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RESEARCH ARTICLE

Pronounced differences in genetic structure despite overall ecological similarity for two *Ambystoma* salamanders in the same landscape

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Abstract Studies linking genetic structure in amphibian species with ecological characteristics have focused on large differences in dispersal capabilities. Here, we test whether two species with similar dispersal potential but subtle differences in other ecological characteristics also exhibit strong differences in genetic structure in the same landscape. We examined eight microsatellites in marbled salamanders (*Ambystoma opacum*) from 29 seasonal ponds and spotted salamanders (*Ambystoma maculatum*) from 19 seasonal ponds in a single geographic region in west-central Massachusetts. Despite overall similarity in ecological characteristics of spotted and marbled salamanders, we observed clear differences in the genetic structure of these two species. For marbled salamanders, we observed strong overall genetic differentiation ($F_{ST} = 0.091$, $F'_{ST} = 0.375$), three population-level clusters of populations ($K = 3$), a strong pattern of isolation by distance ($r = 0.58$), and marked variation in family-level structure (from 1 to 23 full-sibling families per site). For spotted salamanders, overall genetic differentiation was weaker ($F_{ST} = 0.025$, $F'_{ST} = 0.102$), there was no evidence of population-level clustering ($K = 1$), the pattern of isolation by distance ($r = 0.17$) was much weaker compared to marbled salamanders, and there was less variation in family-level structure (from 10 to 36 full-sibling families per site). We suspect that a combination of breeding site fidelity, effective population size, and generation interval is responsible for these marked differences. Our results suggest that marbled salamanders, compared to spotted salamanders, are more sensitive to fragmentation from various land-use activities and would be less likely to recolonize extirpated sites on an ecologically and conservation-relevant time frame.

Keywords Genetic structure · Full-sibling families · *Ambystoma* · Effective number of breeders · Life-history

Introduction

Multi-species conservation planning requires that we understand whether species with similar ecological characteristics interact with the landscape in a similar manner (Nicholson and Possingham 2006; Schwenk and Donovan 2011). It is often assumed that suites of ecologically similar species have similar population responses to the same landscape features (Lambeck 1997; Whiteley et al. 2006; Richardson 2012). One way to test the species-specificity of population responses and to determine the appropriate scale of conservation actions is through examination of the genetic structure of multiple species in the same landscape. Comparisons of the genetic structure of multiple species in the same landscape can be used to test whether certain landscape features have a similar disruptive effect on gene flow across species and whether species-specific ecological

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differences lead to differences in how genetic variation is distributed within and among populations of each species (Whiteley et al. 2004). These comparisons have been made for a growing number of taxonomically diverse sets of species (Turner and Trexler 1998; McDonald et al. 1999; King and Lawson 2001; Dawson et al. 2002; Whiteley et al. 2004; Steele et al. 2009; Delaney et al. 2010; Lai et al. 2011; Richardson 2012). Certain characteristics are key drivers of patterns of genetic structure. In particular, traits related to natal philopatry, dispersal potential, specificity of habitat requirements particularly in relation to breeding, and effective population size can strongly influence genetic structure and have been found to drive species-specific similarities and differences (Bohonak 1999; Whiteley et al. 2004; Steele et al. 2009).

Amphibians, due to widespread population declines, have been the focus of intensive research efforts (Houlahan et al. 2000; Stuart et al. 2004). Several studies have tested for species-specific patterns in genetic structure based on predictions drawn from ecological characteristics (Steele et al. 2009; Goldberg and Waits 2010; Mullen et al. 2010; Richardson 2012; Sotiropoulos et al. 2013). These recent studies have tested for differences in genetic structure for species with different locomotion abilities (e.g. hopping versus crawling, (Goldberg and Waits 2010; Richardson 2012)) or terrestrial versus aquatic metamorphosis (Steele et al. 2009; Sotiropoulos et al. 2013). Differences in genetic structure have generally been in line with predicted differences in dispersal abilities (Steele et al. 2009; Goldberg and Waits 2010; Richardson 2012; Sotiropoulos et al. 2013). This work has been used to urge that, as we start to consider ecosystem conservation approaches, we still consider species-specific interactions with the landscape (Richardson 2012). A next critical step is to test whether species with similar ecological characteristics have similar genetic population structure in the same landscape. It is possible that even species with very similar overall ecological characteristics have widely different genetic structures, which, if true, would further complicate matters for multi-species conservation planning.

In the northeastern United States, marbled (*Ambystoma opacum*) and spotted (*Ambystoma maculatum*) salamanders have many ecological similarities, including dispersal potential, that lead to predictions of minor differences in genetic structure over similar spatial scales. Both species breed in temporary or seasonal ponds, commonly referred to as vernal pools (Petranka 1998). These ponds support the egg and larval stages while upland forests provide habitat for juveniles and adults (Petranka 1998). Adults and juveniles have the same mode of locomotion and similar observed dispersal distances. In a series of 14 seasonal ponds in Massachusetts, estimated dispersal distances for first-time marbled salamander breeders ranged from 142 to 1,297 m (68 % of observations from 200 to 400 m) and from 105 to 439 m for experienced breeders (Gamble et al. 2007). Dispersal among breeding sites has not been rigorously quantified in spotted salamanders, but observed ranges of maximum post-breeding emigration distances to upland sites have ranged from 2 to 467 m in three separate studies (Montieth and Paton 2006; Madison 1997; Veysey et al. 2009). Relative to other comparisons of the effects of ecological characteristics on genetic structure in amphibians, differences between spotted and marbled salamanders appear to be slight and conservation efforts would likely group these organisms together. However, three factors may contribute to greater genetic differentiation of marbled relative to spotted salamanders in the same landscape: (1) greater natal philopatry and habitat specificity related to fall breeding, (2) smaller population sizes, and (3) a shorter generation interval.

First, natal philopatry in marbled salamanders might be greater because they court in the late summer and early fall and subsequently lay eggs terrestrially in receded or dry pond basins (Noble and Brady 1933; Bishop 1941; Gamble et al. 2007). Gamble et al. (2007) observed that 91.0 % of first-time marbled salamander breeders returned to natal ponds. Further, 96.4 % of experienced breeders maintained breeding site fidelity through multiple seasons (Gamble et al. 2007). In contrast, spotted salamanders migrate to already-filled seasonal ponds in late spring (March and April) where courtship and breeding aggregations occur (Husting 1965). Movement to already filled ponds may indicate less breeding habitat specificity and less philopatry compared to marbled salamanders. Based on ecological data spotted salamanders are generally assumed to exhibit strong philopatry, but data are limited (Whitford and Vinegar 1966; Vasconcelos 1999). Limited genetic data have shown weak fine-scale genetic subdivision, which is inconsistent with strong philopatry (Zamudio and Wieczorek 2007; Purrenhage et al. 2009; Richardson 2012).

Second, marbled salamanders appear to generally have smaller local population sizes in Massachusetts—the focal region for our study. Here, breeding populations are small, likely due to proximity to northern range limits (Gamble et al. 2007). This species is listed as "Threatened" under the state Endangered Species Act (M.F.L c.131A and regulations 321 CMR 10.00). Spotted salamanders are generally more widespread and locally abundant (Egan and Paton 2004) and are not considered threatened. If we assume census size reflects effective population size, smaller census size in marbled compared to spotted salamanders could lead to greater effects of genetic drift within local breeding populations and greater allele frequency divergence among populations.

Third, generation length (average age at reproduction) is shorter for marbled (4–5 years; Gamble et al. 2009;

Plunkett 2009) relative to spotted (7–8 years; Flageole and Leclair 1992) salamanders. Shorter generation intervals in marbled salamanders could lead to a more rapid development of genetic structure in response to past fragmentation effects.

In this paper, we compare the genetic structure of spotted and marbled salamanders in a single geographic region in west-central Massachusetts. We used eight microsatellites for each species to examine 974 marbled salamanders from 29 seasonal ponds and 440 spotted salamanders from 19 seasonal ponds. We examined: (1) family- and population-level genetic structure, (2) effective number of breeders (N_b), and (3) the geographic scale of genetic differentiation for both species. We predicted that we would observe little to no differences in population genetic structure between these two species owing to the overall similarity of ecological characteristics. Alternatively, if differences in genetic structure were observed, we predicted that marbled salamanders would exhibit stronger genetic differentiation due to subtle, but potentially important differences in the timing of breeding (and associated habitat specificity), local effective population size, and generation interval.

Methods

Sample Collection

Larval salamanders were collected from seasonal ponds in the Pioneer Valley in west-central Massachusetts (Fig. 1). We collected marbled salamanders from 29 (m1–m29) ponds and spotted salamanders from 19 ponds (s1–s19; Table S1; Fig. 1). Collection occurred during March and April in 2010 for marbled salamanders and July and August in 2007 and 2008 for spotted salamanders. Each pond was sampled by a visual scan of the pond's perimeter at night with a headlamp. We attempted to capture approximately 30 larval salamanders of the focal species around the entire perimeter of each pond, although the ultimate number of individuals captured varied somewhat among ponds due to variation in local population size (Tables 1, 3). If we encountered multiple larval salamanders within one section, we captured only a subset of those salamanders in an effort to minimize the collection of closely related individuals. We then continued around the pond perimeter. A tissue sample (tip of tail) was taken as a source of genetic material and larvae were returned to each pond.

A second, larger sample of marbled salamanders was collected from one of the ponds (m7) on May 7, 2010 (m7.2, Table 1). The pond dried early and recently (within 24 h) deceased larval salamanders were collected and frozen whole until analysis. These deceased salamanders were likely at least 1-month from metamorphosis and emigration (Timm et al. 2007) and therefore do not represent slowly developing individuals within the cohort. Genetic analysis of this single large sample allowed us to assess the effect of sample size on estimates of genetic parameters and family structure for this site. The smaller m7 sample was used for analyses described below, unless otherwise specified.

Genetic analyses

DNA was extracted from each larval tail clip with a standard salt precipitation procedure. For marbled salamanders, we genotyped all individuals at eight microsatellite loci: *AmaD49*, *Aop36*, *AmaD95*, *AmaD184*, *AmaD42*, *AmaD328*, *AjeD23*, and *AmaD321* (Julian et al. 2003a, b; Croshaw et al. 2005). We genotyped all spotted salamanders at the following eight microsatellite loci: *AmaD321*, *AmaD95*, *AmaD287*, *AmaD328*, *AmaC40*, *AjeD23*, *AmaD49*, *AmaD184* (Julian et al. 2003a, b). We used Qiagen multiplex buffer (Qiagen, Inc.) and the manufacturer recommended thermalcycler profile for microsatellite amplification. An Applied Biosystems 3130xl capillary sequencer was used to determine the size of PCR fragments. GeneMapper and PeakScanner (Applied Biosystems) were used to score individual genotypes based on the ROX 500 size standard run with each individual.

We used GENEPOP version 4.0.10 (Rousset 2008) to test for deviations from Hardy–Weinberg (HW) expectations and gametic (linkage) disequilibrium (LD). Because of the large number of assumptions associated with these tests, we used the conservative Bonferroni correction (Rice 1989) to correct for inflated type I error rates due to multiple testing (Narum 2006). For tests of HW expectations, we corrected for the eight locus-tests performed per population sample. For tests of LD, we corrected for the 28 tests per population. We used FSTAT ver. 2.9.3.2 (Goudet 2001) to estimate allele frequencies, observed (H_O) and expected (H_E) heterozygosity per locus and population, mean within-population expected heterozygosity (H_S), mean allelic richness per population (AR; mean number of alleles scaled to the smallest sample size; $N = 11$ for marbled, $N = 12$ for spotted), and F_{IS}.

Family structure within single-cohort samples can cause deviations from HW expectations, elevated LD, and biased analyses of genetic structure (Allendorf and Phelps 1981; Anderson and Dunham 2008; Rodriguez-Ramilo and Wang 2012). To minimize any biases associated with family structure, we first reconstructed full-sibling families within each sample with COLONY version 1.2 (Wang 2004). Second, we randomly selected one individual per family from each population sample to obtain a random subset of

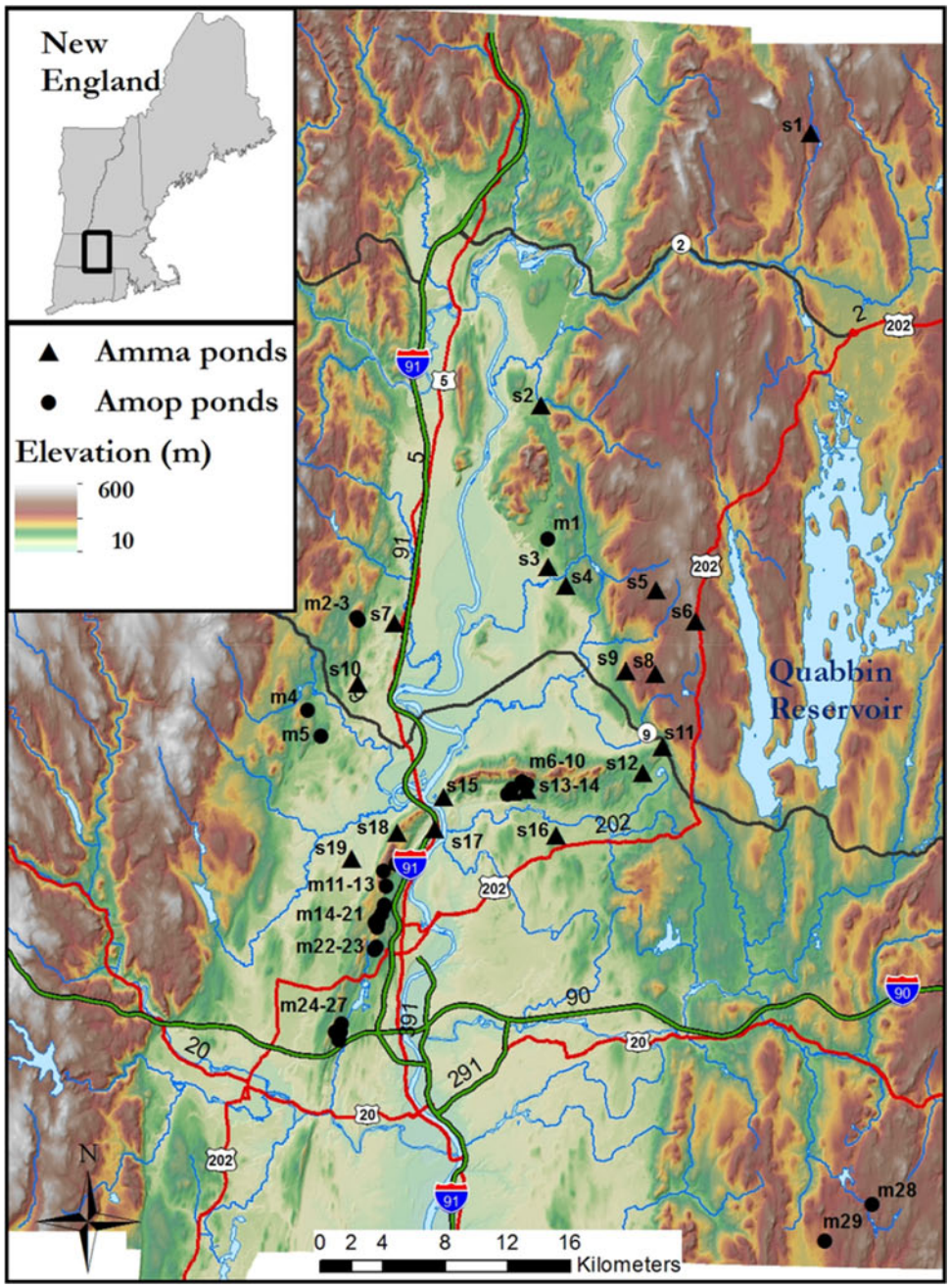


Fig. 1 Map of west-central Massachusetts, USA showing 29 marbled and 19 spotted salamander seasonal ponds examined in this study. Marbled salamander sites are labeled with an 'm' and shown as a *filled circle*. Amop is short for *Ambystoma opacum*. Spotted salamander sites are labeled with an 's' and shown as a filled triangle. Amma is short for *Ambystoma maculatum*

the data that should be free of family structure effects (Rodriguez-Ramilo and Wang 2012). We did not resample the data to form multiple random subsets because we were removing full-siblings that are by definition highly genetically similar and therefore resampled subsets would be assured of producing similar results. We performed analyses with the entire data set and with the randomly chosen subset of the data. To quantify aspects of the distribution of full-sib families within each site, we calculated family evenness (*FE*) for each cohort sample according to the equations: $FE = \frac{H'}{H'_{Max}}$, where $H' = \sum_1^S p_i \ln(p_i)$ and $H'_{Max} = \ln(S)$ (Mulder et al. 2004). *S*, which usually represents the number of species in an evenness calculation, here represented the number of families and p_i represented the proportion of the *i*th family.

We constructed models to further examine widespread signal of deviation from HW proportions and gametic disequilibrium. We predicted that family structure within the cohort-specific samples would be the most likely cause of the large amount of significant HW deviations and gametic disequilibrium, along with factors that influence power (*N* and *AR*). We constructed separate generalized

Table 1 Genetic summary statistics for larval marbled salamanders (*A. opacum*) captured in 29 seasonal ponds in Massachusetts, USA

Site name	*N*	HW	LD	Families	Mean FS	*FE*	A_O
m1	30	1	2	10	3.0	0.953	5.4
m2	30	0	0	18	1.7	0.969	7.1
m3	30	0	6	15	2.0	0.898	7.4
m4	30	0	0	2	15.0	0.837	2.5
m5	29	0	1	17	1.7	0.961	7.5
m6 (UM2)	31	0	5	10	3.1	0.926	6.0
m7 (UM3)	30	1	1	17	1.8	0.954	7.3
m7.2 (UM3)	147	1	12	64	2.3	0.954	8.9
m8 (UM4)	30	0	0	23	1.3	0.982	8.5
m9 (UM5)	30	1	1	15	1.9	0.948	7.9
m10 (UM12)	30	0	1	18	1.7	0.967	7.4
m11	11	1	1	3	3.7	0.943	3.8
m12	30	0	3	12	2.5	0.957	8.1
m13	29	0	0	14	1.6	0.951	8.1
m14	30	6	3	2	15.0	0.469	3.9
m15	30	0	2	21	1.4	0.960	8.5
m16	30	3	7	10	3.0	0.909	9.0
m17	30	0	1	21	1.4	0.958	10.6
m18	29	2	6	13	2.2	0.931	9.0
m19	29	1	2	13	2.2	0.941	9.1
m20	30	0	3	12	2.3	0.924	7.6
m21	30	0	2	19	1.6	0.959	9.1
m22	30	0	4	17	1.8	0.944	8.6
m23	30	1	1	22	1.4	0.981	10.1
m24	30	0	8	7	4.1	0.926	5.1
m25	30	3	11	11	2.7	0.887	8.3
m26	30	4	6	8	3.8	0.964	6.4
m27	20	0	2	13	1.5	0.949	8.4
m28	30	7	0	1	–	–	4.3
m29	30	1	3	8	3.8	0.934	6.8

Site name	A_O-RS	*AR*	H_S	H_S-RS	F_{IS}	F_{IS}-RS	N_b
m1	5.1	4.8	0.654	0.684	0.012	−0.005	16.3 (11.0–24.2)
m2	6.8	5.9	0.725	0.737	−0.006	−0.027	115.8 (64.0–369.4)
m3	6.9	6.2	0.747	0.747	−0.032	−0.016	28.1 (20.2–40.9)
m4	–	2.4	0.479	–	−0.035	–	62.5 (19.2–INF)
m5	6.9	6.2	0.760	0.766	−0.068	−0.069	51.5 (32.4–97.8)
m6 (UM2)	5.3	4.8	0.655	0.703	0.015	0.147	31.3 (21.2–48.8)
m7 (UM3)	7.1	5.9	0.732	0.750	0.032	0.029	49.5 (34.7–76.4)
m7.2 (UM3)	8.3	6.1	0.751	0.747	−0.018	−0.032	67.6 (54.3–84.6)
m8 (UM4)	8.3	7.1	0.819	0.815	0.007	−0.007	1251.6 (208.2–INF)
m9 (UM5)	7.4	6.4	0.776	0.765	−0.003	0.053	71.2 (48.4–120.6)
m10 (UM12)	7.0	6.0	0.720	0.713	−0.001	0.039	49.8 (32.0–90.0)
m11	–	3.8	0.612	–	0.164	–	3.8 (2.9–7.0)
m12	6.9	6.3	0.702	0.718	0.015	−0.030	33.6 (24.7–47.8)
m13	7.8	6.7	0.779	0.783	−0.045	−0.015	79.7 (44.2–240.9)
m14	–	3.3	0.565	–	−0.290	–	24.7 (12.8–52.2)
m15	8.1	6.8	0.766	0.773	−0.022	0.007	48.8 (34.9–73.7)
m16	7.8	7.0	0.773	0.839	0.047	0.106	18.3 (15.4–21.8)

Table 1 continued

Site name	A_O-RS	*AR*	H_S	H_S-RS	F_{IS}	F_{IS}-RS	N_b
m17	10.3	8.1	0.818	0.816	−0.003	0.023	114.3 (73.8–220.8)
m18	8.5	6.9	0.768	0.783	−0.032	0.030	22.1 (17.4–28.4)
m19	8.1	7.4	0.780	0.790	0.084	0.063	35.4 (27.2–47.5)
m20	6.8	6.2	0.772	0.806	−0.048	−0.058	20.3 (16.0–26.0)
m21	8.6	7.4	0.784	0.794	−0.036	−0.035	79.0 (53.9–132.3)
m22	8.0	6.9	0.803	0.816	−0.025	0.018	35.5 (25.9−51.2)
m23	9.1	7.8	0.835	0.838	−0.053	−0.078	127.6 (73.3–352.8)
m24	4.0	4.5	0.638	0.644	−0.065	0.002	9.7 (7.3–12.6)
m25	7.4	6.8	0.798	0.810	−0.039	0.004	18.0 (14.3–22.8)
m26	5.6	5.4	0.712	0.757	−0.053	0.091	21.3 (15.5–29.7)
m27	7.5	7.2	0.826	0.822	−0.058	−0.065	81.7 (52.1–166.3)
m28	–	3.9	0.718	–	−0.324	–	655.7 (52.4–INF)
m29	6.0	5.7	0.756	0.795	−0.097	−0.140	16.6 (12.6–22.0)

Site numbers are preceded with an "m" for marbled, numbers in parentheses for some sites represent numbers used in Gamble et al. (2007) and Gamble et al. (2009). Measures are as follows: number of individuals genotyped (*N*), number of significant departures from Hardy–Weinberg proportions following Bonferroni correction ($\alpha = 0.05$) for eight locus tests within each populations (HW), number of significant tests for LD following Bonferroni correction ($\alpha = 0.05$) for 28 pairwise tests within each populations (LD), number of estimated full-sibling families (Families), mean number of individuals per full-sibling family (Mean FS), family evenness (*FE*), mean number of observed alleles for the entire data set (A_O) and for the random sample (RS) of one full-sib per family (A_O-RS), allelic richness standardized to $N = 11$ (*AR*), mean expected heterozygosity for the entire data set (H_S) and random sample (H_S-RS), F_{IS} for the entire data set and random sample (F_{IS}-RS), and LDNe-based single-sample estimates of the effective number of breeders (with 95 % confidence intervals) that gave rise to the larval cohort examined (N_b). A_O, *AR*, H_S and F_{IS} were not calculated for random samples if sample size was three or lower. Site m7.2 is shown for comparison purposes only, it was not included in the majority of analyses and it should be noted that summary statistics reflect seven instead of eight loci

linear models (GLMs) to relate either counts of significant HW violations per population or counts of significant tests of LD per population (response variables) to variation in family structure (number of full-sib families and evenness of full sib family distributions), sample size, and allelic richness (predictor variables). We used general linear models with a Poisson error structure and a log link function and performed analyses with R version 2.15.0 (R Development Core Team 2006).

We estimated the effective number of breeders (N_b) for the larvae collected at each site. When applied to single-cohort samples, single-sample N_e estimators provide an estimate of the effective number of breeders that gave rise to that cohort (Waples and Do 2010). All N_b estimates were generated using the single-sample linkage disequilibrium method within the program LDNe version 1.31 (Waples and Do 2008). A monogamous mating model was assumed. N_b estimates were derived using a minimum allele frequency cutoff (P_{crit}) of 0.02. $P_{crit} = 0.02$ has been shown to provide an adequate balance between precision and bias across sample sizes (Waples and Do 2008). 95 % confidence intervals were generated using the jackknife approach.

We combined locus-specific exact tests for allele frequency (genic) differentiation implemented in GENEPOP with Fisher's method. This test assumes that, under the null hypothesis of no allele frequency differentiation at any of the eight loci, the quantity $-2\sum \ln P_j$ is distributed *as* χ^2 with d.f. $= 2\ k$, where k is the number of loci and P_j is the P value for the jth locus (Ryman et al. 2006). We used the less conservative B-Y False Discovery Rate (FDR) correction method to control the type I error rate for results from this combined test (Benjamini and Yekutielie 2001; Narum 2006). We used Meirmans and Hedrick's unbiased estimator G''_{ST} (Meirmans and Hedrick 2011) for estimates of overall and pairwise F'_{ST}. F'_{ST} provides a measure of F_{ST} standardized by its maximum possible value for a given level of within-population genetic diversity (Meirmans and Hedrick 2011). We used Nei's unbiased estimator of G_{ST} (Nei 1987) for estimates of overall and pairwise F_{ST}. Both F'_{ST} and F_{ST} were calculated with GENODIVE version 2.0b22 (Meirmans and Van Tienderen 2004).

For analysis of population groups across geographic space, we used STRUCTURE ver. 2.3.1 (Pritchard et al. 2000) to estimate the number of population clusters (K) with the highest log likelihood. For STRUCTURE analyses, we did not incorporate prior population information. We used 200,000 replicates and 50,000 burn-in cycles under an admixture model. We inferred a separate α for each population (α is the Dirichlet parameter for degree of admixture). We used the correlated allele frequencies model with an

initial λ of 1, where λ parameterizes the allele frequency prior and is based on the Dirichlet distribution of allele frequencies. We allowed F to assume a different value for each population, which allows for different rates of drift among populations. We performed ten runs for each of $K = 1$ to the total number of population samples examined for each species ($N = 29$ for marbled and $N = 19$ for spotted salamanders). We calculated mean q-values for each site and considered a population to be assigned to a cluster if the mean q-values for that group exceeded 0.70.

We also tested the relationship between geographic and genetic distance (Isolation By Distance; IBD) for both species. We examined population-level genetic distances (pairwise F'_{ST} and F_{ST}) and individual-level genetic distances (squared Euclidean (Smouse and Peakall 1999); and chord (Cavalli-Sforza and Bodmer 1971)). Following random selection of one individual per full-sib family from each population sample for marbled salamanders, four sites had three individuals or fewer (m4, m11, m14, and m28). We performed the population-level analyses with and without these sites because small sample sizes can have a large influence on genotypic distributions. We calculated genetic distances and perform Mantel tests with GENODIVE. We used Euclidean distances for geographic distance between each pair of sites. Mean geographic distance between pairs of sites for marbled salamanders was 13.4 km (range 0.1–49.8 km) and for spotted salamanders was 16.5 km (range 0.9–55.0 km).

Results

Variation within populations—marbled salamanders

We examined 974 larval marbled salamanders at eight microsatellite loci from 29 ponds. There was pronounced family-level structure in some ponds. The mean number of estimated full-sib families was 12.8 (range 1–23) and the range of mean family size was 1.3–15 (Table 1). Mean family evenness was 0.892 (range 0–0.982). Mean F_{IS} per population ranged from −0.324 to 0.164 (Table 1). Three of the four sites with three or fewer full-sib families were responsible for the greatest absolute values of F_{IS} (m11, m14, and m28; Table 1). Two of these were strongly negative (m28 and m14) while the other (m11) was positive. Estimates of the effective number of breeders (N_b) were consistent with few reproducing individuals and strong family-level structure in some of the ponds. $\hat{N}_b$ for three sites (m4, m8, and m28) included infinity (Table 1). Imprecision was likely due to so few full-sib families for two of these sites (m4 and m28). Site m8 had the most full-sib families. The confidence interval for m8 likely included infinity because $\hat{N}_b$ was large, though the point estimate was unrealistically large ($\hat{N}_b = 1{,}251.6$). Otherwise, point estimates of $\hat{N}_b$ ranged from 3.8 to 127.6 (Table 1).

Family-level structure was the most likely cause of widespread departures from Hardy–Weinberg (HW) proportions. Significant departures from HW proportions occurred in 62 of 232 tests performed ($P < 0.05$), where 12 were expected by chance ($\alpha = 0.05$). Following correction for approximately eight tests within each population, the mean number of HW violations was 1.1 (range 0–7; Table 1). Ponds in which we sampled few full-sib families tended to have the most violations of HW expectations. Following the random selection of one full-sib per family from all sites, significant departures from HW proportions occurred in three of 217 tests performed ($P < 0.05$), where 11 were expected by chance. Our examination of the influence of number of full-sib families, family evenness, N, and AR on deviations from HW proportions revealed that number of full-sib families had the largest relative effect on the number of significant HW violations per population ($z = -3.4$, $P = 0.0008$) followed by allelic richness ($z = 2.8$, $P = 0.005$). Sample size ($z = 1.2$, $P = 0.24$) and family evenness ($z = -0.64$, $P = 0.52$) had small and nonsignificant relative effects. Combined, these four predictors explained a substantial proportion of variation in the number of significant HW tests per population (explained deviance = 59.4 %).

Family-level structure also appeared to cause gametic (linkage) disequilibrium (LD). Significant LD was detected in 248 of 732 (34 %) tests performed ($P < 0.05$), where 37 were expected by chance ($\alpha = 0.05$). Following correction for approximately 28 tests within each population, the mean number of significant tests for LD was 2.8 (range 0–11; Table 1). Only one locus pair (*AmaD49–AmaD184*) had more than five significant tests ($N = 7$) following Bonferroni correction (correcting for 29 populations per locus pair). Following the random selection on one full-sib per family from all sites, significant LD occurred in 14 of 634 tests performed ($P < 0.05$), where 32 were expected by chance. Number of families had the largest relative effect on the number of significant LD tests per population ($z = -5.05$, $P < 0.0001$) followed by allelic richness ($z = 3.78$, $P = 0.0002$). Family evenness ($z = 2.4$, $P = 0.02$) and sample size ($z = 2.3$, $P = 0.02$) had less relative influence. Combined, these four predictors explained a substantial proportion of variation in the number of significant LD tests per population (explained deviance = 42.5 %). Several ponds had very few estimated full-sib families (m4, $N_{fs.fam} = 2$; m11, $N_{fs.fam} = 3$; m14, $N_{fs.fam} = 2$; and m28, $N_{fs.fam} = 1$) and these sites also had very few significant LD tests (mean = 1). With these populations removed, family evenness had the largest

relative effect on the number of significant LD tests per population ($z = -3.5$, $P = 0.0005$) followed by number of full-sib families ($z = -2.2$, $P = 0.03$). Sample size ($z = 0.45$, $P = 0.65$) and allelic richness ($z = 0.35$, $P = 0.73$) had smaller and nonsignificant relative effects. The model based on this subset of the data explained a greater proportion of variation in the number of significant LD tests per population (explained deviance = 70.0 %).

For the entire data set, the mean number of alleles (A_O) per population ranged from 2.5 to 10.6, mean allelic richness (AR; standardized to $N = 11$) ranged from 2.4 to 8.1, and mean expected heterozygosity (H_S) ranged from 0.479 to 0.835 (Table 1). The subset of the data that contained a random selection of one full-sib per family yielded similar estimates of genetic variation within sites as the entire data set (Table 1). The estimated number of families per pond became the sample size of each site (Table 1). Estimates of A_O and H_S were similar in magnitude (Table 1). Mean F_{IS} ranged from −0.140 to 0.147. Large absolute values of F_{IS} tended to occur in sites with fewer families and were likely due to sub-sampling effects because F_{IS} values were smaller for the complete sample for each extreme case (Table 1). We did not calculate summary statistics for sites with three or fewer families, nor did we calculate AR for the random subsample data set because sample sizes for sites with few families were too small.

The large ($N = 147$) sample from site m7 allowed us to assess the effect of sample size on genetic estimates for this site and to more completely examine family structure in this pond (Table 1). Prior to subsampling based on full-sib family membership, this site had one significant test for HW proportions following Bonferroni correction for eight tests within this population. Twelve tests for LD were significant following Bonferroni correction. Following subsampling one full-sib per family, zero HW and one LD tests remained significant. Genetic summary statistics from the smaller m7 ($N = 30$) and larger m7 samples were generally close in value (Table 1). This sample revealed moderate skew in reproductive success. The 64 full-sib families had between one and eight members and mean family size was 2.3 (Fig. 2).

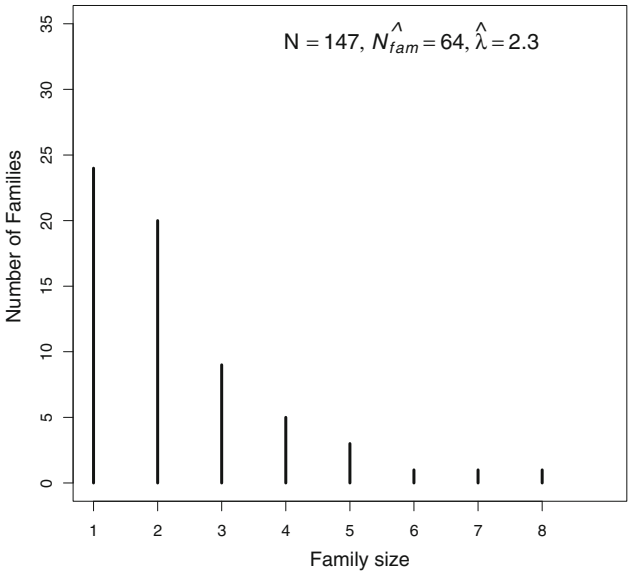


Fig. 2 Family size distribution of a large ($N = 147$) sample of a marbled salamanders collected from site m7. Number of families (N_{fam}) represents the number of full-sibling families estimated from COLONY ver. 1.2. Mean family size (λ) was estimated by fitting a Poisson distribution to these data

Genetic differentiation among populations—marbled salamanders

We found strong overall genetic differentiation, a strong pattern of isolation by distance, three population-level clusters of populations, and marked variation in family-level structure for marbled salamanders. Without taking family structure into account, all of the 406 combined pairwise tests for genic differentiation were significant based on Fisher's method, which tested the joint null hypothesis of no allele frequency differentiation at any of the eight loci, even after controlling the FDR with the B-Y correction method. Overall F'_{ST} was 0.449 (95 % CI 0.343–0.581) and Overall F_{ST} was 0.120 (95 % CI 0.107–0.136). Pairwise F_{ST} ranged from 0.007 to 0.330. Pairwise F'_{ST} ranged from 0.034 to 0.763 (Table 2).

Randomly sampling one full-sib per population sample lowered the signal of genetic differentiation. Based on the random subsample, overall F'_{ST} was 0.375 (0.283–0.509) and overall F_{ST} was 0.091 (0.074–0.108; Table 5). Pairwise F_{ST} ranged from −0.006 to 0.383. Pairwise F'_{ST} ranged from −0.033 to 0.909 (Table 2). Of 406 combined pairwise tests for genetic differentiation among the 29 sites, 373 (92 %) were significant based on Fisher's method and controlling the FDR with the B-Y correction method. Nineteen of the 33 nonsignificant tests (58 %) involved a population with three or fewer full-sibling families as part of the pair. Extremely small samples sizes after random subsampling from full-sib families is likely responsible for the lack of significance despite generally high F_{ST} values for these 19 pairs (mean pairwise $F'_{ST} = 0.441$, mean pairwise $F_{ST} = 0.136$; Table 2). On the other hand, sites m16 through m21 appear to exhibit high gene flow. These sites were located close together (mean pairwise geographic distance = 386.7 m). Of the 15 possible pairwise tests of genetic differentiation among these six sites, 12 (80 %) were non-significant. The mean pairwise F'_{ST} for these six sites was 0.059 (mean pairwise $F_{ST} = 0.012$; Table 2). The results for m16–m21 were not a function of geographic proximity alone. Sites m6–m10 were also

Table 2 Genetic differentiation among 29 seasonal ponds samples of larval marbled salamanders (*A. opacum*) in Massachusetts

	m1	m2	m3	m4	m5	m6	m7	m8	m9	m10	m11	m12	m13	m14	m15
m1	–	**0.066**	**0.109**	**0.240**	**0.126**	**0.151**	**0.136**	**0.090**	**0.102**	**0.051**	**0.150**	**0.113**	**0.078**	**0.121**	**0.077**
m2	**0.229**	–	*0.017*	**0.232**	**0.073**	**0.113**	**0.080**	**0.048**	**0.054**	**0.062**	**0.199**	**0.103**	**0.057**	**0.157**	**0.074**
m3	**0.385**	*0.065*	–	**0.220**	**0.079**	**0.083**	**0.066**	**0.043**	**0.039**	**0.074**	**0.211**	**0.111**	**0.072**	**0.150**	**0.070**
m4	**0.569**	**0.593**	**0.570**	–	**0.247**	**0.285**	**0.213**	**0.180**	**0.181**	**0.191**	**0.324**	**0.221**	**0.196**	*0.307*	**0.169**
m5	**0.457**	**0.293**	**0.324**	**0.655**	–	**0.115**	**0.073**	**0.065**	**0.058**	**0.107**	**0.221**	**0.119**	**0.084**	**0.158**	**0.100**
m6	**0.492**	**0.404**	**0.303**	**0.706**	**0.431**	–	**0.074**	**0.087**	**0.060**	**0.114**	**0.212**	**0.147**	**0.123**	**0.180**	**0.119**
m7	**0.482**	**0.311**	**0.263**	**0.559**	**0.300**	**0.270**	–	**0.036**	**0.034**	**0.109**	**0.207**	**0.113**	**0.093**	**0.183**	**0.105**
m8	**0.361**	**0.216**	**0.198**	**0.523**	**0.310**	**0.362**	**0.164**	–	**0.025**	**0.046**	**0.157**	**0.079**	**0.051**	*0.084*	**0.052**
m9	**0.371**	**0.218**	**0.159**	**0.489**	**0.246**	**0.226**	**0.140**	**0.120**	–	**0.050**	**0.184**	**0.084**	**0.052**	**0.122**	**0.068**
m10	**0.169**	**0.224**	**0.275**	**0.475**	**0.411**	**0.391**	**0.405**	**0.194**	**0.192**	–	**0.130**	**0.092**	**0.053**	**0.066**	**0.051**
m11	**0.427**	**0.617**	**0.665**	**0.697**	**0.715**	**0.629**	**0.658**	**0.560**	**0.604**	**0.388**	–	**0.143**	**0.136**	*0.129*	**0.127**
m12	**0.376**	**0.377**	**0.413**	**0.547**	**0.462**	**0.508**	**0.424**	**0.340**	**0.325**	**0.323**	**0.429**	–	**0.033**	*0.128*	**0.057**
m13	**0.294**	**0.237**	**0.304**	**0.538**	**0.373**	**0.479**	**0.399**	**0.256**	**0.229**	**0.210**	**0.459**	**0.131**	–	**0.102**	**0.035**
m14	**0.376**	**0.538**	**0.525**	*0.677*	**0.569**	**0.593**	**0.653**	*0.341*	**0.449**	**0.219**	*0.357*	*0.423*	**0.386**	–	*0.049*
m15	**0.285**	**0.301**	**0.290**	**0.460**	**0.434**	**0.456**	**0.439**	**0.253**	**0.293**	**0.199**	**0.420**	**0.226**	**0.157**	*0.184*	–
m16	**0.250**	**0.288**	**0.286**	*0.439*	**0.402**	**0.438**	**0.427**	**0.175**	**0.328**	**0.206**	**0.435**	**0.198**	**0.106**	*0.211*	**0.049**
m17	**0.337**	**0.196**	**0.212**	**0.537**	**0.291**	**0.452**	**0.296**	**0.155**	**0.205**	**0.295**	**0.568**	**0.248**	**0.105**	*0.396*	**0.170**
m18	**0.254**	**0.296**	**0.325**	*0.417*	**0.411**	**0.522**	**0.365**	**0.160**	**0.299**	**0.246**	**0.376**	**0.255**	**0.195**	*0.315*	**0.089**
m19	**0.358**	**0.276**	**0.249**	*0.413*	**0.390**	**0.386**	**0.319**	**0.205**	**0.262**	**0.296**	**0.508**	**0.149**	**0.117**	*0.340*	**0.137**
m20	**0.399**	**0.359**	**0.323**	**0.678**	**0.385**	**0.503**	**0.431**	**0.300**	**0.349**	**0.395**	**0.619**	**0.398**	**0.241**	*0.395*	**0.237**
m21	**0.268**	**0.251**	**0.275**	**0.483**	**0.353**	**0.451**	**0.318**	**0.200**	**0.274**	**0.239**	**0.483**	**0.199**	**0.126**	**0.389**	**0.123**
m22	**0.385**	**0.391**	**0.381**	**0.579**	**0.322**	**0.403**	**0.385**	**0.248**	**0.283**	**0.329**	**0.592**	**0.350**	**0.170**	**0.424**	**0.302**
m23	**0.441**	**0.369**	**0.394**	**0.602**	**0.299**	**0.391**	**0.394**	**0.269**	**0.260**	**0.347**	**0.560**	**0.386**	**0.200**	**0.405**	**0.241**
m24	**0.422**	**0.342**	**0.403**	**0.446**	**0.505**	**0.531**	**0.430**	**0.361**	**0.387**	**0.360**	**0.715**	**0.349**	**0.340**	**0.617**	**0.338**
m25	**0.472**	**0.323**	**0.385**	**0.737**	**0.282**	**0.407**	**0.424**	**0.295**	**0.332**	**0.339**	**0.660**	**0.381**	**0.323**	**0.477**	**0.355**
m26	**0.499**	**0.333**	**0.312**	**0.547**	**0.116**	**0.350**	**0.253**	**0.250**	**0.130**	**0.324**	**0.631**	**0.322**	**0.255**	**0.535**	**0.327**
m27	**0.290**	**0.334**	**0.368**	**0.724**	**0.310**	**0.407**	**0.433**	**0.225**	**0.342**	**0.316**	**0.523**	**0.457**	**0.271**	**0.457**	**0.362**
m28	**0.581**	**0.690**	**0.620**	*0.909*	**0.816**	**0.771**	**0.855**	**0.493**	**0.555**	**0.534**	*0.755*	**0.791**	**0.675**	*0.783*	**0.588**
m29	**0.439**	**0.367**	**0.397**	**0.566**	**0.480**	**0.475**	**0.449**	**0.288**	**0.328**	**0.413**	**0.631**	**0.344**	**0.293**	*0.482*	**0.306**

	m16	m17	m18	m19	m20	m21	m22	m23	m24	m25	m26	m27	m28	m29
m1	**0.060**	**0.084**	**0.068**	**0.094**	**0.102**	**0.070**	**0.096**	**0.105**	**0.142**	**0.119**	**0.140**	**0.072**	**0.138**	**0.114**
m2	**0.061**	**0.044**	**0.071**	**0.065**	**0.082**	**0.059**	**0.087**	**0.078**	**0.106**	**0.073**	**0.085**	**0.074**	**0.140**	**0.086**
m3	**0.060**	**0.046**	**0.077**	**0.058**	**0.072**	**0.063**	**0.083**	**0.082**	**0.122**	**0.086**	**0.078**	**0.079**	**0.119**	**0.091**
m4	*0.143*	**0.184**	*0.151*	*0.147*	**0.241**	**0.173**	**0.199**	**0.202**	**0.198**	**0.257**	**0.206**	**0.250**	*0.383*	**0.208**
m5	**0.080**	**0.061**	**0.093**	**0.087**	**0.082**	**0.078**	**0.067**	**0.059**	**0.149**	**0.060**	**0.028**	**0.064**	**0.154**	**0.105**
m6	**0.100**	**0.108**	**0.134**	**0.098**	**0.123**	**0.113**	**0.097**	**0.090**	**0.173**	**0.099**	**0.095**	**0.097**	**0.151**	**0.119**
m7	**0.088**	**0.064**	**0.085**	**0.073**	**0.096**	**0.073**	**0.083**	**0.081**	**0.130**	**0.093**	**0.063**	**0.093**	**0.156**	**0.102**
m8	**0.030**	**0.029**	**0.032**	**0.040**	**0.057**	**0.039**	**0.046**	**0.047**	**0.097**	**0.055**	**0.054**	**0.041**	**0.069**	**0.056**
m9	**0.065**	**0.043**	**0.068**	**0.058**	**0.075**	**0.060**	**0.059**	**0.051**	**0.113**	**0.071**	**0.031**	**0.071**	**0.092**	**0.072**
m10	**0.046**	**0.069**	**0.062**	**0.073**	**0.095**	**0.059**	**0.077**	**0.078**	**0.115**	**0.081**	**0.086**	**0.073**	**0.111**	**0.102**
m11	**0.115**	**0.158**	**0.111**	**0.148**	**0.178**	**0.141**	**0.165**	**0.151**	**0.266**	**0.187**	**0.196**	**0.146**	*0.205*	**0.186**
m12	**0.044**	**0.058**	**0.064**	**0.037**	**0.095**	**0.048**	**0.081**	**0.086**	**0.111**	**0.090**	**0.085**	**0.105**	**0.172**	**0.376**
m13	**0.020**	**0.021**	**0.042**	**0.025**	**0.050**	**0.027**	**0.034**	**0.038**	**0.097**	**0.066**	**0.059**	**0.053**	**0.112**	**0.062**
m14	*0.048*	*0.096*	*0.083*	*0.087*	*0.101*	**0.101**	**0.103**	**0.096**	**0.212**	**0.119**	**0.147**	**0.112**	*0.220*	*0.128*
m15	**0.010**	**0.035**	**0.020**	**0.030**	**0.050**	**0.027**	**0.062**	**0.047**	**0.098**	**0.074**	**0.077**	**0.073**	**0.099**	**0.066**
m16	–	*0.004*	*0.005*	*−0.006*	*0.012*	*−0.002*	**0.034**	**0.025**	**0.075**	**0.064**	**0.067**	**0.047**	*0.005*	**0.042**
m17	*0.023*	–	*0.013*	*0.007*	*0.017*	*0.000*	**0.034**	**0.024**	**0.077**	**0.055**	**0.045**	**0.047**	**0.081**	**0.049**
m18	*0.024*	*0.067*	–	*0.012*	**0.039**	*0.008*	**0.061**	**0.044**	**0.086**	**0.088**	**0.072**	**0.061**	**0.084**	**0.069**

Table 2 continued

	m16	m17	m18	m19	m20	m21	m22	m23	m24	m25	m26	m27	m28	m29
m19	*−0.033*	*0.034*	*0.058*	–	**0.039**	*−0.003*	**0.047**	**0.034**	**0.084**	**0.062**	**0.049**	**0.062**	**0.110**	**0.061**
m20	*0.066*	*0.092*	**0.191**	**0.191**	–	**0.031**	**0.055**	**0.047**	**0.133**	**0.079**	**0.082**	**0.073**	**0.089**	**0.075**
m21	*−0.014*	*0.002*	*0.040*	*−0.014*	**0.155**	–	**0.040**	**0.033**	**0.073**	**0.066**	**0.065**	**0.057**	**0.118**	**0.061**
m22	**0.199**	**0.183**	**0.307**	**0.237**	**0.290**	**0.208**	–	*0.009*	**0.117**	**0.048**	**0.061**	**0.036**	**0.085**	**0.068**
m23	**0.155**	**0.141**	**0.234**	**0.184**	**0.263**	**0.178**	*0.054*	–	**0.117**	**0.044**	**0.052**	**0.038**	**0.097**	**0.074**
m24	**0.294**	**0.289**	**0.301**	**0.299**	**0.486**	**0.262**	**0.439**	**0.454**	–	**0.126**	**0.105**	**0.141**	**0.228**	**0.122**
m25	**0.363**	**0.295**	**0.432**	**0.311**	**0.409**	**0.335**	**0.254**	**0.247**	**0.462**	–	**0.036**	**0.033**	**0.117**	**0.087**
m26	**0.330**	**0.212**	**0.312**	**0.216**	**0.374**	**0.289**	**0.284**	**0.254**	**0.350**	**0.167**	**–**	**0.069**	**0.124**	**0.095**
m27	**0.277**	**0.261**	**0.308**	**0.320**	**0.395**	**0.298**	**0.198**	**0.223**	**0.529**	**0.178**	**0.327**	–	**0.085**	**0.078**
m28	*0.054*	**0.610**	**0.538**	**0.763**	**0.568**	**0.730**	**0.637**	**0.701**	**0.858**	**0.823**	**0.751**	**0.581**	–	*0.089*
m29	**0.233**	**0.253**	**0.327**	**0.294**	**0.379**	**0.299**	**0.353**	**0.404**	**0.436**	**0.443**	**0.423**	**0.407**	*0.492*	–

F'_{ST} is below the diagonal. F_{ST} is above the diagonal. Bold values were significant following Fisher's method for combining P values across the eight exact tests for each of the eight loci tested per population pair and following FDR correction (B-Y FDR correction for 406 tests, nominal $P = 0.0076$). Italicized values were not significant following Fisher's method for combining P values and FDR correction (same nominal P value)

geographically nearby (mean pairwise geographic distance = 778.8 m) and all pairwise test of genic differentiation were significant.

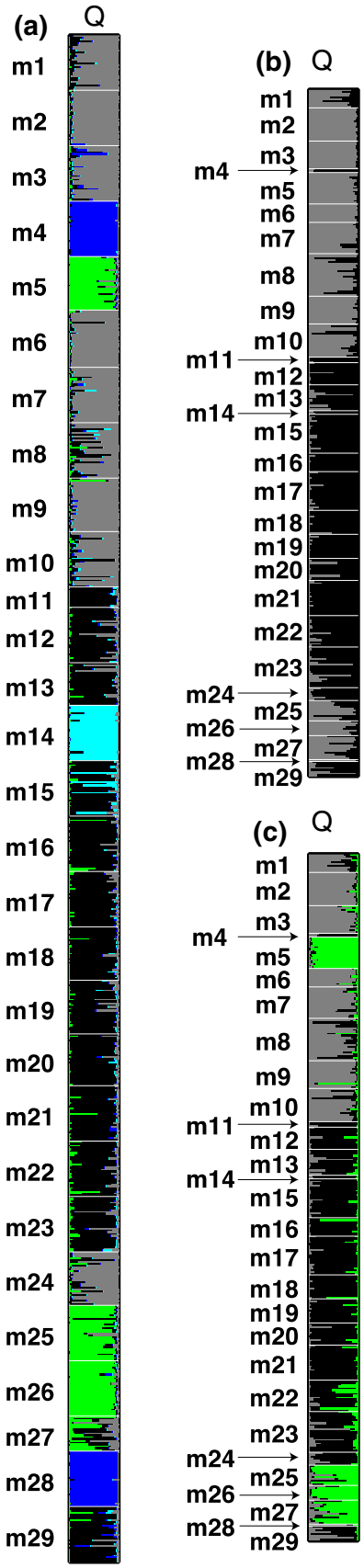
Family-level structure had a large influence on genetic clusters revealed by STRUCTURE. There was evidence for at least five clusters (Fig. S1), but it was difficult to distinguish between population-level and family-level structure when all individuals were included. Furthermore, a pattern of IBD can also make K sensitive to sampling effects. For the $K = 5$ model, two of the clusters corresponded to sites with the fewest full-sib families. Sites m4 ($N_{fs.fam} = 2$) and m28 ($N_{fs.fam} = 1$) formed one cluster (dark blue; Fig. 3a). Site m14 ($N_{fs.fam} = 2$) formed another cluster (light blue; Fig. 3a). The remaining sites formed three clusters and appeared to reflect population-level structure independent of family structure. One cluster included m1, m2, m3, m6–m9, m10, and m24 (grey; Fig. 3a). A second cluster included m5, m25, and m26 (light green, Fig. 3a). The third cluster included m11–m13, m15–m23, and m29. Sites m8, m10 and m15 had mean q-values <0.75 for the most likely group and therefore had relatively high admixture (Table S2). Site m27 had high levels of admixture with mean $q < 0.40$ for all groups (Table S2).

Analysis of the random subsample of the data with one individual per full-sib family further supported the three-cluster population-level inference. For models with this subset of the data, estimated STRUCTURE log-likelihoods increased from $K = 1$ to $K = 3$, after which estimated log-likelihoods declined and variance among the ten runs increased markedly (Fig. S1). The model with $K = 2$ had a first cluster that included m1–3, m5–10, m25–m29 (grey; Fig. 3b). Sites m4, m11–m24, and m29 formed the second cluster (black; Fig. 3b). Sites m1, m25, m27 and m29 had relatively high admixture (q-values <0.80 for the most likely cluster; Table S2). The model with $K = 3$ had stronger support than the $K = 2$ model. In the $K = 3$ model, the first cluster included m1–m3, and m6–m10 (grey; Fig. 3c). The second cluster included m11–m24, and m29 (black; Fig. 3c). The third cluster included m5, and m25–m27 (light green; Fig. 3c). Sites m1, m8, m22, m27, and m29 exhibited the strongest signal of mixed ancestry (q-values <0.75 for the most likely cluster; Table S2; Fig. 3c).

There was a strong pattern of IBD for marbled salamanders. The strong pattern was evident with both F'_{ST} ($r = 0.50$, $P < 0.001$) and F_{ST} ($r = 0.36$, $P = 0.003$). We also performed analyses without the four populations that contained three or fewer full-sib families (m4, m11, m14, and m28). This increased the IBD relationship for both F'_{ST} ($r = 0.58$, $P < 0.001$; Table 5; Fig. 4) and F_{ST} ($r = 0.52$, $P < 0.001$). IBD also occurred for individual squared Euclidean ($r = 0.08$, $P = 0.003$) and chord ($r = 0.10$, $P < 0.001$) distances.

Variation within populations—spotted salamanders

We examined 440 larval spotted salamanders at eight microsatellite loci from 19 ponds. For the entire data set, the mean number of alleles (A_O) per population ranged from 6.1 to 8.1, mean allelic richness (AR; standardized to $N = 12$) ranged from 5.3 to 6.6, and mean expected heterozygosity (H_S) ranged from 0.691 to 0.777 (Table 3). Mean F_{IS} ranged from −0.069 to 0.037. The mean number of estimated full-sib families was 17.2 (range 10–36) and the range of mean family size was 1.1–1.7 (Table 3). Mean



◀ **Fig. 3** Proportion of the genome (*Q*) of each individual assigned by STRUCTURE to each population sample for marbled salamanders. Results correspond to models with the entire data set (**a**) and for the subset of the data with one randomly sampled full-sib per family from all sites (**b**, **c**). In **a** the best-supported STRUCTURE model with $K = 5$ is shown. In **b** $K = 2$, and in **c** $K = 3$. Each *row* corresponds to an individual and sample locations are separated by *horizontal bars*. Each of the clusters was given a *separate color*

family evenness was 0.974 (range 0.958–0.988). Point estimates of effective number of breeders (N_b) revealed large and difficult to estimate N_b in most sites. Thirteen of 19 sites had confidence intervals that included infinity. Negative point estimates in three cases indicated that the effect of small sample size overwhelmed the LD signal. For the six sites with non-infinite confidence intervals, point estimates of ranged from 28.3 to 72.2 (Table 3).

Significant departures from Hardy–Weinberg (HW) proportions occurred in eight of 152 (5 %) tests performed ($P < 0.05$), with the same number expected by chance ($\alpha = 0.05$). One test for one of the loci (*AmaD32*1) remained significant following Bonferroni correction for 19 sites per locus ($\alpha = 0.05$). One test in each of two populations (s3 and s8; Table 3) remained significant following Bonferroni correction for eight loci per population ($\alpha = 0.05$). Significant linkage disequilibrium (LD) was detected in 31 of 518 (6 %) tests performed ($P < 0.05$; Table 3), with 26 by chance ($\alpha = 0.05$). Following Bonferroni correction for approximately 28 tests within each population, five tests in four different populations remained significant ($\alpha = 0.05$).

Family-level structure was much less pronounced in spotted salamanders than marbled salamanders, but we conservatively took a subset of the data that contained only one randomly selected individual per full-sibling family and used this for some analyses. This subset of the data contained a total of $N = 323$ individuals. The estimated number of families per pond became each site's sample size (Table 3). The random subset of the data that included one individual per full-sibling family yielded similar results for tests of both HW proportions and LD. Significant departures from Hardy–Weinberg (HW) proportions occurred in seven of 152 tests performed ($P < 0.05$), fewer than expected by chance (eight at $\alpha = 0.05$). Significant linkage disequilibrium (LD) was only detected in 11 of 518 (2 %) tests performed ($P < 0.05$). This subset of the data yielded similar estimates of genetic variation within sites (Table 3).

Genetic differentiation among populations spotted salamanders

We found weaker overall genetic differentiation in spotted compared to marbled salamanders. The pattern of isolation

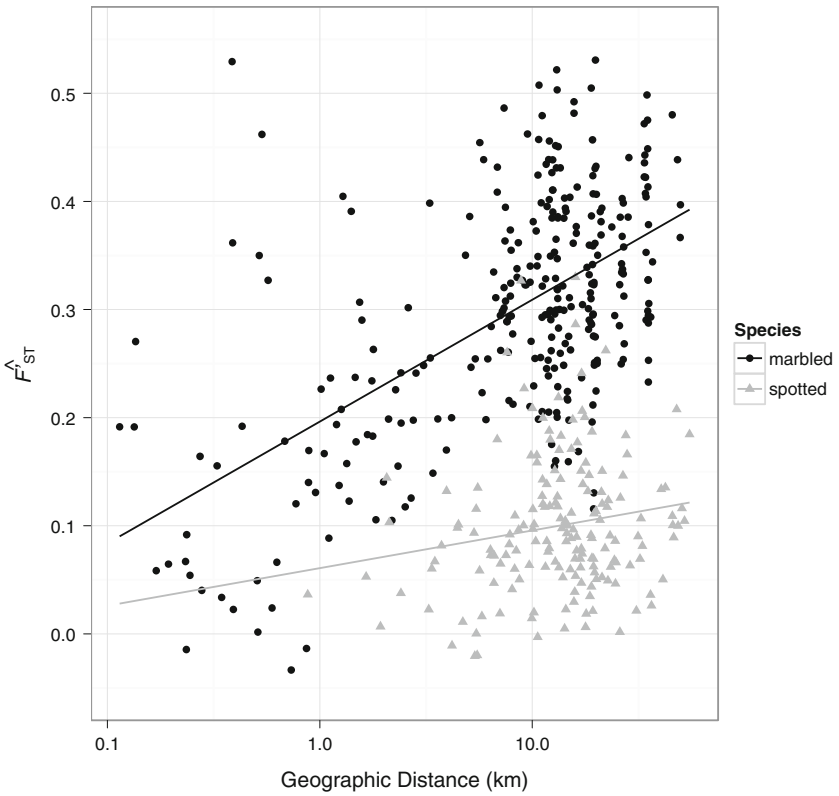


Fig. 4 Genetic versus geographic distance for marbled and spotted salamanders in west-central Massachusetts. Marbled salamanders are shown as *filled circles*, spotted as *grey triangles*. F'_{ST} values for both species are based on a subset of the data with one randomly sampled full-sibling per family from all sites. The four sites that contained three or fewer full-sib families (m4, m11, m14, and m28) were also removed from the marbled salamander analysis. Note the log-transformation of geographic distance values on the *x*-axis

by distance was weaker, there was little variation in family structure within ponds, and there was no evidence for population-level clustering for spotted salamanders. Without taking family structure into account, 156 of the 171 (91 %) combined pairwise tests for genetic differentiation were significant based on Fisher's method after controlling the FDR with the B-Y correction method. Overall F'_{ST} was 0.131 (95 % CI 0.108–0.166) and overall F_{ST} was 0.033 (95 % CI 0.027–0.041). Pairwise F'_{ST} ranged from 0.005 to 0.33. Pairwise F_{ST} ranged from 0.005 to 0.085 (Table 4).

Randomly sampling one full-sibling per population sample slightly lowered the signal of genetic differentiation. Overall F'_{ST} was 0.102 (95 % CI 0.080–0.130) and overall F_{ST} was 0.025 (95 % CI 0.017–0.036; Table 5). For the random subset of the data, 78 of 171 (46 %) tests were significant based on Fisher's method after controlling the FDR with the B-Y correction method. Sites s1–s9 exhibited particularly low genetic differentiation. Of the 36 pairwise comparisons for these nine sites, 27 (75 %) were non-significant for pairwise test of genetic differentiation (mean pairwise F'_{ST} for s1–s9 = 0.036, mean pairwise $F_{ST} = 0.009$).

The STRUCTURE model with the greatest support was $K = 1$ for the entire data set and with the random subset (Fig. S1). Further, IBD was weaker for spotted compared to marbled salamanders. The relationship between genetic and geographic distance for spotted salamanders was positive but not significant for F'_{ST} ($r = 0.17$, $P = 0.08$; Table 5; Fig. 4) and F_{ST} ($r = 0.16$, $P = 0.10$). IBD was not evident for individual squared Euclidean ($r = -0.01$, $P = 0.40$) or chord ($r = 0.01$, $P = 0.35$) distances.

Discussion

Despite overall similarity in ecological characteristics of spotted and marbled salamanders, we observed clear differences in the genetic structure of these two species in west-central Massachusetts. For marbled salamanders, we observed strong overall genetic differentiation, three population-level clusters of populations, a strong pattern of isolation by distance, and marked variation in family-level structure. For spotted salamanders, there was no evidence of population-level clustering, the pattern of isolation by distance was much weaker compared to marbled salamanders, and there was little variation in family-level structure. We suspect that a combination of factors is responsible for these marked differences, namely natal philopatry and breeding site fidelity, effective population size, and generation interval.

Natal philopatry

Pond-breeding amphibians are often classified as poor dispersers that are closely tied to water and their natal

Table 3 Genetic summary statistics for larval spotted salamanders (*A. maculatum*) captured in 19 seasonal ponds in Massachusetts, USA

Site name	*N*	HW	LD	Families	Mean FS	*FE*	A_O	A_O-RS	*AR*	*AR*-RS	H_S	H_S-RS	F_{IS}	F_{IS}-*RS*	N_b
s1	27	0	2	18	1.5	0.967	7.5	7.4	6.1	6.1	0.736	0.738	−0.057	−0.091	59.9 (39–108.8)
s2	30	0	0	24	1.3	0.983	7.4	7.1	6.2	5.9	0.751	0.756	0.001	−0.012	394.4 (123.3–INF)
s3	20	1	0	12	1.7	0.961	5.9	5.8	5.3	5.5	0.713	0.74	0.027	0.085	40.7 (28.2–65.2)
s4	12	0	0	10	1.2	0.979	5.9	5.6	5.9	5.6	0.748	0.744	0.025	−0.008	133.5 (49.3–INF)
s5	20	0	0	16	1.3	0.971	7.4	7.1	6.5	6.2	0.757	0.766	0.019	0.052	135.6 (60–INF)
s6	20	0	0	13	1.5	0.958	6.1	6	5.6	5.6	0.723	0.74	0.015	−0.001	52.8 (32.5–109.7)
s7	20	0	0	16	1.3	0.98	7.4	7.3	6.3	6.1	0.743	0.746	−0.035	−0.058	251.9 (92.5–INF)
s8	20	1	1	18	1.1	0.988	7.9	7.8	6.9	6.6	0.777	0.781	0.027	0.031	−1026.5 (170.5–INF)
s9	19	0	0	14	1.4	0.977	7.1	6.8	6.4	6.2	0.773	0.775	−0.029	−0.025	262.5 (83.6–INF)
s10	30	0	0	19	1.6	0.971	8	7.8	6.5	6.4	0.777	0.795	0.013	0.007	128.4 (67.2–556.2)
s11	20	0	1	14	1.4	0.978	6.5	6.5	5.9	6	0.76	0.771	0.037	0.05	153 (72.2–9011.4)
s12	20	0	1	14	1.4	0.958	6.4	6.1	5.7	5.6	0.715	0.735	0.013	0.004	78 (38.4–471.6)
s13	50	0	0	36	1.4	0.975	7.8	7.6	6.2	6.1	0.741	0.738	−0.069	−0.04	1932.8 (280.4–INF)
s14	32	0	0	26	1.2	0.984	8.1	7.9	6.4	6.1	0.731	0.734	0.017	−0.003	2225.7 (210.5–INF)
s15	20	0	0	17	1.2	0.984	7.4	7.4	6.5	6.4	0.772	0.776	0.013	0.033	−422.5 (261.7–INF)
s16	20	0	0	13	1.5	0.969	5.9	5.8	5.4	5.4	0.691	0.695	−0.076	−0.092	595.6 (86–INF)
s17	20	0	0	15	1.3	0.969	6.3	6	5.6	5.4	0.723	0.726	−0.011	0.001	−346.1 (104.9–INF)
s18	20	0	0	15	1.3	0.969	7.3	7.1	6.6	6.4	0.771	0.778	−0.013	0.004	173.4 (80.0–INF)
s19	20	0	0	13	1.5	0.969	6.8	6.5	6	5.9	0.739	0.762	−0.006	0.053	138.7 (59.7–INF)

Site numbers are preceded with an "s" for spotted. Measures are as follows: number of individuals genotyped (N_G), number of significant departures from Hardy–Weinberg proportions following Bonferroni correction ($\alpha = 0.05$) for eight locus tests within each populations (HW), number of significant tests for LD following Bonferroni correction ($\alpha = 0.05$) for 28 pairwise tests within each populations (LD), number of estimated full-sibling families (Families), mean number of individuals per full-sibling family (mean FS), family evenness (*FE*), mean number of observed alleles for the entire data set (A_O) and for the random sample (RS) of one full-sib per family (A_O-RS), allelic richness standardized to $N = 12$ for the entire data set (*AR*), and for the random sample of one full-sib per family (*AR*-RS; standardized to $N = 10$), mean expected heterozygosity for the entire data set (H_S) and random sample (H_S-RS), and LDNe-based single-sample estimates of the effective number of breeders that gave rise to the larval cohort examined (N_b), with 95 % confidence intervals

ponds. Natal philopatry should lead to elevated fine-scale genetic structure in these taxa, as it does in others (e.g. salmonids; Taylor 1991). Overall, our genetic results for marbled salamanders are consistent with strong rates of natal philopatry. Our results are similar to one other study of fine-scale genetic structure in marbled salamanders (Greenwald et al. 2009). In addition to multiple geographically cohesive genetic clusters within our study area, the overall 92 % of significant pairwise tests for genetic differentiation in our analysis suggests that the local breeding pond tends to be the scale at which populations are genetically independent, even, in some cases, among nearby sites such as m6–m10 (mean pairwise geographic distance = 778.8 m). The exception occurred with the cluster of nearby sites m16–m21 (mean pairwise geographic distance = 386.7 m) where multiple ponds appear to consist of one panmictic population.

Detailed demographic data are available for some of our marbled salamander sites. Gamble et al. (2007) demonstrated high rates of natal philopatry for sites included in our study. In a series of 14 ponds (interpond distances ranged from 50 to 1,500 km), including m6–m10 examined here, 91 % of 395 first-time marbled salamander breeders returned to natal ponds. Therefore, 9.0 % of first-time breeders dispersed to new breeding sites. Further, 96 % of experienced breeders maintained breeding site fidelity through multiple seasons (Gamble et al. 2007). The overall F_{ST} we observed for sites m6–m10 was 0.07, which is consistent with approximately three migrants per generation under an island model at equilibrium (Wright 1969). While the island model makes many simplifying assumptions (Whitlock and McCauley 1999), this large discrepancy between demographic estimates of dispersal and estimates of gene flow suggests that the results from Gamble et al. (2007) overestimate the number of successfully reproducing dispersers for these sites and "realized" natal philopatry may be more pronounced than those authors estimated. Local adaptation, also often associated with strong philopatry and habitat specificity (Whiteley et al. 2004), could be responsible for low effective dispersal rates among this set of ponds, however local adaptation has not been demonstrated in marbled salamanders.

Table 4 Genetic differentiation among 19 seasonal pond samples of larval spotted salamanders (*A. maculatum*) in Massachusetts

	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	s13	s14	s15	s16	s17	s18	s19
s1	–	**0.022**	**0.028**	*0.021*	*0.005*	*0.018*	*0.013*	*0.009*	*0.006*	**0.025**	**0.033**	**0.036**	**0.026**	**0.024**	**0.024**	**0.059**	**0.031**	**0.025**	**0.046**
s2	**0.086**	–	**0.022**	**0.035**	**0.012**	*0.020*	*0.017*	*0.008*	*0.001*	**0.027**	**0.022**	**0.017**	**0.012**	**0.017**	*0.000*	**0.050**	**0.043**	*0.0185*	**0.022**
s3	**0.107**	**0.086**	–	*0.014*	*0.005*	**0.030**	**0.043**	*0.015*	*0.015*	**0.024**	**0.045**	**0.023**	**0.027**	**0.025**	**0.019**	**0.058**	**0.035**	**0.017**	**0.029**
s4	*0.081*	**0.138**	*0.053*	–	*0.004*	**0.025**	**0.051**	*0.010*	*0.017*	**0.023**	**0.046**	**0.032**	**0.057**	**0.028**	**0.023**	**0.093**	**0.053**	**0.045**	**0.065**
s5	*0.021*	**0.052**	*0.019*	*0.016*	–	*0.006*	*0.015*	*−0.005*	*−0.005*	**0.011**	**0.023**	*0.021*	**0.022**	**0.010**	*0.010*	**0.041**	**0.027**	*0.011*	**0.030**
s6	*0.069*	*0.079*	**0.115**	**0.096**	*0.023*	–	*0.005*	*−0.003*	*0.000*	*0.018*	**0.027**	**0.027**	**0.029**	*0.014*	*0.014*	**0.050**	**0.040**	*0.015*	**0.036**
s7	*0.050*	*0.069*	**0.165**	**0.200**	*0.061*	*0.021*	–	*0.011*	*0.014*	**0.023**	**0.022**	**0.038**	**0.037**	**0.033**	*0.015*	**0.067**	**0.045**	*0.015*	**0.042**
s8	*0.036*	*0.035*	*0.064*	*0.041*	*−0.021*	*−0.011*	*0.047*	–	*0.002*	**0.019**	*0.002*	*0.019*	**0.031**	**0.018**	*0.007*	**0.047**	**0.034**	*0.015*	**0.028**
s9	*0.026*	*0.006*	*0.060*	*0.072*	*−0.020*	*0.000*	*0.060*	*0.007*	–	**0.016**	**0.026**	*0.012*	*0.004*	*−0.001*	*0.001*	**0.031**	**0.017**	*0.004*	*0.012*
s10	**0.109**	**0.120**	**0.105**	**0.100**	**0.051**	*0.079*	**0.101**	**0.089**	**0.074**	–	**0.025**	**0.016**	**0.035**	**0.028**	**0.022**	**0.073**	**0.038**	**0.035**	**0.027**
s11	**0.134**	**0.091**	**0.183**	**0.188**	**0.097**	**0.109**	**0.091**	*0.011*	**0.116**	**0.114**	–	*0.036*	**0.056**	**0.052**	*0.005*	**0.087**	**0.050**	**0.036**	**0.031**
s12	**0.135**	**0.068**	**0.086**	**0.121**	*0.086*	**0.101**	**0.147**	0.078	*0.047*	**0.070**	*0.144*	–	*0.019*	*0.020*	**0.016**	*0.051*	*0.026*	*0.022*	*0.016*
s13	**0.101**	**0.046**	**0.101**	**0.219**	**0.089**	**0.112**	**0.143**	**0.128**	*0.014*	**0.151**	**0.227**	**0.073**	–	0.010	**0.023**	**0.019**	**0.020**	**0.012**	**0.020**
s14	**0.089**	**0.066**	**0.093**	**0.107**	**0.039**	*0.054*	**0.127**	**0.076**	*−0.003*	**0.118**	**0.209**	**0.077**	*0.036*	–	**0.024**	**0.038**	**0.037**	**0.022**	**0.036**
s15	**0.100**	*0.002*	**0.077**	**0.097**	*0.044*	*0.057*	*0.062*	*0.030*	*0.005*	**0.100**	*0.023*	**0.067**	**0.093**	**0.098**	–	**0.069**	**0.026**	*0.018*	*0.015*
s16	**0.208**	**0.184**	**0.206**	**0.330**	**0.151**	**0.177**	**0.241**	**0.180**	**0.117**	**0.286**	**0.327**	**0.180**	**0.067**	**0.132**	**0.260**	–	**0.024**	*0.005*	**0.032**
s17	**0.116**	**0.166**	**0.132**	**0.201**	**0.105**	**0.151**	**0.170**	**0.139**	**0.070**	**0.158**	**0.198**	**0.098**	**0.073**	**0.135**	**0.103**	**0.083**	–	*0.009*	*0.015*
s18	**0.104**	*0.063*	**0.111**	**0.187**	*0.049*	*0.062*	*0.061*	*0.069*	*0.019*	**0.165**	**0.158**	*0.090*	**0.050**	**0.092**	*0.082*	*0.020*	*0.038*	–	*0.014*
s19	**0.185**	**0.090**	**0.117**	**0.262**	**0.126**	**0.146**	**0.171**	**0.124**	*0.052*	**0.120**	**0.131**	*0.062*	**0.079**	**0.143**	*0.065*	**0.118**	*0.058*	*0.060*	–

F'_{ST} is below the diagonal. F_{ST} is above the diagonal. Bold values were significant following Fisher's method for combining P values across the eight exact tests for each of the eight loci tested per population pair and following FDR correction (B-Y FDR correction for 171 tests, nominal $P = 0.0087$). Italicized values were not significant following Fisher's method for combining P values and FDR correction (same nominal P value)

Table 5 Summary of overall genetic differentiation and isolation by distance for marbled and spotted salamanders in western Massachusetts

Species	Overall differentiation		Isolation by distance	
	F'_{ST}	F_{ST}	F'_{ST}	F_{ST}
marbled	0.375 (0.283–0.509)	0.091 (0.074–0.108)	0.58*	0.52*
spotted	0.102 (0.080–0.130)	0.025 (0.017–0.036)	0.17	0.16

F'_{ST} and F_{ST} are reported for overall genetic differentiation after one full-sibling was randomly sampled per family at each site, 95 % confidence intervals are in parentheses. Mantel Test correlation coefficients (r-values) are shown for the relationship between geographic distance and both F'_{ST} or F_{ST}. Asterisks indicate level of significance ($P < 0.001$), no asterisk for correlation values indicates $P > 0.05$. Correlation values for marbled salamanders exclude outlier ponds with three or fewer full-sibling families

It is also worth noting that not all marbled salamander ponds exhibited such strong fine-scale genetic differentiation (e.g. sites m16–m21).

We hypothesize that natal philopatry and breeding site fidelity during subsequent reproductive bouts are generally pronounced in marbled salamanders due to habitat specificity associated with their reproductive timing. They court in the late summer and early fall and subsequently lay eggs terrestrially in receded or dry pond basins (Noble and Brady 1933; Bishop 1941). Eggs hatch only if they are inundated by rising pond water in the subsequent weeks or months (Kaplan and Crump 1978; Petranka 1998). Natal philopatry and pond-specific local adaptations should be favored when reproduction occurs in dry pond basins that must later fill for successful reproduction.

Spotted salamanders are also generally assumed to exhibit strong natal philopatry and site fidelity (Zamudio and Wieczorek 2007; Richardson 2012), but the data are less comprehensive. Spotted salamanders have the ability to return to a breeding pond when experimentally displaced (Whitford and Vinegar 1966; Shoop 1968). In a single-pond study of spotted salamanders in Massachusetts, 76.8 % of tagged spotted salamanders returned to the same breeding pond after 1 year and 66.0 % returned after 2 years (Whitford and Vinegar 1966). Tagged individuals were not detected in nearby breeding ponds within a 1 km radius although less effort was used to detect dispersing compared to homing individuals (Whitford and Vinegar 1966). Vasconcelos and Calhoun (2004) observed strong site fidelity in seasonal ponds in Maine for a subset of tagged individuals, but 43 % of their animals were not recovered and were therefore potential dispersers. Further, low natal philopatry has been observed for this species following site disturbance (e.g. fish invasion; Petranka et al. 2004).

Spotted salamanders migrate to already-filled seasonal ponds in late spring (March and April) where courtship and breeding aggregations occur (Husting 1965). We hypothesize that movement to already-filled ponds creates a weaker link between natal philopatry and reproductive success for spotted relative to marbled salamanders. Relatively high "straying" rates are consistent with our overall F_{ST} (0.025) and our STRUCTURE results ($K = 1$). Our results are also similar to other fine-scale genetic structure analyses of spotted salamanders in Ohio ($F_{ST} = 0.050$), New York ($F_{ST} = 0.073$), and Connecticut/Massachusetts ($F_{ST} = 0.033$) (Zamudio and Wieczorek 2007; Purrenhage et al. 2009; Richardson 2012). Aside from subtle regional differences, the weight of evidence suggests that natal philopatry at the breeding pond level in spotted salamanders is weaker than previously assumed. Furthermore, while $K = 1$ was the STRUCTURE model with the most support in our analysis, significant allele frequency divergence in 46 % of the pairwise tests for genic differentiation and a weak but nonsignificant pattern of IBD reveals that populations were not panmictic across our study region. However, clusters of nearby ponds were panmictic (e.g., 75 % of the pairwise tests for genic differentiation nonsignificant for s1–s9), suggesting substructure results are scale-dependent. Our results suggest that spotted salamanders may be philopatric to groups of neighboring ponds among which gene flow tends to be high, as proposed by Petranka et al. (2004). A surprising aspect of these results was that sites on the opposite side of the Connecticut River, which should serve as a strong barrier to spotted salamander dispersal, did not exhibit significant allele frequency differences in some cases. This suggests that other aspects of the biology of spotted salamanders, namely effective population size and generation interval, may interact with site fidelity in mediating its genetic structure.

Population size

Marbled salamanders had generally low estimates of N_b and had marked variation in the number of full-sib families within ponds. Small N_b for the marbled salamander populations examined here (mean $\hat{N}_b = 90.9$, extreme m8 value omitted, mean = 46.3) suggests that marbled salamander populations also have small generational N_e. The relationship between N_b and N_e cannot be directly determined for iteroparous organisms with age structure (Waples 2010). The median N_b/N_e ratio for seven amphibian species examined by Waples et al. (2013) was 0.73 (SD = 0.34). If we assume that generational N_e is also small, these populations have experienced elevated allele frequency change due to genetic drift and an increased probability of inbreeding, especially in the smallest breeding aggregations. Small effective size likely has contributed to extant patterns of strong population genetic divergence. Some of

the ponds examined had three or fewer estimated full-sib families. Genetic monitoring of these sites, in particular, will be needed to determine if they are in jeopardy of extirpation.

It is possible that our estimates of N_b are biased low due to small sample sizes (Whiteley et al. 2012). Our large sample of site m7 suggests that this bias is present but not severe [$\hat{N}_b = 67.6$ ($N = 147$) vs. 49.5 ($N = 30$)]. A strong correlation ($r = 0.99$) between $\hat{N}_b$ and available abundance estimates ($\hat{N}_C$) for five of our sites ($\hat{N}_{Cm6}$= 23, $\hat{N}_{Cm7} = 30.2$, $\hat{N}_{Cm8} = 421.2, \hat{N}_{Cm9} = 53.5, \hat{N}_{Cm10} = 46.6$) (Plunkett 2009) further suggests that number of adults breeding in a pond is closely related to N_b for that site. In addition to the number of breeders at a site, N_b can also be influenced by the number of families produced, variation in family size, and family-dependent survival from fertilization through sampling (Waples and Do 2010; Christie et al. 2012). The relationship between N_b and recruitment makes it useful for genetic monitoring for iteroparous organisms with overlapping generations, even if N_b cannot be easily translated to generational N_e (Waples 2010). Recently, there has been concern that N_b estimates can be biased if population substructure is not accounted for (Neel et al. 2013). Specifically, there are concerns that arise if the sampling area is greater than the breeding neighborhood. We do not believe this is the case for marbled salamanders because the sampling unit (each pond) and breeding neighborhood appear to be concordant.

Spotted salamanders had much larger N_b estimates than marbled salamanders (mean $\hat{N}_b = 422.3$). Estimates of large N_b tend to be imprecise and biased low, especially when sample size is small relative to the value being estimated (Tallmon et al. 2010). This is reflected in the large number of estimates with upper confidence intervals that included infinity and three negative point estimates, which indicates that N_b is large but inestimable because the genetic drift signal (LD) is smaller than the sample size correction (Waples 2006). Our results are very similar to those from Richardson (2012), where nine of 22 estimates for spotted salamanders were infinity and mean was 909.4. Together, these results suggest that effective size of spotted salamander populations is large and that genetic drift will generally be less influential in spotted relative to marbled salamander populations.

A comparison of N_b estimates and estimates of abundance (N_C) in spotted salamander breeding aggregates offers insight on the spatial scale to which N_b estimates apply. Estimates of pond-specific N_C based on egg masses (a surrogate of female breeding abundance) range from fewer than 10 to 747 (mean 42) in Rhode Island (Egan and Paton 2004). Cook (1978) used egg mass counts and estimates of eggs per mass and eggs per female to obtain estimates of the minimum number of breeding females from 2 to 315 for ponds in Massachusetts. Another Massachusetts study estimated 1,311 and 1,674 individuals migrating to a large pond in successive years (Jackson 1990). Given the large variance in abundance estimates, large estimates of N_b for this species could correspond to single ponds in some cases. In other cases, localized gene flow might be great enough (>approximately 10 %; Waples and England 2011) that the large $\hat{N}_b$ correspond to the effective number of breeders in groups of neighboring ponds.

Generation interval

Increased generation length should increase N_e (Nunney 1993) and therefore slow drift and genetic divergence. Shorter generation length for marbled (4–5 years) compared to spotted (7–8 years) salamanders would contribute to more rapid development of genetic structure for marbled salamanders in response to past demographic disturbance. Uncertainty in estimates of generation intervals for each species should be acknowledged. We used estimates of juvenile and adult marbled salamander survival based on demographic analyses conducted in some of our sample sites (Gamble et al. 2009; Plunkett 2009) to construct a life table. We assumed fecundity (in this case the number of metamorphs per female) was age-independent because of large variation in juvenile survival rates among ponds that overwhelms size- or age-dependent fecundity variation (Plunkett 2009). Positive age-dependent fecundity would increase generation interval, but over the range of observed fecundity values (Plunkett 2009), generation length does not increase beyond 5 years. Further, we assumed age at maturity is 3 years for marbled salamanders. Earlier age at maturity is possible (Plunkett, pers. comm.), but this would lead to a decreased generation length. The estimate of generation length for spotted salamanders is based on a skeletochronology study from Québec (Flageole and Leclair 1992). We used the observed number of individuals at each age to determine the average age of reproducing individuals (age 3 or greater) for males and females separately and then found the average for both sexes. Generation length might be longer in Québec relative to Massachusetts but we are unaware of estimates for more southern populations. A latitudinal gradient in generation length for spotted salamanders would lessen the species-specific effects of generation time on genetic structure that we propose.

Regional history

The three factors just discussed (natal philopatry, effective population size, and generation interval) must be

considered in conjunction with regional history. New England was largely deforested in the late 1700 and early 1800s (Foster 1992). Both marbled and spotted salamanders should have been similarly influenced by this extreme land-use and it is likely that most if not all populations of both species were extirpated from much of the landscape during the period of high-intensity agriculture. By 1850 most farms were abandoned and reversion to forest began (Foster 1992). Thus, it is likely that the ponds we examined have been recolonized in the last 160 years. Since both salamander species have similar overall habitat requirements, it is not likely that differential historical effects are responsible for the differences in genetic structure we observed. This would require that marbled salamander populations were able to persist, likely as small bottlenecked populations, while spotted salamander populations were not. This scenario would then posit that the minimal genetic structure observed for spotted salamanders is due to recent regional colonization. However, this scenario is unlikely. Of the two species, larger population sizes and less specificity of breeding habitat requirements (i.e. they breed in a wider range of ponds that vary more in aspects such as hydroperiod) for spotted salamanders lead to the speculation that spotted rather than marbled salamanders would have been more likely to persist during the deforested period. We favor the hypothesis that both species recolonized the region in the last 160 years and that natal philopatry, N_e, and generation interval have been critical factors related to the development of their extant genetic structure. All of these factors work in favor of a slower development of structure in spotted compared to marbled salamanders.

An additional historical factor associated with the recolonization process could be responsible for elevated genetic structure in marbled salamanders. Marbed salamanders are at the northern periphery of their range in Massachusetts (Petranka 1998). Uni-directional range expansions can be associated with strong genetic drift in populations located at the edge of the expansion (Excoffier and Ray 2008). If this kind of genetic "surfing" effect led to elevated genetic structure upon recolonization in marbled salamanders, the maintenance of this elevated structure would still depend on the ecological factors we describe. This kind of enhanced drift effect during range expansion would not be expected for spotted salamanders, since Massachusetts is more central to the species range (Petranka 1998) and recolonization likely proceeded from multiple fronts.

Conservation implications

We propose that the combination of greater natal philopatry, smaller N_e, and shorter generation interval, along with possible differences in colonization history are responsible for the extant elevated fine-scale genetic structure we have documented for marbled compared to spotted salamanders in Massachusetts. Differing population responses by similar species within the same landscape provides challenges for scaling up from single-species management approaches. Our results suggest that marbled salamanders are more sensitive to fragmentation from various land-use activities and would be less likely to recolonize extirpated sites on an ecologically and conservation-relevant time frame. Spotted salamanders, due to observed clusters of panmixia and weak IBD relationship, in our study and others (e.g. Purrenhage et al. 2009), may be less susceptible to fragmentation and would be more likely to recolonize vacant habitats on a conservation-relevant time scale. The differences in genetic structure we observed are all the more striking because of the overall similarities between the two focal species in their ecological characteristics. Our work extends past research that has focused on amphibian dispersal potential due to locomotion or aquatic versus terrestrial metamorphosis (Steele et al. 2009; Goldberg and Waits 2010; Richardson 2012; Sotiropoulos et al. 2013) and identifies additional characteristics that must be considered for attempts to predict differences in genetic structure and ultimately species sensitivity to anthropogenic disturbance.

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