Am. Midl. Nat. 157:149–161

Phylogeography of the Blue-spotted Salamander, Ambystoma laterale (Caudata: Ambystomatidae)

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ABSTRACT.—The blue-spotted salamander (*Ambystoma laterale*) has the northern-most distribution of any North American salamander and primarily occurs in areas that were once covered by the southern Laurentide Ice Sheet during the last glacial maximum. We examine the phylogeography of this primary post-glacial invader of northern North America. Mitochondrial DNA sequence data indicate a clade comprised of populations from the western portion of the species' range. Data from six nuclear DNA microsatellite loci support this finding. Taken together, the genetic data suggest rejection of the existing hypothesis of a single, western glacial refugium for this species in favor of two alternative hypotheses.

INTRODUCTION

It is widely accepted that the biological effects of Pleistocene events on northern North America were both dramatic and pervasive (Pielou, 1991). Organisms that are currently distributed across previously glaciated areas must have re-colonized these areas from refugia in unglaciated areas, and this re-colonization often proceeded at a relatively rapid rate (Hewitt, 2000). It is often assumed that these refugia were located to the south, coinciding with major shifts of floral communities as indicated from palynological studies (Webb *et al.*, 2004a). Many northern taxa have lower genetic diversity than do their southern counterparts, presumably because of both the rapidity and the recent nature of these post-glacial recolonizations (Hewitt, 1996). Until recently, this low genetic variability has hampered our ability to detect the subtle genetic structure that may be present within taxa of higher latitudes.

Molecular techniques such as microsatellite analysis and sequencing of rapidly evolving mitochondrial DNA (mtDNA) have made it possible to detect very slight differences among individuals and has made it possible to address questions such as: (1) Did organisms retreat to a single refugium or were they isolated in multiple areas of favorable habitat? and (2) Did different species respond to these glacial advances in a concerted fashion or were their responses independent (*e.g.*, Sullivan *et al.*, 2000)?

For example, using these techniques, several studies have detected the presence of multiple southern Pleistocene refugia in vertebrates, with subsequent secondary contact among cryptic lineages within and among species (Arbogast *et al.*, 2001; Austin *et al.*, 2002; Church *et al.*, 2003; Zamudio and Savage, 2003). Another study has even supported the possibility of a northern North American refugium (Rowe *et al.*, 2004), the existence of which has been suggested by recent paleobotanical studies (Jackson *et al.*, 2000; Stewart and Lister, 2001).

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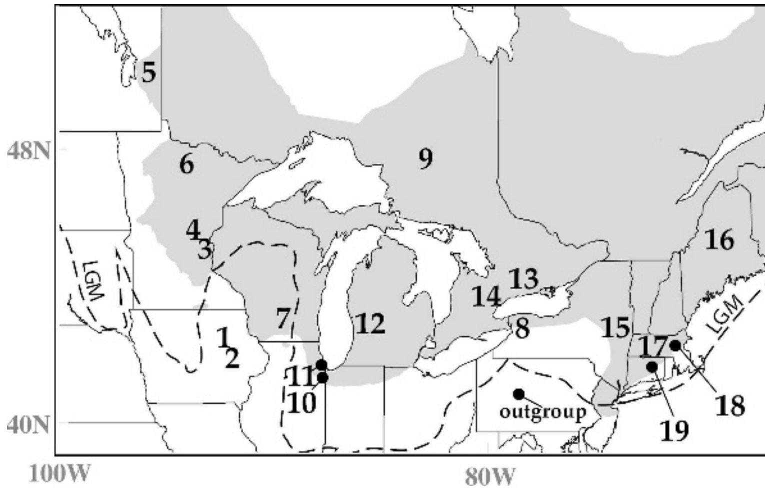

FIG. 1.—Collecting localities for ingroup taxa (1–19) and the outgroup (*Ambystoma jeffersonianum*). Dashed line depicts the approximate extent of the last glacial maximum (LGM; Mickelson and Colgan, 2004)

The blue-spotted salamander (*Ambystoma laterale*) has one of the northern-most distributions of any amphibian in North America (Conant and Collins, 1991). During the last glacial maximum (LGM) of the Wisconsinan (ca. 18,000 y ago), the Laurentide Ice Sheet extended over almost the entirety of the blue-spotted salamander's current distribution (Fig. 1). Therefore, *A. laterale* can be considered a prime example of a post-glacial colonizer and is considered a primary invader of post-glacial areas (Holman, 1995). Although paleontological material is scarce, *A. laterale* is present in the Sheriden Pit herpetofauna of northwestern Ohio with a carbon-14 date of 11,700 b.p. (Holman, 1995). Clear evidence that the range of *A. laterale* shifted with the cyclical advances and retreats of ice is supported by the presence of fossil material in Nebraska, west of its present range, coinciding with the extensive glaciations of the mid-Pleistocene (Holman, 1995).

Herein, we examine the phylogeography of *Ambystoma laterale* across much of its range in northern North America in an attempt to determine whether the current distribution of this species is the result of colonization from a single refugium or from multiple refugia. Recolonization from a single refugium likely would result in a geographic pattern with low levels of variation (consistent with rapid colonization; Hewitt, 1996) or in a pattern consistent with an isolation-by-distance model (Endler, 1977). Multiple refugia, on the other hand, likely would result in geographic breaks in any clinal pattern of genetic variation, with the breaks representing zones of secondary contact between the previously isolated populations (Endler, 1977; Avise *et al.*, 1987).

MATERIALS AND METHODS

SPECIMENS EXAMINED

For our phylogeographic analysis of *Ambystoma laterale*, mtDNA sequence data (41 specimens representing 19 localities) and six microsatellite loci (40 specimens from 18 localities) were examined from across the range of the species in the United States and southern

TABLE 1.—Locality information for specimens of *Ambystoma laterale* and *A. jeffersonianum* (outgroup) included in this study. Locality numbers correspond to those in figures

Locality number	Locality	Sample size	Specimen ID[2]
1	IOWA: Black Hawk Co., George Wyth State Park	n = 5	JSE 15, 22, 23, 24, 26,
2	IOWA: Linn Co., Behrens Ponds	n = 5	JSE 33, 35, 36, 48, 58
3	MINNESOTA: Anoka Co.	n = 5	JSE 78–82
4	MINNESOTA: Anoka Co., Bethel	n = 1	JSE 77
5	MANITOBA: Whiteshell Provincial Park	n = 2	ROM 17029, 17574
6	MINNESOTA: Itasca Co.	n = 1[1]	JPB 33051
7	WISCONSIN: Ozowkee	n = 1	ROM LL579
8	NEW YORK: Erie Co., West Seneca, 100 m S of N branch of Smokes Creek	n = 1	ANS 12088
9	ONTARIO: Cochrane District, Kapuskasing	n = 5	ROM 17023–17027
10	ILLINOIS: Will Co., NE Crete Township	n = 2	INHS 11949, 11950
11	ILLINOIS: Cook Co., Country Lane Woods	n = 1	INHS 11814
12	MICHIGAN: Muskegon Co.	n = 2	ANS 10776, 10777
13	ONTARIO: Haliburton Co. Lake Kashagawigamog	n = 1	ROM 21418
14	ONTARIO: Halton Co., Third Side Road at Twiss Road	n = 1	ANS 12060
15	NEW YORK: Albany Co., intersection routes 156 and 307	n = 1	ANS 12023
16	MAINE: Aroostook Co., Cyr Township	n = 1	MCZ 110601
17	MASSACHUSETTS: Hampdon Co. Wilbraham, 2400 m S route 20	n = 1	ANS 12069
18	MASSACHUSETTS: Worcehester Co., E. Brookfield	n = 3	AMNH 129381, 129382, 129385
19	CONNECTICUT: Windham Co., Plainfield	n = 2	AMNH 129439, 129440
outgroup	PENNSYLVANIA: Chester Co. Warwick Township	n = 2	LSUMZ H-1207, 1208

[1] No data were generated for microsatellite analysis because of a lack of available DNA

[2] Disposition of vouchers: AMNH = American Museum of Natural History; ANS = Academy of Natural Sciences; INHS = Illinois Natural History Survey; JPB = J Bogart, U. Guelph; JSE = University of Northern Iowa; LSUMZ = Louisiana State University Museum of Zoology; MCZ = Museum of Comparative Zoology; ROM = Royal Ontario Museum

Canada (Table 1; Fig. 1). Two specimens of *A. jeffersonianum* were used as outgroup taxa for the mtDNA analyses (Table 1). Genomic DNA was isolated from excised toes or organ tissues using either standard phenol-chloroform procedures or the DNeasy™ Tissue Kit (Qiagen, Valencia, California).

ANALYSIS OF MITOCHONDRIAL DNA

A 534 base-pair (bp) region of mtDNA, including all of the tRNA for proline, an intergenic spacer (IGS) potentially unique to ambystomatids (Shaffer and McKnight, 1996), and 220 bp of the control region, was amplified by the polymerase chain reaction (PCR) using *Thermus aquaticus* DNA polymerase (Promega, Madison, WI; Saiki *et al.*, 1986, 1988). Double -stranded amplifications of this fragment and subsequent cycle sequencing reactions were performed using the primers THR and DL3 (Shaffer and McKnight, 1996).

Double-stranded PCR amplifications were performed in 50 μl EasyStart tubes (Molecular Bioproducts, Inc., San Diego, CA) with the addition of 3 μl of each primer (10 μM) and 1 unit of *Taq* DNA polymerase (Promega, Madison, WI). Thirty-five PCR cycles were performed with the following parameters: 1 min denaturation at 94 C, 1 min annealing at 44 C and 1 min extension at 72 C. This was followed by a final 10 min extension at 72 C.

Prior to sequencing, the double-stranded PCR product was purified using the Quickstep™ PCR Purification Kit (Edge Biosystems, Gaithersburg, Maryland). Sequencing was performed at the Iowa State University Sequencing and Synthesis Facility, Ames, Iowa. Both DNA strands were sequenced for all PCR products and Sequencher version 4.2 was used to edit sequences (Gene Codes Corporation, Ann Arbor, MI). The resulting sequences were aligned using Clustal X (Thompson *et al.*, 1997) and deposited in GenBank (GenBank accession numbers AY939928–AY939951 and DQ453754–DQ453772).

To generate the maximum likelihood tree, Modeltest (Version 3.6; Posada and Crandall, 1998) was used to examine the fit of 56 models of nucleotide substitution to the sequence data. Models of evolution providing the best approximation of the data using the fewest parameters were chosen for subsequent analyses according to the Akaike Information Criterion (AIC; Huelsenbeck and Rannala, 1997; Posada and Buckley, 2004). A full heuristic maximum likelihood search was conducted with the preferred model in PAUP* 4.0b10 and 10 randomized input orders (Swofford, 2002). Equal-weighted parsimony also was conducted using a heuristic search with 10 random stepwise additions. A full heuristic bootstrap (1000 pseudoreplicates) was performed using both ML and parsimony criteria. Alternate tree topologies were examined using a Kishino-Hasegawa Test (Kishino and Hasegawa, 1989) and likelihood ratio tests.

Using mtDNA, Hedges *et al.* (1992) demonstrated that *Ambystoma laterale* and *A. jeffersonianum* are very distinct relative to each other and to the unisexual populations that occur sympatrically in portions of both species' ranges. Therefore, mtDNA data was also used to insure that no hybrid individuals were included in the subsequent nuclear DNA (nucDNA) analyses. During the course of this project, two specimens from Illinois previously identified as *A. laterale* (INHS 11822 and 11823) were found to be of hybrid origin and were discarded from the study.

MICROSATELLITE ANALYSIS

Six microsatellite loci were examined: AjeD75, AjeD94, AjeD283, AjeD346, AjeD422 (Julian *et al.*, 2003b) and AmaD367 (Julian *et al.*, 2003a). Each locus exhibits a tetranucleotide repeat motif $(TAGA)_n$. Thermal parameters for amplification were as follows: initial denaturation occurred at 95 C for 2 min; 36 cycles followed, consisting of denaturation at 95 C for 1 min, primer annealing at either 55 C (AjeD283, AjeD346, and AmaD367) or 60 C (AjeD75, AjeD94 and AjeD422) for 1 min, and nucleotide extension at 72 C for 1 min; a final extension period of 30 min at 72 C was conducted to both promote the completion of PCR products and alleviate some of the ambiguity in allele scoring (Culjkovic *et al.*, 2003). Length of the amplified DNA was determined electrophoretically by the Iowa State University Sequencing and Synthesis Facility, Ames, Iowa. The program Genographer (Benham, 2001) was used for genotypic scoring of individuals.

A Mantel's test (Mantel, 1967) was used to test for the presence of a pattern of isolation by distance in the microsatellite data. This procedure tests the hypothesis that the pattern of distances in one matrix (pair-wise geographic distances of populations) is independent of the pattern of distances in the second matrix (genetic distances) by comparing the sum of cross-products for all pairs of distances between the two observed matrices (the Mantel statistic [$\underline{Z}$]) to those of randomly shuffled matrices (Hope, 1968; Schnell *et al.*, 1985). One

thousand permutations were performed using the Mantel 3.0 program in the R-package (Legendre and Vaudor, 1991). The Mantel statistic ($\underline{Z}$) was converted to a standardized form ($\underline{r}$; Smouse *et al.*, 1986) and is equivalent to a Pearson correlation coefficient between the values of the two matrices (Legendre and Vaudor, 1991). A pair-wise geographic-distance matrix was generated from the map coordinates of the sample populations using the program GeoDistances (Legendre and Vaudor, 1991). Populations that lacked original GPS coordinates in their locality data were georeferenced using the online version of the program GEOLocate (Rios and Bart, 2002). A matrix of pairwise F_{ST} distances between population pairs was generated by Arlequin (Schneider *et al.*, 2000) using the *D* estimator (Weir and Cockerham, 1984). Genetic partitioning among populations was determined by F_{CT} and global F_{ST}, respectively, in Arlequin (Schneider *et al.*, 2000). F_{CT} measures the component of divergence that is attributable only to genetic structure between defined groups.

Although there are limitations to using distance-based techniques to examine allelic data in a geographic context (Pritchard *et al.*, 2000), in order to facilitate a visual comparison of the two data sets (mtDNA and nuclear microsatellites) with regard to geography, a neighbor-joining (NJ) tree was constructed for the microsatellite data using the F_{ST} distance matrix. This NJ tree was constructed using PHYLIP (Felsenstein, 1993). Richarde and Thorpe, (2001) demonstrated that distances based on variance in allele frequency such as F_{ST}–based measures, outperformed measures based on variance in repeat numbers (such as R_{ST}) when they were used in phylogeny reconstruction. FSTAT was used *post hoc* to compare gene diversity per locus and allelic richness of the primary groups that were recovered by PHYLIP (Felsenstein, 1993). Insofar as these groups may represent highly genetically structured meta-populations, inbreeding coefficients and measures of conformity to Hardy-Weinberg equilibrium were not considered.

The model-based clustering method implemented by the program Structure (Pritchard *et al.*, 2000) was used to determine fine-scale population structure in the microsatellite data by assigning individuals to groups without prior knowledge of the collecting locality for the individuals. The number of assumed populations (K) was determined by the results of the mtDNA analysis and consequently two values were tested (K = 2 and K = 3). Burn in was set for 10,000 and the simulation was run for 10^6 Markov Chain Monte Carlo iterations. Both the ''no admixture model'' and the ''admixture model'' of ancestry were used in the analyses.

RESULTS

The 534 base pairs of mtDNA sequenced yielded 61 parsimony-informative sites. Only four of these sites, however, are potentially informative for the ingroup; the remaining 57 provide support for the monophyly of *Ambystoma laterale.* Of the four informative sites within the ingroup, three of these are found in the IGS; the remaining site is found in the proline t-RNA region. In addition to the four informative sites, there are five autapomorphies. There is no apparent homoplasy within the ingroup. Five indels were present in the IGS resulting in *A. jeffersonianum* yielding a fragment of different length than that of the ingroup (652 bp). These indels were omitted from the phylogenetic analysis.

The transitional model with gamma distribution (TIM + G) was chosen for the maximum-likelihood analysis of mtDNA (Rodriguez *et al.*, 1990). The resulting maximum-likelihood tree (-lnL = 1023.8689; Fig. 2) depicts three groups although only a western clade is well supported (three synapomorphies). Five localities (Connecticutt, Maine, eastern New York and two from Massachusetts) form an east-coast group (Fig. 2), which is divergent from the

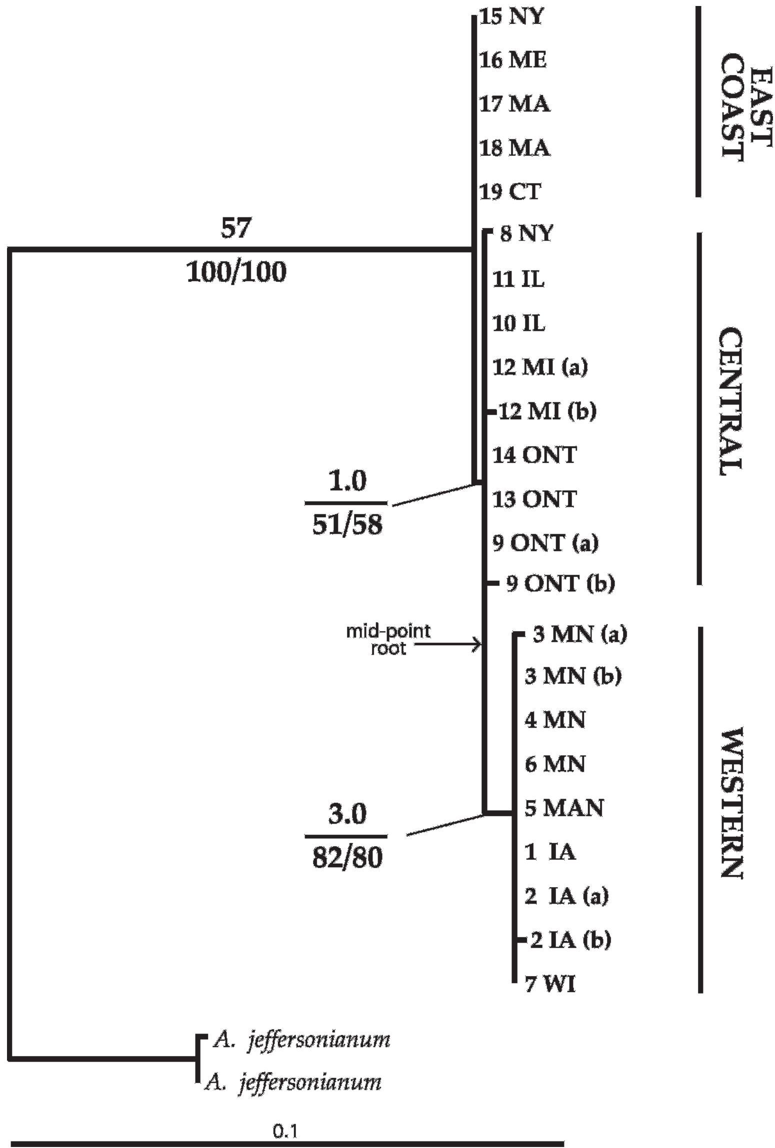

FIG. 2.—Maximum likelihood tree for 19 sampled populations of *Ambystoma laterale* and the outgroup *A. jeffersonianum* based on 534 bp of mtDNA. Numbers above branches are absolute branch lengths, and below depict bootstrap values (ML/parsimony). Parenthetical letters in taxon names indicate haplotypes of a polymorphic population

central group at a single nucleotide site. Low bootstrap values for the central and east-coast groups are not surprising given the paucity of informative sites. Constraining the root for the ingroup taxa to the mid-point of the longest inter-nodal branch (-lnL = 1025.5097) is not significantly different than the outgroup rooted tree when tested using either the

TABLE 2.—Gene diversity and allelic richness per locus for each geographic group (Figs. 1, 2). Values for allelic richness are adjusted for unequal sample sizes

	Gene diversity		Allelic richness	
locus	east/central	western	east/central	western
AjeD75	0.91	0.78	12.60	7.00
AjeD94	0.61	0.00	6.71	1.00
AjeD283	0.86	0.88	10.79	10.00
AjeD346	0.93	0.88	12.60	10.00
AmaD367	0.93	0.25	13.52	3.00
AjeD422	0.95	0.93	18.11	16.00

Kishino-Hasegawa Test (one-tailed and fully optimized distribution; $P = 0.21$; Kishino and Hasegawa, 1989) or a log likelihood ratio test ($P = 1.0$).

The distribution of private microsatellite alleles between the two mitochondrial groups was heavily skewed. For the east/central group, 43 private alleles were observed (or 7.2 per locus), whereas 14.4 private alleles (corrected for unequal sample sizes) were detected in the western clade (or 2.4 alleles per locus). In fact, only 34 of 90 alleles were observed in both groups. These data substantiate both a significantly high level of interpopulational structure (global $F_{ST} = 0.349$; $P < 0.001$) and a substantial level of genetic differentiation between the east/central and western groups ($F_{CT} = 0.084$; $P < 0.005$), further indicating that these groups are distinct.

Gene diversity calculated using FSTAT for the western group (Figs. 1, 2) as compared to the east/central group was lower for all alleles except AjeD283 (Table 2). Allelic richness was lower in the western group for all alleles examined (Table 2).

Within the western samples (localities 1–7; Fig. 1), microsatellite locus AjeD94 was monomorphic for allele A (148 bp, $n = 19$), yet four additional alleles were detected among samples in the east/central group ($n = 21$). The common allele (A) was shared between both groups, while four alleles (B, C, D, and E, 144 bp, 152 bp, 156 bp and 184 bp) were unique to the east-coast/central group. Because the E allele (184 bp) fell well outside the range of variation of the other alleles examined (144 bp–156 bp), it was suspected that allele E may not solely be the result of processes affecting tandem nucleotide repeats. However, sequence analysis of these alleles indicated that the size variation is the result of an increase in repeat number.

There was no significant correlation between geographic distance and genetic distance derived from the microsatellite data among all 18 populations (Mantel test, $P = 0.22$), nor within the western group ($P = 0.28$) or the east/central group ($P = 0.07$). Therefore, there is not a clear pattern of isolation by distance in these microsatellite data although the correlation between geography and genetics is relatively high within the east/central group. An unrooted neighbor-joining tree based on the microsatellite data (Fig. 3) does not tightly correspond to geography, but does recover the western mtDNA clade as a distinct cluster.

The model-based analysis of microsatellite data (Structure; Pritchard *et al.*, 2000) yielded results that were concordant with the mtDNA data. Using the admixture model, only five of the 40 individuals sampled were not assigned to the expected east or west mitochondrial group (K set to 2) at a probability of 0.7 or higher. Two of these individuals were from the east/central group (from populations 15NY and 9ONT; Fig. 1) and three individuals were from the west (all individuals from 7WI and 5MAN; Fig. 1). When admixture was not included in the model the results were identical with the exception of one of the individuals

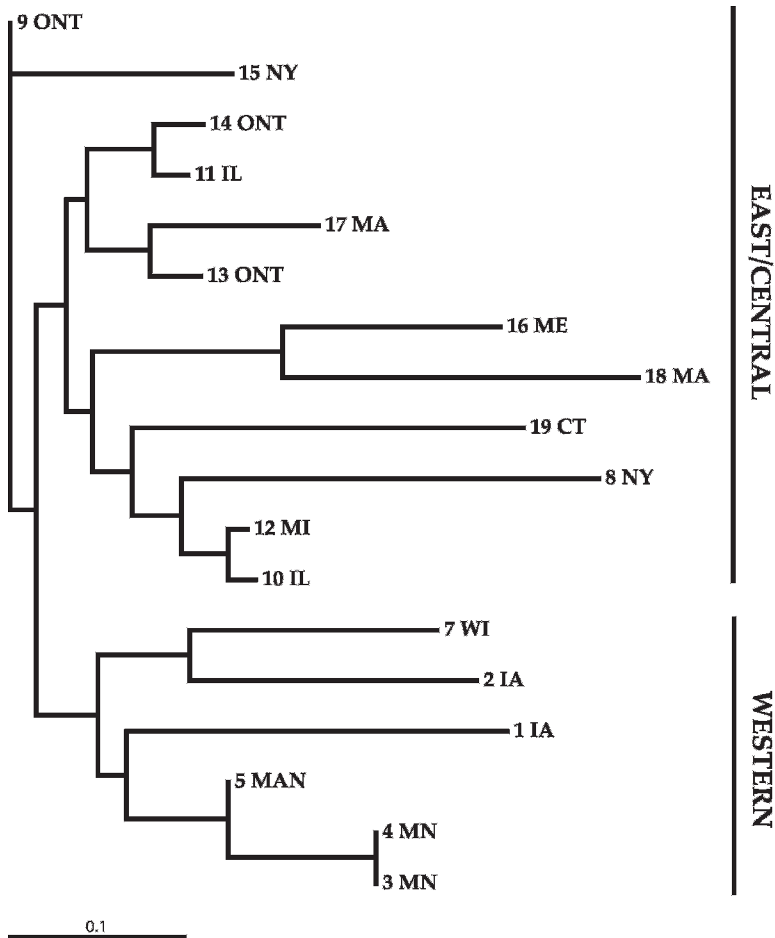

FIG. 3.—Unrooted neighbor-joining tree for 18 sampled populations of *Ambystoma laterale* based on an F_{ST} distance matrix derived from 6 microsatellite loci

from 5MAN being assigned to the west, resulting in four individuals not being assigned to the expected mtDNA group. Interestingly, when three groups were assumed (K = 3) both ancestry models formed a third population consisting of the populations from Manitoba and Minnesota.

DISCUSSION

It is noteworthy that for 534 nucleotides of predominantly non-protein coding mtDNA, salamanders collected in Manitoba are identical to those in Iowa despite geographic separation of over 1100 km. Similarly, specimens from near Chicago, Illinois differ from those in Northern Maine (>1500 km) by a single nucleotide. This extremely low level of variability in a non-protein-coding region of mtDNA suggests recent, rapid re-colonization consistent with what one would expect to see in recolonization following the LGM. Low

levels of genetic variation should not be surprising given that 18,000 y ago most of the present-day range of *Ambystoma laterale* lay beneath the Laurentide Ice Sheet.

Uzzell (1964) hypothesized that the Paleozoic Plateau of Iowa (northeast Iowa) likely served as the glacial refugium for the ancestral populations that led to the entire present-day distribution of *Ambystoma laterale.* (Menzel and Goellner, 1976) refined Uzzell's ideas based on more recent geologic data, and stated that the ancestral populations of *A. laterale* likely were found slightly farther south and west, nearer the present-day populations in Iowa (localities 1 and 2, Fig. 1). Therefore, authors of both studies assumed a single, western refugium for ancestral populations of *A. laterale.* Authors of both studies speculated that the vicariant event that led to the formation of the sister taxa, *A. laterale* and *A. jeffersonianum,* occurred during the most recent glaciation. Our mtDNA evidence (divergence of 10.7%–11.1% uncorrected *p*, excluding the indels) suggests a speciation event that may pre-date the Wisconsinan, perhaps by several million years. For example, using the same segment of mtDNA (plus an additional 308 bp of the control region), Shaffer and McKnight (1996) calculated that lineages of *A. tigrinum* were diverging at a rate of 1–1.5% per million years.

The existence of a single western refugium (Uzzell, 1964; Menzel and Goellner, 1976) seems unlikely based on our genetic data. Instead, the genetic data suggest two alternative hypotheses: (1) a multiple refugia hypothesis or (2) an eastern refugium hypothesis. As we will outline below, either of these hypotheses is better supported than is the hypothesis of a single western refugium.

The alternative hypothesis of multiple refugia is worthy of consideration given the clear segregation of *Ambystoma laterale* populations into two distinct groups as indicated by the M–L tree (mtDNA), NJ tree (nucDNA), model-based genetic structure analysis and measures of F_{CT}. Such clear segregation is not expected from a species that radiated out from a single center of origin (Hewitt, 2001). Midpoint rooting of the maximum likelihood tree (Fig. 1) is consistent with this scenario. (Arbogast and Kenagy, 2001) pointed out the power of a comparative phylogeographic approach to understanding historical biogeography, and there are recent phylogeographic studies available for other species in the area surrounding the Great Lakes. For example, the present-day distributions of two other species of ambystomatids, *A. tigrinum* and *A. maculatum,* have been shown to be the result of expansion out of separate eastern and western glacial refugia although the east-west phylogeographic discontinuities for these two species are shifted eastward relative to that of *A. laterale* (Church *et al.*, 2003; Zamudio and Savage, 2003). Furthermore, the eastern chipmunk (*Tamias striatus*) has been shown to exhibit an east-west genetic break near the Wisconsin-Illinois border (Rowe *et al.*, 2004), coinciding with the break seen in *A. laterale.* Therefore, the factors that led to an east-west genetic break in *A. laterale* could be the same factors that led to similar breaks in *A. tigrinum, A. maculatum* and *T. striatus.* There is ample evidence supporting the presence of suitable refugial habitat in both eastern and western regions of the present-day distribution of *A. laterale* (Anderson, 1983; Jackson *et al.*, 2000; Webb *et al.*, 2004b).

A second alternative to the hypothesis of a single western refugium involves westward colonization from a single, eastern refugium; the genetic data could also be viewed as consistent with this hypothesis. Given that the western samples exhibited fewer private alleles, lower allelic richness and lower gene diversity (Table 2), a central or eastern center of origin is more likely than is a western center of origin. Outgroup rooting of the maximum likelihood tree (Fig. 2) is consistent with this hypothesis, where the western clade is nested within the east/central clade. If the refugium for *Ambystoma laterale* was in the east, subsequent colonization then would have proceeded northward into the previously

glaciated areas of New England and Quebec and westward, eventually moving north of the Great Lakes (and south of ancient Lake Ojibway). The reduced allelic richness and gene diversity (Table 2) in the western clade (relative to the east/central group) could, therefore, be the result of a very recent and rapid colonization (Hewitt, 1996) from the north and east as the species completed its ring-like colonization of areas surrounding the Great Lakes. Austin *et al.* (2002) demonstrated that the post-glacial history of northern populations of the spring peeper (*Pseudacris crucifer*) very likely mirrored these events. Their data strongly suggest that populations of *P. crucifer* originating from a refugium in the vicinity of the Northern Appalachians re-colonized New England and Nova Scotia, then moved westward across Ontario, and finally moved southward into present-day Wisconsin. The distribution of haplotypes of *P. crucifer* closely resembles that of *A. laterale* (Fig. 1).

Clearly, more extensive population-level sampling of *Ambystoma laterale* is needed to better test the two hypotheses discussed above. Of particular interest would be the geographic zone north of Lake Superior (Fig. 1) where the distribution of *A. laterale* is constricted; this area may represent a zone of secondary contact for the two putative expanding refugial populations. The maintenance of a distinctive contact zone would support the hypothesis of multiple refugia, whereas the lack of a distinct break would support the hypothsis of a single eastern refugium. Sampling of the northeastern reaches of the species distribution also could reveal additional geographic structure. Importantly, with intensive sampling, techniques based on coalescent theory could be brought to bear on the questions of range expansion and timing of divergence, and evidence for the position of the root of the *A. laterale* population-level phylogeny could be strengthened.

Additional studies of a diversity of vertebrate organisms would also greatly enhance our ability to complete a thorough comparative study of responses to the LGM in the areas that were most dramatically affected by glaciation, thereby allowing us to determine how widespread faunal responses to the LGM were in this region. Several tetrapod species have distributions that are similar to that of *Ambystoma laterale*; among these are the southern bog lemming (*Synaptomys cooperi*), the woodland jumping mouse (*Napaeozapus insignis*) and the wood turtle (*Glyptemys insculpta* [currently under study]; Hall, 1981; Conant and Collins, 1991). The painted turtle (*Chrysemys picta*) and the chorus frog (*Pseudacris triseriata*) also have similar distributions with subspecies boundaries near the Wisconsin/Illinois border (Conant and Collins, 1991), potentially indicating genetic discontinuity within the range of these species as well.

Acknowledgments.—We thank the following persons and institutions for graciously supplying tissues for this study: J. LeClere; H. Hadow; C. Phillips, Illinois Natural History Survey; R. Murphy, Royal Ontario Museum; Louisiana State University Museum of Natural Science; J. Bogart; and the Academy of Natural Sciences of Philadelphia. B. Arbogast, P. Beerli, J. Bogart,T. Spradling, and two anonymous reviewers provided many useful comments on the manuscript.

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SUBMITTED 5 DECEMBER 2005 ACCEPTED 5 JUNE 2006