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The Biogeographic Origins and Trophic Ecology of Maine's Island Red-Backed Salamanders (Plethodon cinereus)

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THE BIOGEOGRAPHIC ORIGINS AND TROPHIC ECOLOGY OF MAINE'S

ISLAND RED-BACKED SALAMANDERS (*Plethodon cinereus*)

By

Nikko-Ideen Shaidani

B.S. The University of Maine, 2012

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Zoology)

The Graduate School

The University of Maine

May 2017

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THE BIOGEOGRAPHIC ORIGINS AND TROPHIC ECOLOGY OF MAINE'S ISLAND RED-BACKED SALAMANDERS (*Plethodon cinereus*)

By

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An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
(in Zoology)
May 2017

Island populations of terrestrial species have an increased potential, compared to mainland populations, to adapt and diverge, as these populations often are isolated with respect to gene flow from other populations and may be subjected to novel pressures. Indeed, extended isolation of individuals can elicit dramatic changes within populations and is recognized as a common driver of speciation. It is for these reasons that island populations are often a priority for conservation. Plethodontid salamanders are among the most terrestrial of Maine's amphibians and are not tolerant of prolonged exposure to seawater, and yet, they are found on a number of Maine's coastal islands. Here I present findings of the first study of the population genetic structure of Maine's island red-backed salamanders to elucidate their probable origins. I collected samples from 12 coastal island and 10 mainland sites. Using nine microsatellite loci, I found that 199 of 210 pairwise comparisons showed significant multilocus differentiation. Island populations had fewer alleles and lower heterozygosity than most mainland populations.

Islands farthest from shore displayed particularly strong divergence from all other sites, a finding consistent with ancient colonization. Phylogenetic and Bayesian structure analyses supported weak regional affiliation, which may be the result of extended isolation and divergence of distant island populations. Salamanders from island sites displayed no overall pattern of isolation by distance, whereas, those from mainland sites showed a weakly positive trend, implying a greater dispersal limitation among island populations.

Isotopic analyses revealed that Maine's coastal islands vary from one another and from mainland systems in productivity baselines and trophic structure, indicating potential differences in sources of primary productivity and food web structure. Alternative ecological dynamics such as these have the potential to instigate adaptive divergence of island salamanders from their mainland counterparts. Consistent with this idea, red-backed salamanders on Maine's coastal islands appear to feed at a higher mean trophic level than mainland salamanders, with smaller, seabird nesting islands featuring the highest estimated trophic position. Salamander populations varied widely in body size and morphological trait allometry suggesting potentially widespread trait divergence that may be linked to local adaptations. Within this general population variation, island populations featured larger head proportions (snout-gular length) for their body size, a pattern that may be adaptive given the higher mean trophic position of these populations. These findings suggest some island populations of *P. cinereus* likely represent isolated and potentially unique components of post-glacially derived variation within this widespread species and support the operation of mechanisms generating at least limited phenotype-environment associations in Maine's red-back salamanders. Indeed, Maine's island red-backed salamanders encourage a closer look at other widespread species on these coastal islands for the possibility that they similarly represent cryptic components of Maine's biodiversity.

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MAINE'S ISLAND RED-BACKED SALAMANDERS (*Plethodon cinereus*)

ABSTRACT

Island populations of terrestrial species have an increased potential, compared to

mainland populations, to adapt and diverge, as these populations often are isolated with respect

to gene flow from other populations and may be subjected to novel pressures. Indeed, extended

isolation of individuals can elicit dramatic changes within populations and is recognized as a

common driver of speciation. It is for these reasons that island populations are often a priority

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amphibians and are not tolerant of prolonged exposure to seawater, and yet, they are found on a

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genetic structure of Maine's island red-backed salamanders to elucidate their probable origins. I

collected samples from 12 coastal island and 10 mainland sites. Using nine microsatellite loci, I

found that 199 of 210 pairwise comparisons showed significant multilocus differentiation.

Island populations had fewer alleles and lower heterozygosity than most mainland populations.

Islands farthest from shore displayed particularly strong divergence from all other sites, a finding

consistent with ancient colonization. Phylogenetic and Bayesian structure analyses supported

weak regional affiliation, which may be the result of extended isolation and divergence of distant

island populations. Salamanders from island sites displayed no overall pattern of isolation by

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dispersal limitation among island populations. These findings suggest some island populations

of *P. cinereus* likely represent isolated and potentially unique components of post-glacially derived variation within this widespread species. Indeed, these results from Maine's island red-backed salamanders encourage a closer look at other widespread species on these coastal islands for the possibility that they similarly represent cryptic components of Maine's biodiversity.

INTRODUCTION

Populations that are geographically distant from each other often are exposed to different environmental conditions and experience adaptive divergence. Geographic separation of populations often is associated with vicariance, the fragmentation and isolation of populations by physical barriers. Restrictions to gene flow owing to vicariance can further facilitate adaptive and neutral divergence in isolated populations by eliminating the homogenizing effects of gene flow (Wiley 1988; Bohonak 1999; Vences et al. 2003). As a result, isolated populations have greater potential to diverge genetically from one another under the joint influences of natural selection and genetic drift (Wright 1943; Dillon 1984; Sexton et al. 2014). These interactions between vicariance and adaptation have long been important to models of allopatric speciation (Ronquist 1997) and often are envisioned at large geographic and temporal scales implied by processes like continental drift and the formation of volcanic island chains. However, for certain organisms, vicariance and divergence might be predicted over much smaller spatial and temporal scales.

Island systems provide some of the most-compelling cases of vicariance, natural selection, and associated adaptive radiations. Radiations of Galápagos finches (*Geospiza*) and tortoises (*Chelonoidis*) (Boag and Grant 1981; Grant and Grant 1992 and 2002; Caccone et al. 1999 and 2002), Anolis (*Anolis sagrei*) lizards in the Caribbean (Losos 1990; Losos et al. 1997

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and 1998), and tree snails (*Achatinella*; *Patrulina*; *Perdicella*; *Newcombia*) in Hawaii (Holland

and Hadfield 2002 and 2004), among others (reviewed in Schluter 2000), amply demonstrate

how populations of terrestrial species can adapt to new niches on islands. However, not all

adaptations to islands require millions of years or thousands of kilometers of isolation. The

limited dispersal abilities and specialized habitats of some organisms may lead to detectable

divergence from mainland populations over much shorter distances and time frames (Cody and

Overton 1996; Martinez-Solano and Lawnson 2008).

Red-backed salamanders (*Plethodon cinereus*) occur on many of Maine's coastal islands

(Shurtleff, 1862; True, 1897 and 1909 Maine Amphibian and Reptile Atlasing Project

(MARAP): accessed 10/11/2016). The presence of salamanders on these islands is noteworthy in

several ways. First, red-backed salamanders are intolerant of seawater (Feder 1983), and thus,

they are less likely to move through marine environments and immigrate from mainland

populations. Also, *P. cinereus* is largely found in cool, moist forest environments (Heatwole

1962; Herbeck and Larsen 1999; Jordan et al. 2009), yet some of Maine's islands possess few

trees and the fallen woody debris that typically provide microhabitat for this species (Jaeger

1980). Despite these potential habitat limitations, red-backed salamanders, unlike most other

amphibians, do not possess an aquatic larval stage and thus do not require access to a freshwater

source to reproduce (Petranka 1998). This terrestrial adaptation may facilitate their persistence

on Maine's smallest coastal islands that lack standing bodies of fresh water.

Despite being one of the most widely distributed and abundant salamanders in North

America (Burton and Likens 1975), previous work suggests that genetic divergence within the

species can occur at very modest geographic scales. Wiens et al. (2006) determined that

diversification can occur within the genus when populations remain at different elevation forests,

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and Cabe et al. (2007) identified genetic structural differences between red-backed populations within 200m of each other. This in part may be the result of the limited dispersal capabilities of amphibians (Larson et al. 1984; Beebee 2005), in general, and red-backed salamanders in particular (deMaynadier and Hunter 2000; Noël et al. 2007; Marsh et al. 2008). Though *P. cinereus* may be more mobile than previously thought (Gibbs 1998; Marsh et al. 2004; Smith and Green 2005), they likely are susceptible to vicariant barriers.

Historic landscape change presents alternative hypotheses for the origins of these island salamander populations. Post-glacial rebound caused sea water levels to be nearly 60 meters below current conditions around 11,000 YBP (Kelley et al. 1992; Barnhardt et al. 1995). During low water conditions, red-backed salamanders may have colonized coastal plain regions of Maine that became Maine's coastal islands roughly 6,000 years later. Salamanders could also have passively colonized islands by rafting in large woody debris washed out to sea from rivers and coastal regions or rare transport by predatory birds. Colonization during this period may also have occurred from one or more glacial refugia. If some island or mainland sites were colonized from different refugia, then divergence among those populations might be much greater than expected for a post-glacial time window, as in the case of divergent red-back salamander populations on Long Island, NY (Fisher-Reid et al. 2013). Alternatively, these islands may have been colonized during more modern times as a byproduct of island inhabitation and trade following European colonization of the region. Some of the larger islands in the Gulf of Maine have supported permanent human populations, whereas, smaller islands were inhabited seasonally, used for grazing of livestock, or maintaining lighthouses. Regardless of their colonization process, present-day island populations of red-backed salamanders are expected to have been substantially isolated from one another and mainland sources in the absence of

ongoing human-mediated dispersal. Here I used common measures of genetic diversity and differentiation across nine microsatellite loci, combined with Bayesian cluster analysis and phylogenetic inference, to examine relative support for three alternative, yet not mutually exclusive, biogeographic hypotheses: post-glacial single refugium, post-glacial multiple refugia, contemporary colonization. Support for these alternatives is based on several biogeographic predictions.

If island and mainland populations were anciently colonized from a shared glacial refuge, then one would predict that a comparison between two or more islands would show greater divergence among islands over similar geographic scales than among multiple mainland populations (Ricketts 2001; Sexton et al. 2014). One would also predict weak, if any, isolation-by-distance metrics for among-island comparisons owing to greater isolation among islands than for mainland populations where migration would have more capacity to constrain the genetic independence of populations diverging under drift. Under this scenario one might also predict that islands further from the mainland would be the most divergent from all other sites owing to earlier isolation during sea level changes, greatly reduced odds of receiving immigrants from mainland sources during the ensuing millennia, and different selection pressures (Kadman and Pulliam 1993; Johnson et al. 2000). Finally, under this colonization scenario phylogenetic and clustering analyses might suggest a coarse regional affiliation of island and mainland populations, however, with relatively weak divergence among regions, assuming islands once shared some ancestry with neighboring mainland sites or continue to receive small numbers of migrants from a regional pool. Alternatively, if islands or mainland sites were post-glacially colonized from more than one glacial refuge, one would expect alternative regional patterns for the most divergent populations or a phylogenetic and clustering pattern suggestive of distinct

population groupings with strong separations (e.g., long tree branches) between regional groupings (Fisher-Reid et al. 2013).

Lastly, if island populations were colonized in recent times, then they would be expected to show divergence equal to or less than that of mainland populations over similar geographic scales (assuming equal environmental conditions and selection pressures), particularly if island colonization was dependent upon ship transport from a limited number of regional ports. Additionally, distant islands would not be expected to show greater divergence from mainland populations than coastal populations. Indeed, the more distant islands in this study have had relatively large human populations compared with the smaller coastal islands and would have thus imported more materials from the mainland that might serve to transport salamanders. Likewise, whereas naturally colonizing populations might be expected to show evidence of reduced genetic variation from bottlenecks, repeated introductions by humans would limit such losses or even lead to greater allelic diversity than in native populations (Kolbe et al. 2004; Voisin et al. 2005; Kolbe et al. 2007).

METHODS

Study Sites

I surveyed for red-backed salamanders at 26 mainland and island sites across the state of

Maine, USA (Table 1.1; Figure 1.1).
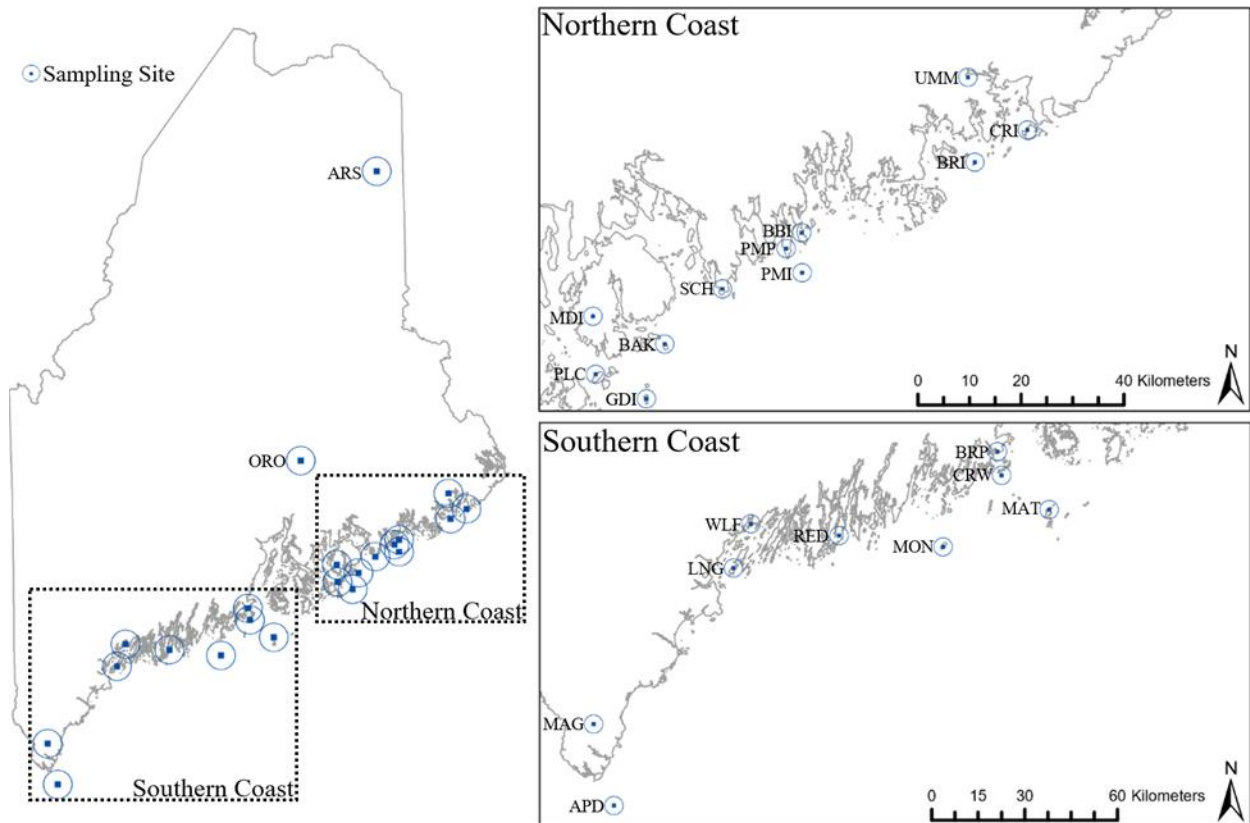
Figure 1.1. Sampling localities across the state of Maine. Sites where samples were collected are denoted by closed circles.

Table 1.1. Sites surveyed for red-backed salamanders (*Plethodon cinereus*) during 2012-2014 across the state of Maine. Approximate area for island sites, euclidean distance to Maine's nearest coast (km), and the dominant vegetation type for both island (I) and mainland (M) sites are presented. Samples were collected from 22 of the 26 sites visited.

Site	Abbreviation	Habitat Type	Area* (ha)	Distance to Coast (km)	Dominant Vegetation	Samples Collected
Appledore Island	APD	I	40	10	Coniferous	34
Aroostook State Park	ARS	M	10000	200	Coniferous	17
Baker Island	BAK	I	3	5	Coniferous	30
Bois Bubert Island	BBI	I	600	1	Coniferous	30
Birch Point State Park	BRP	M	200	<0.5	Deciduous	20
Cross Island	CRI	I	700	1	Coniferous	31
Crow Island	CRW	I	5	5	Coniferous	30
Great Duck Island	GDI	I	80	10	Coniferous	30
Isle au Haut	IHT	I	2800	5	Coniferous	0
Long Island	LNG	I	600	5	Coniferous	30
Mount Agamenticus	MAG	M	500	8	Deciduous	30
Matinicus Island	MAT	I	325	23	Coniferous	32
Metinic Island	MET	I	150	8	Coniferous	0
Monhegan Island	MON	I	250	17	Coniferous	30
Mount Desert Island	MDI	M	1000	4	Deciduous	19
Orono	ORO	M	200	50	Coniferous	20
Outer Green Island	OGI	I	2	8	Grass/ Shrub	0
Placentia Island	PLC	I	200	4	Coniferous	27
Petit Manan Island	PMI	I	4	4	Grass/ Shrub	37
Petit Manan Point	PMP	M	500	1	Grass/ Shrub	16
Reid State Park	RED	M	500	<0.5	Coniferous	33
Schoodic Education and Research	SCH	M	80	<0.5	Coniferous	23
Smuttynose Island	SMI	I	15	10	Grass/ Shrub	0
University of Maine at Machias	UMM	M	2000	5	Coniferous	22
Western Brothers Island	BRI	I	5	5	Grass/ Shrub	31
Wolfe's Neck Woods State Park	WLF	M	60	<0.5	Coniferous	29

*Mainland site area was estimated by the amount of continuous forest surrounding the sampling area. Estimates were made using polygon measurements in Google Earth.

All mainland sites, with the exception of Aroostook State Park and Orono, were selected given their proximity to shore and island sites (Table 1.1). Aroostook State Park and Orono were selected to account for potential genetic variation farther inland, whereas, island sites were

chosen to provide a thorough sampling of the entire coast of Maine while constrained by accessibility.

Salamander Capture

During 2012-2014, I collected salamanders from 22 of 26 survey sites (Table 1.1) by lifting natural cover objects and sifting through leaf litter. Captured individuals were retained in a ventilated, shaded, moist container for processing. Although I found salamanders on Isle au Haut, their small numbers (i.e., <5 salamanders) precluded collection. Capture and handling methods were approved by the University of Maine Institutional Animal Care and Use Committee (IACUC; A2012-04-05).

Sedation and Tissue Collection

Salamanders were anesthetized with tricaine methanesulfonate (MS-222; 2g/l solution buffered with sodium bicarbonate). The tail tip (3-5 mm) from 601 salamanders was removed with forceps, preserved (90% ethanol), and a benzalkonium chloride antiseptic was applied to the wound. Forceps were cleaned aseptically between salamanders with a dip in 95% ethanol, flamed with a portable lighter, rinsed again in ethanol, and then air dried. Individuals recovered from anesthesia in a water bath at ambient temperature prior to release at the capture site.

DNA Extraction and Microsatellite Analysis

Tail samples were subdivided to reserve tissue for multiple re-extractions. DNA was extracted with the Qiagen® DNeasy Blood & Tissue Kit (Qiagen®, Valencia, CA) with modified guidelines (presented here). Tissue was digested in proteinase K for 8-12 hours at 56°C; vortexed, and combined with 200 µl of AL lysis buffer. DNA concentration and quality were quantified with a NanoDrop® spectrophotometer (NanoDrop® Technologies Inc., Wilmington, DE).

I examined genetic variation at nine microsatellite loci previously identified for this species (Connors and Cabe 2003; Fisher-Reid et al. 2013) (Table 1.2). A GTTT pigtail was added to reduce stutter (Brownstein et al. 1996; Faircloth et al. 2009) for primers lacking a fluorescent dye on the 5' end. I modified the standard touchdown thermal-cycler program (Fisher-Reid et al. 2013) by reducing the number of cycles and increasing the increments at which the annealing temperature decreased each cycle to -1.0ºC. This was necessary to accommodate my multiplex design (Table 1.3).

I optimized the selected loci for multiplex PCR by initially evaluating optimal PCR conditions and visualizing locus-specific fragment sizes on agarose gels containing ethidium bromide and a 100 bp ladder. Based on locus-specific allele size ranges, I constructed two multiplex PCR reactions containing MS1718, MS3241, MS3544, MS4953, PcXF08, MS5155, MS6162, PcJX06, and PcLX23 fluorescently labeled loci. All PCR reactions included water, a combination of primers, dNTPs, DNA template, Promega GoTaq® DNA Polymerase, 5x reaction buffer, and $MgCl_2$ (Promega Corporation, Madison, WI) (Table 1.3). Multiplexed PCR products were then electrophoresed on an ABI 3730 sequencer at the University of Maine DNA Sequencing Facility and scored relative to a Genescan™ – 500 LIZ® size standard using Geneious, version 7.1.7 (http://www.geneious.com, Kearse et al. 2012). Ten percent of all genotypes were re-amplified and re-scored to ensure accuracy in allele frequency estimation.

Table 1.2. Selected microsatellite loci and primers designed for *Plethodon cinereus* and their published source. Fluorescent tags and GTTT pigtails reflect changes made within this study.

Locus	Primer sequence with fluorescent tag or pigtail (5' → 3')	Published source
MS1718	F: [PET]ATTCGGCATATTTTTCACTCTAA R: GTTTGCAGCAGGTAGGTCTATCACG	Fisher-Reid et al. 2013
MS3241	F: [VIC]GGTCCCCTTTGTATCTACTTTG R: GTTTGCCGCCTAATTGCATTCC	Fisher-Reid et al. 2013
MS3544	F: [6FAM]GCACAAAGCAAAGAGGAGAAA R: GTTTCATTCATGTCTGAACTAGTGTG	Fisher-Reid et al. 2013
MS4953	F: [PET]TGTGCGTCCTAAAAGCCATC R: GTTTCACCTAGCTCACTTGCCC	Fisher-Reid et al. 2013
MS5155	F: GTTTGCTTGAGGAGCTAAGTGATG R: [6FAM]GCATGTGGCACGTAAAACAG	Fisher-Reid et al. 2013
MS6162	F: [PET]GGTCTTTTTGGAGCATGTTGA R: GTTTGAGGAACTCAAACCAGCAA	Fisher-Reid et al. 2013
PcJX06	F: GTTTCCCTCCTAGAACCCAGAACC R: [NED]TGGGTTAGGAAGGGAGAAATG	Connors and Cabe 2003
PcLX23	F: [VIC]TTCTCTCAAAATGCATTAGACTGG R: GTTTCCTAGTATGCCCCTTATCTGG	Connors and Cabe 2003
PcXF08	F: GTTTAAGAACTCAGATTTTCCCTGTTTC R: [NED]GGCAGGAATAACAAATGTACAAG	Connors and Cabe 2003

Table 1.3. PCR multiplex runs for 25 µl reactions per individual and annealing temperatures. Five loci were featured within multiplex 1, while four loci were featured within multiplex 2.

Multiplex	Loci	Annealing temperature	Water	Reaction buffer	25mM MgCl$_2$	2mM dNTPs	5u/µl Taq	5uM Primer	DNA template
Panel 1	MS1718 MS3241 MS3544 MS4953 PcXF08	55→50ºC	2.9 µl	5 µl	3 µl	2 µl	0.1 µl	10 µl	2 µl
Panel 2	MS5155 MS6162 PcJX06 PcLX23	61→51ºC	4.8 µl	5 µl	3 µl	2 µl	0.2 µl	8 µl	2 µl

Analyses

I evaluated the assumption of Hardy-Weinberg equilibrium (HWE) with GENEPOP, Version 4.3 (Raymond and Rousset 1995), and I estimated *P*-values with a Markov chain method exact test (Guo and Thompson 1992) with 10,000 batches and 100,000 iterations. I tested statistical significance of each comparison with α = 0.05 and a sequential Bonferroni correction (Rice 1989). I evaluated linkage disequilibrium with GENEPOP using a Markov chain method exact test with 500 batches and 50,000 iterations. Loci were checked for null alleles using

FreeNA (Chapuis and Estoup 2007). Mean number of alleles (k), allelic richness (A), observed heterozygosity (H_O), and expected heterozygosity (H_E) were measured with FSTAT, version 2.9.3.2 (Goudet 2002). Pairwise F_{ST} values (Weir and Cockerham 1984) were computed and tested for significance with an exact G-test with 10,000 batches and 10,000 iterations in GENEPOP. The web-based program SMOGD (Crawford 2010) was used to estimate G'_{ST}, a standardized measure of differentiation (Hedrick 2005). These values were then averaged across all loci. I used nonmetric multidimensional scaling (NMDS) in SPSS Statistics 20 to plot pairwise F_{ST} values and reduce them to two dimensions.

Isolation by genetic distance was analyzed by regressing pairwise genetic distance ($F_{ST}/[1 - F_{ST}]$) against Euclidian geographic distance (km) to reveal an universal pattern of isolation by distance. Island and mainland sites were plotted concurrently and individually, reflecting my expectation of a greater correlation between genetic and geographic distance for mainland populations. I compared genetic and geographic distance with a mantel procedure (Mantel 1967) in R, version 3.0.3 (The R Project for Statistical Computing, http://www.r-project.org) with the package Vegan, Version 2.3 (Oksanen 2008).

I evaluated population structure with Bayesian cluster analysis (STRUCTURE version 2.34; Pritchard et al. 2000; Falush et al. 2003). I analyzed microsatellite data with a 50,000 burn-in-period, 150,000 Markov chain-Monte Carlo iterations, and 21 possible populations (K) replicated for 10 runs, assuming an admixture model of ancestry with and without location priors. The best supported number of genetic clusters were determined by the rate of change of the likelihood function (ΔK) (Evanno et al. 2005) with STRUCTURE HARVESTER, web version 0.694 (Earl and vonHoldt 2011) and CLUMMP (Jakobsson and Rosenberg 2007). The final bar plot was visualized with DISTRUCT (Rosenberg 2004).

I constructed an unrooted neighbor-joining (Saitou and Nei 1987) tree with Cavalli-Sforza and Edwards (1967) chord distances in the phylogenetic inference program PHYLIP, Version 3.695 (Felsenstein 2004). I resampled 10,000 datasets with SEQBOOT, and I computed genetic distances with GENDIST. I used the NEIGHBOR package to produce several neighbor joining trees and CONSENSE to generate a consensus tree with the Majority Rule method, which was then visualized using the package DRAWTREE.

I differentiated patterns among island and mainland populations with hierarchical analyses of molecular variance (AMOVA) (Arlequin version 3.5; Excoffier and Lischer 2010). I grouped island and mainland sites for AMOVA 1 and grouped the four most distant islands separately from the remaining islands to compare with mainland sites for AMOVA 2. AMOVA 3 featured 9 groupings as extracted from my previous phylogenetic analysis.

Estimating Island Vicariance

I estimated time of isolation for Maine's islands by applying projections of sea level change along Maine's coast over the last 12,000 years (Barnhardt et al. 1995) to 10 m contour bathymetry lines estimated for the Gulf of Maine (http://www.maine.gov/megis/catalog/metadata/bathym100.htm; access date 11/1/2013). I converted the bathymetry polylines to raster format with the 3D Analyst tool 'Topo to Raster', which creates a hydrologically correct elevation model from topographic contours (ArcMap 10.1; ESRI, Inc). I partitioned depth ranges with initial natural breaks 60 m below present sea level, the estimated sea level of approximately 10,800 YBP (Barnhardt et al. 1995) when sea level along Maine's coastline was predicted at its lowest point following glaciation and current mean sea level (Kelley et al. 1992; Barnhardt et al. 1995). Areas < 60 m in depth were labeled "exposed", whereas, areas ≥ 60 m in depth were assigned "submerged." Current distribution of

Maine's exposed islands was represented by an island shapefile (polygon vector data) (Linda Welch, U.S. Fish and Wildlife Service, Coastal Islands Refuge, ME). Subsequent to this initial analysis, I estimated the time before present that various islands would have become isolated by sea level changes using depths extracted from Barnhardt et al. (1995) at 500-year increments moving from the peak low-water period to present-day sea levels.

RESULTS

Assumptions and Genetic Diversity

Tests for Hardy-Weinberg equilibrium revealed 10 out of 189 tests with an assumed excess of heterozygotes ($P = 0.021$), and linkage disequilibrium was identified in only 29 out of 792 combinations (3.6%). Deviations and disequilibrium were spread across all loci and sampling sites, and all tests were found to be in equilibrium after Bonferroni correction. I found less evidence for null alleles than expected across 8/9 loci and 14/21 sites, thus these no action was taken. Moderate variation was observed in gene diversity, with the lowest diversity values for populations inhabiting the most distant marine islands (APD, MAT, MON) (Table 1.4).

Table 1.4. Genetic diversity for 21 sites where red-backed salamanders (*Plethodon cinereus*) were captured during 2012-2014 in Maine. *Ho* = observed gene diversity within individuals, and *He* = expected gene diversity within individuals. The mean number of alleles across all sites for each locus are displayed as a footnote.

Site	Sample Size *n*	Mean number of alleles *k*	Allelic richness *A*	H_O	H_E
APD	34	4.11	3.65	0.529	0.522
ARS	17	5.11	5.03	0.627	0.635
BAK	30	6.33	5.46	0.667	0.691
BBI	30	6.88	5.84	0.707	0.732
BRP	20	5.33	5.10	0.672	0.681
CRI	31	6.11	5.23	0.692	0.714
GDI	30	4.88	4.17	0.570	0.578
LNG	30	6.44	5.42	0.637	0.686
MAG	30	7.11	5.99	0.685	0.701
MAT	32	3.88	3.31	0.392	0.416
MON	30	3.77	3.44	0.574	0.548
MDI	19	5.55	5.35	0.661	0.722
ORO	20	5.66	5.41	0.656	0.682
PLC	24	5.55	5.00	0.718	0.689
PMI	37	5.88	5.09	0.685	0.688
PMP	16	5.00	5.00	0.729	0.699
RED	32	6.55	5.34	0.563	0.606
SCH	23	5.66	5.18	0.715	0.690
UMM	23	5.77	5.36	0.671	0.703
BRI	30	5.77	5.06	0.670	0.657
WLF	29	6.11	5.42	0.724	0.677

k across loci: 4.52 for MS3544, 4.14 for MS3241, 8.09 for PcXF08, 4.61 for MS1718, 9.52 for MS4953, 7.23 for MS5155, 3.38 for PcLX23, 5.85 for PcJX06, and 3.00 for MS6162.

Population Differentiation

There was strong multi-locus differentiation among all sites, with island sites featuring the greatest differentiation compared to mainland sites (Island global F_{ST} = 0.133; Mainland global F_{ST} = 0.054). Eleven of 189 pairwise F_{ST} values were not significant (Table 1.5).

Salamanders from the most distant offshore islands (APD, GDI, MAT, and MAT) were most differentiated from all other sites, and Matinicus Island (approximately 23 km from the current mainland coastline) salamanders were most distinct when compared to all sites (mean F_{ST} = 0.273). This pattern was supported by a NDMS plot based on pairwise F_{ST} values (Figure 1.4.). Standardized differentiation values (G'_{ST}) that account for the greater heterozygosity of microsatellite loci further support marked divergence of the distant island populations from all

others (mean G'_{ST}: APD = 0.33 ± 0.099, GDI = 0.35 ± 0.086, MAT = 0.51 ± 0.07, MON = 0.33 ± 0.097; all others (excluding distant islands) = 0.12 ± 0.07).

Table 1.5. Pairwise F_{ST} values (below central diagonal; Weir and Cockerham 1984) and standardized pairwise G'_{ST} values (above central diagonal; Hedrick 2005) for 571 red-backed salamander (*Plethodon cinereus*) samples collected in Maine from 2012-2014. Non-significant pairwise values are bolded for Weir and Cockerham F_{ST} ($P > 0.05$).

Site	APD	ARS	BAK	BBI	BRP	CRI	GDI	LNG	MAG	MAT	MON	MDI	ORO	PLC	PMI	PMP	RED	SCH	UMM	BRI	WLF
APD	---	0.31111	0.24336	0.25838	0.26825	0.27542	0.5782	0.33079	0.37344	0.60294	0.43741	0.33478	0.33329	0.22972	0.30081	0.25953	0.31773	0.28916	0.29266	0.28165	0.24766
ARS	0.1755	---	0.13363	0.15257	0.09011	0.15326	0.34524	0.17042	0.15138	0.56097	0.248	0.13295	0.16659	0.1815	0.25282	0.14307	0.12384	0.20409	0.15875	0.24809	0.1568
BAK	0.1166	0.0803	---	0.02768	0.06175	0.03388	0.30946	0.07472	0.07063	0.5374	0.31974	0.00765	0.18695	0.04689	0.12629	0.09747	0.24846	0.08387	0.03043	0.14432	0.06955
BBI	0.1112	0.0673	0.0085	---	0.02622	-0.0234	0.26234	0.04816	0.03921	0.43705	0.28132	-0.0087	0.15359	0.00858	0.08887	0.02312	0.16581	0.04315	-0.0056	0.11033	0.06474
BRP	0.1266	0.0499	0.0224	**0.0077**	---	0.01778	0.26454	0.04368	0.05026	0.47307	0.24894	0.0378	0.18101	0.07807	0.1389	0.04793	0.07117	0.13011	0.03864	0.1381	0.0533
CRI	0.1205	0.074	0.0126	**-0.008**	**0.0072**	---	0.27257	0.03299	0.03512	0.47121	0.28217	-0.0262	0.13321	0.00943	0.08744	0.05529	0.18337	0.06073	-0.0184	0.09044	0.04719
GDI	0.3038	0.1681	0.1278	0.1039	0.114	0.1093	---	0.28349	0.26498	0.4887	0.45571	0.28861	0.44358	0.30826	0.39785	0.31026	0.31245	0.32134	0.28112	0.41207	0.30922
LNG	0.1614	0.0956	0.0304	0.0183	0.0185	0.0132	0.1165	---	0.03555	0.54862	0.31095	0.0338	0.15438	0.06212	0.12843	0.10749	0.16375	0.09848	0.06698	0.14635	0.02314
MAG	0.1785	0.0726	0.0272	0.0151	0.0224	0.0154	0.1085	0.013	---	0.47014	0.29906	0.00576	0.09371	0.08951	0.17378	0.10653	0.16912	0.09402	0.06424	0.22869	0.06782
MAT	0.3763	0.3022	0.2625	0.2115	0.2464	0.2358	0.2847	0.2718	0.2299	---	0.67455	0.51166	0.4803	0.47486	0.57534	0.37975	0.47883	0.43695	0.46857	0.62728	0.57979
MON	0.2396	0.1121	0.1423	0.1158	0.1139	0.1196	0.2306	0.1413	0.131	0.3905	---	0.29963	0.38321	0.32968	0.29891	0.28844	0.21991	0.30236	0.32199	0.34522	0.27791
MDI	0.1537	0.0653	**0.0027**	**-0.004**	0.0121	**-0.0075**	0.1153	0.0138	**0.0033**	0.2573	0.1287	---	0.1092	0.0545	0.1011	0.07976	0.21963	0.07957	0.00314	0.1448	0.06309
ORO	0.1963	0.0748	0.0728	0.054	0.0723	0.0501	0.1919	0.059	0.0397	0.2694	0.1798	0.0355	---	0.1286	0.25828	0.19096	0.27715	0.15658	0.15791	0.30704	0.14851
PLC	0.1063	0.0905	0.0174	**0.0034**	0.0313	0.0041	0.1376	0.0257	0.0341	0.2551	0.147	0.0193	0.0544	---	0.12921	0.06721	0.22479	0.0662	-0.0062	0.10775	0.06302
PMI	0.1352	0.1122	0.0424	0.0255	0.0504	0.0276	0.1647	0.0426	0.0589	0.2803	0.1324	0.0314	0.095	0.0444	---	0.09859	0.2202	0.14944	0.11279	0.11387	0.15492
PMP	0.1271	0.0696	0.0354	0.0109	0.0228	0.0238	0.1352	0.0449	0.0427	0.2058	0.1423	0.0298	0.0797	0.0301	0.0331	---	0.13443	0.11454	0.07606	0.13605	0.13744
RED	0.1647	0.0626	0.1071	0.0724	0.0331	0.0779	0.1471	0.0754	0.0852	0.2681	0.0991	0.0953	0.1398	0.1025	0.096	0.0705	---	0.23477	0.20557	0.24168	0.17288
SCH	0.1394	0.0926	0.0317	0.0148	0.0464	0.0202	0.1379	0.0366	0.0318	0.2509	0.1433	0.0274	0.061	0.0248	0.0497	0.0472	0.1031	---	0.08401	0.22899	0.16129
UMM	0.1337	0.0786	0.0099	**-0.0037**	0.0133	**-0.0065**	0.1202	0.0249	0.0249	0.2428	0.1406	**-0.0016**	0.0593	**-0.0009**	0.0356	0.0281	0.0947	0.0324	---	0.07855	0.06453
BRI	0.1258	0.1215	0.0542	0.0405	0.0615	0.0355	0.1898	0.0615	0.0961	0.3319	0.1555	0.0549	0.1285	0.0421	0.0417	0.0592	0.1217	0.0872	0.0338	---	0.16776
WLF	0.1357	0.0908	0.0274	0.0225	0.0204	0.0154	0.1246	0.0076	0.0295	0.288	0.1277	0.0211	0.0666	0.0241	0.0551	0.059	0.0778	0.0614	0.0219	0.065	---

Figure 1.2. NMDS plot using pairwise F_{ST} values with Euclidean distance for 21 sites where red-backed salamanders (*Plethodon cinereus*) were captured during 2012-2014 in Maine (Table 1.1; Figure 1.1). Distant island sites (□) show a pattern of overall greater divergence from all other sites (○) with the MAT populations dominating along Dimension 1.

Isolation by Distance

I found no universal pattern of isolation by distance among all sites (Mantel: $r = -0.044$, $P = 0.92$; $R^2 = 0.0028$, $F_{1,208.} = 0.574$, $P = 0.449$; Figure 1.3 **A**). Likewise there was no evidence of isolation by distance among island sites (Mantel: $r = -0.08$, $P = 0.935$; $R^2 = 0.0027$, $F_{1,53.} =$

0.141, $P = 0.708$; Figure 1.3 **B**). There was no correlation of genetic with geographic distance among mainland sites with all sites included (Mantel: $r = -0.06$, $P= 0.853$; $R^2 = 0.0482$, $F_{1,43.}=$ 2.175, $P = 0.147$; Figure 1.3 **C**). However, with exclusion of Reid State Park samples, there is a suggestion of a trend of weak isolation by distance among remaining mainland populations (Mantel: $r = 0.05$, $P= 0.199$; $R^2 = 0.1108$, $F_{1,35.}= 4.359$, $P = 0.044$; Figure 1.3 **D**).
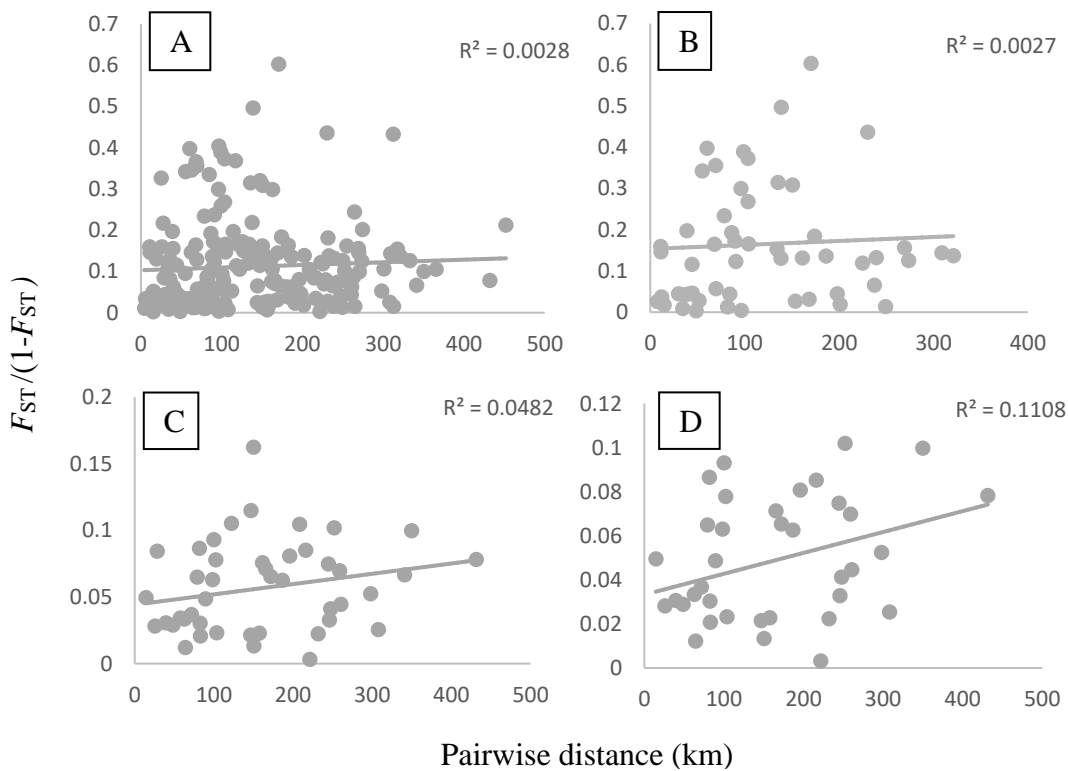
Figure 1.3. Genetic distance ($F_{ST}/1$-F_{ST}) plotted against Euclidian geographic distance (km) for 21 sites where red-backed salamanders (*Plethodon cinereus*) were captured during 2012-2014 in Maine.   Four site groupings are detailed: **(A)** All sites, **(B)** island sites, **(C)** mainland sites, and **(D)** mainland sites excluding Reid State Park. R^2 values are displayed for each plot.

Phylogenetic Analysis

Phylogenetic analysis resulted in a tree with low bootstrap values on the majority of branches but increased support for several genetic clusters on external branches (Figure 1.4). The greatest frequency of partitioning the genetic variation occurred among BRI-PMI (41.9%),

PLC-UMM (49.5%), and GDI-MAT (71.0%). There was broad structure reflecting the study area's geographic landscape. For example, Long Island and Wolfe's Neck Woods State Park, Petit Manan Point and Petit Manan Island, and Mount Desert Island and Baker Island are each examples of sites sharing general proximity, which is indicated in the neighbor joining tree (Figure 1.4). Great Duck Island and Matinicus Island showed the greatest bootstrap support (71.0%), indicating relatively strong confidence in genetic clustering of these distant, offshore populations.
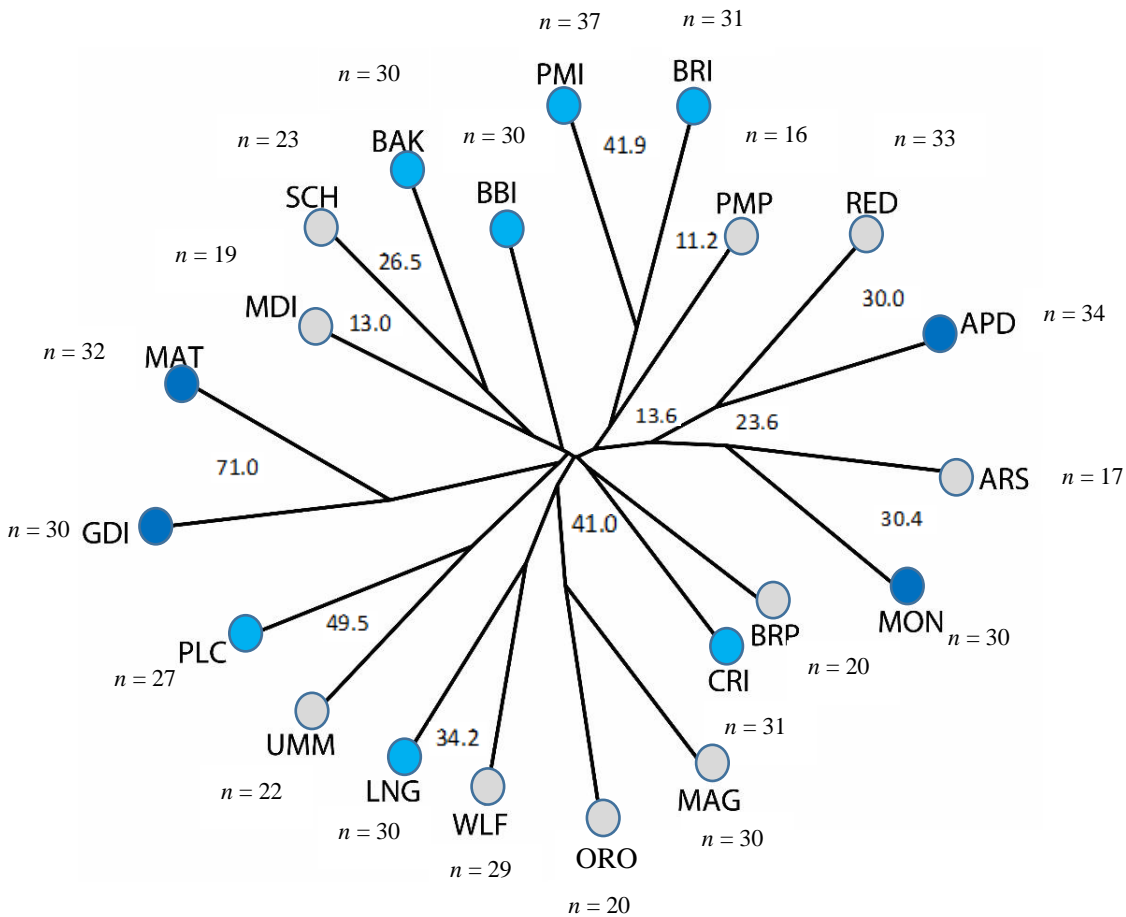
Figure 1.4. Neighbor joining tree using Cavalli-Sforza and Edwards (1967) chord distances for 21 *Plethodon cinereus* sampling sites in Maine. Bootstrap percentages are displayed for 10,000 permutations; those with low confidence (<10%) are excluded from the tree. Branch lengths correspond with genetic distance. Abbreviations are defined in table 1.1 and sites are mapped in Figure 1.1. Mainland sites (grey circles), island sites (light blue circles), and distant island sites (dark blue circles).

Bayesian Structure Analysis

Results from the structure analysis assuming an admixture model, correlated allele frequencies, and no location priors found that $K = 3$ was the most supported cluster value (ΔK_{max} of 86.03). There was support for $K = 7$ on the basis of log likelihood values (Mean $LnP(K) = -14486.02$). An analysis with location priors yielded ΔK values 6.18 (K=3) and 5.03 (K=8). There was improved support for $K = 8$ under log-likelihood criteria (Mean $LnP(K) = -14031.37$). Matinicus Island had distinct structure among all four bar plots (Figure 1.5). Other distant offshore islands (APD: K=3; GDI: K=7; MON; K=8) also had high probabilities of being defined by a particular cluster. Western Brothers Island (K=7) and Petit Manan Island (K=8) showed similar probabilities of belonging to their own cluster.
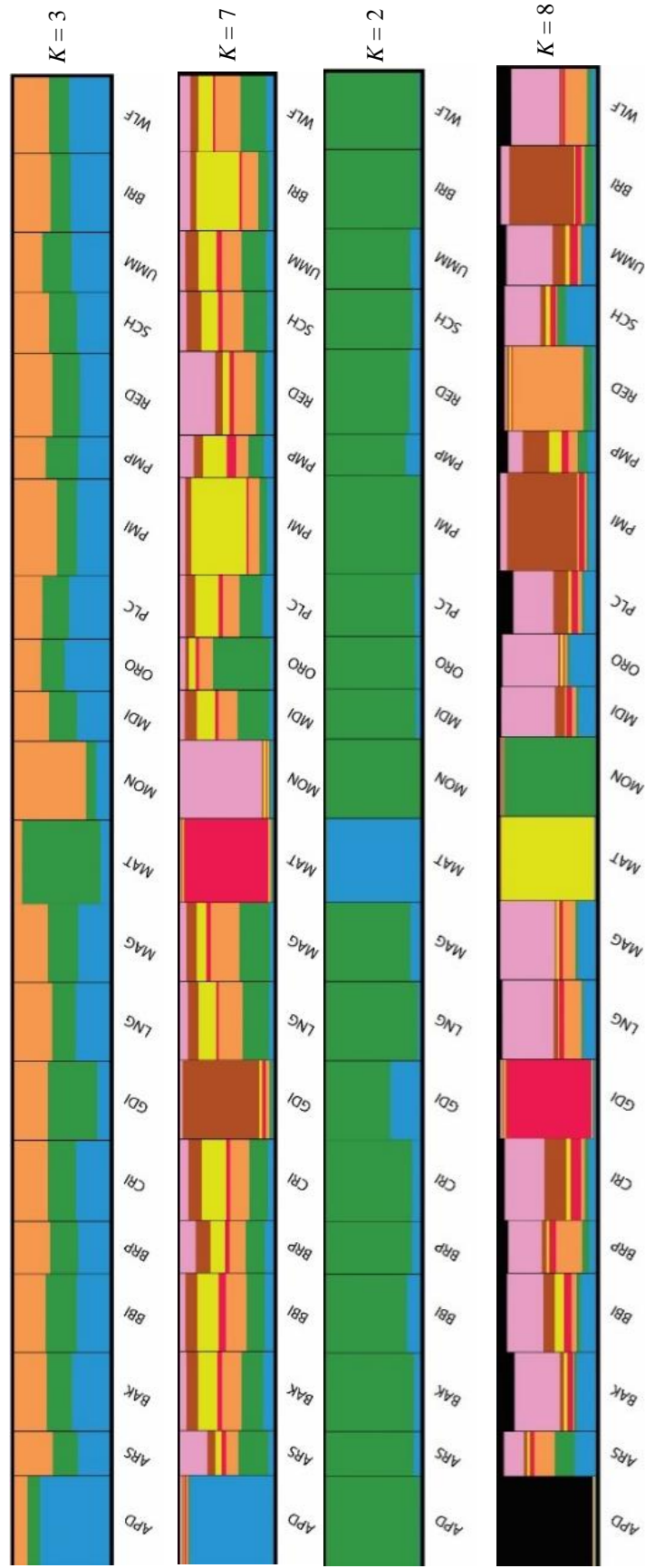
Figure 1.5. Bayesian cluster analysis for $K = 3$ and 7 assuming no location priors and $K = 2$ and 8 assuming location priors. 21 *Plethodon cinereus* sampling sites in Maine during 2012-2014. Optimal cluster values were determined using ΔK_{max} and log likelihood criteria.

Analysis of Molecular Variance

Within-population variation explained the majority of molecular variance (Table 1.6).

Model 1, which compared all island sites with all mainland sites, indicated the least amount of

variance explained by the 'among groups' factor of any of the AMOVA models (-0.34%).

Model 2, which distinguished distant islands, other islands and the mainland populations in the

among-groups term, also did not indicate significant among-group variation (0.58%). The

AMOVA model employing nine groupings representing the major clades determined by my

phylogenetic analysis provided the strongest support for an among-group term (3.83%).

Table 1.6. Analysis of molecular variance (AMOVA) for 21 sites where red-backed salamanders
(*Plethodon cinereus*) were captured during 2012-2014 in Maine. Three different grouping
comparisons are included.

Grouping	Source of Variation	d.f.	% variation	Variance Components	P
1. Mainland	Among Groups	1	-0.34	-0.01	0.88
2. Island	Among Populations within groups	19	10.22	0.33	
1. Mainland	Among Groups	2	0.58	0.02	0.13
2. Island	Among Populations within groups	18	9.61	0.31	
3. Distant Islands					
Phylogenetic Tree	Among Groups	8	3.83	0.12	<0.001
(9 Groups)	Among Populations within groups	12	6.46	0.21	

Estimating Island Vicariance

Isolation of the most distant islands predated those closer to the mainland (Table 1.7).

Matinicus Island and Monhegan Island became isolated approximately 10,000 YBP; Great Duck

Island became isolated approximately 9,000 YBP; and, Appledore Island became isolated nearly

6,000 YBP. All other islands became isolated as recently as 4,000 YBP (Table 1.7).

Table 1.7. Estimated timing of island isolation along Maine's coast based on Barnhardt et al. (1995) modified sea-level curve applied to estimated bathymetry (http://www.maine.gov/megis/catalog/metadata/bathym100.htm; access date 11/1/2013).

Estimated Years Before Present (YBP)	Island	Distance from present day coastline (km)
10,000 YBP	Matinicus Island	23
10,000 YBP	Monhegan Island	17
9,000 YBP	Great Duck Island	10
6,000 YBP	Appledore Island	10
4,000 YBP	All Other Sampled Islands	1-5

DISCUSSION

Genetic patterns observed in populations of *Plethodon cinereus* from Maine's islands and mainland are most consistent with predictions for a scenario of ancient colonization of Maine's coastal islands from a single regional refugia, followed by extended isolation, particularly for distant island populations. Specifically, island populations showed markedly less genetic diversity than mainland populations, were more divergent from each other than mainland populations, and showed less evidence for isolation by distance than mainland populations. At the same time, islands showed broad regional affinities in phylogenetic and cluster analyses, and the greater magnitude of divergence shown by distant islands was consistent with expectations based on reconstruction of their likely earlier post-glacial isolation due to sea level change and lower odds of receiving migrants via rare dispersal processes.

Less Genetic Diversity on Islands

Basic principles of island biogeography (MacArthur and Wilson 1963, 1967) predict less community, or genetic, diversity at equilibrium on islands than among mainland locations and less genetic diversity on distant islands than those near the mainland (Jaenike 1973; Frankham

24

1997). Microsatellite variation in Maine's island salamanders illustrated this pattern; more

distant islands showed the least allelic richness and heterozygosity, whereas, some inshore

islands had values similar to or exceeding some mainland populations. Models of island

biogeography suggest that equilibrium conditions may require thousands of years (Cowie 1995;

Heaney 2000; Whittaker and Fernandez-Palacios 2007); however, it is not necessary to invoke

complete equilibrium to produce such patterns on post-glacial time scales. Indeed, a similar

pattern would be predicted if distant islands were isolated earlier than inshore islands owing to

sea level changes, and thus experienced more time for loss of alleles under founder effects and

genetic drift in the absence of constraining gene flow.

Ancient Colonization and Divergence

Support for an ancient origin of Maine's island salamanders also comes from the

observed scale of population divergence. Divergence for distant island populations of red-

backed salamanders in particular is comparable to or greater than that from studies of other

populations of other vertebrates that were anciently colonized following deglaciation, such as

Atlantic salmon (*Salmo salar*, F_{ST} 0.11-0.13; Säisä et al. 2005) and Wood frogs (*Lithobates*

sylvaticus, F_{ST} 0.52-0.83; Lee-Yaw et al. 2008). Theoretical maximum F_{ST} values for highly

variable loci like microsatellites are expected to be much less than that expected for diallelic loci

(1.0; Hedrick 1999; Bernatchez et al. 2002). The average gene diversity across all sites and loci

in this study would permit a maximum predicted F_{ST} of approximately 0.35. As such, the global

F_{ST} for all island sites of 0.133, approaches 37.5% of the predicted maximum, while the mean

pairwise F_{ST} of 0.189 for the four most-distant islands (APD, GDI, MAT, and MON) is roughly

53% of the theoretical maximum. Pairwise G'_{ST} estimates similarly account for the high

heterozygosity of microsatellite loci and thus reflect divergence on a scale of 0-1 (Hendrick

1999; Bernatchez et al. 2002), with the Matinicus population commonly exceeding values of 0.5 to 0.6 in pairwise comparisons. The substantial divergence of the Matinicus population is consistent with the strong separation it shows from all other populations in the NMDS analysis of F_{ST} values, albeit the distance in the NDMS should probably not be interpreted as proportional. This considerable divergence provides further motivation to explore distant offshore islands in Maine as there potentially are other populations of salamanders exhibiting a similar or greater degree of divergence.

There was no universal pattern of isolation by distance, independent of environmental conditions, which is expected if gene flow is largely absent among islands at all scales, leading to their largely independent divergence owing to drift. Interestingly, there was, at most, weak evidence of isolation by distance among mainland sites. This suggests that even many of the mainland populations are largely isolated from one another over the geographic scales we collected and indeed, the global G'_{ST} among mainland sites was 0.11. *Plethodon cinereus* is characterized by localized dispersal and home ranges, leading to genetic divergence over very short spatial scales. For example, Cabe et al. (2007) found genetic differentiation at distances between 200m and 2 km. Indeed, if isolation by distance does occur over such fine scales, population structure could exist within the geographic scope of the larger islands sampled in this study.

A Single Glacial Refugium

The strong divergence among many populations contributed to different outcomes for the Bayesian structure and neighbor joining analyses. The Bayesian structure analyses confirmed the divergence of distant island populations, and, like the F_{ST} (and G'_{ST}) analyses, confirmed that these populations are likely have been isolated for an extended period. In contrast, the neighbor

joining analysis yielded a tree with weakly supported lineages branching from shallow

divergences near the center of the tree. Such a pattern is consistent with a rapid post-glacial

radiation of populations from a shared ancestral source followed by strong isolation and drift in

likely small local demes. Hence, the strong divergence among all populations obscured robust

population affinities. Nonetheless, the neighbor joining analysis indicated broad geographic

groupings also supported by AMOVA.

Interestingly, the strongest supported lineage was for two of the distant islands, MAT

and GDI, that showed some of the greatest divergence in terms of F_{ST} and G'_{ST} from all other

islands and each other. These two islands are not in close proximity to one another, but could

have shared some common colonization history. It is possible that one island (e.g., MAT) could

have been colonized via debris drifting from the other (e.g., GDI) or transport via seabirds

moving between these nesting islands, or humans visiting both islands. Alternatively, the

apparent affinity of these populations may reflect changing genotype dynamics on the mainland

during the thousands of years between when these populations were isolated and the isolation

time of many other islands. It is not likely that these two populations represent colonization

from a distinct glacial refuge source compared to all other populations, because support for their

grouping is only modest, and the branch length separating them from other populations is not

much longer than others in the tree.

Mainland Divergence

In addition to detecting potentially important genetic diversity in island populations, this

study also identified an ostensibly divergent 'mainland' population in Reid State Park (RED).

This population showed somewhat greater pairwise F_{ST} and G'_{ST} values from other mainland

sites and was also supported in two of the Bayesian cluster analyses with and without spatial

priors. This greater divergence may be facilitated by geographic isolation of this site on what actually represents a very large coastal island (Georgetown Island), separated from the mainland by a narrow marine channel (Back River). The sample from Reid State Park had a notably larger proportion of lead-backed salamanders (53%) relative to other populations that were predominantly of the red-backed color morph. Notably, six out of the nine primers used in this study were developed with *P. cinereus* from Long Island, NY, where investigators found multilocus differentiation among red and lead morphs. Although this population was less divergent than the distant island populations, the divergence it shows is perhaps worthy of further future consideration given the greater proximity to other coastal populations and the high frequency of an alternate color morph.

Modes of Dispersal

While colonization appears to have been ancient, multiple mechanisms may have been at play in that process. The potential for a combination of rare dispersal mechanisms, such as rafting in forest debris carried from the mainland in river outflow that merged with coastal currents or transport by birds, may increase the odds of colonization, leading to widespread distribution of red-backed salamanders on Maine's coastal islands (Gillespie et al. 2012). Storms may transport debris, such as logs, containing salamanders across the ocean (Queiroz 2005), and this may be a mechanism for transport of Plethodontid salamanders, which lay eggs in decaying logs (Petranka 1998). Indeed, the shorelines of many of Maine's coastal islands are heavily littered with driftwood even today. In addition to these chance natural mechanisms, we cannot exclude the possibility of purposeful or accidental transport of salamanders to some islands by humans. Historically, Native Americans inhabited some of Maine's coastal islands and may have accessed many to collect resources such as seabird eggs (Conkling 1981; Bourque 2004).

Given that Native Americans have occupied this region for at least 500 years, any island populations they might have facilitated would likely show ancient colonization to other mechanisms.

More recent establishment of island populations associated with European colonization would not be unexpected, however, this is not consistent with my data. Large islands (e.g., Matinicus, Monhegan) were first extensively occupied by Europeans in the mid-1600s for fishing, fur trading, and residence. In the last two centuries, islands such as Petit Manan Island (USFWS ownership) and Western Brothers Island (MDIFW ownership) have been used seasonally or year-round for maintaining light houses or for grazing livestock. Although Native Americans often visited islands for hunting and gathering and pirates used islands for meetings and materials, European settlement of the islands was more permanent and involved importation of large amounts of materials from the mainland such as wood, soil and animal feed (e.g., hay) that could have harbored salamanders. However, the pattern of genetic divergence expected under such recent and extensive colonizations would be different than what I found.

Specifically, the substantial genetic divergence of salamanders on the most distant islands from mainland salamanders is unlikely to have arisen over only a few hundred years. In addition, extensive and repeated introductions of salamanders under such conditions would tend to minimize or erase signatures of founder effects, bottlenecks and drift relative to single rare colonization events (Kolbe et al. 2004; Voisin et al. 2005; Kolbe et al. 2007). And yet, the most distant island populations in this study showed the least within-population genetic diversity, a pattern consistent with predictions from island biogeography. Indeed, persistence of such a pattern may indicate that salamanders accidentally or purposefully introduced by Europeans may have been relatively poorly adapted to island conditions compared to the local populations.

Challenges of Island Size

The small size of coastal islands may present a challenge for long-term persistence of island populations without some dispersal from other sources. The larger size of the distant islands in this study might be associated with larger salamander populations and in turn greater resilience against stochastic extinctions and inbreeding. Some smaller islands may not be so buffered, and I did not find salamanders on all islands (e.g., Outer Green Island and Metinic Island). Likewise, smaller islands frequently lack the preferred forest habitat of *P. cinereus,* as in the case of Western Brothers Island where the species appears to be at low abundance. However, these challenges of smaller islands may in part be offset by their closer proximity to the mainland and thus greater odds of receiving occasional dispersal from the mainland. Again, this would fit with the fact that many of the populations on small islands had more and not less genetic diversity than those on large distant islands.

All that said, the actual census and effective population sizes of island salamander populations are currently unknown. Obtaining this information may be a priority, as it would inform estimates of both divergence times (Hey and Nielson 2004) and the relative risks of extinction from demographic stochasticity (Engen et al. 2003) or inbreeding depression (Charlesworth and Charlesworth 1987; Keller and Waller 2002; Frankham 2005). Islands surveyed in this study are not managed for red-backed salamanders, however, several are extensively managed for seabird conservation, including shrub removal and vegetation burning, which may further compromise habitat for the species.

Concluding Remarks

Management of island populations can be challenging without a thorough understanding of the ecological and evolutionary processes shaping them. This study provided a general

assessment of populations previously unstudied and found that there was significant genetic differentiation occurring among the majority of populations. Given the strong isolation and unique environments afforded by islands, and what we know of the role of islands in generating biodiversity, it is interesting to speculate that the observed divergence of some *P. cinereus* populations may be a precursor to eventual speciation. Indeed, this system may provide an opportunity to study allopatric divergence and the processes that lead to early stages of reproductive isolation (Orr and Smith 1998). Regardless, Maine's island salamanders represent unique genetic repositories within this otherwise widespread species and may even be hypothesized to play an important ecological role in island ecosystems. Recognizing this unique diversity in red-backed salamanders may even serve a further role. Specifically, geographic features, like islands, that lead to isolated and divergent populations of one species may be associated with isolated and divergent populations of other species. In this respect, island biogeography of red-backed salamanders may serve as indicator of islands affording unique genetic diversity in many other taxa. Such literal and figurative islands of biodiversity may be particularly important resources with respect to preserving Maine's natural heritage.

CHAPTER TWO:

THE ECOLOGICAL INTERACTIONS AND LOCAL ADAPATATIONS OF

MAINE'S ISLAND RED-BACKED SALAMANDERS (*Plethodon cinereus*)

ABSTRACT

Common mainland species colonizing relatively young archipelagos potentially provide useful insights into the incipient stages of island adaptive radiations. I used stable isotope and morphological analyses to examine the potential trophic dependences and associated phenotype correlations of red-backed salamanders (*Plethodon cinereus*) on Maine's post-glacially formed coastal islands. Isotopic analyses revealed that Maine's coastal islands vary from one another and from mainland systems in productivity baselines and trophic structure, indicating potential differences in sources of primary productivity and food web structure. Alternative ecological dynamics such as these have the potential to instigate adaptive divergence of island salamanders from their mainland counterparts. Consistent with this idea, red-backed salamanders on Maine's coastal islands appear to feed at a higher mean trophic level than mainland salamanders, with smaller, seabird nesting islands featuring the highest estimated trophic position. Salamander populations varied widely in body size and morphological trait allometry suggesting potentially widespread trait divergence that may be linked to local adaptations. Within this general population variation, island populations featured larger head proportions (snout-gular length) for their body size, a pattern that may be adaptive given the higher mean trophic position of these populations. These findings support the operation of mechanisms generating at least limited phenotype-environment associations in Maine's red-back salamanders, which may in turn reflect the early stages of an island-mainland adaptive radiation.

INTRODUCTION

Island communities and ecosystems often differ markedly from those on neighboring mainlands due their geographic isolation and dependence on surrounding aquatic systems (Wright 1940). The combination of relative isolation, different communities, and different resource availability on islands can in turn be a potent driver of location adaptation and ecological speciation (Losos and Ricklefs 2009) that further shape species interactions and contribute to the unique characteristics of such communities and ecosystems. While ancient island systems often provide remarkable examples of these interactions, and their fragility (Boag and Grant 1981; Caccone et al. 1999; Holland and Hadfield 2002), we know much less about the earliest stages of these processes and how they unfold. Studies of the ecological dependences and emerging adaptations of common mainland species colonizing recently formed archipelagos may provide these insights. In this study, I used stable isotope and morphological analyses to examine the potential trophic dependences and associated adaptations of red-backed salamanders on Maine's post-glacially formed coastal islands.

Island biogeography theory highlights the roles of island size and distance on island communities (MacAuthur and Wilson, 1963, 1967) as drivers of both colonization and extinction rates. This general theory, however, is only a coarse predictor of factors influencing the presence, absence, and sustainability of the diversity of biota on islands. Other factors play additional roles in determining the structure and function of these communities. For example, the exploitation ecosystems hypothesis (Oksanen et al. 1981) emphasizes productivity and its importance for supporting communities with higher trophic levels. Primary production is the foundation of any ecosystem (Boullion et al. 2002), and thus ecosystems featuring different types or amounts of primary production may support different consumers and more or less trophic

levels. Along these lines, arthropod abundance and community composition on islands can be strongly influenced by terrestrial plant community composition (Siemann et al. 1999; Schaffers et al. 2008) and the potential for productivity inputs from other resource pools (Polis and Hurd 1996). Differences in arthropod community composition and abundance are in turn expected to potentially influence the presence and trophic specializations of their consumers, including vertebrates.

Estimating productivity on islands can be challenging. However, island size affects local productivity (Schoener 1989; Holt 1996), and smaller islands may not support multiple consumer levels (Polis et al. 2004; Holt and Hoopes 2005), including vertebrate predators (Terborgh et al. 1997). By extension, such small islands may be much more dependent on allochthanous sources of production than larger island and mainland systems. Marine islands can indeed be very dependent on marine derived resources (Croll et al. 2005). Island systems, such as those found on Maine's coast, receive limited resource inputs, or subsidies, from the mainland production and often receive substantial subsidies, from marine sources. Marine-derived carbon and nitrogen have been found to contribute considerably to insular terrestrial communities (Polis and Hurd 1996; Barrett et al. 2005; Paetzold 2008; Spiller et al. 2010). Indeed, allochthonous input from the ocean can shape food-web interactions occurring in terrestrial environments. Nesting seabirds influence primary production and resource availability on marine islands (Anderson and Polis 1999; Mulder and Keall 2001; Ellis et al 2006; Caut et al. 2012) by supplying nutrients through colony death, feces, and residual food matter (e.g., guano and eggs shells) (Heatwole 1971; Sanchez-Piñero and Polis 2000; Ellis et al. 2006). This is often reflected in island plants having enriched delta N values from these avian deposits (Wainright et al. 1998; Ellis et al. 2006; Caut et al. 2012). This bottom level marine nutrient input can in turn propagate through the

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system to varying degrees depending on community structure and interactions of higher level organisms such as invertebrates (Fukami et al. 2006; Bassett et al. 2014) and vertebrates (Polis and Strong 1996; Pace et al. 1999; Schmitz et al. 2000; Halaj and Wise 2001).

Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are an informative tool to infer and quantify sources of primary productivity (Fogel et al. 1993; Hobson et al. 1995) and food web interactions (Peterson and Fry 1987; Layman et al. 2012), dynamics (Vander Zanden et al. 1999), and trophic position (Kelly 2000; Estrada et al. 2003). The low fractionation rate of $\delta^{13}C$ (<1‰) (Rounick and Winterbourn 1986; Peterson and Fry 1987; France and Peters 1997) enables identification of a species direct or indirect dependence on different sources of primary productivity, whereas, the estimated fractionation rate of $\delta^{15}N$ 3-4‰ (DeNiro and Epstein 1981; Minagwa and Wada 1984; Peterson and Fry 1987) allows for an estimation of trophic position (Post 2002). This predictable movement of carbon and nitrogen through ecosystems reveals a consumer's trophic dependences in the local environment which may in turn be potent drivers of local adaptation adaptive radiation.

Adaptive radiation results from habitat and resource differences promoting phenotypic differentiation among populations that in turn promote further reproductive isolation and speciation (Schulter 2000). Phenotype-environment correlations are prevalent (Kahilainen et al. 2007; Harrod et al. 2010), and in some cases system productivity itself is associated with patterns of population trait variation (Tuckett et al. 2014). Island habitats can affect trait variation in body size, body shape, and color (Lomolino 1985; King 1993; Garcia-Verdugo 2014). One obvious mechanism for this is the capacity for distinct island communities and ecosystems to affect diets and in turn the particular morphological and physiological character values that aid trophic efficiency (Lomolino 2005; Herrel et al. 2008). The vegetation and geology of islands

can also differ enough from mainland sites to influence physical aspects of habitat structure affecting locomotion and crypsis (Losos et al. 2000; King and Lawson 1995).

Maine's coast contains thousands of islands, ranging from non-forested islands less than 6 hectares (Western Brothers Island) to islands of several thousand hectares in old-growth forest (Isle au Haut). These islands were formed following the last major glaciation. After the ice sheets initially retreated and the regional land mass rose under isostatic rebound, today's islands were hills on large coastal plain until between 10,000 and 4000 YBP when sea levels rose dramatically and isolated them from the present-day mainland (Kelley et al. 1992; Barnhardt et al. 1995). Red-backed salamanders (*Plethodon cinereus*), a common forest species of eastern North America (Burton and Likens 1975), likely colonized Maine's islands during the initial low-sea water period or via later rare transport (e.g., rafting in wood or dropped by birds). Populations of these salamanders currently occupy a majority of Maine's islands that possess even limited areas of woody terrestrial vegetation (Maine Amphibian and Reptile Atlasing Project (MARAP): accessed 10/11/2016). These highly terrestrial salamanders are intolerant of long term exposure to fresh or saltwater (Wells 2010), and Maine's island populations are extensively isolated from their mainland conspecifics and one another (Chapter 1).

Red-backed salamanders are often characterized as being dependent on dense forest environments (Heatwole 1962; Herbeck and Larsen 1999; Jordan et al. 2009) and fallen woody debris that provides microhabitat and resources for their preferred prey (Jaeger 1980). Maine's islands expose red-backed salamanders to a much wider variety of habitats with potentially different food web structures and dependence on marine inputs. Although the species has a widely conserved phenotype (Wake 1963; Highton 1977), prior studies show its morphology can vary with local ecological conditions (e.g., character displacement) and resource use (Adams and

Rohlf 2000; Adams 2004; Maerz et al. 2006; Adams et al. 2007). Considering these factors, Maine's island salamanders represent an interesting opportunity to study phenotype-environment associations and the potential early stages of island-driven adaptive radiation.

In this study, I used natural abundances of C and N heavy isotopes to compare red-backed salamander food web relationships among islands and mainland sites and also compared red-backed salamander morphological measurements among populations to assess possible phenotype-environment associations. I hypothesized that C and N heavy isotope signatures of salamanders and other food web constituents on small islands would be increased relative to mainland populations owing to potentially greater dependence of islands, particularly those with little or no forest cover, on allochthonous marine productivity (Polis and Hurd 1996). I further predicted that differences in community and ecosystem structure among mainland and island sites would influence the relative trophic dependences of island and mainland salamanders, including their trophic position. Finally, I predicted that the differences in the diets, habitat structure, and competition of island and mainland salamanders has led to morphological divergence consistent with ecologically mediated adaptive divergence (Adams and Rohlf 2000; Claude et al. 2004; Harmon et al. 2005; Maerz et al. 2006).

METHODS

Study Sites

I surveyed for red-backed salamanders at 26 mainland and island sites in Maine, USA (Table 1.1; Figure 1.1) during 2012-2014. Islands sites were selected to span the entire Maine coast with some constraints imposed by accessibility by boat or plane. Most mainland sites occurred along the Maine coast in an overlapping distribution with corresponding island sites to

ensure broadly similar geology, climate and phylogenetic history (Table 1.1). Two mainland

populations, Aroostook State Park and Orono, were selected to account for potential habitat and

genetic variation further inland.

Table 2.1. Sites surveyed for red-backed salamanders (*Plethodon cinereus*) during 2012-2014
across the state of Maine. Approximate area for island sites, euclidean distance to Maine's
nearest coast (km), and the dominant vegetation type for both island (I) and mainland (M) sites
are presented. Samples were collected from 22 of the 26 sites visited.

Site	Abbreviation	Habitat Type	Area* (ha)	Distance to Coast (km)	Dominant Vegetation	Samples Collected
Appledore Island	APD	I	40	10	Coniferous	34
Aroostook State Park	ARS	M	10000	200	Coniferous	17
Baker Island	BAK	I	3	5	Coniferous	30
Bois Bubert Island	BBI	I	600	1	Coniferous	30
Birch Point State Park	BRP	M	200	<0.5	Deciduous	20
Cross Island	CRI	I	700	1	Coniferous	31
Crow Island	CRW	I	5	5	Coniferous	30
Great Duck Island	GDI	I	80	10	Coniferous	30
Isle au Haut	IHT	I	2800	5	Coniferous	0
Long Island	LNG	I	600	5	Coniferous	30
Mount Agamenticus	MAG	M	500	8	Deciduous	30
Matinicus Island	MAT	I	325	23	Coniferous	32
Metinic Island	MET	I	150	8	Coniferous	0
Monhegan Island	MON	I	250	17	Coniferous	30
Mount Desert Island	MDI	M	1000	4	Deciduous	19
Orono	ORO	M	200	50	Coniferous	20
Outer Green Island	OGI	I	2	8	Grass/ Shrub	0
Placentia Island	PLC	I	200	4	Coniferous	27
Petit Manan Island	PMI	I	4	4	Grass/ Shrub	37
Petit Manan Point	PMP	M	500	1	Grass/ Shrub	16
Reid State Park	RED	M	500	<0.5	Coniferous	33
Schoodic Education and Research	SCH	M	80	<0.5	Coniferous	23
Smuttynose Island	SMI	I	15	10	Grass/ Shrub	0
University of Maine at Machias	UMM	M	2000	5	Coniferous	22
Western Brothers Island	BRI	I	5	5	Grass/ Shrub	31
Wolfe's Neck Woods State Park	WLF	M	60	<0.5	Coniferous	29

*Mainland site area was estimated by the amount of continuous forest surrounding the sampling area. Estimates
were made using polygon measurements in Google Earth.

Salamander Capture

During 2012-2014, I collected salamanders from 22 of the 26 survey sites (Table 1.1) by lifting natural cover objects and sifting through leaf litter. Captured individuals were retained in a ventilated, shaded, moist, container for transport and processing. I did not encounter salamanders during the few hours of sampling afforded on three of the remaining sites. Although I found red-backed salamanders on Isle au Haut, I encountered too few (i.e., < 5) to proceed with further sampling.

Sedation and Image Capture

Captured salamanders were anesthetized with tricaine methanesulfonate (MS-222; 2g/l solution buffered with sodium bicarbonate). A subsample of salamanders from each site were lethally collected for isotopic analysis. Euthanized individuals were brought to the lab and immediately placed in a -20°C freezer for extended storage. Color pattern (primarily red-backed or lead-backed) was noted for each individual, as color forms can vary widely in frequencies and have been proposed to be a potential habitat adaptation. To determine sex, I employed the candling method outlined by Gillette and Peterson (2001) and inspected nasolabial grooves. Dorsal and ventral images were captured with a Canon PowerShot SX150 IS camera and a 6.35 mm background grid to provide a known scale.

Measurements

Field measurements using calipers were performed for total length (TL) and snout-vent length (SVL). Mass was taken using an Ohaus® digital scale. Digital linear measurements were performed on dorsal and ventral images using ImageJ, version 1.8.0_60 (http://imagej.nih.gov/ij/, Rasband 2016). I utilized the measurements defined by Good and Wake (1992), with some exclusions and modifications (Figure 2.1). *Ex situ* SVL measurements were avoided as image

quality left the vent indistinguishable for the majority of individuals. Hence, field measurements

of SVL were used with adjusted values as determined by the difference between *in situ* and *ex*

situ TL measurements.
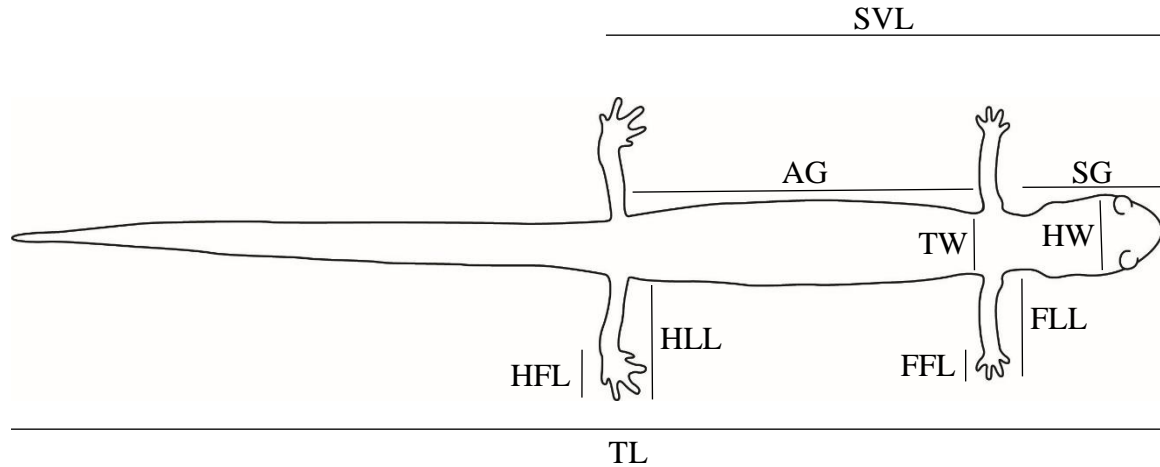
Figure 2.1. Linear measurements of body proportions used to assess morphological variation. Measurements include: total length (TL), snout-gular (head) length (SG), head width (HW), axilla-groin (trunk) length (AG), trunk width (TW), forelimb length (FLL), hind limb length (HLL), front foot length (FFL), and hind foot length (HFL).

Stable Isotope Preparation

Red-backed salamanders (*Plethodon cinereus*) from four of the mainland sites (ARS,

MAG, ORO, and RED) and six of the island sites (APD, BRI, CRI, GDI, MAT, and PMI) were

rinsed with deionized water and weighed using an Ohaus® Adventurer scale to 0.000g precision.

Individuals were pinned to a plate of plexi-glass and an incision was made ventrally from the

gular to the cloaca. Internal organs inferior to the heart and superior to the cloaca were removed

to reduce the likelihood of recent diet biasing the isotope signature as a result of recent feeding

activity (Post et al. 2007; Milanovich and Maerz 2012). Additionally, the tail of each individual

was removed, as approximately 66% of the lipids in a plethodontid salamander are stored in the

tail (Maiorana 1975). Isotopic baselines included both terrestrial and marine plant material and invertebrates collected at mainland and island sites. Plant material included members of Asteraceae, Rosaceae, Cyperaceae, and Poaceae. Terrestrial invertebrates included snails (Zontidae) and slugs (Limacidea). Invertebrates such as marine snails (*Littorina spp.*) and blue mussels (*Mytilus spp.*) were removed from their calcified shells and guts were retained.

Samples from salamanders and baselines were then weighed and placed in a drying oven at 60°C for > 60 hours. The dried tissue was then pulverized into a homogeneous powder via mortar and pestle, transferred into pre-weighed glass shell-vials, and stored in a desiccator. For a select number (7) of *P. cinereus* specimens, methanol chloroform lipid extraction was performed to assess potential bias, as lipids have been found to be depleted in C (Post 2002; Dalerum and Angerbjorn 2005; Post et al. 2007; Tarroux et al. 2010). Isotope samples were analyzed with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer by the University of California Davis Stable Isotope Facility.

Trophic Position

I estimated *Plethodon cinereus* trophic position as ($\lambda + (\delta^{15}N_{sc} - \delta^{15}N_{base})/\Delta_n$), assuming a mean tropic fractionation rate of $\delta^{15}N$ of 3.4‰ (Post 2002). In this model, λ is the trophic position of the organism used to estimate $\delta^{15}N_{base}$ ($\delta^{15}N$ the baseline organism), $\delta^{15}N_{sc}$ is the $\delta^{15}N$ of the consumer trophic level being estimated, in this case red-backed salamanders, and Δ_n is the enrichment of $\delta^{15}N$ per trophic level (3.4‰). Site specific primary producers (e.g., grassy and herbaceous plants; $\lambda=1$) and primary consumers (e.g., gastropods; $\lambda=2$) were used as isotopic baselines for each red-backed salamander. Trophic position was then calculated for salamanders at each site using plant and gastropod baselines. These two estimates from different baselines were then averaged for each individual.

Statistical Analyses

I used JMP statistical software version 10 (SAS Institute Inc., Cary, NC) to conduct a hierarchical analysis of variance (ANOVA), with red-backed salamander population as a factor nested within habitat type (island or mainland) for $\delta^{15}N$, $\delta^{13}C$, trophic position and salamander size traits (SVL, TL and Mass). Population and habitat type (island and mainland) differences of salamander morphological measurements were summarized and tested in an allometric framework, using univariate analyses of covariance between log10 trait values and log10 snout vent length (SVL). An initial model tested for allometric trait divergence among all the salamander populations assayed, irrespective of their particular island or mainland identity:

$$\text{log10Trait} = \text{log10SVL} + \text{Population (fixed)} + \text{log10SVL*Population}$$

When the interaction term was not significant in these models (>0.05), it was dropped to retest the independent effects of log10SVL and Population. In addition to this assessment of overall population divergence, I further assessed whether divergence was associated with island or mainland habitat types. For this allometric analysis I treated populations as a random term nested within habitat type using the following extended model:

$$\text{logTrait} = \text{log10SVL} + \text{Habitat Type (fixed)} + \text{Population [random nested within Habitat Type]} + \text{log10SVL* Habitat Type}$$

Again, when the interaction term was not statistically significant, it was dropped to test the independent log10SVL and Habitat Type effects. In this respect, my first allometric model

tests whether there is evidence of morphological divergence and adaptive radiation of salamander populations in general, and the extended model tests whether such a radiation is associated with a generalizable island versus mainland habitat dichotomy.

RESULTS

Trophic Position

Lipid-extracted samples differed in $\delta^{15}N$ and $\delta^{13}C$ values from unextracted samples by < 5%, therefore, I did not adjust natural abundance values for lipid content. Stable isotope signatures of red-backed salamanders, marine and terrestrial invertebrates, and primary producers from both mainland and island sites were separated in isotope space (Figure 2.2). Marine primary producers were enriched in both $\delta^{15}N$ and $\delta^{13}C$ relative to terrestrial primary producers, and consumers were enriched in both heavy isotopes relative to primary producers. Salamanders had the greatest mean $\delta^{15}N$ and $\delta^{13}C$ values in their respective terrestrial habitat type (island or mainland).

Comparing island and mainland salamanders, island populations were significantly enriched in $\delta^{15}N$ relative to mainland populations (mainland, 4.86 ± 0.226; island, 8.57 ± 0.465) whereas, mainland populations were significantly more enriched in $\delta^{13}C$ signatures (mainland, -23.75 $\pm$ 0.086; Island, -24.65 $\pm$ 0.149) (Table 2.2; Figure 2.3). There was also significant among site variation in isotopic enrichment of salamander tissues within the island and mainland groups (Figure 2.3). Across all sites, Petit Manan Island salamanders had the largest mean $\delta^{15}N$ signatures (12.47 ± 0.400); whereas, Reid State Park had the largest mean $\delta^{13}C$ signatures (-23.22 $\pm$ 0.176) (Figure 2.3).
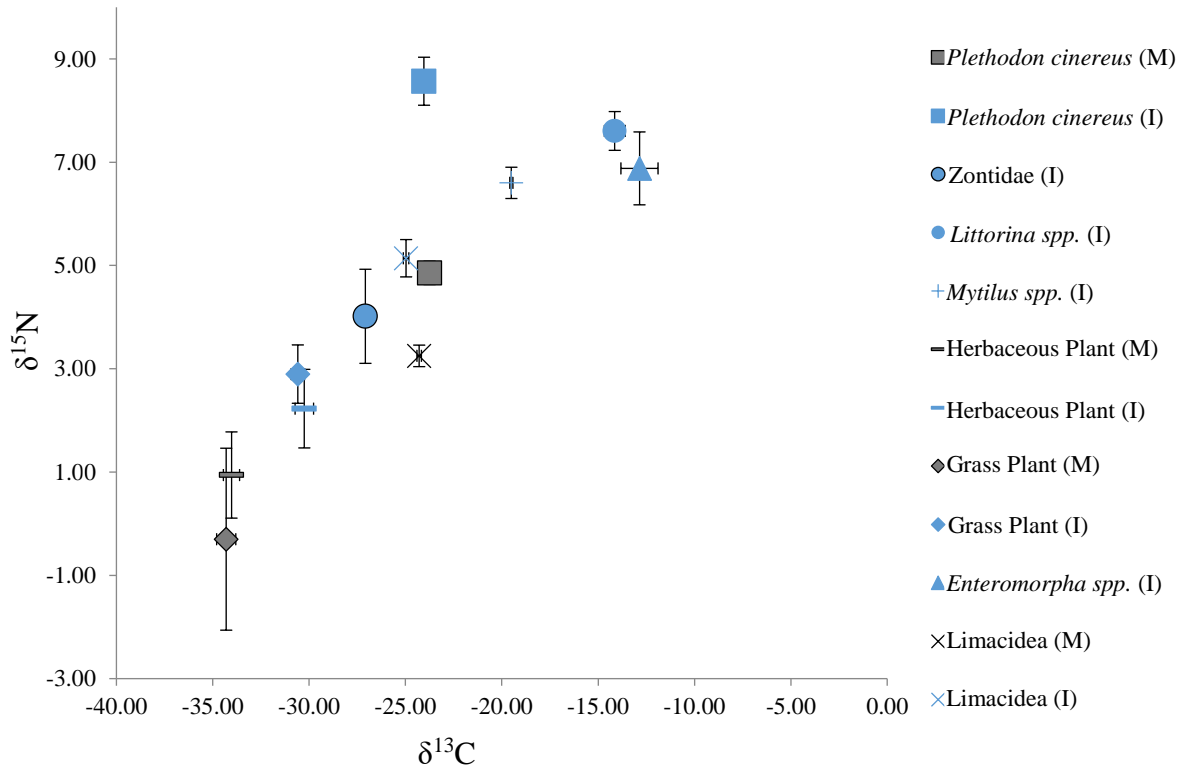
Figure 2.2. Natural $\delta^{15}N$ and $\delta^{13}C$ values (Mean± 1SE) of red-backed salamanders and baseline organisms. Includes: mainland (M, gray symbols) red-backed salamanders (*Plethodon cinereus*, n =31), island (I, blue symbols) red-backed salamanders (*Plethodon cinereus*, n =40), marine snails (*Littorina spp.*, n =12), island terrestrial snails (Zontidae, n =15), marine mussels (*Mytilus spp.*, n =3), mainland (n = 15) and island (n = 16) forbs (e.g., Asteraceae, Rosaceae), mainland (n = 7) and island (n = 13) grasses (e.g., Cyperaceae, Poaceae), *Enteromorpha spp.* (n = 12), and mainland (n = 16) and island (n = 7) terrestrial slugs (Limacidea) sampled during 2012-2014 in Maine from four mainland (ARS, MAG, ORO, and RED) and six island (APD, BRI, CRI, GDI, MAT, and PMI) sites.
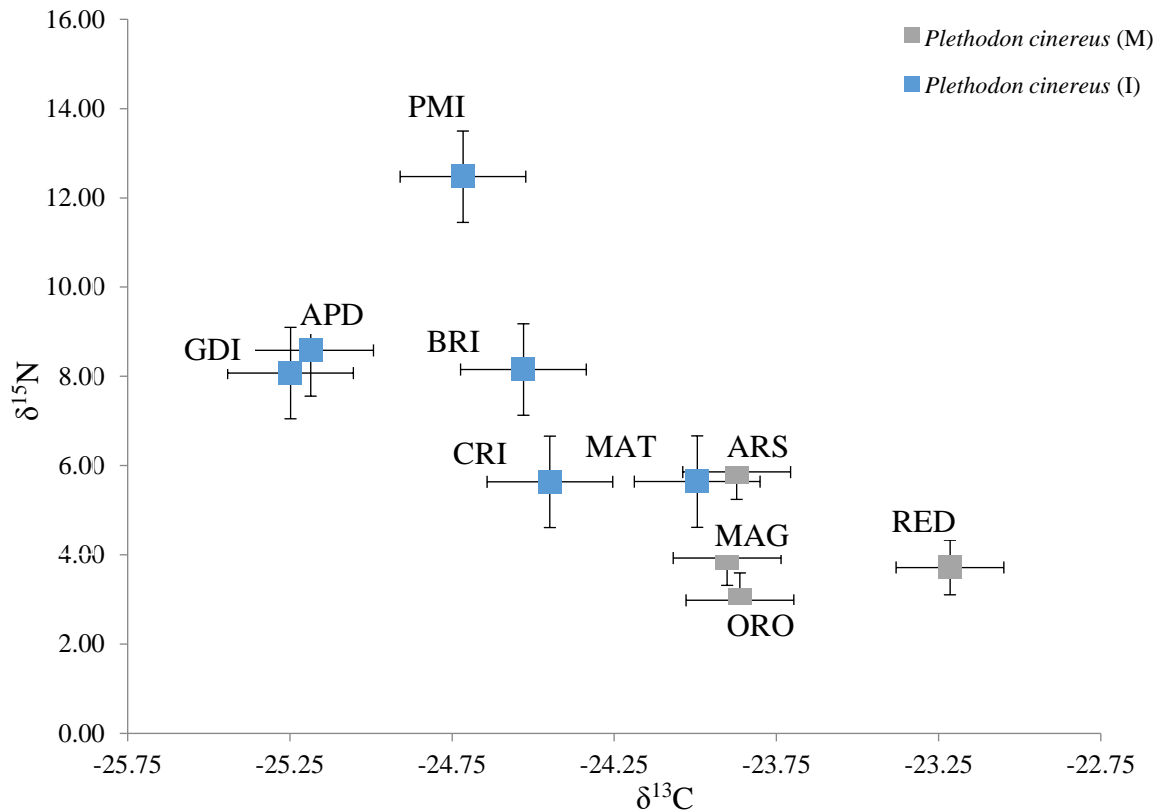
Figure 2.3. Population specific values of natural $\delta^{15}N$ and $\delta^{13}C$ values (Mean± 1SE) of mainland (M, grey symbols) red-backed salamanders (*Plethodon cinereus*, n =31) and island (I, blue symbols) red-backed salamanders (*Plethodon cinereus*, n =40) from Maine. Red-backed salamanders were sampled from four mainland sites (ARS n = 16, MAG n = 5, ORO n = 4, and RED n = 6) and six island sites (APD n = 5, BRI n = 6, CRI n = 6, GDI n = 5, MAT n = 7, and PMI n = 11) during 2012-2014.

Table 2.2. Effect tests for *Plethodon cinereus* $\delta^{15}N$ signatures, $\delta^{13}C$ signatures, and estimated trophic position for nested ANOVA. Populations are nested within their respective habitat type (island/mainland).

Isotopic Feature	Source	df	SS	F	P
$\delta^{15}N$	Island vs. Mainland	1	206.81	180.98	< 0.0001*
	Population within Habitat Type	8	314.72	34.46	< 0.0001*
$\delta^{13}C$	Island vs. Mainland	1	13.69	45.85	< 0.0001*
	Population within Habitat Type	8	8.72	3.65	0.0016*
Trophic Level	Island vs. Mainland	1	4.82	48.89	< 0.0001*
	Population within Habitat Type	8	22.11	27.99	< 0.0001*

Table 2.3. Mean trophic position for mainland and island red-backed salamanders calculated as trophic position $= \lambda + (\delta^{15}N_{sc} - \delta^{15}N_{base})/\Delta_n$, assuming enrichment of $\delta^{15}N$ 3.4‰ per trophic level (Post 2002). Bold text indicates small (<100 hectares) island sites with nesting seabird populations.

Site	Habitat Type	Trophic Position (±SD)
ARS	Mainland	2.19 ± 0.183
MAG	Mainland	1.93 ± 0.388
ORO	Mainland	1.55 ± 0.258
RED	Mainland	2.54 ± 0.242
APD	**Island**	**2.87 ± 0.140**
BRI	**Island**	**3.24 ± 0.205**
CRI	Island	1.83 ± 0.217
GDI	**Island**	**3.75 ± 0.665**
MAT	Island	2.28 ± 0.242
PMI	**Island**	**3.18 ± 0.372**

Estimated trophic position for island and mainland populations of red-backed salamanders ranged from 1.55 ± 0.258 to 3.75 ± 0.665 (Table 2.3), where a trophic position of 3.00 is predicted if salamanders are exactly two trophic levels above the primary producers. Mean trophic position for island sites (2.86 ± 0.111) was significantly greater than that for mainland sites (2.05 ± 0.062) (Table 2.2). The highest estimated trophic positions were for the four smallest islands with nesting seabird populations (Appledore Island, Western Brothers Island, Great Duck Island, and Petit Manan Island).

Phenotypic Variation

Morphological trait means, including body lengths, mass and linear traits (Table 2.4) varied extensively among the 22 populations. In addition to significant population-to-population variation in mean snout-to-vent lengths (SVL) and total lengths (TL), island and mainland populations were significantly different with island salamanders having 8% longer SVL (Table 2.4). Likewise, mean mass of island and mainland salamanders differed. After allometrically accounting for size (SVL; Tables 2.5, 2.6), mass differed significantly among populations overall. The heaviest salamanders for their length were found at Matinicus Island, and the lightest for their length at Petit Manan Point. However, further analysis by habitat types, showed

that island populations were not significantly heavier for their length than their mainland counterparts (Table 2.7).

Allometric analysis of the 10 linear trait measures relative to snout-vent-length (univariate ANCOVAs; Tables 2.5, 2.6), showed that the majority of individual variation in salamander trait sizes was associated with individual size, as the effects of SVL or SVL-by-population interactions were significant for all traits. Analyses considering all populations irrespective of their habitat types further showed evidence of divergence in the slopes or elevations of all the traits for varying subsets of populations (Table 2.6). However, this broad-scale allometric divergence among populations did not correspond with consistent differences between island and mainland habitats for the majority of morphological traits. The one exception to this was for snout-gular (SG) length, wherein island populations had significantly longer heads for a given SVL than their mainland counterparts (Table 2.5).

Seventy of 601 red-backed salamanders were lead-backed morph, possessing no dorsal stripe. Lead-backed morphs were present at 13 of the 22 sites with full samples of salamanders, without strong bias by habitat types (7 mainland, 6 island) (Table 2.8).

Table 2.4. Untransformed means of 10 morphological characteristics (mm) (Figure 2.1) and mass (mg) of red-backed salamanders (*Plethodon cinereus*) by capture site in Maine. Sites are displayed by abbreviation alphabetically (Table 2.1).

Site	TL	SVL	TW	SG	HW	AG	FLL	FDL	HLL	HDL	Mass (mg)
APD	61.3	35.4	3.3	7.6	4.8	19.8	6.5	1.7	7.6	2.4	496.5
ARS	59.6	36.2	3.6	8.0	5.0	20.2	6.4	1.5	7.5	2.2	671.8
BAK	84.9	48.4	4.3	9.8	6.1	27.2	7.7	2.0	9.2	2.8	1068.0
BBI	83.6	45.2	4.0	9.4	5.8	26.1	7.6	2.3	9.0	3.3	939.3
BRI	88.3	51.5	4.9	10.3	6.6	29.7	11.5	2.4	10.1	3.2	1464.0
BRP	69.1	39.0	3.7	8.3	5.1	22.0	6.6	1.9	7.9	2.5	649.5
CRI	88.9	46.8	4.1	9.5	5.8	27.4	7.6	2.2	9.1	3.0	1165.5
CRW	80.9	45.5	4.5	9.6	5.9	26.2	7.7	2.1	8.8	2.8	1123.3
GDI	77.1	43.6	4.1	9.3	5.8	24.6	7.7	1.9	9.1	2.6	918.3
LNG	70.1	39.5	3.7	8.7	5.3	22.1	7.0	1.8	8.1	2.4	676.0
MAG	80.6	43.2	4.2	9.0	5.6	24.6	7.3	2.0	8.6	2.6	921.7
MAT	80.8	46.2	4.4	9.7	6.0	26.3	7.9	2.1	9.5	2.8	1113.8
MDI	81.6	44.4	4.2	9.0	5.7	25.6	7.4	2.2	8.8	3.3	926.8
MON	78.5	42.6	3.9	9.1	5.5	24.4	7.2	1.8	8.6	2.6	848.3
ORO	74.4	42.9	3.8	8.8	5.6	24.6	7.2	1.9	8.7	2.5	806.5
PLC	81.4	44.2	4.0	9.4	5.8	24.8	7.6	1.9	8.9	2.6	966.2
PMI	100.3	52.7	5.2	10.4	6.5	31.6	8.1	2.6	9.4	3.5	1923.1
PMP	75.6	42.7	4.2	8.7	5.4	24.1	6.9	1.9	8.2	2.7	899.4
RED	69.4	40.5	3.6	8.5	6.5	22.9	7.0	1.7	8.3	2.5	677.0
SCH	80.1	40.9	3.9	8.6	5.3	24.2	6.6	2.0	8.0	2.9	813.9
UMM	76.1	43.3	3.9	9.0	5.4	24.5	7.4	2.0	8.7	2.7	738.6
WLF	74.8	42.8	3.9	9.0	5.7	24.3	7.5	1.9	9.0	2.7	825.9
Total	78.7	43.8	4.1	9.1	5.7	25.0	7.5	2.0	8.7	2.8	959.9

Table 2.5. Effect tests for *Plethodon cinereus* size traits (total length, snout vent length, and mass). Populations are nested within their respective habitat type (Island/Mainland).

Size Trait	Source	df	SS	*F*	*P*
TL	Island vs. Mainland	1	7065	39.43	< 0.0001*
	Population within Habitat Type	20	43092	12.02	< 0.0001*
SVL	Island vs. Mainland	1	1699	50.49	< 0.0001*
	Population within Habitat Type	20	9092	13.51	< 0.0001*
Mass	Island vs. Mainland	1	9549899	74.74	< 0.0001*
	Population within Habitat Type	20	52259421	20.45	< 0.0001*

Table 2.6. Parameter estimates for population comparisons across eight morphological characters (Figure 2.1). Output from univariate analysis of covariance (ANCOVA). Significant values ($P < 0.05$) are denoted with an asterisk. Red-backed salamanders (*Plethodon cinereus*) were captured during 2012-2014 in Maine from 22 sites. All tests were conducted on log10 transformed SVL and trait values.

Morphological Characteristic	Parameter	df	SS	*F* Ratio	*P*
TW					
	SVL	1	0.88031	730.49	<.0001 *
	Population	21	0.15524	6.1341	<.0001 *
	SVL*Population	21	0.02384	0.9422	0.5358
SG					
	SVL	1	0.8476	2364.35	<.0001 *
	Population	21	0.02366	3.1433	<.0001 *
	SVL*Population	21	0.01525	2.026	0.0046 *
HW					
	SVL	1	0.78031	1177.78	<.0001 *
	Population	21	0.03597	2.5856	0.0002 *
	SVL*Population	21	0.01379	0.9911	0.4725
AG					
	SVL	1	1.79252	3862.73	<.0001 *
	Population	21	0.0141	1.4465	0.0905
	SVL*Population	21	0.02363	2.4247	0.0004 *
FLL					
	SVL	1	0.59653	827.055	<.0001 *
	Population	21	0.07279	4.8057	<.0001 *
	SVL*Population	21	0.02152	1.4205	0.1015
FFL					
	SVL	1	0.74266	276.999	<.0001 *
	Population	21	0.31086	5.5212	<.0001 *
	SVL*Population	21	0.0496	0.8809	0.6167
HLL					
	SVL	1	0.61008	952.601	<.0001 *
	Population	21	0.06675	4.9628	<.0001 *
	SVL*Population	21	0.02105	1.5653	0.0524
HFL					
	SVL	1	0.69019	315.621	<.0001 *
	Population	21	0.39285	8.5547	<.0001 *
	SVL*Population	21	0.06134	1.3358	0.1451
Mass					
	SVL	1	10.5458	2195.78	<.0001 *
	Population	21	0.56177	5.5699	<.0001 *
	SVL*Population	21	0.27017	2.6788	<.0001 *

Table 2.7. Parameter estimates for island and mainland habitat type comparisons across eight morphological characters and overall body mass (Figure 2.1). Output from univariate analysis of covariance (ANCOVA) with SVL as the covariate. Significant values ($P < 0.05$) are denoted with an asterisk. All tests were conducted on log10 transformed SVL and trait values.

Morphological Characteristic	Parameter	SE	df	t Ratio	P
TW					
	Intercept	0.038451	580.4	-16.99	<.0001 *
	Habitat Type	0.00425	20.66	0.68	0.5062
	SVL	0.023432	588	32.82	<.0001 *
SG					
	Intercept	0.020601	465.9	-10.86	<.0001 *
	Habitat Type	0.001433	21.33	2.2	0.0393 *
	SVL	0.0126	474.2	57.24	<.0001 *
HW					
	Intercept	0.027203	413.4	-14.7	<.0001 *
	Habitat Type	0.001746	20.77	1.62	0.1214
	SVL	0.016643	420.2	42.23	<.0001 *
AG					
	Intercept	0.0235	447.7	-16.58	<.0001 *
	Habitat Type	0.001582	21.48	-0.17	0.8693
	SVL	0.014375	455.4	75.75	<.0001 *
FLL					
	Intercept	0.0295	544	-5.55	<.0001 *
	Habitat Type	0.00251	21	1.89	0.0723
	SVL	0.018022	554	34.85	<.0001 *
FFL					
	Intercept	0.057206	577.6	-15.66	<.0001 *
	Habitat Type	0.006104	20.04	0.94	0.3599
	SVL	0.034875	586	20.83	<.0001 *
HLL					
	Intercept	0.028023	560.3	-5.11	<.0001 *
	Habitat Type	0.00257	21.21	1.14	0.2673
	SVL	0.017109	569.9	38.6	<.0001 *
HFL					
	Intercept	0.052633	586.6	-14.43	<.0001 *
	Habitat Type	0.006927	20.04	0.04	0.9713
	SVL	0.031991	591	22.85	<.0001 *
Mass					
	Intercept	0.07892	576.1	-18.33	<.0001 *
	Habitat Type	0.008225	20.8	1.14	0.2681
	SVL	0.048125	584.6	55.58	<.0001 *

Table 2.8. Percentage of lead-backed salamanders collected at each field site. Six-hundred and one individuals were collected from 22 mainland and island sites.

Population	Habitat Type	Lead-backed (%)	Sample Size
Birch Point State Park	Mainland	5	20
Western Mount Desert Island	Mainland	21	19
Petit Manan Point	Mainland	13	16
Reid State Park	Mainland	55	33
Schoodic Education and Research Center	Mainland	43	23
University of Maine at Machias	Mainland	13	22
Wolfe Neck Wood's State Park	Mainland	3	29
Baker Island	Island	10	30
Bois Bubert Island	Island	33	30
Western Brothers Island	Island	23	31
Cross Island	Island	23	31
Great Duck Island	Island	3	30
Long Island	Island	10	30

DISCUSSION

Coastal island ecosystems in Maine vary in their isotopically inferred productivity baselines and trophic structure from one another and from mainland systems, suggesting potential differences in sources of primary productivity and food web structure. These differences have the potential to serve as the ecological basis for adaptive divergence of island salamanders from their mainland counterparts. Consistent with this possibility, *Plethodon cinereus* on Maine's coastal islands appear to feed at a higher mean trophic level than mainland salamanders, especially on smaller islands with seabird breeding colonies. Salamander populations varied widely in phenotypic size and morphological trait allometry, suggesting potentially widespread divergence that may be linked to local adaptations. Within this general population variation, island salamanders are significantly larger and have proportionally larger heads than their mainland counterparts, a pattern that potentially is adaptive given the higher mean trophic position of these populations. This combination of findings supports the operation of mechanisms generating at least limited phenotype-environment associations in Maine's red-

back salamanders, which may in turn reflect the incipient stages of an island-mainland adaptive radiation.

Island versus Mainland Ecology

Natural abundances of $\delta^{15}N$ were enriched on islands compared to the mainland. This was reflected in higher baselines in primary producers and higher values in consumers, including red-backed salamanders. This pattern was most prevalent on smaller islands that support seabird breeding colonies; whereas, larger islands with forest dominated habitat had nitrogen values overlapping with mainland population signatures. Interestingly, even accounting for the higher baselines of islands, salamanders on seabird islands had higher estimated trophic levels. In mainland sites, salamanders were effectively a single trophic level removed from primary producers. In contrast, populations on seabird islands averaged effectively one or more trophic levels higher than mainland populations. This suggests that island salamanders either consume other predators of herbivorous insects, such as ants and spiders, or are partly linked into an allochthanous food chain that has an inflated baseline from avian inputs. Regardless, this supports my hypothesis that islands systems in Maine have different community and ecosystem structure that reaches all of the way up to salamanders as secondary, or even tertiary or higher consumers. This is also consistent with previous studies where coastal subsidies and seabird input have led to reptiles operating at higher trophic levels (Barrett et al. 2005).

Analysis of carbon stable isotopes did not provide evidence for dependence of island communities on marine derived carbon with its higher 13C ratios. Indeed, quite the opposite, salamanders from island populations showed slightly depleted 13C signatures relative to mainland populations. This result is unlikely to result from the modest amount of trophic fractionation that sometimes occurs with 13C (Peterson and Fry 1987; Post 2002) as that would

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favor higher values on islands. These differences in δ^{13}C may relate to differences in plant communities supporting primary production, but this was not supported by differences in baseline signatures of the herbaceous and grassy plants sampled, as both were biased in the opposite direction (i.e., mainland samples with more depleted signatures). The pattern for salamanders, was however, analogous to that for island and mainland slugs, a likely prey item of red-backed salamanders (Maglia 1996). Ultimately, the span of salamander δ^{13}C values across island and mainland systems was very modest at only about 2‰, particularly compared to that between taxa and between terrestrial and marine baselines. Hence, while there were some differences between island and mainland sites in salamander 13C signatures, both systems appear to be predominately supported by terrestrial carbon production.

Linear Measurements and Morphometrics

All morphological characters, with the exception of trunk length, significantly differed among populations in allometric analysis (Table 2.6), suggesting that *P. cinereus* may show wide-spread local adaptation, although other contributions to phenotypic variation cannot be excluded (e.g. phenotypic plasticity). Discussion of all of the pairwise trait differences among populations in an adaptive context would require a large amount of speculation; however, two traits are illustrative of how such patterns might be adaptive. Three populations (APD, BRI, WLF) differed in head widths (HW), with Western Brothers Island and Wolfe Neck Wood's State Park having larger widths for their size. Head and gape width is a common trophic specialization, and future analyses of local diets might confirm such a connection. Limb and foot measurements (FLL, FFL, HLL, HFL) were highly variable among populations. Limb patterning in *Plethodon cinereus* is a function of original limb development rather than alternative patterning stimulated by regeneration (Dinsmore and Hanken 1986), and one might

presume that limb dimensions could represent adaptations to locomotion in habitats with different structural demands. For example, limb length varies in lizard hatchlings exposed to habitats with different woody habitat structure (Losos et al. 2000), and shorter limbs facilitate accelerated movement (Losos et al. 2000). Five populations (GDI, MAT, PMI, SCH, WLF) differed significantly in limb length (FLL, HLL), with Petit Manan Island and Schoodic Education and Research Center populations having smaller limbs relative to their size. During visits to Petit Manan Island (PMI), salamanders were found moving through grass in the early morning, a behavior that was not observed at any other site.

In addition to the observed wide range of overall population trait variation, I did find support for a generalized pattern of island versus mainland divergence in both mean body size (length) and in relative head length, or more specifically snout-gular length for a given SVL. Both of these traits are interesting in conforming to predictions for how populations should diverge with respect to trophic position and in representing repeated parallel patterns of trait divergence, given that geography and population genetics suggest islands from different regions of the Maine coast were likely independently colonized (Chapter 1). As noted, differences in head proportions, such as head length or width, are often associated with differences in diet. My isotopic analyses suggest that island populations may forage at a higher trophic level, and higher trophic levels tend to consist of individuals and species of larger average size (Layman et al. 2005; Arim et al. 2010). Larger mean size would also presumably aid in capture and consumption of larger prey. Moreover, repeated parallel trait divergence is unlikely to arise under random processes like drift and is thus commonly considered an indicator of adaptive divergence or plasticity (West-Eberhard 2005; Schluter 2009; Pfennig et al. 2010).

The fact that I found some potential morphological support for trophic divergence suggests grounds for more detailed future analyses. Tools such as landmark-based shape analyses have become increasingly powerful for assessing how morphological traits covary with abiotic and biotic variables (Rohlf and Marcus 1993; Adams 1999; Adams et al. 2004; Slice 2007). Unlike linear measurements, geometric morphometric methods are capable of accounting for the integrated geometry of an organism and additional dimensions of size (Adams et al. 2004). Some previous morphological studies of trophic divergence in red-backed salamanders have used lateral landmarks of the skull and jaw that require a stereomicroscope (Adams 2004; Maerz et al. 2006; Adams et al. 2007). High resolution lateral images were not unobtainable in this study owing to photographing salamanders in the field where they could be subsequently released. This was deemed a conservative measure in absence of knowledge of the abundance and resilience of island populations. Nonetheless, future detailed measurements of cranial and jaw characters could provide a more powerful assessment of the suggested influence of island size and community structure as drivers of trophic adaptation in red-backed salamanders. Likewise, functional feeding studies could be conducted in the lab to determine the relative capacity for island and mainland populations to capture and handle prey of different sizes and trophic levels.

Color Phase

P. cinereus has two color morphs (red-backed and lead-backed; Burger 1935; Highton 1959), and the frequency and proportion of these morphs vary geographically, such that certain populations are polymorphic while others feature only one morph (Highton 1975; Petranka 1998; Fisher-Reid et al. 2013). Beyond these genetically determined morphs, pigmentation also varies temporally with season and age (Highton 1959; Kraemer et al. 2012) and, more recently, other

rarer color phenotypes have been described (Moore and Ouellet 2014). Previously it was believed that lead-backed salamanders were more abundant in warmer regions (Lotter and Scott 1977; Gibbs and Karraker 2006), however, recent surveys indicate no correlation between phase abundance and climate (Moore and Ouellet 2015). Lead-backed individuals also have more circulating leukocytes, a common proxy for stress (Davis and Milanovich 2010) from threats such as predation, competition, or resource limitation. Indeed, lead-backed salamanders have higher incidences of tail autotomy when compared to red-backed individuals (Venesky and Anthony 2007).

A total of 11.6% of the red-backed salamanders captured in this study were the lead-backed morph, and they were collected at more than half of the study sites, sometimes at high frequencies (e.g., Reid at 55%), but also commonly at low frequencies of < 10%. The numbers of sites with these animals did not differ appreciably between mainland and island populations (7 vs 6). In truth, lead-backed forms may be present in many of my sampled populations at frequencies too low for me to consistently detect with my modest sample sizes. However, the smaller size and isolated nature of island populations is predicted to increase the risk of loss or fixation of this form due to drift. Interestingly, the most genetically isolated and divergent of my island study populations (APD, MAT, MON, GDI – Chapter 1), had no lead-backed morphs or the lowest detectable amounts (3%).

Insular Gigantism?

Ecological processes such as predation pressure (Foster 1964, Heaney 1978, Crowell 1986, Adler and Levins 1994) and resource availability (Foster 1964, Case 1978, Lawlor 1982, Lomolino 1985) may influence adaptive patterns of life history and size. On islands this can result in patterns of insular dwarfism or gigantism (Palkovaks 2003). The body size of island

herpetofauna often exceeds that of mainland herpetofauna (Soulé 1966, Rand et al. 1975), and consistent with that pattern, island salamander populations were 35% heavier than that of their mainland counterparts. I collected the largest red-backed salamanders from some of the smallest islands, Petit Manan Island and Western Brothers Island.

   Salamanders from these two small islands with seabird colonies had some of highest estimated trophic positions, suggesting different resource use by red-backed salamanders at these sites. Moreover, these same islands are non-forested habitats dominated by grasses and herbaceous species presenting potentially different opportunities and challenges foraging, locomotion and predation risks than typical mainland habitats. Anecdotally, I observed red-backed salamanders on Petite Manan actively moving through grass habitat during the day, an atypical behavior that suggests island populations face very different foraging opportunities and predation risks than their mainland counterparts. However, while it is interesting to speculate that Maine's island salamander radiation may entail a degree of insular gigantism, the genetic basis of this pattern would need to be tested with controlled rearing studies.

Management Implications

   P. cinereus is widespread and abundant, giving little cause for conservation focus on this common species as a whole, yet the isolation, distinct habitat and trait values of coastal island populations of this species merit an examination of potentially important intraspecific variation. Considering the protected status of the closely related *Plethodon shenandoah* (Highton 1967; Jaeger 1980; Carpenter et al. 2001; Sites Jr. et al. 2004), the rate of diversification that previously has occurred within the genus *Plethodon* (Wiens et al. 2006), as well as the high divergence values that I observed, Maine island populations of *P. cinereus* may harbor potentially unique contributions to the adaptive diversity of this species. Recognizing this diversity is the first

critical step to assessing its potential value and threats compared to other populations. Unfortunately, island populations have a poor record of persistence in the face of anthropogenic pressures, and since 1600 AD, seventy-five percent of all documented animal extinctions have taken place on islands (Groombridge 1992).

Beyond intraspecific diversity, there are other reasons for preserving island populations of the species. Gaston and Fuller (2007) stressed the importance of preserving common species that are fundamental components of the ecosystem, both from their source as a genetic resource as well as their buffering contribution to ecosystem stability. Red-backed salamanders play a key role in the food chain as they are generalist feeders hypothesized to be efficient at converting food into energy for nutrient cycling (Wyman 1998; Homyack et al. 2010). Although we do not have abundance estimates from islands, Red-backed salamanders are one of the most abundant vertebrates in northern forests and may be particularly important to the function of these ecosystems by merit of their sheer biomass. If island salamander abundances are anywhere near those of many mainland sites, red-backed salamanders may be especially important to island ecosystems where they might easily represent the dominant vertebrate biomass that actually forages on the islands themselves.

Although Maine's coastal islands have supported reproductive colonies of seabirds for centuries, the distribution and abundance of these colonies and their reproductive success have long been affected by hunting, industrial pollution, overfishing, and predation (Kadlec and Durly 1968). These fluctuations and declines have initiated a concerted effort by state and federal agencies to manage to ensure their continued presence. While seabird presence has been heavily monitored on these islands, the relationships between seabird colony presence and populations of other island biota, including amphibian populations, has largely been overlooked. The result of

changes in breeding colonies (either location, size, or year to year variation) could have

important and cascading effects on the rest of the island ecosystems. Establishing monitoring

and survey programs to examine linkages among island food web components, such as *P.*

cinereus, would be a positive step in managing their populations and their island ecosystems for

long-term persistence.

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