1-1-2008

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THE "WHITE-EYED" EASTERN TOWHEE: A MOLECULAR ASSESSMENT OF THE VALIDITY OF *PIPILO ERYTHROPHTHALMUS ALLENI*

by

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Bachelor of Science
College of Charleston, Charleston, South Carolina
2001

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science Degree in Biological Sciences
School of Life Sciences
College of Sciences

Graduate College
University of Nevada, Las Vegas
December 2008

UNLV
UNIVERSITY OF NEVADA, LAS VEGAS

Thesis Approval

The Graduate College
University of Nevada, Las Vegas

October 2, 20 08

The Thesis prepared by

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Entitled

The "White-eyed" Eastern Towhee: A Molecular Assessment of the Validity of Pipilo erythrophthalmus alleni

is approved in partial fulfillment of the requirements for the degree of

Master of Science in Biological Sciences

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ABSTRACT

The "White-eyed" Eastern Towhee: A Molecular Assessment of the Validity of *Pipilo erythrophthalmus alleni*

by

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Exploring the factors that influence the distribution and diversification of organisms is essential to the field of evolutionary biology. Molecular examination across a species' distribution may result in the discovery of differentiated populations that, when interpreted in the context of past events (e.g. climate change), may elucidate the causal mechanisms. Comparative phylogeographic studies have revealed both similar and disparate genetic patterns among co-distributed organisms, although similar geographic patterns may differ greatly in temporal scale and thus in the magnitude of genetic differentiation. Studies of diverse taxa occurring across the southeastern United States have revealed several common patterns of intraspecific divergence- defined mainly by river and mountain boundaries.

The Eastern Towhee (*Pipilo erythrophthalmus*) is a sparrow found in scrub,

thicket, and forest edge habitat across the eastern United States. Eastern towhee consists of three main phenotypic groups: red-eyed continental forms, "white-eyed" Florida peninsular forms (referred to in the text as yellow-eyed), and a putative hybrid form with variable eye-color. I sequenced the complete ND2 mitochondrial DNA gene to determine whether these morphologically separable groups are also genetically distinct. Reciprocal monophyly is lacking between red and yellow-eyed birds. However, several statistically significant analyses support genetic differentiation of yellow-eyed populations. This divergence between red-eyed and yellow-eyed populations is estimated to have originated late in the Pleistocene epoch. Very low estimates of migration are consistent with a scenario of incomplete lineage sorting rather than ongoing gene flow. Overall, these results reveal a common finding of intraspecific genetic divergence in a southeastern U.S. taxon. Such differentiation is rare however within avian taxa. In addition, this study supports an uncommon geographic pattern of divergence, that is, divergence between peninsular Florida and mainland populations.

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CHAPTER 1

INTRODUCTION AND SIGNIFICANCE

The analysis of highly variable molecular markers, particularly mitochondrial DNA (mtDNA), provides researchers the ability to detect genetic structure among closely related individuals and populations (Avise et al., 1987, Avise, 2000). The processes that have impacted the evolutionary history of the intraspecific lineages can often be inferred when these genetic data are examined with respect to geological, paleontological, and paleoclimatic studies. Fueled by advances in molecular techniques, the rapid growth of this field of intra-specific (and sometimes interspecific) biogeography, termed "phylogeography" by Avise *et al.* (1987), has led to the generation of hundreds of independent studies across a wide array of taxa. Regional phylogeographic studies of unrelated and co-distributed taxa provide an opportunity to detect congruent patterns of genetic structure. Similar patterns of genetic structure have been observed across groups of taxa, reflecting a common geologic and evolutionary history in several regions of North America, including the Northwest U.S. (Carstens et al., 2005), Baja California peninsula (Riddle et al., 2000), and the Southeast U.S. (Avise, 1992, Soltis et al., 2006). These modern studies of "comparative phylogeography" are similar in spirit to earlier biogeographic studies that constructed area cladograms of co-distributed species groups to elucidate patterns of endemism, dispersal, and vicariance (Platnick and Nelson, 1978, Cracraft, 1986).

Eastern U.S. Phylogeographic Studies

The pioneer North American phylogeographic study was performed prior to direct DNA sequencing methods, using restriction fragment length polymorphisms (RFLPs) of mtDNA. This investigation revealed two distinct lineages of the southeastern pocket gopher (*Geomys pinetus*), split across northern Florida (Avise et al., 1979). Since then, taxa of the eastern United States have remained a popular focus of molecular genetic studies, providing an ever-increasing amount of data for phylogeographic comparisons (Avise, 1992, Avise, 1996, Soltis et al., 2006).

In a recent review of eastern U.S. molecular studies, Soltis *et al.* (2006) demonstrated that several plant species share phylogeographic patterns also present in animals, and summarized those that were most common. Six different patterns of genetic discontinuities were recognized which vary in the location of the genetic break. The patterns were named according to landscape features present at or near the genetic split (Fig. 7), 1) Atlantic vs. Gulf coast, 2) the Apalachicola River (Central Florida Panhandle), 3) the Tombigbee River (Coastal Alabama), 4) the Appalachian Mountains, 5) the Mississippi River, and 6) both the Mississippi and Apalachicola Rivers (Soltis *et al.* 2006).

Genetic Structure in Birds

Although birds are well represented in the total number of eastern North American studies, the majority (15 of 19) of these avian studies do not reveal any major genetic breaks (Soltis et al., 2006, Buerkle, 1999, Mock, 2002, Mc Cracken, 2001). Only two species display pronounced phylogeographic structure along with reciprocal

monophyly, one of which is the Carolina Chickadee (*Parus carolinensis*) which exhibits an east/west split oriented across Alabama's Tombigbee River (Gill et al., 1993). The second species, the coastally distributed Seaside Sparrow (*Ammodramus maritimus*), consists of Atlantic and Gulf of Mexico lineages that reflect a small disjunction in the distribution (Avise and Nelson, 1989).

Less defined genetic structure is evident in some other eastern North America avian taxa. Allopatric populations of endangered Florida Grasshopper Sparrow (*Ammodramus savannarum floridanus*) (Bulgin et al., 2003) and Prairie Warbler (*Dendroica discolor*) (Buerkle, 1999), lack monophyly but display significant population level differentiation. Within the Mottled Duck (*Anas fulvigula*), a maritime species, divergent but non-monophyletic mtDNA haplotypes are also found between allopatric populations (Florida and coastal Texas/Louisiana), a pattern that may have resulted from secondary contact (Mc Cracken et al., 2001). An intraspecific study of Wild Turkey (*Meleagris gallopavo*) is unique in uncovering differentiation, based on Φ_{ST} values, in a continuously distributed species (Mock et al., 2002). However, although the Florida subspecies was significantly differentiated from the eastern subspecies, the pairwise Φ_{ST} value was not significant after a Bonferroni correction.

In summary, although several avian species display population genetic structure among allopatric populations, available evidence indicates that reciprocal monophyly among non-allopatric populations has been detected in only one eastern bird, the Carolina Chickadee. Thus, intraspecific structure appears rare for birds in the eastern U.S. and particularly so within continuously distributed species.

Here I use phylogenetic and coalescent analyses to assess the geographic distribution of genetic variation within the Eastern Towhee (*Pipilo erythrophthalmus)* and interpret it in light of an existing eastern U.S. phylogeographical framework. I also assess levels of gene flow and the genetic relationships among the four described subspecies. These analyses allow the estimation of the evolutionary history of the species. In addition, I am able to comment on the processes that likely shaped this evolutionary history and how they resemble and differ from those processes influencing the structure of co-distributed species.

CHAPTER 2

THE "WHITE-EYED" EASTERN TOWHEE: A MOLECULAR ASSESSMENT OF *PIPILO ERYTHROPHTHALMUS ALLENI*

Introduction

The Eastern Towhee, *Pipilo erythrophthalmus*, is a sparrow in the New World family Emberizidae. Unlike most other northern emberizids, towhees are boldly patterned, relatively large, and have long tails. This species prefers areas of secondary to mid-successional growth, dense leaf litter, and forest edges (Greenlaw, 1996). The year-round range of the Eastern Towhee (Figure 1) encompasses the eastern U.S., stretching northward to southern Canada, and westward across the Mississippi River to the Great Plains where they hybridize with Spotted Towhee (*Pipilo maculatus*) (Greenlaw, 1996). In a study of approximately 2,300 museum specimens, Dickinson (1952) found diagnosable differences in several morphological characters among groups of populations. Adult iris color is essentially a discrete character, being red in the northern part of the range and yellow in and around the Florida peninsula. Eye color varies in the relatively narrow hybrid zone between yellow and red-eyed populations centered on the Florida/Georgia border. Clinal variation was demonstrated in other characters, the most significant of which is a north to south decrease in wing length and tail spot size (Dickinson, 1952).

Based on Dickinson's descriptions, four subspecies (Figure 1) of Eastern Towhee are currently recognized (American Ornithologists' Union, 1957). The subspecies *Pipilo erythrophthalmus erythrophthalmus* has a red iris, a relatively short bill, relatively large wing length, and large white tail spot area. It contains the most migratory populations. Individuals of the similar *P. e. canaster* usually have a red iris, but differ from *P. e. erythrophthalmus* by having a relatively large bill and a variable iris color near the southeastern boundary of its range (Greenlaw, 1996). The non-migratory Florida peninsula subspecies, *P. e. alleni*, has a pale to deep yellow iris, relatively short wing length, and the smallest and fewest tail feather spots (Dickinson, 1952). The transition across the boundary of these so-called "white-eyed" towhees marks discontinuous variation in iris color and tail spot area. The fourth subspecies, *P. e. rileyi*, is morphologically intermediate between *P. e. canaster* and *P. e. alleni*, and has been considered a putative hybrid between these red and yellow-eyed birds. Populations containing orange-eyed birds and individuals having a yellow iris with a small red ring (hereafter referred to as mixed) occur in the relatively narrow area (Figure 1) comprising *P. e. rileyi's* distribution.

Although moderate vocal variation exists within the Eastern Towhee, yellow-eyed *P. e. alleni* birds produce the most distinct song compared to *P. e. erythrophthalmus* (Greenlaw, 1996). This Florida subspecies also has a larger repertoire and higher levels of song sharing, compared to northern migratory populations, which may result from more time shared with neighboring males (Ewert and Kroodsma, 1994). These behavioral and morphological differences could be the result of isolation of red-eyed and yellow-

eyed populations and warrant an assessment for genetic differentiation within Eastern Towhee.

Objectives

The main objective of this study was to assess genetic structure within the Eastern Towhee (P. erythrophthalmus) in the context of known biogeographic patterns in the eastern U.S. I explored three alternative hypotheses to explain the observed pattern of phenotypic differentiation of eye and plumage color with respect to genetic differentiation.

1) **Post-Pleistocene Differentiation** - Recent expansion of populations from a single Pleistocene distribution or refugium and rapid post-Pleistocene (Holocene) differentiation of eye and plumage color. Red and yellow-eyed populations will have evolved from fairly unstructured Pleistocene populations. Such an expansion from a single ancestral range area would result in low genetic structure outside of refugial areas.
2) **Historical Isolation** - Pleistocene or older differentiation of populations due to isolation in two historical ancestral areas or refugia, most likely Florida and along the Gulf Coast, with subsequent secondary contact. The past isolation and morphological differentiation would result in geographically structured genetic divergence that is concordant with phenotypic divergence and retained despite post-Pleistocene secondary contact.
3) **Multiple Isolation Events** - Multiple events of isolation, divergence and secondary contact have occurred with different subsets of populations, perhaps involving multiple refugia. In this case, some but not all events contributing to the

genetic differentiation will be concordant in time or geographic location with the events that contribute to phenotypic divergence.

Phenotypic divergence could be due to ecological or sexual selection or drift under the hypotheses of isolation (Historical Isolation and Multiple Isolation Events) but would require strong divergent natural or sexual selection under a hypothesis of Post-Pleistocene Differentiation from a single ancestral population. In all scenarios more northern populations will be expected to have less genetic structure due to Post-Pleistocene expansion and due to their tendency for migratory behavior. In addition, all scenarios have the possibility of secondary contact and introgression possibly creating a cline of phenotypes and haplotypes.

Materials and Methods

Sampling

Tissue samples of the four subspecies of Eastern Towhees were obtained during the breeding seasons of 2004 and 2005 and supplemented by museum tissue loans. In all, 119 individuals were sampled from across much of the breeding range. Sampling efforts were focused in the southeastern U.S., especially across northern Florida, where several subspecies ranges converge (Figure 1). Samples of approximately ten individuals per location were obtained wherever possible for population genetic analyses. Birds collected were prepared as voucher specimens. Study skins and tissue specimens from these birds were deposited in the Marjorie Barrick Museum of Natural History (MBM) on the campus of the University of Nevada, Las Vegas. Frozen tissue samples are also housed in MBM. I obtained additional samples from several institutions: Field Museum of Natural

History, Chicago (Illinois, n=10, Florida, n=3, Minnesota, n=3), United States National Museum, Washington, D.C. (Virginia, n=10, Florida, n=4), and the American Museum of Natural History, New York (New York, n=3, Connecticut, n=2, Rhode Island, n=1). I also included in the data set two previously collected MBMNH samples from Pennsylvania and Minnesota.

Laboratory Methods

Total genomic DNA was extracted from tissue samples using the DNeasy tissue extraction kit, following the manufacturer's instructions (Qiagen, Valencia, California). All 1,041 base pairs of the protein-coding mtDNA gene dehydrogenase 2 (ND2) were amplified via the polymerase chain reaction (PCR), and sequenced using the following primers: L5215 (5'-TATCGGGCCCATACCCCGAATAT-3' (Hackett, 1996) and either H6313 (5'-CTCTTATTTAAGGCTTTGAAGGC-3' (Johnson and Sorenson, 1998) or HTrpC (5'-CGGACTTTAGCAGAAACTAAGAG-3') (Smithsonian Tropical Research Institute). All fragments were amplified in 12.5 uL reactions under the following conditions: denaturation at 94 °C, followed by 40 cycles at 94 °C for 30 seconds, 54 °C for 45 seconds, and extension at 72 °C for 1 minute. This was followed by a 10-minute final extension at 72 °C and a 4 °C soak. Products were purified with Exosap-IT (USB Corporation, Cleveland, Ohio) purification following the manufacturer's directions. I performed 20 uL BigDye (Applied Biosystems, Foster City, California) DNA sequencing reactions using 20 ng of purified and concentrated PCR product following the manufacturer's protocol. Sequencing reactions were then purified using a magnetic bead clean-up procedure (Agencourt Biosciences, Beverly, Massachusetts) and run on an ABI 3100-*Avant* automated sequencer (Applied Biosystems, Foster City, California).

Sequences were aligned, verified by eye, and checked for the absence of internal stop codons using the program SEQUENCHER 4.2 (Gene Codes Corporation, Ann Arbor, Michigan).

Phylogenetic Analyses

I used PAUP 3.1 (Swofford, 1991) to construct a phylogenetic tree using all samples (n = 119). Only those clades with bootstrap percentages of 70% or above are considered significantly supported. I used Modeltest 2.2 to select the best-fit model of nucleotide substitution for the data (Posada, 2001). Modeltest identified GTR + I as the appropriate model. I also used the program Network 3.1.1 (Bandelt et al., 1999) to construct a median joining haplotype network. Standard and molecular diversity indices were both calculated using DnaSP 4.10.9 (Rozas et al., 2003).

Population Structure

To analyze the overall pattern of genetic structure, I performed an analysis of molecular variance (AMOVA) (Excoffier et al., 1992), as implemented in the program ARLEQUIN (Schneider et al., 2000). AMOVA uses the frequencies and pairwise differences of haplotypes to estimate and test the significance of molecular variance that is partitioned among certain components of genetic structure. These variance components are called Φ-statistics, and are analogous to conventional F-statistics (Excoffier et al., 1992). I performed several non-hierarchical AMOVA analyses using both population samples and iris phenotypes as "populations". These runs generated the Φ_{ST} statistic, based on the correlation of haplotypes within a population to haplotypes sampled from the total, i.e. the proportion of the total genetic variance that is due to differences among populations (Excoffier et al., 1992). To further examine genetic structure, I used

ARLEQUIN (Schneider et al., 2000) to generate and test the significance of pairwise Φ_{ST} values for all pairs of populations. Rather than subjectively defining groups of populations (e.g. subspecies) to perform a hierarchical AMOVA, I performed a spatial analysis of molecular variance to genetically define groups of populations using the program SAMOVA (Dupanloup et al., 2002). Similar to a standard AMOVA, SAMOVA uses the geographical coordinates of sampled populations and assigns them to groups based on genetic similarity. SAMOVA does this by maximizing the Φ_{CT} statistic, which is the correlation of haplotypes from within a group of populations to haplotypes sampled from the total, i.e. proportion of the total genetic variance explained by differences among groups of populations. The user specifies the number of groups that populations are sorted into (typically performed with a range, e.g. 2-5). Although SAMOVA tends to group geographically adjacent populations together, it is not confined to doing so (Dupanloup et al., 2002). SAMOVA also plots a line representing an inferred barrier to gene flow. To further analyze the geographical distribution of genetic variation I constructed a haplotype map using ArcView GIS 3.2 (Environmental Systems Research Institute Inc., Redlands, California).

To investigate the inferred history of population size, stability, and genetic isolation I used ARLEQUIN 2.0 (Schneider et al., 2000) to calculate mismatch distributions (Slatkin and Hudson, 1991, Rogers and Harpending, 1992), Fu's F_s (Fu, 1997), and Tajima's D (Tajima, 1989a, Tajima, 1989b). The significance of F_s and D values, which are used to test for selective neutrality of the genetic samples, were estimated through the use of 1,000 simulation replicates. To determine whether the data fit a pattern of isolation by distance, I used ARLEQUIN to calculate the Mantel

correlation coefficient (Mantel, 1967) between matrices of both geographic and genetic distance, based on pairwise Φ_{ST} values. I assessed the significance of the Mantel correlation using 1,000 simulation replicates.

Coalescent Analyses- Isolation-With-Migration

Because shallow genetic structure can be attributed to either recent divergence or ongoing gene flow, I used the Bayesian and coalescent theory based IM (Isolation with Migration) program (Nielsen and Wakeley, 2001, Hey and Nielsen, 2004) to estimate migration rates, divergence time, and effective population size. The IM program requires several basic assumptions, including selective neutrality, no recombination within loci, and that the compared samples have a 'sister' relationship. The IM program uses MCMC methods to simultaneously estimate the marginal posterior probability densities of six model parameters related to the splitting of an ancestral population into two daughter populations (Hey and Nielsen, 2004, Nielsen and Wakeley, 2001). These parameters are: 1) effective population size of the ancestral population, 2-3) effective population sizes of the two daughter populations, 4) time since divergence of the two populations, 5-6) migration rates of each population into the other.

Specifically, I used the IM program to estimate: θ_{red} (effective population size of female red-eyed eastern towhees), θ_{yellow} (effective population size of yellow-eyed females), θ_A (effective population size of ancestral females prior to population divergence), m_1 (effective number of female migrants from yellow-eyed to red-eyed populations), m_2 (effective number of female migrants from red-eyed to yellow-eyed populations), and t (time of divergence of red-eyed and yellow-eyed populations). The aforementioned six parameters, which are scaled to the neutral mutation rate, were

converted to estimates of actual demographic parameters using a mutation rate of 2.95% / Myr (see Mutation Rate Calibration section) and the formulas from the IM manual.

I estimated the population migration rate of females (M) using the formula $M = \theta m/2$ where θ is the effective number of gene copies and m is the migration rate per gene copy (Hey and Nielsen, 2004, Nielsen and Wakeley, 2001). The coalescent calculations also required an estimate of the mutation rate per generation. To correct generation time for survival rate I used the equation $T = \alpha + [s/(1-s)]$, where α is the age at maturity and s is the annual adult survival rate (Lande et al., 2003). Using a survivorship rate of 58% (Greenlaw, 1996) we find the corrected generation time to be $T = 1 + [0.58/(0.42)]$, or 2.38 years.

Mutation Rate Calibration

Although the use of molecular clocks is problematic due to many factors and assumptions (e.g. calibration points, variation across genes and taxa), they are necessary to estimate divergence times that can then be correlated with vicariant and climatic events. Potassium-Argon (K-Ar) dating of the Hawaiian Islands have been used in conjunction with ctyochrome-b (cyt-b) data to estimate an uncorrected sequence divergence of 1.9%/ Myr or 0.0095 substitutions/per site/per Myr for Hawaiian Drepanidines (Fleischer et al., 1998). To convert this rate for use with the relatively more quickly evolving ND2 gene I used the program MEGA 3.1 (Kumar et al., 2004) to compute mean pairwise differences of 0.060 and 0.093 for cyt-b and ND2, respectively, using complete cyt-b and ND2 sequence data from six individuals of *Pipilo maculatus* (Batten, unpublished data). This suggests that ND2 evolves at a rate ~1.55 times that of cyt-b (0.093/0.060=1.55), and that it evolves at a rate of approximately 2.95% / Myr.

Potential problems arise, however, when using a mutation rate calibrated from interspecific data for an intraspecific study. Mutation rates calculated with recent calibration points (e.g. radiocarbon dating) tend to be higher than those estimated with relatively older points (e.g. fossil dating) (Ho et. al., 2005). This may be due to the sampling of mildly deleterious alleles, present in recently separated groups but absent in deeply divergent systems (Ho et. al., 2005). It is possible then, that the actual mutation rate of *P. erythrophthalmus* may be much higher, resulting in an overestimation of divergence times.

Results

Sequence Diversity

In the sample of 119 individuals of Eastern Towhee I found 24 unique mtDNA haplotypes, which differed on average by 1.23 base pairs. Within the 1,041 bp of the ND2 mtDNA gene, I identified 21 polymorphic sites consisting of nine phylogenetic informative and 12 singleton sites. There were 22 total mutations consisting of 16 synonymous and six replacement substitutions. No insertions or deletions were present. The total G+C content was 0.46 and the overall haplotype diversity was 0.76.

Phylogenetic Analyses

An unresolved phylogeny was generated by PAUP (Figure 2). The haplotypes representing the different iris phenotypes (which largely reflect subspecies boundaries) did not segregate into monophyletic lineages. In the haplotype network (Figure 3) there are two common and widespread haplotypes that include birds with both iris colors. There are 3 yellow-eyed and 45 red-eyed individuals with one haplotype and 15 yellow-

eyed and 17 red-eyed individuals of the second haplotype. Two additional haplotypes were shared by six individuals each, one haplotype by three individuals, and four haplotypes were shared by two individuals each. The remaining 15 haplotypes were unique to single individuals, referred to as private alleles (Slatkin, 1985). Four of these unique haplotypes were from the peninsular population (*P. e. alleni*) and an additional four were found in the putative hybrid zone (*P. e. rileyi*).

The haplotype distribution map (Figure 4) supported the results of the haplotype network and showed two widespread haplotypes, represented by large pie slices of either dark or light gray. The 'dark gray' haplotype is common and the 'light gray' haplotype rare in the yellow-eyed populations. Small uniquely shaded slices representing private haplotypes occur in most populations.

Population Structure

While most of the genetic variation was found within populations (78.8%, Table 2), the AMOVA showed that a significant portion of the total genetic variance is due to differences among populations (Φ_{ST}= 0.21, p <0.0001). A slightly higher proportion of total genetic variation is explained when grouping birds by iris phenotypes (Φ_{ST} = 0.248). To investigate the contribution of the yellow-eyed subspecies (*P. e. alleni*) to the Φ_{ST} statistic, two additional non-hierarchical AMOVAs were also performed, the first removing the *P. e. alleni* population and the second the nearby nFL and GA populations of *P. e. rileyi*. Removing these populations had the effect of decreasing the Φ_{ST} value to 0.134 and 0.053, respectively.

The SAMOVA results (Table 3) indicate that the maximum Φ_{CT} for two groups (k=2) is 0.253 and corresponds to differences between the three southeastern most

populations (pFL, nFL, and GA) and all other populations (Φ_{CT} = 0.25, p= 0.003). The results for k=3 were similar, except that the Northern Florida population (nFL) was separated from the other yellow-eyed populations and constituted its own group (Φ_{CT} = 0.27, p= 0.001). Setting higher values of k tended to further divide the southeastern group. An increase in K is expected to increase Φ_{CT} because of the corresponding decrease in Φ_{SC} (the percent of total variation due to differences among populations within each group), which drops as the number of populations in each group becomes fewer. The variance proportion Φ_{CT} thus increases due to the relationship $(1-\Phi_{ST}) = (1-\Phi_{SC})(1-\Phi_{CT})$ (Dupanloup et al., 2002).

Partitioning the molecular variation in pairwise comparisons of populations into within-population and total-variance components yielded pairwise Φ_{ST} values (Table 4). All Φ_{ST} values statistically significant after a false discovery rate (FDR) correction are those of comparisons of red-eyed populations with other populations that contained 'pure' yellow-eyed individuals the Florida peninsular population (pFL). The highest Φ_{ST} values of 0.58 and 0.50 are observed in comparisons with the populations of Louisiana and Mississippi, respectively. All other significant Φ_{ST} values are for comparisons involving the Florida panhandle (nFL) and Georgia (GA), the two (i.e. individuals with an iris phenotype identical to that of peninsular birds).

Table 1 shows no difference in haplotype or nucleotide diversity among the populations. All populations, with the exception of Illinois, West Virginia, Louisiana and Mississippi, contain at least one private haplotype, although Louisiana and Mississippi contain exactly one private haplotype shared between them. Mismatch distributions for all samples (Figure 5) as well as those performed for individual populations (not shown)

indicated that the observed distributions of pairwise differences were all unimodal and not significantly different from the expected model of exponential growth. Tajima's *D* values were not significant (Table 1), although significant Fu's F*s* values were obtained for three populations.

Coalescent Analyses- Isolation-With-Migration

Coalescent analyses (Table 5) suggested that red-eyed and yellow-eyed eastern towhees diverged approximately 18,644 ybp (t = 0.275) (range = 11,864 ybp - 598,305 ybp). The effective number (N_e) of yellow-eyed females was estimated at 50,647 individuals (θ_{yellow} = 7.0906) (range = 24,222 - 508,675 females). The effective number (N_e) of red-eyed females was estimated to be 374,350 individuals (θ_{red} = 52.4090) (range = 154,144 - 4,117,852). Ancestral effective population size (N_e) of 15,414 was calculated (θ_A = 2.1580) (range = 5,137 - 1,254,440 females). Migration estimates were calculated as the population migration rate (θ **x** *m*/2). The number of female migrants into the yellow-eyed range ($2N_1m_1$) was estimated at 0.062 per generation (m_1= 0.0175) (range = 0.047 –147.15). Finally, the estimated number of female migrants into the red-eyed range ($2N_2m_2$) was 0.197 individuals per generation (m_2= 0.0075) (range = 0.189 – 1303.61).

Discussion

Phylogenetic Analyses

Because genetic differentiation was limited, with six base pairs being the maximum difference between individual haplotypes of Eastern Towhee, PAUP generated an unresolved phylogeny (Figure 2). Although some clades were present in the

phylogenetic analysis, none of the clades were significantly supported (i.e. with bootstrap values > 70%), with the exception of the pairing of two haplotypes of yellow-eyed birds from the northern Florida (nFL) locality. The results of the phylogenetic tree refute my second hypothesis, Historical Isolation. The poor resolution and lack of monophyletic clades in the phylogeny of mtDNA haplotypes (Figure 2) are not consistent with long-term (i.e. persistence throughout multiple glacial cycles) isolation of red and yellow-eyed populations of *P. erythrophthalmus.* Similarly, the haplotype network (Figure 3) reveals extensive sharing of one of the common haplotypes among all populations of the four subspecies. Despite this sharing, the network reveals a large difference in the relative frequencies of these haplotypes in red and yellow-eyed towhee populations.

Population Structure and Phylogeography

Despite the lack of phylogenetic structure, the yellow-eyed Eastern Towhee populations have significantly different haplotypes frequencies and are genetically differentiated from the red-eyed populations. The AMOVA indicates that 21.2 % ($\Phi_{ST} = 0.21$ $p<0.0001$) of the total genetic variation is found among populations. When populations containing only red-eyed individuals are analyzed with AMOVA, the among-population value decreases to 5.3 % and is non-significant ($\Phi_{ST}= 5.3$, $p= 0.087$). Taken together, these analyses suggest that the contrast of the yellow-eyed and red-eyed populations is responsible for the significant among-population AMOVA value. Similarly, the SAMOVA (K=2) reveals that 25.3% ($\Phi_{CT} = 0.253$) of the total genetic variation is found between the group of populations containing yellow-eyed forms (nFL, pFL, and GA) and the group with only red-eyed birds (the remaining 8 populations). The amount of variation due to differences among populations within groups was 4.3 %

(Φ_{SC} = 0.043). Thus, differences between yellow and red-eyed forms greatly exceed differences between other randomly grouped populations. A Mantel test correlation coefficient revealed a weakly positive but non-significant relationship between genetic and geographic distance (r = 0.29, p= 0.088). This suggests that differentiation in *P. erythrophthalmus* cannot be explained solely with isolation by distance. The lack of a relationship between genetic and geographic distance is clearly due to the fact that yellow-eyed Florida populations are more differentiated from the more proximate southern populations of Louisiana and Mississippi than they are from the more distant Illinois, Virginia and Arkansas populations.

Whereas Florida and the Gulf Coast are probably important areas of population stability for several eastern taxa (Swenson and Howard, 2005), a simple scenario of recolonization from two southern refugia (Hypothesis 2- Historical Isolation) does not fully explain the Eastern Towhee genetic data. This study reveals two widespread haplotypes consistent with a recent shared history and/or high levels of ongoing gene flow. The significant genetic differentiation between red and yellow-eyed birds (AMOVA) is mainly driven by the large differences in frequency of these widespread sequences. Conversely, many populations contain one or more private haplotypes, which suggests at least partial separation and/or low gene flow (Slatkin, 1985). While private haplotypes in the extreme southern portion of the range (Florida and the Gulf Coast) are consistent with isolation in ancestral areas or refugia, it is unlikely that the red-eyed haplotypes unique to more northerly samples (e.g. North Carolina, Arkansas, Kansas, Virginia) could have accrued in the short time span since post-Pleistocene expansion from Gulf Coast refugia. A Mantel test performed with only red-eyed populations has a

correlation of zero and indicates that the diversity among red-eyed populations is not explained by isolation by distance, although levels of genetic differentiation among red-eyed populations may be too low for the analysis.

At least two scenarios may explain the diversity among red-eyed populations of Eastern Towhee. First, it is possible that there were two southern ancestral areas or refugia and the northern private alleles are the result of a complex interplay of founder effects, differential colonization, and genetic drift. Alternatively, multiple refugia (or isolated populations within a single large 'refugium') may have persisted in unglaciated areas throughout the last glacial maximum or longer, not only in Florida and along the Gulf Coast, but also in more northerly areas. For example, post-glacial expansion from the Appalachians has been proposed for the Spring Peeper (*Pseudacris crucifer*) (Austin *et al.*, 2004), the Northern Short-tailed Shrew (*Blarina brevicauda*) (Brant and Orti, 2003), Red Maple (*Acer rubrum*) (McLachlan *et. al.,* 2005), and Eastern Tiger Salamander (*Ambystoma tigrinum tigrinum*) (Church *et. al.*, 2003). Thus, whereas for certain species the Appalachian Mountains have served as a barrier to gene flow, the southern Appalachians are emerging as an important glacial refugium for other taxa (Soltis *et. al.*, 2006). Similarly, the private alleles may have originated from differential colonization of the north from genetically differentiated populations in Pleistocene ancestral areas such as Florida, the Gulf Coast, and the Appalachians.

Of the three hypotheses considered: 1) Post- Pleistocene Differentiation, 2) Historical Isolation, and 3) Multiple Isolation Events, the data most strongly support Multiple Isolation Events. In accordance with this hypothesis, diversity among red-eyed populations, based on genetic divergence across the entire southern region and a high

frequency of private haplotypes, suggests that Eastern Towhee may have persisted in multiple locations, including the Southern Appalachian Mountains, at least throughout the last glacial maximum. Therefore, the genetic differentiation among some red-eyed populations is not concordant with major phenotypic divisions in the Eastern Towhee, that is, divergence of red and yellow-eyed forms. Empirical and simulation studies have shown an inverse relationship between the number of private alleles and levels of gene flow (Slatkin, 1985). In general, the widespread presence of private alleles in the Eastern Towhee suggests limited movement of individuals among many populations.

Coalescent Analyses- Isolation-With-Migration

The divergence time for red and yellow-eyed *P. erythrophthalmus* populations is ca. 12, 000 to 600, 000 ybp, with a mean estimate of ca. 18, 000 ybp, based on a rate of 2.95% / Myr. Thus available evidence suggests that the two Eastern Towhee groups diverged during the late Pleistocene time period and possibly as recently as the Last Glacial Maximum time period.

Mean estimates of gene flow were very low (less than one female per generation). The estimated mean number of female migrants into the red-eyed populations, 0.197 (range = 0.189 – 1303.61), was roughly three times that of the estimated number of female migrants into the yellow-eyed range, 0.062 (range = 0.047 –147.15). Because both gene flow estimates include a 95% low posterior parameter greater than zero I cannot statistically reject gene flow (in either direction) (Omland, 2006). Overall, however, the very low magnitude of the gene flow estimates is not consistent with ongoing gene exchange but rather suggests a lack of lineage sorting due to a recent separation.

Refugia

In Europe, comparative studies of diverse plant and animal species have revealed the importance of peninsulas (e.g. Iberian, Italian, Balkan) as areas of refuge during glacial maximum (Hewitt, 1996, Hewitt, 2000). As previously stated, *P. e. alleni* is most strongly differentiated from the *P. e. canaster* samples from Louisiana ($\Phi_{ST} = 0.588$) and Mississippi ($\Phi_{ST} = 0.503$). Although isolation in refugia is not necessary for differentiation, I suggest that two potential refugia for the Eastern Towhee are the Florida peninsula and the Gulf Coast area (Swenson and Howard, 2005). Given the lack of significant differentiation (AMOVA) between the two red-eyed subspecies, *P. e. erythrophthalmus* and *P. e. canaster*, post-glacial expansion across the Eastern U.S. may have occurred primarily from the Gulf Coast. The suggestion of both peninsular Florida and the Gulf Coast as glacial refugia has been explicitly proposed for at least two additional taxa, Yellow Poplar (*Liriodendron tulipifera*) (Sewell et al., 1996, Parks, 1994), and the Spring Peeper Frog (*Pseudacris crucifer*) (Austin et al., 2002, Austin et al., 2004). In addition, Swenson and Howard (2005) noted a contact zone "hot spot" in Alabama, shared by several species, consistent with secondary contact of populations expanding from areas along the Gulf Coast and Florida.

Causes of Phenotypic Divergence

While the actual mechanism driving and maintaining divergence between red and yellow eye-color groups is unknown, it seems reasonable to invoke a combination of both ecological and sexual selection. While all floral communities were compressed towards the equator during glacial advance, peninsular Florida underwent a dramatic conversion and to widespread desert scrub, very similar to the central and coastal scrub highlands

that exist today (Graham 1999). Peninsular towhees would have been subjected to new environmental conditions because paleontological records confirm the presence of the Eastern Towhee in the Florida peninsula during the late-Pleistocene (Emslie, 1998). However, predicting abundance and distribution from fossil data is problematic, particularly given the relatively poor avian fossil record (Emslie, 1998). Although yellow-eyed towhees are not presently limited to scrub habitat, they clearly thrive in this habitat and are more abundant there than anywhere else throughout their range (Patuxent Wildlife Research Center, U.S. Geological Survey).

It has even been suggested that the Eastern Towhee prefers dense secondary growth, open forest, and edge habitat outside of scrub areas because these habitats mimic the essential elements of scrub by providing both dense cover and open ground (Dickinson, 1952). If peninsular towhees became adapted to desert scrub, it could contribute to ecological isolation among peninsular and other southern populations. If eye-color is used in assortative mate choice, then the evolution of a pale iris in Florida populations would have contributed to behavioral or pre-mating isolation that would limit introgression. Finally, the retention and fixation of these or any other traits which evolved in peninsular populations could have been influenced by the physiography of the peninsula itself, enhanced by the same forces (e.g. reduced immigration) thought to create peninsular effects (Simpson, 1964). These hypothetical scenarios do not, of course, preclude the possibility of the existence of post-mating isolating mechanisms that could contribute to population differentiation.

Eastern U.S. Biogeography

Of the six geographic patterns of genetic structure described for eastern U.S. taxa (Fig. 7) by Soltis *et. al.* (2006), the genetic structure revealed within Eastern Towhee would be placed in the Apalachicola River discontinuity category (Central Florida Panhandle). However, this is because the Apalachicola river pattern is the same pattern assigned by Soltis *et. al.* (2006) to those taxa with genetic divergence between peninsular Florida and the mainland, i.e. Yellow Poplar (*Liriodendron tulipifera*) (Sewell et al., 1996) and Striped Mud Turtle (*Kinosternon baurii*) (Walker and Avise, 1998), presumably because of the relative rarity of the latter pattern. I suggest that the actual pattern of diversification for Eastern Towhee as well as Yellow Poplar and Striped Mud Turtle is best described as divergent northern and southern groups split approximately between the Florida Peninsula and the continental U.S.

It is important to distinguish between these two patterns. In addition to being geographically different, the Florida/mainland pattern may also result from a different biogeographic history, e.g. isolation in, but no expansion from, the Florida peninsula. For example, highland areas of the Florida peninsula existed as an island archipelago during most of the Pliocene time period, isolated from the mainland by a intermittent seaway feature known as the Suwannee Strait (Gilbert, 1987). Therefore, differentiation of some peninsular populations may have resulted from isolation due to this insularization process. The Siren Salamander (*Pseudobranchus striatus*) is another species placed in the Apalachicola River discontinuity category (Soltis et. al., 2006). However, it is not the Apalachicola River, but the ancient seaway that is believed to have caused the major phylogeographic divisions within this salamander (Liu et al., 2006). Thus, whereas the

modern phylogeographic pattern of a taxon may correspond to a particular landscape feature, the feature itself may not function at all as a barrier to gene flow, or perhaps may have served as such only recently. Eastern Towhee diversity was once thought to have been shaped by this Pre-Pleistocene seaway (Greenlaw, 1996; Remington, 1968). The relatively young divergence time in Eastern Towhee clearly shows that it was not. Regardless, the Eastern Towhee and Siren Salamander studies serve to highlight how apparent geographic patterns of diversification may differ from causative mechanisms.

Subspecies Validity

There is at least some support for the distinction of three of the four subspecies of Eastern Towhee. The peninsular Florida (pFL) sample of *P. e. alleni* is significantly differentiated from all populations except Georgia based on pairwise Φ_{ST} values. *P. e. alleni* is most strongly differentiated from the *P. e. canaster* samples from Louisiana ($\Phi_{ST} = 0.588$) and Mississippi ($\Phi_{ST} = 0.503$). Two lines of evidence show differentiation of the Florida panhandle (nFL) sample of *P. e. rileyi*. First, the nFL sample is significantly different from the pFL sample ($\Phi_{ST} = 0.193$), despite its proximity to the pFL population. In addition, the nFL is the first population to fall out as its own group in the SAMOVA (k = 3). I found no evidence for differentiation between the two red-eyed subspecies *P. e. erythrophthalmus* and *P. e. canaster*.

I conclude that while red and yellow-eyed towhee populations are not phlyogenetically distinct, population-level statistical analyses support the genetic uniqueness of Eastern Towhee populations of the Florida peninsula (*P. e. alleni*) and nearby coastal plain (*P. e. rileyi*). Like some other species, towhee populations may have persisted farther north throughout the last glacial maximum than previously thought.

Divergence between red and yellow-eyed towhees is not consistent with ancient insularization or vicariance but is incipient, occurring as recently as the last glacial maximum or later. This study provides further support for a commonly observed pattern of intraspecific differentiation in eastern U.S. taxa. However, it reveals a relatively uncommon geographic pattern of differentiation that does not match most other taxa , thus emphasizing the complexity and variability of the eastern biotic assemblage as a whole. This study also provides a rare example of genetic divergence, albeit shallow, in a continuously distributed eastern U.S. avian taxon, demonstrating that differentiation can occur very rapidly, even in an organism with potentially high vagility.

REFERENCES

American Ornithologists' Union (1957) *Check-list of North American birds,* Baltimore, MD, Am. Ornithol. Union.

Austin, J, Lougheed S & Boag P (2004) Discordant temporal and geographic patterns in maternal lineages of eastern north American frogs, *Rana catesbeiana* (Ranidae) and *Pseudacris crucifer* (Hylidae). *Molecular Phylogenetics and Evolution,* 32, 799-816.

Austin, J, Lougheed S, Neidrauer L, Chek A & Boag P (2002) Cryptic lineages in a small frog: the post-glacial history of the spring peeper, *Pseudacris crucifer* (Anura : Hylidae). *Molecular Phylogenetics and Evolution,* 25, 316-329.

Avise, J (1992) Molecular Population-Structure and the Biogeographic History of a Regional Fauna - A Case-History with Lessons for Conservation Biology. *Oikos,* 63, 62-76.

Avise, J (1996) Towards a Regional Conservation Genetics Perspective: Phylogeography of Faunas in the Southeastern United States. IN AVISE, J. & HAMRICK, J. (Eds.) *Conservation Genetics: Case Histories from Nature.* New York, Chapman and Hall.

Avise, J (2000) Phylogeography: The history and formation of species.

Avise, J, Arnold J, Ball R, Bermingham E, Lamb T, Neigel J, Reeb C & Saunders N (1987) Intraspecific Phylogeography - The Mitochondrial-DNA Bridge Between

Population-Genetics and Systematics. *Annual Review of Ecology and Systematics,* 18, 489-522.

Avise, J, Giblindavidson C, Laerm J, Patton J & Lansman R (1979) Use of Restriction Endonucleases to Measure Mitochondrial-DNA Sequence Relatedness in Natural-Populations.2. Mitochondrial-DNA Clones and Matriarchal Phylogeny within and among Geographic Populations of the Pocket Gopher, *Geomys pinetus*. *Proceedings of the National Academy of Sciences of the United States of America,* 76, 6694-6698.

Avise, J & Nelson W (1989) Molecular Genetic-Relationships of the Extinct Dusky Seaside Sparrow. *Science,* 243, 646-648.

Bandelt, H, Forster P & Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution,* 16, 37-48.

Brant, S V & Orti G (2003) Phylogeography of the Northern short-tailed shrew, *Blarina brevicauda* (Insectivora : Soricidae): past fragmentation and postglacial recolonization. *Molecular Ecology,* 12, 1435-1449.

Buerkle, C (1999) The historical pattern of gene flow among migratory and nonmigratory populations of prairie warblers (Aves: parulinae). *Evolution,* 53, 1915-1924.

Bulgin, N, Gibbs H, Vickery P & Baker A (2003) Ancestral polymorphisms in genetic markers obscure detection of evolutionarily distinct populations in the endangered Florida grasshopper sparrow (Ammodramus savannarum floridanus). *Molecular Ecology,* 12, 831-844.

Carstens, B, Brunsfeld S, Demboski J, Good J & Sullivan J (2005) Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: Hypothesis

testing within a comparative phylogeographic framework. *Evolution,* 59, 1639-1652.

Church, S A, Kraus J M, Mitchell J C, Church D R & Taylor D R (2003) Evidence for multiple pleistocene refugia in the postglacial expansion of the eastern tiger salamander, *Ambystoma tigrinum tigrinum. Evolution,* 57, 372-383.

Cracraft, J (1986) Origin and Evolution of Continental Biotas - Speciation and Historical Congruence within the Australian Avifauna. *Evolution,* 40, 977-996.

Dickinson, J (1952) Geographic Variation in the Red-eyed Towhee of the Eastern United States. *Bulletin of the Museum of Comparative Zoology,* 107.

Dupanloup, I, Schneider S & Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology,* 11, 2571-2581.

Emslie, S (1998) Avian Community, Climate, and Sea-Level Changes in the Plio-Pleistocene of the Florida Peninsula. *Ornithological Monographs No. 50.*

Ewert, D & Kroodsma D (1994) Song Sharing and Repertoires among Migratory and Resident Rufous-Sided Towhees. *Condor,* 96, 190-196.

Excoffier, L, Smouse P & Quattro J (1992) Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes - Application to Human Mitochondrial-DNA Restriction Data. *Genetics* 131, 479-491.

Fleischer, R, Mcintosh C & Tarr C (1998) Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Molecular Ecology,* 7, 533-545.

Fu, Y (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics,* 147, 915-925.

Gilbert, C R (1987) Zoogeography of the Fresh-Water Fish Fauna of Southern Georgia and Peninsular Florida. *Brimleyana*, 25-54.

Gill, F, Mostrom A & Mack A (1993) Speciation in North American Chickadees .1. Patterns of mtDNA Genetic-Divergence. *Evolution,* 47, 195-212.

Graham A (1999) *Late Cretaceous and Cenozoic History of North American Vegetation North of Mexico,* New York, Oxford University Press.

Greenlaw, J (1996) *Eastern Towhee (Pipilo erythrophthalmus).* The Academy of Natural Sciences, Philadelphia and The American Ornithologists' Union, Washington, D. C.

Hackett, S (1996) Molecular phylogenetics and biogeography of tanagers in the genus Ramphocelus (Aves). *Molecular Phylogenetics and Evolution,* 5, 368-382.

Hewitt, G (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society,* 58, 247-276.

Hewitt, G (2000) The genetic legacy of the Quaternary ice ages. *Nature,* 405, 907-913.

Hey, J & Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics,* 167, 747-760.

Ho, S, Phillips M, Cooper A & Drummond A (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution,* 22, 1561-1568.

Huelsenbeck, J P & Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics,* 17, 754-755.

Johnson, K & Sorenson M (1998) Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome b and ND2) in the dabbling ducks (Tribe : Anatini). *Molecular Phylogenetics and Evolution,* 10, 82-94.

Kumar, S, Tamura K & Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics,* 5, 150-163.

Lande, R, Engen S & Saether B (2003) Stochastic Population Dynamics in Ecology and Conservation.

Liu, F G R, Moler P E & Miyamoto M M (2006) Phylogeography of the salamander genus Pseudobranchus in the southeastern United States. *Molecular Phylogenetics and Evolution,* 39, 149-159.

Mantel, N (1967) Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Research,* 27, 209-&.

Mc Cracken, K, Johnson W & Sheldon F (2001) Molecular population genetics, phylogeography, and conservation biology of the mottled duck (*Anas fulvigula*). *Conservation Genetics,* 2, 87-102.

Mclachlan, J S, Clark J S & Manos P S (2005) Molecular indicators of tree migration capacity under rapid climate change. *Ecology,* 86, 2088-2098.

Mock, K, Theimer T, Rhodes O, Greenberg D & Keim P (2002) Genetic variation across the historical range of the wild turkey (*Meleagris gallopavo*). *Molecular Ecology,* 11, 643-657.

Nielsen, R & Wakeley J (2001) Distinguishing migration from isolation: A Markov chain Monte Carlo approach. *Genetics,* 158, 885-896.

Omland, K, Baker J & Peters J (2006) Genetic signatures of intermediate divergence: population history of Old and New World Holarctic ravens (Corvus corax). *Molecular Ecology,* 15, 795-808.

Patuxent Wildlife Research Center, U.S. Geological Survey, http://www.mbr-pwrc.usgs.gov/id/framlst/i5870id.html

Platnick, N & Nelson G (1978) Method of Analysis for Historical Biogeography. *Systematic Zoology* 27, 1-16.

Posada, D (2001) The effect of branch length variation on the selection of models of molecular evolution. *Journal of Molecular Evolution,* 52, 434-444.

Riddle, B, Hafner D & Alexander L (2000) Comparative phylogeography of baileys' pocket mouse (*Chaetodipus bailey*) and the *Peromyscus eremicus* species group: Historical vicariance of the Baja California Peninsular desert. *Molecular Phylogenetics and Evolution,* 17, 161-172.

Rogers, A & Harpending H (1992) Population-Growth Makes Waves in the Distribution of Pairwise Genetic Differences. *Molecular Biology and Evolution,* 9, 552-569.

Rozas, J, Sanchez-Delbarrio J, Messeguer X & Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics,* 19, 2496-2497.

Schneider, S, Roessli D & Excoffier L (2000) Arlequin: A software for population genetics data analysis Ver. 2.000. *Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.*

Sewell, M, Parks C & Chase M (1996) Intraspecific chloroplast DNA variation and biogeography of North American Liriodendron L (Magnoliaceae). *Evolution,* 50, 1147-1154.

Simpson, G. G. 1964. Species density of North American recent mammals. - Systematic Zoology, 13: 57-73.

Slatkin, M (1985) Rare Alleles as Indicators of Gene Flow. *Evolution,* 39, 53-65.

Slatkin, M & Hudson R (1991) Pair-wise Comparisons of Mitochondrial-DNA Sequences in Stable and Exponentially Growing Populations. *Genetics* 129, 555-562.

Soltis, D, Morris A, Mclachlan J, Manos P & Soltis P (2006) Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology,* 15, 4261-4293.

Swenson, N & Howard D (2005) Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *American Naturalist* 166, 581-591.

Swofford, D L (1991) PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1. Champaign, Illinois, Computer program distributed by the Illinois Natural History Survey.

Tajima, F (1989a) The Effect of Change in Population-Size on DNA Polymorphism. *Genetics,* 123, 597-601.

Tajima, F (1989b) Statistical-Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. *Genetics,* 123, 585-595.

Walker, D & Avise J (1998) Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. *Annual Review of Ecology and Systematics,* 29, 23-58.

Table 1. Intrapopulation genetic diversity. Column values correspond to sample size (*n*), number of unique haplotypes (H), number of private haplotypes (pri.), nucleotide diversity (π), Tajima's *D*, Fu's *Fs*, and significance of the mismatch distribution (MM; ns= not significantly different from a model based on exponential population growth). Significant *D* and *Fs* values at $\alpha \leq 0.05$ are in boldface.

Sample	***n***	**H**	**Pri.**	**π**	**Tajima's *D***	**Fu's *Fs***	**MM**
Florida Peninsula	15	5	2	0.000842	-0.922	-1.864	ns
Florida Panhandle	10	6	3	0.001430	-0.632	**-2.647**	ns
Georgia	9	5	1	0.001014	-1.149	**-2.360**	ns
North Carolina	10	5	3	0.001217	-1.136	**-1.671**	ns
Mississippi	10	3	0	0.000534	-0.691	-0.594	ns
Louisiana	13	3	0	0.000419	-0.910	-0.790	ns
Arkansas	9	4	2	0.001281	-0.382	-0.450	ns
Kansas	6	3	1	0.001473	-0.676	0.540	ns
Illinois	10	4	0	0.000897	-0.431	-1.020	ns
West Virginia	7	2	0	0.000549	1.342	0.856	ns
Virginia	10	4	2	0.000918	-1.245	-0.971	ns

Table 2. Analysis of molecular variance (AMOVA) results. Statistically significant Φ_{ST} values at $\alpha = 0.05$ are in boldface.

Grouping	**Source of Variation**	**% Total Variation**	**Φ_{ST}**	***P*-value**
All populations (n=11)	Within populations	78.8	**0.212**	<0.0001
Iris color (n=3) (red, yellow, mixed)	Within populations	75.2	**0.248**	<0.0001
FL peninsula birds removed	Within populations	86.6	**0.134**	<0.0001
Red-eyed only	Within populations	94.7	0.053	0.087

Table 3. Spatial analysis of molecular variance (SAMOVA) results. *K* indicates the number of groups to which populations were assigned. Groups are separated by slash marks. Statistically significant Φ_{CT} values at $\alpha = 0.05$ are shown in boldface.

K	**Grouping Result**	**Φ_{CT}**	***P*- value**
2	{pFL+nFL+GA} / other samples	**0.253**	0.003
3	nFL / {pFL+GA} / other samples	**0.270**	0.001
4	pFL / nFL / {GA+NC} / other samples	**0.270**	<0.001
5	pFL / GA / nFL / NC / other samples	**0.270**	0.003

Table 4. Pairwise Φ_{ST} values for all population comparisons. Statistically significant values at $\alpha = 0.05$ after a FDR (false discovery rate) multiple hypothesis testing correction are shown in boldface.

		pFL	**nFL**	**GA**	**LA**	**MS**	**AR**	**NC**	**IL**	**VA**
Peninsular Florida	(pFL)	—								
North Florida	(nFL)	**0.193**	—							
Georgia	(GA)	0.069	0.051	—						
Louisiana	(LA)	**0.583**	**0.451**	**0.471**	—					
Mississippi	(MS)	**0.503**	**0.377**	**0.375**	-0.086	—				
Arkansas	(AR)	**0.341**	0.169	0.187	0.056	0.005	—			
North Carolina	(NC)	**0.154**	0.056	0.004	0.282	0.187	0.049	—		
Illinois	(IL)	**0.306**	0.183	0.161	0.082	0.019	-0.035	0.032	—	
Virginia	(VA)	**0.444**	**0.336**	**0.320**	-0.027	-0.050	0.002	0.158	-0.020	—

Table 5. Population parameters from the Isolation with Migration program (IM). Rows correspond to the six parameters. IM was used to estimate the parameter value, which was converted to a demographic value (# of individuals) using formulas provided by Hey and Nielsen, 2004. Values in boldface have the highest posterior probability and are provided with 95% confidence intervals.

Parameter	**Parameter Value**	**Demographic Value**
N_e yellow-eyed females	**7.0906** (3.391 - 71.214)	**50,647** (24,222 - 508,675)
N_e red-eyed females	**52.4090** (21.580 - 576.499)	**374,350** (154,144 - 4,117,852)
N_e ancestral females	**2.1580** (0.7193 - 175.621)	**15,414** (5,137 - 1,254,440)
Years since divergence	**0.2750** (0.175- -8.825)	**18,644** (11,864 - 598,305)
N_e migrant females per gen. into yellow-eyed range ($2N_1m_1$)	**0.0175** (0.0275 - 4.132)	**0.062** (0.047 - 147.15)
N_e migrant females per gen. into red-eyed range ($2N_2m_2$)	**0.0075** (0.0175 - 4.522)	**0.197** (0.189 - 1303.61)

Figure 1. The year-round distribution of *Pipilo erythrophthalmus* (modified from Dickinson, 1954). The range of *Pipilo e. erythrophthalmus* is in waved pattern, *P. e. canaster* in horizontal lines, *P. e. rileyi* in dark gray, and *P. e. alleni* in light gray. Numbers indicate sample size and sampling locality.

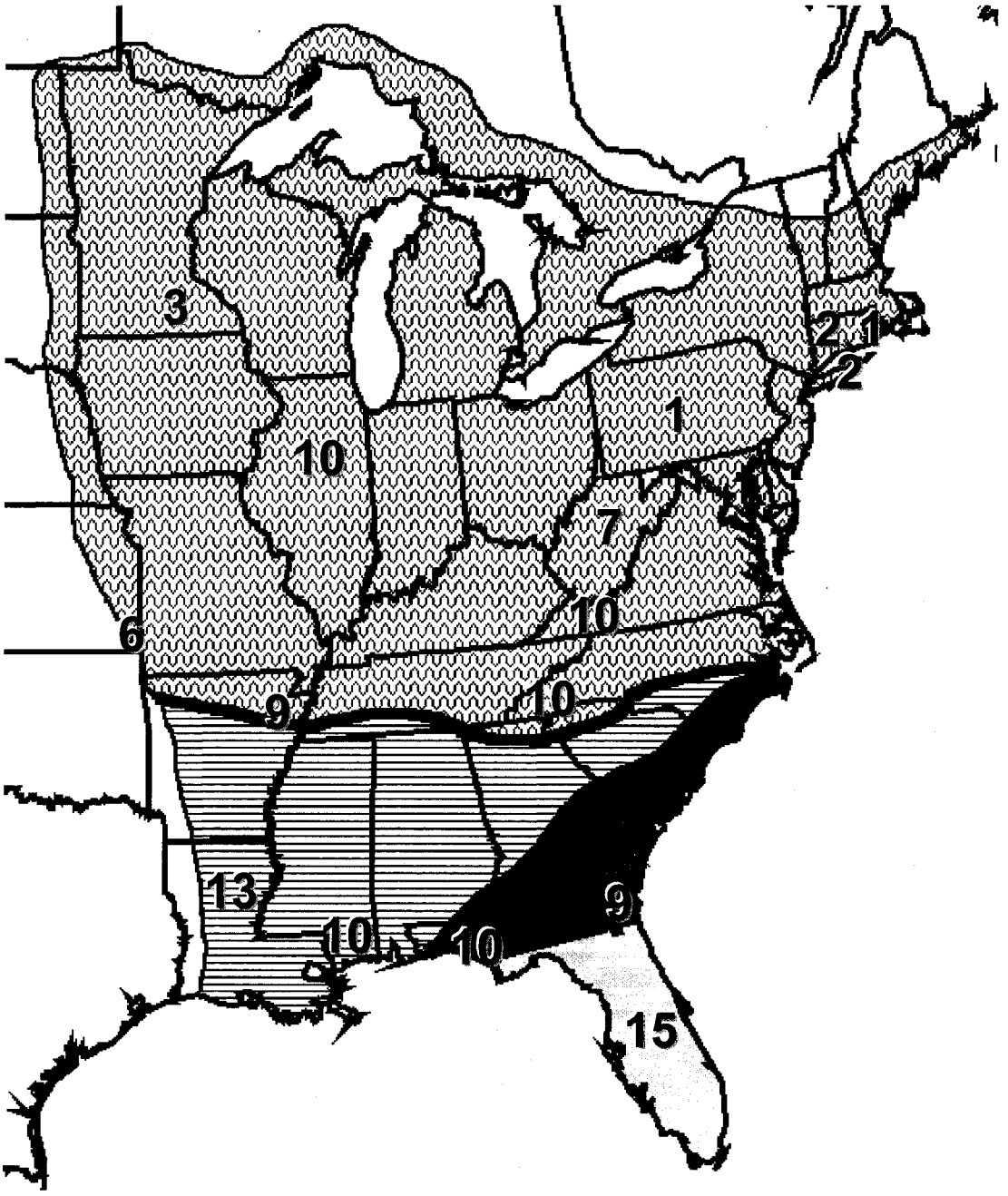


Figure 2. Likelihood phylogenetic tree created with PAUP using the 24 unique haplotypes. Sample abbreviations correspond with U.S. states listed in Table 4.

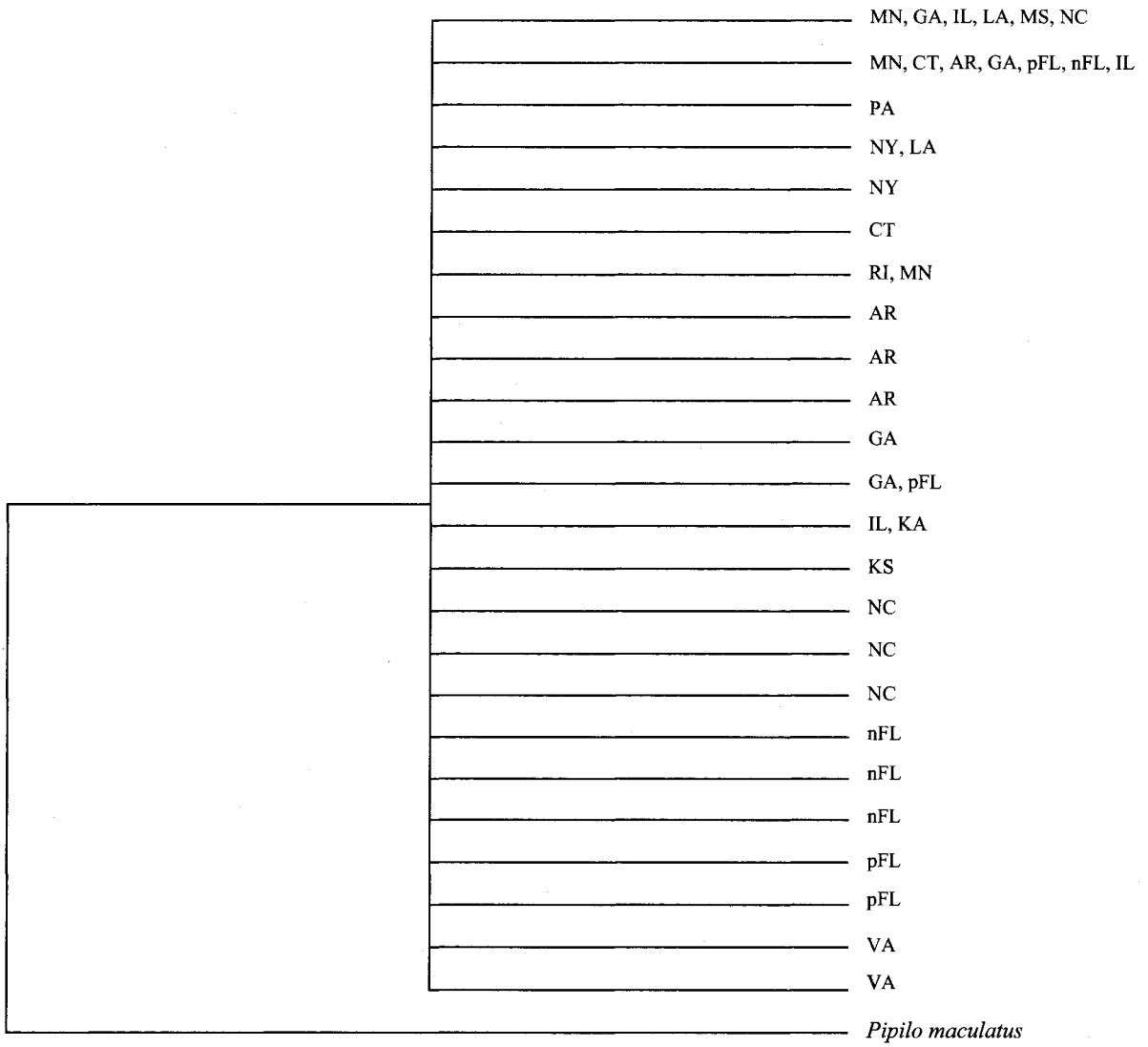


Figure 3. Haplotype network. The 24 unique haplotypes are shown as circles and are proportional to the number of individuals sharing the haplotype. Circle shade corresponds to iris phenotype: black (red-eyed), white (yellow-eyed), and gray (intermediate). The two open circles are median vectors inferred as transitional states between haplotypes.

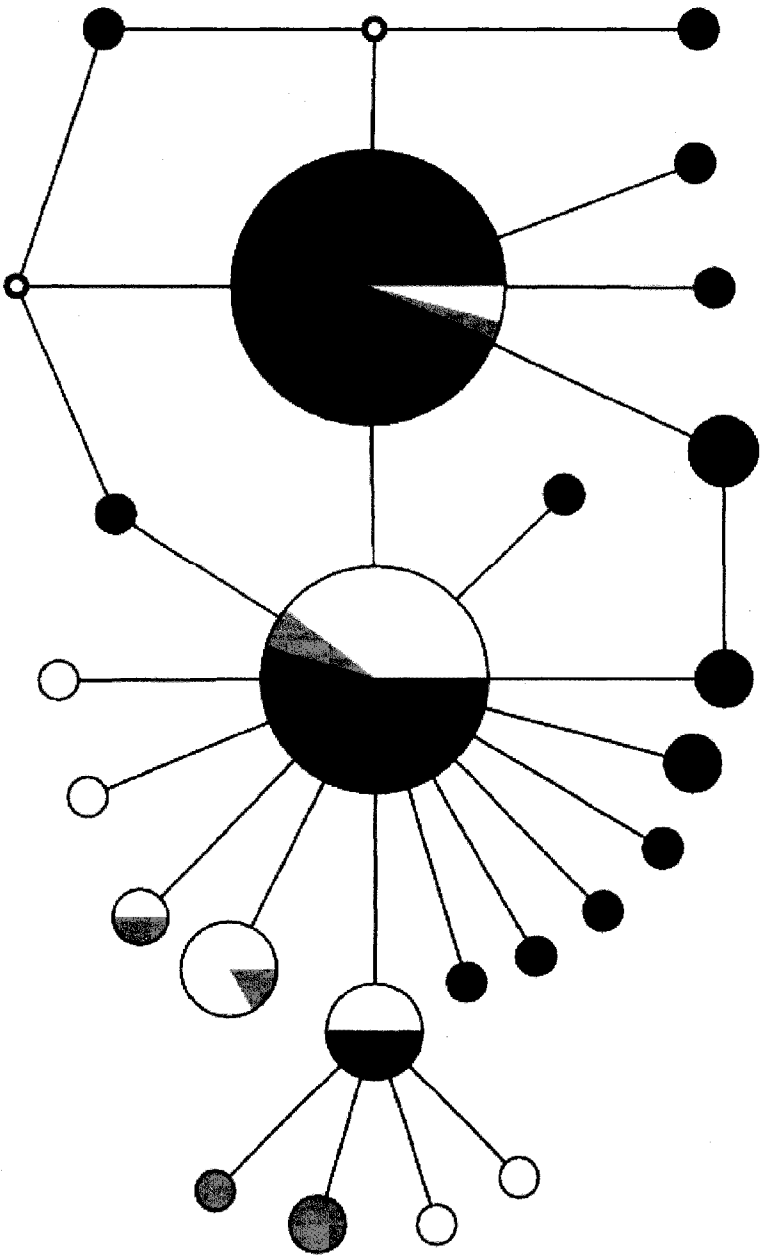


Figure 4. Frequency and distribution of haplotypes. A unique shade of gray represents each of the 24 haplotypes. Charts are positioned at approximate sampling sites and the size of the circle is proportional to sample size, with the smallest representing 1 sample and the largest indicating 15. The range of *P. erythrophthalmus* is shown in light gray.

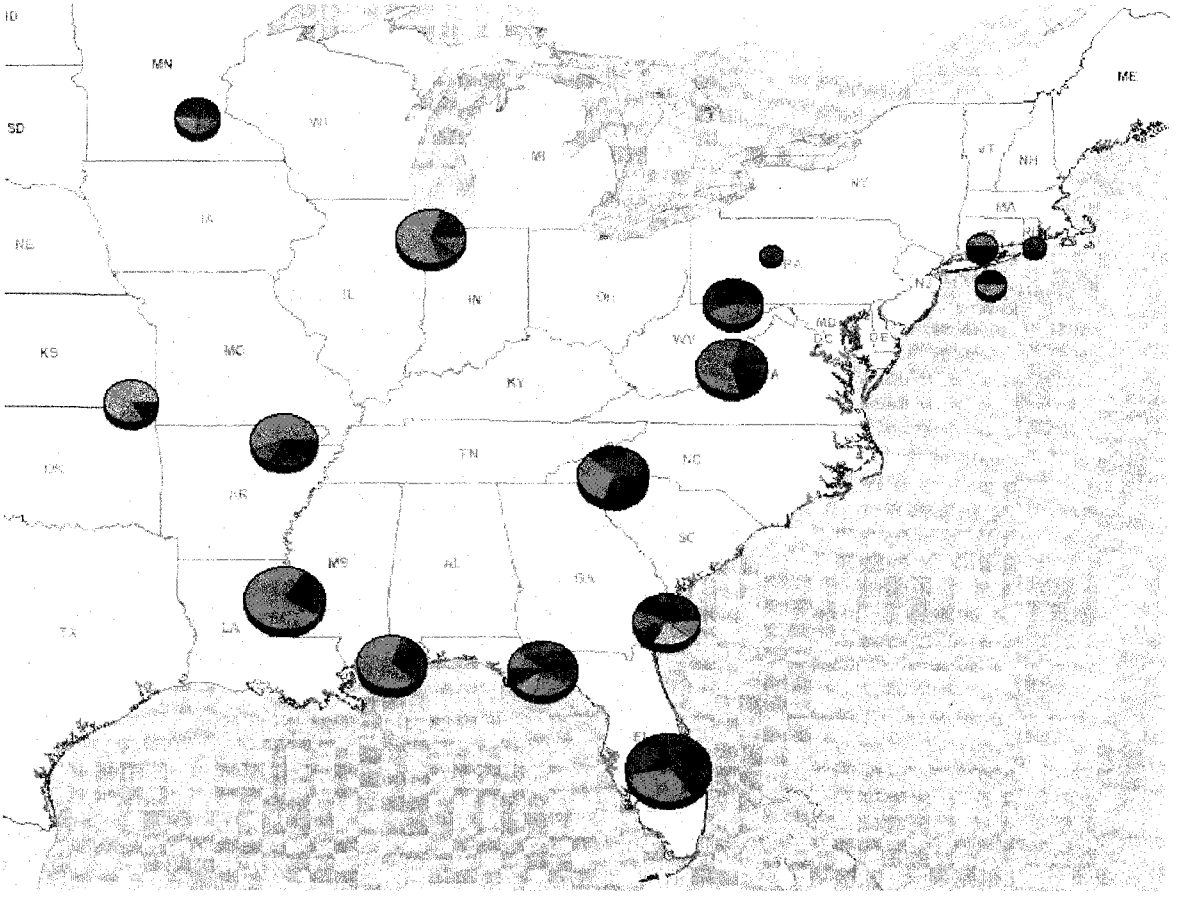


Figure 5. Mantel Test of genetic vs. geographic distance using all pairwise Φ_{ST} values ($n = 36$).

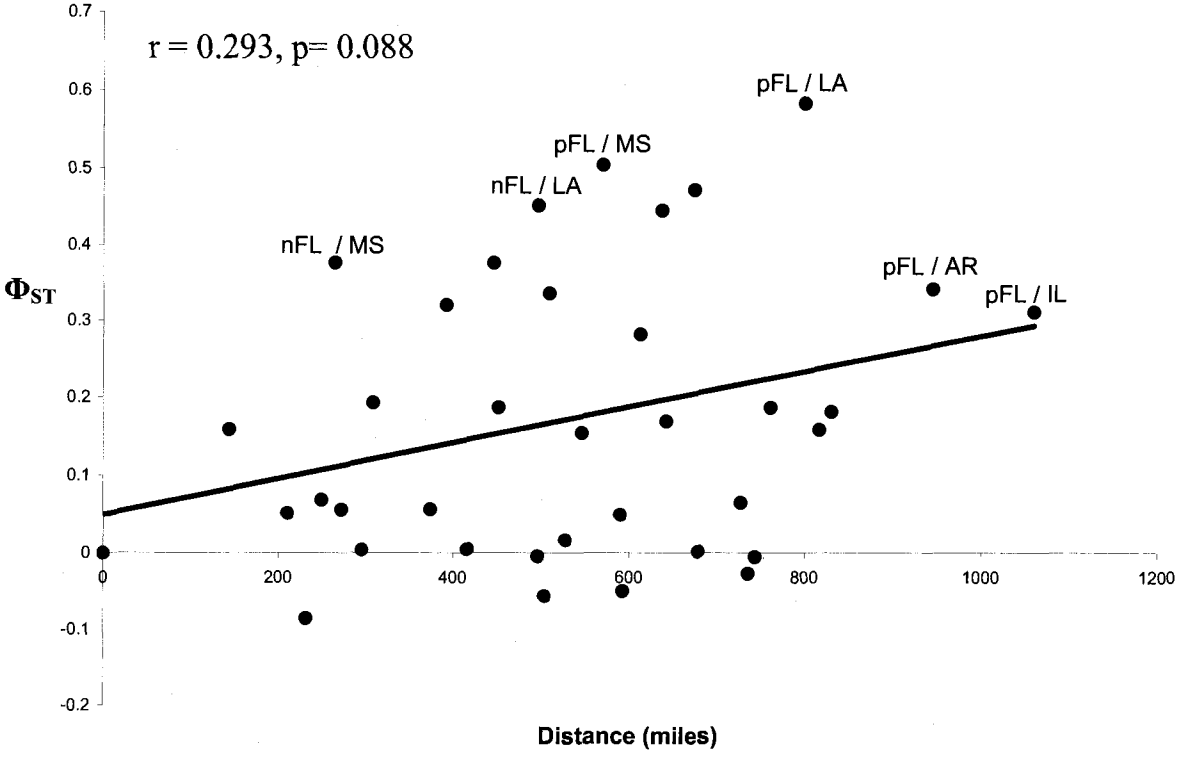


Figure 6. Mismatch Distribution. Number of pairwise differences plotted against frequency in pairwise comparisons (n = 119).

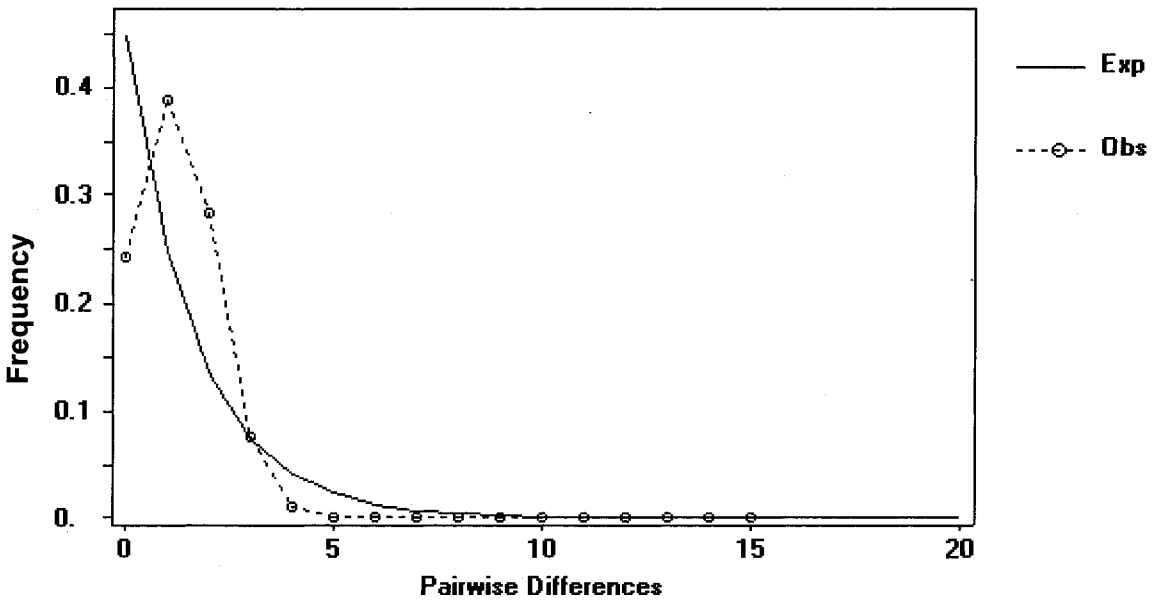
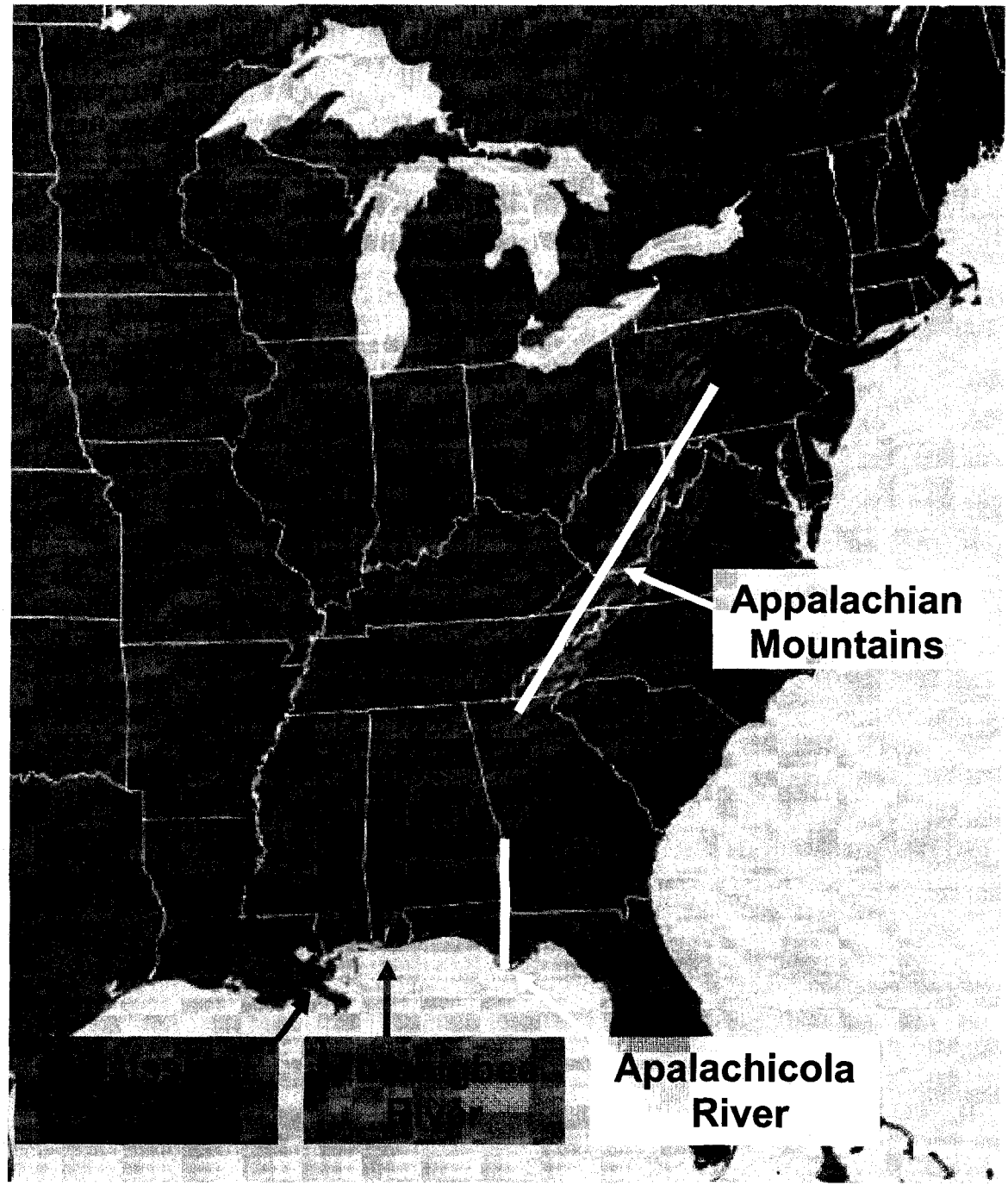


Figure 7. Some physical features of the eastern United States that correspond to shared genetic divisions within multiple 'species'. Adapted from Soltis *et. al.* (2006).



APPENDIX SPECIMEN DATA

Genus	Species	Museum #	State	Specific Locality	Lat Long	Iris
Pipilo	*erythrophthalmus*	MBM16258	AR	Harold Alexander Wildlife Management Area	36°14.26'N, 91°26.23'W	red
Pipilo	*erythrophthalmus*	MBM16256	AR	Harold Alexander Wildlife Management Area	36°14.26'N, 91°26.23'W	red
Pipilo	*erythrophthalmus*	MBM16257	AR	Harold Alexander Wildlife Management Area	36°14.26'N, 91°26.23'W	red
Pipilo	*erythrophthalmus*	MBM16255	AR	Harold Alexander Wildlife Management Area	36°14.26'N, 91°26.23'W	red
Pipilo	*erythrophthalmus*	MBM16259	AR	Harold Alexander Wildlife Management Area	36°14.26'N, 91°26.23'W	red
Pipilo	*erythrophthalmus*	MBM16469	AR	Harold Alexander Wildlife Management Area	36°14.26'N, 91°26.23'W	red
Pipilo	*erythrophthalmus*	MBM16260	AR	Harold Alexander Wildlife Management Area	36°14.26'N, 91°26.23'W	red
Pipilo	*erythrophthalmus*	MBM16261	AR	Harold Alexander Wildlife Management Area	36°14.26'N, 91°26.23'W	red
Pipilo	*erythrophthalmus*	MBM16262	AR	Gulf Mountains Wildlife Management Area	35°33.18'N, 92°39.50'W	red
Pipilo	*erythrophthalmus*	AMNH GFB1076	CT	Guilford, Falkner Island		red
Pipilo	*erythrophthalmus*	AMNH GFB1099	CT	Guilford, Falkner Island		red
Pipilo	*erythrophthalmus*	MBM571	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	red
Pipilo	*erythrophthalmus*	MBM14327	FL	Apalachicola National Forest	30°16.82'N, 84°40.55'W	yellow
Pipilo	*erythrophthalmus*	MBM14326	FL	Apalachicola National Forest	30°16.82'N, 84°40.55'W	yellow
Pipilo	*erythrophthalmus*	MBM14325	FL	Apalachicola National Forest	30°16.82'N, 84°40.55'W	mixed
Pipilo	*erythrophthalmus*	MBM14329	FL	Apalachicola National Forest	30°16.82'N, 84°40.55'W	mixed
Pipilo	*erythrophthalmus*	MBM14328	FL	Apalachicola National Forest	30°16.82'N, 84°40.55'W	yellow
Pipilo	*erythrophthalmus*	MBM14322	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	MBM14318	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	MBM14863	FL	Apalachicola, 5 mi W on US 98	29°43.16'N, 85°04.10'W	yellow
Pipilo	*erythrophthalmus*	MBM14483	FL	Apalachicola National Forest	30°18.85'N, 84°50.18'W	mixed
Pipilo	*erythrophthalmus*	MBM14317	FL	Three Lakes WMA	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	MBM14323	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	MBM14321	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	MBM14320	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	MBM14324	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	MBM14718	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	MBM14542	FL	Apalachicola National Forest	30°16.82'N, 84°40.55'W	Mixed

Genus	Species	Museum #	State	Specific Locality	Lat Long	Iris
Pipilo	*erythrophthalmus*	MBM14319	FL	Three Lakes Wildlife Management Area	27°54.35'N, 81°08.54'W	yellow
Pipilo	*erythrophthalmus*	USNM430803	FL	Archbold Biological Station		red
Pipilo	*erythrophthalmus*	USNM430804	FL	Avon Park Bombing Range		red
Pipilo	*erythrophthalmus*	USNM430805	FL	State Route 8, N of SR 70		red
Pipilo	*erythrophthalmus*	USNM626393	FL	MacDill AFB, Coon Hammock Creek	275124N,0823001W	red
Pipilo	*erythrophthalmus*	USNM626394	FL	MacDill AFB, Coon Hammock Creek	275124N,0823001W	red
Pipilo	*erythrophthalmus*	USNM626425	FL	Tyndall AFB, Horseshoe Lake	300418N,0853358W	red
Pipilo	*erythrophthalmus*	USNM626427	FL	Tyndall AFB, Horseshoe Lake	300418N,0853358W	red
Pipilo	*erythrophthalmus*	USNM622564	FL	Eglin AFB	303208N,0862004W	red
Pipilo	*erythrophthalmus*	MBM14857	GA	Oster Drive	31°47'N, 81°22'W	red
Pipilo	*erythrophthalmus*	MBM14554	GA	Richmond Hills Wildlife Management Area	31°47.07'N, 81°12.98'W	mixed
Pipilo	*erythrophthalmus*	MBM14717	GA	Richmond Hills Wildlife Management Area	31°47.07'N, 81°12.98'W	red
Pipilo	*erythrophthalmus*	MBM14552	GA	Richmond Hills Wildlife Management Area	31°47.07'N, 81°12.98'W	mixed
Pipilo	*erythrophthalmus*	MBM14556	GA	Richmond Hills Wildlife Management Area	31°47.07'N, 81°12.98'W	mixed
Pipilo	*erythrophthalmus*	MBM14557	GA	Richmond Hills Wildlife Management Area	31°47.07'N, 81°12.98'W	yellow
Pipilo	*erythrophthalmus*	MBM14558	GA	Richmond Hills Wildlife Management Area	31°47.07'N, 81°12.98'W	mixed
Pipilo	*erythrophthalmus*	MBM14553	GA	Richmond Hills Wildlife Management Area	31°47.07'N, 81°12.98'W	yellow
Pipilo	*erythrophthalmus*	MBM14555	GA	Richmond Hills Wildlife Management Area	31°47.07'N, 81°12.98'W	yellow
Pipilo	*erythrophthalmus*	FM329760	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	FM350337	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	FM350658	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	FM365132	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	FM389560	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	FM389561	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	FM435159	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	FM435160	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	FM435649	IL	Winfield		red
Pipilo	*erythrophthalmus*	FM436964	IL	Chicago, McCormick Place		red
Pipilo	*erythrophthalmus*	MBM16101	KS	Elk City Wildlife Management Area	37°15.34'N, 95°46.65'W	red
Pipilo	*erythrophthalmus*	MBM16278	KS	Big Hill Wildlife Management Area	37°15.53'N, 95°25.70'W	red
Pipilo	*erythrophthalmus*	MBM16279	KS	Big Hill Wildlife Management Area	37°15.53'N, 95°25.70'W	red

Genus	Species	Museum #	State	Specific Locality	Lat Long	Iris
Pipilo	*erythrophthalmus*	MBM16280	KS	Big Hill Wildlife Management Area	37°15.53'N, 95°25.70'W	red
Pipilo	*erythrophthalmus*	MBM16276	KS	Big Hill Wildlife Management Area	37°15.53'N, 95°25.70'W	red
Pipilo	*erythrophthalmus*	MBM16277	KS	Big Hill Wildlife Management Area	37°15.53'N, 95°25.70'W	red
Pipilo	*erythrophthalmus*	MBM16254	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16098	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16099	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16100	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16097	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16093	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16096	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16092	LA	Buckthorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16094	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM16095	LA	Buckhorn Wildlife Management Area	32°02.69'N, 91°21.87'W	red
Pipilo	*erythrophthalmus*	MBM14476	LA	Three Rivers Wildlife Management Area	31°02.98'N, 91°34.75'W	red
Pipilo	*erythrophthalmus*	MBM14578	LA	Pointe-au-Chien Wildlife Management Area	29°28.29'N, 90°32.28'W	red
Pipilo	*erythrophthalmus*	MBM14577	LA	Pointe-au-Chien Wildlife Management Area	29°28.29'N, 90°32.28'W	red
Pipilo	*erythrophthalmus*	FM397005	MN	T104N 6W Sec 35		red
Pipilo	*erythrophthalmus*	FM430521	MN	Baxter		red
Pipilo	*erythrophthalmus*	FM437261	MN	Staples, Rte 1		red
Pipilo	*erythrophthalmus*	MBM14595	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	MBM14599	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	MBM14596	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	MBM14594	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	MBM14593	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	MBM14592	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	MBM14591	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	MBM14600	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	mixed
Pipilo	*erythrophthalmus*	MBM14598	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	MBM14597	MS	Ward Bayou Wildlife Management Area	30°32.98'N, 88°38.13'W	red
Pipilo	*erythrophthalmus*	AMNH JMB2137	NY	Great Gull Island		red
Pipilo	*erythrophthalmus*	AMNH JMB2141	NY	Great Gull Island		red

Genus	Species	Museum #	State	Specific Locality	Lat Long	Iris
Pipilo	*erythrophthalmus*	AMNH PRS189	NY	Long Island		red
Pipilo	*erythrophthalmus*	MBM16197	NC	Waynesville 6 mi SE, Lake Logan	35°25.18'N, 82°55.40'W	red
Pipilo	*erythrophthalmus*	MBM16201	NC	Brevard ~6.5 mi WNW, FR 475	35°15.67'N, 82°50.37'W	red
Pipilo	*erythrophthalmus*	MBM16200	NC	Brevard ~6.5 mi WNW, FR 475	35°15.67'N, 82°50.37'W	red
Pipilo	*erythrophthalmus*	MBM16199	NC	Waynesville ~8.5 mi SE, Little E Fork Rd (S end)	35°24.31'N, 82°53.93'W	red
Pipilo	*erythrophthalmus*	MBM16198	NC	Waynesville 6 mi SE, Lake Logan	35°25.18'N, 82°55.40'W	red
Pipilo	*erythrophthalmus*	MBM16357	NC	Brevard ~7.0 mi NW, fire rd 475b	35°19.92'N, 82°47.86'W	red
Pipilo	*erythrophthalmus*	MBM16356	NC	Brevard ~7.0 mi NW, fire rd 475b	35°19.92'N, 82°47.86'W	red
Pipilo	*erythrophthalmus*	MBM16202	NC	Brevard ~7.0 mi NW, FR 475b	35°19.92'N, 82°47.86'W	red
Pipilo	*erythrophthalmus*	MBM12792	PA	10mi South of Clearfield Rt. 153	78°25'N/41°05 'W	red
Pipilo	*erythrophthalmus*	AMNH JP1239	RI	Flagg Road- URI campus		red
Pipilo	*erythrophthalmus*	USNM601429	VA	Ryder Gap, 1 mi E, 4 mi N, FR55 G. Wash. NF	381107N,0795517W	red
Pipilo	*erythrophthalmus*	USNM601415	VA	Warm Springs, 7 MI N, 1.5 M E., G. Wash. NF	380858N,0794555W	red
Pipilo	*erythrophthalmus*	USNM601414	VA	Warm Springs, 7 MI N, 1.5 M E., G. Wash. NF	380858N,0794555W	red
Pipilo	*erythrophthalmus*	USNM601490	VA	Fort Blackmore, 6.5 MI N; Jefferson NF FR 2610	365202N,0823515W	red
Pipilo	*erythrophthalmus*	USNM601583	VA	Trout Dale, 2.25 MI S, 2.75 MI W; Jefferson NF	364011N,0812913W	red
Pipilo	*erythrophthalmus*	USNM601584	VA	Trout Dale, 2.25 MI S, 2.75 MI W; Jefferson NF	364011N,0812913W	red
Pipilo	*erythrophthalmus*	USNM601585	VA	Trout Dale, 2.25 MI S, 2.75 MI W; Jefferson NF	364011N,0812913W	red
Pipilo	*erythrophthalmus*	USNM601586	VA	Trout Dale, 2.25 MI S, 2.75 MI W; Jefferson NF	364011N,0812913W	red
Pipilo	*erythrophthalmus*	USNM601473	VA	Gilley, 1/3 MI N 1/4 MI W; Jefferson NF	370617N,0824055W	red
Pipilo	*erythrophthalmus*	USNM601578	VA	Chilhowie, 6.5 MI S, 7.75 MI E; Jefferson NF		red
Pipilo	*erythrophthalmus*	MBM16203	WV	Elleber Knob, Bartow ~8.5 mi SE, FR 1681	38°24.75'N, 79°42.60'W	red
Pipilo	*erythrophthalmus*	MBM14490	WV	Near State Road 72 x US 50, Erwin	39°19.17'N, 79°40.92'W	red
Pipilo	*erythrophthalmus*	MBM14856	WV	Near State road 72 x US 50, Erwin	39°19.17'N, 79°40.92'W	red
Pipilo	*erythrophthalmus*	MBM14608	WV	Near state road 72 x US 50, Erwin	39°19.17'N, 79°40.92'W	red
Pipilo	*erythrophthalmus*	MBM14609	WV	Near state road 72 x US 50, Erwin	39°19.17'N, 79°40.92'W	red
Pipilo	*erythrophthalmus*	MBM14492	WV	Near State Road 72 x US 50, Erwin	39°19.17'N, 79°40.92'W	red
Pipilo	*erythrophthalmus*	MBM14491	WV	Near State Road 72 x US 50, Erwin	39°19.17'N, 79°40.92'W	red

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