*, **16**, 1559–1578.

Lebigre C, Alatalo R, Siitari H (2010) Female-biased dispersal alone can reduce the occurrence of inbreeding in black grouse (*Tetrao tetrix*). *Molecular Ecology*, **19**, 1929–1939.

Lecomte N, Gauthier G, Giroux J, Milot E, Bernatchez L (2009) Tug of war between continental gene flow and rearing site philopatry in a migratory bird: the sex-biased dispersal paradigm reconsidered. *Molecular Ecology*, **18**, 593–602.

Liebgold EB, Jaeger RG (2007) Juvenile movements and potential inter-age class associations of red-backed salamanders. *Herpetologica*, **63**, 51–55.

Liebgold EB, Cabe PR, Jaeger RG, Leberg PL (2006) Multiple paternity in a salamander with socially monogamous behaviour. *Molecular Ecology*, **15**, 4153–4160.

Marsh DM, Thakur KA, Bulka KC, Clarke LB (2004) Dispersal and colonization through open fields by a terrestrial salamander. *Ecology*, **85**, 3396–3405.

Mathis A (1990) Territoriality in a terrestrial salamander: the influence of resource quality and body size. *Behaviour*, **112**, 162–175.

Mathis A (1991a) Large male advantage for access to females – evidence of male–male competition and female discrimination in a territorial salamander. *Behavioral Ecology and Sociobiology*, **29**, 133–138.

Mathis A (1991b) Territories of male and female terrestrial salamanders: costs, benefits, and intersexual spatial associations. *Oecologia*, **86**, 433–440.

Mathis A, Jaeger RG, Keen WH *et al.* (1995) Aggression and territoriality by salamanders and a comparison with the territorial behaviour of frogs. In: *Amphibian Biolog* (eds Heatwole H, Sullivan BK), pp. 633–676. Surrey Beatty and Sons, Chipping Norton, New South Wales.

Ng MY, Wilbur HM (1995) The cost of brooding in *Plethodon cinereus*. *Herpetologica*, **51**, 1–8.

Noël S, Ouellet M, Galois P, Lapointe F (2007) Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander. *Conservation Genetics*, **8**, 599–606.

Ousterhout B, Liebgold EB (2010) Dispersal versus site tenacity of adult and juvenile red-backed salamanders (*Plethodon cinereus*). *Herpetologica*, **63**, 269–275.

Palo J, Lesbarreres D, Schmeller D, Primmer C, Merila J (2004) Microsatellite marker data suggest sex-biased dispersal in the common frog *Rana temporaria*. *Molecular Ecology*, **13**, 2865–2869.

Pardini E, Hamrick J (2008) Inferring recruitment history from spatial genetic structure within populations of the colonizing tree *Albizia julibrissin* (Fabaceae). *Molecular Ecology*, **17**, 2865–2879.

Peakall R, Smouse P (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution*, **57**, 1182–1195.

Perrin N, Goudet J (2001) Inbreeding, kinship and the evolution of natal dispersal. In: *Dispersal* (eds Clobert J, Danchin E, Dhondt A, Nichols J), pp. 123–142. Oxford University Press, Oxford.

Perrin N, Mazalov V (1999) Dispersal and inbreeding avoidance. *American Naturalist*, **154**, 282–292.

Perrin N, Mazalov V (2000) Local competition, inbreeding and the evolution of sex-biased dispersal. *American Naturalist*, **155**, 116–127.

Petranka JW (1998) *Salamanders of the United States and Canada*, U.S.A. edn. Smithsonian Institution Press, Washington, D.C.

Pough FH, Andrews RM, Cadle JE *et al.* (2004) *Herpetology*. Pearson Education, Inc., Upper Saddle River, New Jersey.

Pusey A (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology and Evolution*, **2**, 295–299.

Raymond M, Roussett F (1995) GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ryman N, Palm S, André C *et al.* (2006) Power for detecting genetic divergence: differences between statistical methods and marker loci. *Molecular Ecology*, **15**, 2031–2045.

SAS Institute Inc. (2004) SAS version 9.1, http://support.sas.com/onlinedoc/913/docMainpage.jsp. Cary, NC.

Sayler A (1966) The reproductive ecology of the red-backed salamander, *Plethodon cinereus*, in Maryland. *Copeia*, **1966**, 183–193.

Schiegg K, Daniels S, Walters J, Priddy J, Pasinelli G (2006) Inbreeding in red-cockaded woodpeckers: Effects of natal dispersal distance and territory location. *Biological Conservation*, **131**, 544–552.

Smith M, Green D (2006) Sex, isolation, and fidelity: unbiased long-distance dispersal in a terrestrial amphibian. *Ecography*, **29**, 649–658.

Smouse P, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.

Stow AJ, Sunnucks P, Briscoe D, Gardner M (2001) The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology*, **10**, 867–878.

Temple H, Hoffman J, Amos W (2006) Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the white-breasted thrasher, *Ramphocinclus brachyurus*. *Molecular Ecology*, **15**, 3449–3458.

Vignieri S (2007) Cryptic behaviours, inverse genetic landscapes, and spatial avoidance of inbreeding in the Pacific jumping mouse. *Molecular Ecology*, **16**, 853–866.

Yurewicz K, Wilbur HM (2004) Resource availability and costs of reproduction in the salamander *Plethodon cinereus*. *Copeia*, **2004**, 28–36.