

GEOGRAPHIC VARIATION IN THE YELLOW-THROATED WARBLER

*(DENDROICA DOMINICA)*

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GEOGRAPHIC VARIATION IN THE YELLOW-THROATED WARBLER

*(DENDROICA DOMINICA)*

Bailey D. McKay

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THESIS ABSTRACT

GEOGRAPHIC VARIATION IN THE YELLOW-THROATED WARBLER

(*DENDROICA DOMINICA*)

Bailey D. McKay

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I examined how variation within the yellow-throated warbler (*Dendroica dominica*) relates to geography. The yellow-throated warbler is a common neotropical migrant with a breeding range confined to the southeastern United States. It is divided into three continental subspecies that differ in ecology, morphology, and migratory behavior. In the first chapter, I used mitochondrial control region sequences to test whether eastern and western yellow-throated warbler subspecies showed temporal division consistent with a phylogeographic break, the Tombigbee River Discontinuity, that is known to have affected the phylogeographic patterns of several vertebrates in the southeast. Considerable genetic variation was uncovered in the yellow-throated warbler, but most of this variation was found within rather than between populations or subspecies. A shallow phylogenetic tree, star-like haplotype network, and unimodal mismatch

distribution all suggested a recent expansion. Coalescent modeling indicated that modern yellow-throated warbler populations are derived from a single common ancestral population and that differences between subspecies in morphology, ecology, and migratory pathways are the result of recent evolution. Some avian subspecies were described with insufficient evidence and do not reflect biological reality, so in the second chapter I performed a range-wide reassessment of the phenotypic differences between yellow-throated warbler subspecies to determine if there was a discrepancy between mtDNA and morphology. Results indicated much overlap in the morphological characters most important in diagnosing subspecies: bill length and proportion of yellow in lore, and discriminant function analysis failed to correctly assign most individuals especially those collected near the subspecies' border. There was a strong west to east clinal change in bill length and proportion of yellow in lore and no evidence of discrete morphological groups. I recommend eliminating the subspecies *D. d. albilora* and *D. d. stoddardi* because they can not be reliably diagnosed by either morphology or mtDNA.

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**CHAPTER 1. EVOLUTIONARY HISTORY AND RAPID  
DIFFERENTIATION IN THE YELLOW-THROATED WARBLER  
(*DENDROICA DOMINICA*)**

## **ABSTRACT**

Molecular tools are reshaping many traditional paradigms concerning the timeframe of avian diversification in North America. Phylogeographic studies of unsurveyed taxa in little-studied regions are essential for guiding the emerging paradigms regarding the geological events that shaped modern taxa. The southeastern United States is one region where the tempo and mode of recent diversification is poorly understood. One phylogeographic break in particular, the Tombigbee River Discontinuity, is mysterious and requires further study. The distributions of morphological subspecies of the yellow-throated warbler suggest that this bird was affected by the Tombigbee River Discontinuity. To determine whether the phylogeographic patterns of the yellow-throated warbler are consistent with this vicariant event, I analyzed control region sequences of 118 yellow-throated warblers from across the species' range. Considerable variation was uncovered, but most of this variation was found within rather than between populations or subspecies. A shallow phylogenetic tree, star-like haplotype network, and unimodal mismatch distribution all suggested a recent expansion from a common ancestral population. Coalescent modeling indicated that modern populations are derived from a single common ancestral population and that differences between subspecies in morphology, ecology, and migratory pathways are the result of recent evolution. The implications of these results for understanding comparative phylogeography in the southeastern United States and for defining taxonomic groups for conservation are discussed.

## INTRODUCTION

Climatic cycles in the late Pleistocene were long viewed as the major events shaping songbird diversification in North America (Mengel 1964). However, when the divergences of sister songbird taxa were dated with molecular tools, their level of differentiation was found to be much larger than expected under a model of late Pleistocene speciation and the Pliocene was implicated as a period of substantial avian diversification (Klicka and Zink 1997; 1999; Zink *et al.* 2004). Subsequent studies using different datasets and methods of comparing speciation patterns confirmed a non-trivial role for the Pleistocene in driving avian diversification (Avice and Walker 1998; Johnson and Cicero 2004; Weir and Schluter 2004), and a debate ensued over what period produced exceptional diversity (given a background rate of speciation and extinction). New paradigms, assimilating the best of both sides of the debate, are emerging (Lovette 2005), and molecular data are profoundly reshaping ideas concerning the timeframe for songbird diversification in North America. For example, it is now evident that many of the east-west splits, at least at lower latitudes, diverged earlier, whereas in some regions, such as the boreal forests (Weir and Schluter 2004), diversification has occurred more recently. Detailed phylogeographic studies of both unsurveyed species and poorly studied regions are essential for correctly guiding the formation of new paradigms.

The timeframe for songbird diversification is especially poorly understood in the eastern United States. A review of comparative phylogeography in North American birds found little evidence for common phylogeographic divisions east of the Rocky Mountains (Zink 1996). However, at least one vicariant event, the “Tombigbee River Discontinuity,” is an exception that involves at least one bird, the Carolina chickadee (*Poecile*

*carolinensis*; Gill *et al.* 1993; 1999). Along with the Carolina chickadee, a number of other vertebrates including several fish species (Wiley and Mayden 1985; Bermingham and Avise 1986), water snakes (Lawson 1987), and possibly swamp rabbits (Chapman and Feldhamer 1981) show discreet eastern and western phylogroups divided roughly along the Tombigbee River drainage in western Alabama (Soltis *et al.* 2006). Eastern and western phylogroups are estimated to have diverged during the early to mid Pleistocene, between 1.5 and 1 million years ago. Common phylogeographic patterns in such varied taxa suggest that a vicariant episode is responsible, but exactly what caused this vicariance is unclear. It is known, however, that range compression during glacial maxima forced many taxa into refugia in the lowlands of the southeast (Pielou 1991) and that current rivers were often much larger during periods of glacial maxima due in part to glacial run-off. Given this, Gill *et al.* (1999) have advanced the leading hypothesis for this vicariant event. They suggest that the Tombigbee River, which would have been much larger during the glacial maxima, divided lowland refugia and served as a barrier to gene flow. Genetic surveys of taxa likely affected by the Tombigbee River Discontinuity will help gauge the timeframe of this vicariant event and determine whether the observed pattern is consistent with the large river hypothesis advanced by Gill *et al.* (1999).

The yellow-throated warbler (*Dendroica dominica*) is another bird species that appears to have been influenced by the Tombigbee River Discontinuity. This common neotropical migrant has a breeding range confined to the southeastern United States where it is divided into three continental subspecies differing in morphology, ecology, and migratory behavior (Hall 1996). The two widespread subspecies represent eastern (*D. d. dominica*) and western (*D. d. albilora*) forms separated roughly along the Tombigbee

River in Alabama (Fig. 1). The eastern *dominica* has a relatively long bill, has a yellow lore, prefers small pockets of loblolly pine stands within deciduous forests, and migrates southeast to its wintering grounds in peninsular Florida and the Caribbean (Hall 1996). In contrast, *albilora* has a relatively shorter bill, has a white lore, prefers sycamore bottomland forests, and migrates southwest to its wintering grounds in Central America (Hall 1996). In addition, a third more restricted subspecies (*D. d. stoddardi*) is confined to coastal Alabama and the Florida panhandle (Fig. 1). *Stoddardi* has a more slender bill that averages longer than *dominica*, has a yellow lore, prefers habitats similar to *dominica*, and is probably non-migratory (Hall 1996). There is also a distinct form confined to the Delaware-Maryland-Virginia (Delmarva) peninsula (Ficken *et al.* 1968) that is similar in appearance to *stoddardi* (Stevenson 1982). These differences suggest significant and possibly long-standing differentiation between these forms, but a genetic examination of this species is currently lacking.

In this paper, variation in mtDNA sequences was utilized to investigate the evolutionary history of the yellow-throated warbler. The aim was to test the hypothesis that eastern and western subspecies constitute phylogroups that show a pattern consistent with the Tombigbee River Discontinuity, specifically, that these two subspecies began diverging during the early to mid Pleistocene (approximately 1.5 to 1 million years ago). Alternative hypotheses are that eastern and western populations are the product of earlier diversification, such as during the Pliocene (Zink *et al.* 2004), or that the observed morphological, ecological, and behavioral differences have arisen more recently (i.e. in approximately the last 200,000 years ago). A secondary objective was to determine if two distinct but geographically restricted populations of the yellow-throated warbler, namely

the subspecies *stoddardi* and the Delmarva population, constitute evolutionary significant units (ESUs; Mortiz 1994), information critical to any future conservation plans regarding this species.

## **METHODS**

### *Sampling*

Tissue samples from 98 individuals collected at 10 geographic localities across the continental breeding range of the yellow-throated warbler were obtained during the 2006 breeding season (Fig. 1; Table 1). In addition, 20 tissue samples were obtained from museums for a total of 118 samples. Tissue samples were preserved in 100% ethanol and stored at -20°C. All recognized continental subspecies of the yellow-throated warbler as well as a distinct population on the Delmarva peninsula are represented in this study.

### *Molecular lab techniques*

Whole genomic DNA was extracted following a standard phenol-chloroform protocol followed by ethanol wash (modified from Quinn and White 1987). DNA was re-suspended in 1xTE (0.01M Tris, 0.001M EDTA, pH 8.0) and stored at -20°C. Domain I of the mitochondrial control region was amplified in 10µl reactions on a MJ Research PTC-100 thermocycler using the primers Dpdl-L5 and Dpdl-H4 (Milot et al. 2000) and a thermal profile of 95°C for 30 s, 55°C for 30 s, and 70°C for 90 s. Extension time was lengthened by 4 s each cycle for 35 cycles. Primers and excess dNTPs were removed from the PCR product with ExoSAP-IT® (USB Corporation) following the manufacturer's instructions. The ExoSAP-IT® treated PCR products were then used as templates in dideoxy-termination cycle sequencing reactions using the CEQ™ DTCS Quick Start Kit (Beckman Coulter) and the sequencing primers Passerine ContReg For



(5'-TAC CTA GGA GGT GGG CGA AT-3'; R. T. Brumfield, unpublished data) and Passerine ContReg Rev (5'-CCC AAA CAT TAT CTC CAA AA-3'; R. T. Brumfield, unpublished data). Sequencing reaction products were purified by ethanol precipitation and sequenced on a Beckman CEQ™ 8000 sequencer. All DNA sequences were sequenced in both directions and complementary strands were unambiguously aligned and edited using SEQUENCHER v. 4.6 (GeneCodes Corporation, Ann Arbor, Michigan). Sequences were inspected individually using the raw spectrograph data and every point mutation was checked for authenticity.

#### *Genetic differentiation and population structure*

Nucleotide diversity ( $\pi$ ), haplotype diversity ( $h$ ), and neutrality statistics (Tajima's  $D$  (Tajima 1989) and Fu's  $F_s$  (Fu 1997)) were estimated at three levels: 1) for each population separately, 2) for each subspecies, and 3) for all samples combined. Neutrality statistics were computed in Arlequin v. 3.01 (Excoffier *et al.* 2005), whereas  $\pi$  and  $h$  were computed with DnaSP v. 4.0 (Rozas *et al.* 2003). Neutrality statistics are used to test the assumption of selective neutrality, and they can also be informative about demographic forces affecting populations with Fu's  $F_s$  being particularly sensitive to population demographic expansion (Fu 1997). The significance of the neutrality statistics was tested with 10,000 coalescent simulations.

Overall genetic structure of populations was tested with an analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) as implemented in Arlequin. In the AMOVA,  $\Phi$ -statistics were used to examine the contribution of molecular variance at three levels: (i) among the three subspecies ( $\Phi_{ct}$ ); (ii) among populations within subspecies ( $\Phi_{sc}$ ); and (iii) among individuals within populations ( $\Phi_{st}$ ). Mismatch distributions were compared

with expectations of a sudden-expansion model (Rogers 1995) and a model of constant population size (Slatkin and Hudson 1991) as implemented in DnaSP. Populations that have experienced a sudden demographic expansion are expected to show a unimodal mismatch distribution, whereas populations that have been in equilibrium are expected to show a bimodal or ragged distribution (Slatkin and Hudson 1991).

#### *Coalescent-based analyses*

To explicitly test whether eastern and western yellow-throated warbler subspecies diverged in early to mid Pleistocene, a model was used that generates estimates of divergence time independent of the gene-migration rates between two populations (Nielson and Wakeley 2001). This was done using a web-based version of the program MDIV (available at <http://cbsuapps.tc.cornell.edu/>; Nielson and Wakeley 2001), which utilizes a Monte Carlo Markov chain (MCMC) method to estimate the time since two populations diverged (T), the migration rate (M), and the population parameter theta ( $\Theta$ ; twice the effective female population size (N<sub>fe</sub>) times the mutation rate ( $\mu$ )). Three independent runs were performed under the finite sites model using the same starting conditions (M<sub>max</sub> = 50, T<sub>max</sub> = 10, chain length =  $5 \times 10^6$ , burn-in =  $5 \times 10^5$ ) and different random seeds. Based on the theoretical work of Lande et al. (2002), Milá et al. (2007) used a generation time of 1.8 years for another warbler species (*Dendroica coronata*), so this estimate of generation time was used to convert number of generations into years.

#### *Phylogenetic analysis*

Along with population level analyses, a maximum-likelihood (ML) phylogeny was constructed using the program PAUP\* v. 4.0b10 (Swofford 2001). Nucleotide

substitution model parameters were selected using a hierarchical likelihood ratio test (hLRT) with the outgroup sequence removed as implemented in MODELTEST v. 3.7 (Posada and Crandall 1998). Published *Dendroica coronata* sequences (GenBank ascensions DQ855191 and DQ855190) were used as an outgroup due to *D. coronata*'s phylogenetic proximity to *D. dominica* (Lovette and Bermingham 1999). Because a bifurcating tree may not accurately represent an intraspecific phylogeny (Templeton *et al.* 1992), a haplotype network (excluding outgroups) was also constructed under the parsimony-based algorithm developed by Templeton *et al.* (1992) and implemented in the program TCS 1.21 (Clement *et al.* 2000). To separate population history from population structure (Templeton 1998), nested clade analysis (NCA; Templeton *et al.* 1995) was performed. Prior to the NCA, reticulations in the haplotype spanning network were resolved following the methods of Crandall *et al.* (1994), and the haplotypes were nested according to the procedures of Crandall (1996). The NCA was conducted in GeoDis v. 2.5 (Posada *et al.* 2000).

## **RESULTS**

### *Phylogenetic analysis*

A total of 399 bp from Domain I of the control region was sequenced for all 118 individuals. These sequences yielded 40 variable and 20 parsimony-informative sites resulting in 47 haplotypes (Fig. 3). The average uncorrected pairwise sequence distance (p) between ingroup samples was 0.9%. The control region is located in an area of the mitochondrial genome that is prone to produce nuclear copies of mitochondrial genes (numts; Sorenson and Quinn 1998). However, several lines of evidence support a mitochondrial origin for the sequences presented here. For example, no insertions or

deletions (indels) were observed. These sequences also aligned with the control region of the chicken genome and aligned without indels with other published *Dendroica* control region sequences. In addition, a large number of haplotypes, which is inconsistent with numts, was uncovered (Zhang and Hewitt 1996).

The hLRT suggested the Hasegawa-Kishino-Yano model with a proportion of invariable sites plus unequal rates among sites (HKY+I+G) as the model of molecular evolution that best fit the data. Maximum likelihood analysis produced a phylogenetic tree ( $-\ln=1080.65$ ; not shown) with short branch lengths and low overall bootstrap support. The haplotype network (Fig. 2) also suggested little genetic structure and was characterized by a large number of haplotypes nested within loops in the network. The NCA resulted in no significant associations between clades and geography.

#### *Genetic variability and population structure*

Overall nucleotide diversity was low (0.00887) and was similar among all populations (Table 2). Overall haplotype diversity was high (0.92) and ranged from 0.61 in the Maryland west coast population to 0.99 in the eastern Alabama population. Neutrality statistics (Tajima's  $D$  and Fu's  $F_s$ ) suggested no deviation from neutral expectations in any single population (Table 2) but were significantly negative for the dominica ( $D = -1.88, p < 0.007$ ;  $F_s = -9.49, p < 0.007$ ) and albilora ( $D = -1.60, p < 0.03$ ;  $F_s = -18.81, p < 0.001$ ) subspecies and when all samples were combined ( $D = -1.94, p < 0.003$ ;  $F_s = -25.65, p < 0.001$ ). These patterns suggest either deviations from neutral evolution in a population with a stable size or population growth.

AMOVA indicated that most of the genetic variation was found within populations (96.9%;  $\Phi_{st} = 0.03$ ;  $p < 0.007$ ). Small and non-significant amounts of

variation were partitioned among populations within subspecies (2.7%;  $\Phi_{sc} = 0.03$ ;  $p < 0.14$ ) and among subspecies (0.4%;  $\Phi_{ct} = 0.00$ ;  $p < 0.26$ ; see Table 3). The mismatch distribution (Fig. 3) was distinctly unimodal with a low average number of differences between haplotype pairs (4.42) and did not differ significantly from the expected model of sudden expansion.

#### *Coalescent-based analyses*

Posterior probability distributions of time since divergence between eastern and western subspecies produced by MDIV were almost uniform which suggests little evolutionary difference between these subspecies. The estimated migration rate between eastern and western subspecies was extremely high ( $M = 26.8$ ) and converts to approximately 14.9 migrant female individuals per year. The estimate of theta was low ( $\Theta = 9.91$ ) which, given a high mutation rate in the control region, probably indicates a relatively low long-term effective population size.

## **DISCUSSION**

#### *Genetic differentiation and population expansion*

Although there are high levels of genetic variation within populations, the results presented here demonstrate a complete lack of genetic differentiation between yellow-throated warbler populations and subspecies. The shallow phylogenetic tree and star-like haplotype network (Fig. 2) show no obvious correlates with geography, and nested clade analysis did not report any significant associations between nested clades and their geographical locations. AMOVA reported non-significant proportions of genetic variation among subspecies and among populations within subspecies, indicating that most (97%) genetic variation could be found within populations.

Several lines of evidence suggest a recent population expansion, which would be one cause for the observed homogeneity across populations. For example, high haplotype diversity and low nucleotide diversity are expected in population with a recent expansion (Avice 2000). Neutrality statistics can indicate demographic expansion, and the significantly negative Tajima's  $D$  and Fu's  $F_s$  reported here for the *dominica* and *albilora* subspecies as well as for all samples combined are consistent with past population expansion. Fu's  $F_s$  is more sensitive to departures from population equilibrium (Fu 1997) and in every significant instance this figure was lower than Tajima's  $D$ . Most populations also have negative neutrality statistics, and some of these might be non-significant due to small sample sizes. The distinctly unimodal mismatch distribution (Rogers and Harpending 1992) did not differ significantly from a model of sudden expansion, and the average number of pairwise differences was low for control region sequences indicating the expansion was relatively recent. The observed homogeneity across populations and lack of any association between genetic variation and geography suggests also that the yellow-throated warbler expanded from a single refugium. A recent expansion is most likely the result of the colonization of suitable habitat following glacial retreats from a more limited yellow-throated warbler distribution during glacial maximums (Pielou 1991). It would be interesting to determine whether other southeastern birds exhibit similar patterns.

#### *Comparative phylogeography of the southeastern United States*

In the yellow-throated warbler, there is little or no support for the kind of genetic discontinuity reported in the Carolina chickadee (Gill *et al.* 1993, 1999) or other taxa associated with the Tombigbee River Discontinuity (Soltis *et al.* 2006). Coalescent-based

analysis suggests that there has not been a genetic separation between eastern and western yellow-throated warbler subspecies during the evolutionary history of the species and that there has been substantial gene flow between these subspecies. Therefore, it appears that there is a discrepancy between eastern and western morphological subspecies, which spatially conform to the reciprocally monophyletic units uncovered in the Carolina chickadee, and the genetic pattern of the yellow-throated warbler, in which there is no evidence for the kind of temporal division reported in eastern and western phylogroups of Carolina chickadees.

There are at least three ways to reconcile these observations. First, it is possible that chickadees and yellow-throated warblers underwent the same vicariant event but, following dissolution of the vicariant force, the resident chickadee maintained genetic distinction but higher gene flow between populations of the migratory warbler expunged evidence of a genetic break. If a large glacial river was the geographic boundary between populations, then it makes sense that a neotropical migrant could more readily cross the boundary than a small resident forest bird. Under this scenario, there would need to be strong natural selection in order to preserve the differences found between eastern and western warbler subspecies in the presence of such gene flow.

Second, if the vicariant episode was cyclic (as might be the case under the scenario of a major river system proposed by Gill *et al.* 1999), then the warbler and chickadee may have experienced the same vicariant event but at two different periods. For example, chickadee populations may have been isolated during an earlier formation of the vicariant event, and their isolation maintained (maybe in part due to their static propensity) during weaker vicariant episodes. In contrast, the yellow-throated warbler

may have only been affected by a more recent vicariant maximum, such as during the Wisconsinian glacial maximum (110 to 10 thousand years ago). In this case, eastern and western warbler subspecies could be evolutionary lineages whose control region haplotypes have not completely sorted. Bill shape has been shown to be highly heritable in some songbirds (Schluter and Smith 1986), so morphology could have evolved faster than mtDNA leaving a pattern of morphological differentiation without mtDNA differentiation. This scenario might also explain why the phylogeographic patterns of other organisms that show genetic discontinuity around the Tombigbee River differ slightly in their spatial and temporal patterns.

A third possibility is that eastern and western yellow-throated warbler subspecies are not evolutionary lineages, as has been demonstrated for many other avian subspecies (Ball and Avise 1992; Zink 2004), and the apparent concordance of morphological features of yellow-throated warblers with the Tombigbee River Discontinuity is coincidental. It is well established that some characters used to delineate avian subspecies can be influenced by the rearing environment (James 1983). If bill length and lore color are phenotypically plastic traits in the yellow-throated warbler, these traits would be expected to vary clinally rather than to form discrete clusters. In a companion study of phenotypic variation within the yellow-throated warbler (Chapter 2), it was found that bill length and the proportion of yellow coloration in the lore showed west to east clinal change. The observation of clinal variation in morphology supports scenario three, though more study is needed. Transplant experiments, such as those performed by James (1983) with red-winged blackbirds (*Agelaius phoeniceus*), would help gauge the heritability of bill length and lore color in the yellow-throated warbler.



### *Conservation genetics*

While some workers maintain that evolutionary significant units should be based on genetic differentiation or reciprocal monophyly (Moritz 1994; Zink 2004), others have suggested that ESU designation should be based on ecologically important traits even if neutral genetic markers show little or no differentiation (Crandall *et al.* 2000; Fraser and Bernatchez 2001). The present study does not support the recognition of any of the three continental yellow-throated warbler subspecies or the long-billed population on the Delmarva peninsula as ESUs under genetic criteria alone. More study on the amount and distribution of potentially ecologically important traits would be needed before ESU assignment by any other criteria could be made. Based on these genetic data, however, it seems that, at best, the conservation priority of any one yellow-throated warbler population is low.

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**Table 1.** Morphological, ecological, and behavioral characteristics of distinct yellow-throated warbler groups.

	Breeding range	Migration behavior	Lore color	Bill length (mm; mean $\pm$ SD)*	Habitat
<i>dominica</i>	Eastern southeast	East	Yellow	9.55 $\pm$ 0.59	Predominately mixed forest stands
<i>albilora</i>	Western southeast	West	White	9.22 $\pm$ 0.47	Predominately Sycamore bottomland
<i>stoddardi</i>	Coastal Alabama and the Florida panhandle	Resident?	Yellow	10.07 $\pm$ 0.44	Mixed forest or Loblolly pine stands
Delmarva population	Delmarva peninsula	East	Yellow	10.91 $\pm$ 0.49	Loblolly pine stands

\* measured from nare to tip with digital calipers and based on male specimens used in this study: *dominica* ( $n = 45$ ), *albilora* ( $n = 34$ ), *stoddardi* ( $n = 9$ ), Delmarva population ( $n = 8$ )



**Table 2.** Population information and intrapopulation statistics for each yellow-throated warbler populations separately, for each subspecies, and for all samples combined (total).

Map No.	Population	State	Subspecies	$n$	$nh$	$h$	$\pi$	$D$	$F_s$
1	AL-West	Alabama	<i>albilora</i>	8	7	0.964	0.00627	0.17	-2.63*
2	AL-East	Alabama	<i>dominica</i>	14	13	0.989	0.00832	-0.90	-8.14***
3	NC	North Carolina	<i>dominica</i>	9	6	0.889	0.00418	-1.07	-2.67*
4	MD-West coast	Maryland	<i>dominica</i>	8	3	0.607	0.00512	0.08	1.06
5	MD-Delmarva	Maryland	<i>dominica</i>	10	7	0.867	0.01216	-1.21	2.90
6	FL-East	Florida	<i>dominica</i>	10	7	0.867	0.00691	-0.98	-2.29
7	LA	Louisiana	<i>albilora</i>	17	12	0.934	0.00208	-0.72	-5.06**
8	MO	Missouri	<i>albilora</i>	20	11	0.874	0.00650	-0.84	-4.63**
9	OH	Ohio	<i>albilora</i>	13	9	0.936	0.00802	-1.42	-3.12*
10	<i>stoddardi</i>	Florida	<i>stoddardi</i>	9	5	0.806	0.00752	-0.43	0.05
			<i>albilora</i> (pooled)	58	29	0.920	0.00711	-1.60*	-18.81***
			<i>dominica</i> (pooled)	51	26	0.893	0.00891	-1.88**	-9.49**
			total	118	47	0.922	0.00887	-1.94**	-25.65***

$n$ , sample size;  $nh$ , number of haplotypes;  $h$ , haplotype diversity;  $\pi$ , nucleotide diversity.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

**Table 3.** Analysis of molecular variance (AMOVA) for yellow-throated warbler mtDNA haplotype data.

Source of variation	d.f.	Sum of squares	Variance component	% variation	$\Phi$ -statistic
Among subspecies	2	6.435	0.01263	0.57	$\Phi_{ct} = 0.00$
Among populations within subspecies	7	19.804	0.05757	2.59	$\Phi_{sc} = 0.03$
Within populations	108	232.278	2.15072	96.84	$\Phi_{st} = 0.03^{**}$
Total	117	258.517	2.22091		

\*\*  $P < 0.01$

## FIGURE CAPTIONS

**Figure 1.** Breeding range of the yellow-throated warbler (shaded area; adapted from Dunn and Garrett 1997) and geographic locations of sampled population in this study (black circles). Numbers correspond to populations in Table 1. The light gray area represents the range of *abilora*. Light blue area represents the range of *dominica*. Dark gray area represents the range of *stoddardi*.

**Figure 2.** Minimum-spanning network for the yellow-throated warbler mtDNA control region haplotypes obtained in this study. Each circle represents a haplotype, and the size of the circles is proportional to its frequency. Small black circles represent unsampled haplotypes.

**Figure 3.** Mismatch distribution showing the significant correlation between observed (solid line) and expected frequencies under a model of sudden expansion (dotted line) for the number of pairwise differences in mitochondrial control region sequences of yellow-throated warblers. The expected frequency distribution for a model of constant population size is also shown (dashed line).

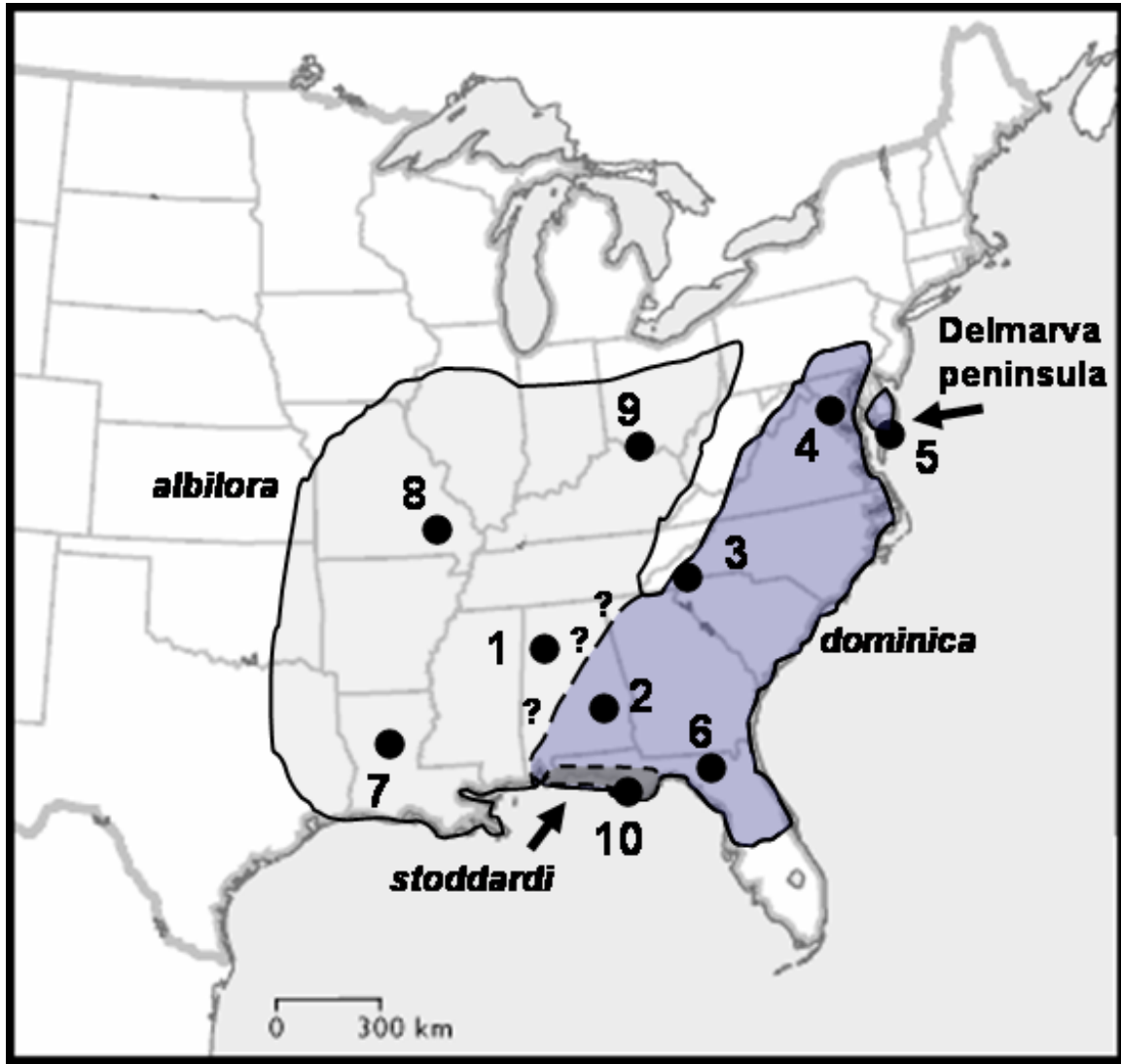


Figure 1.

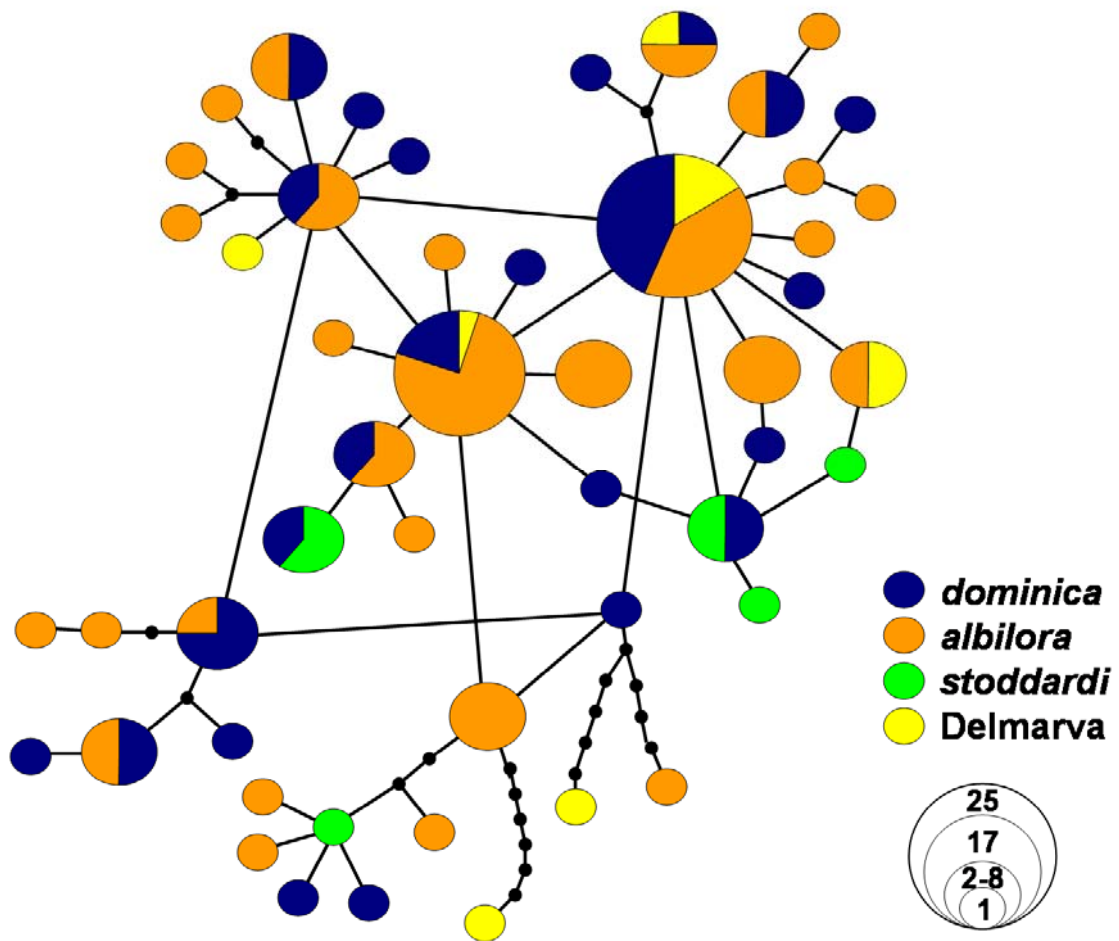
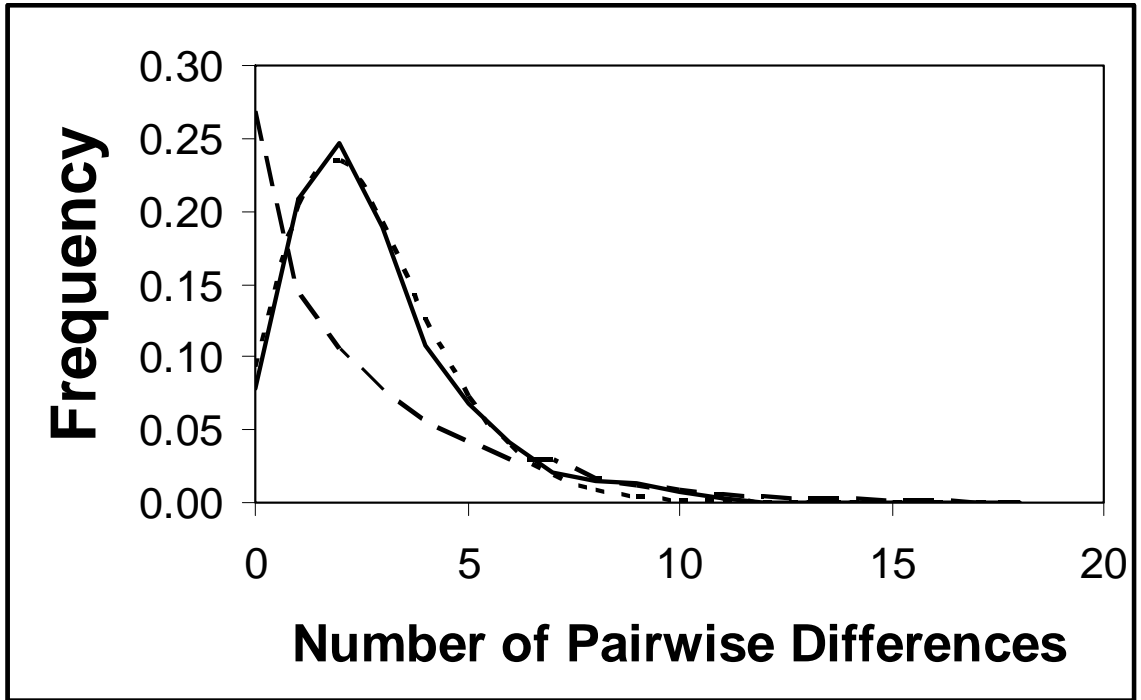


Figure 2.



**Figure 3.**

**CHAPTER 2. PHENOTYPIC VARIATION IN THE YELLOW-THROATED  
WARBLER (*DENDROICA DOMINICA*)**

## ABSTRACT

Subspecies are assumed to have unique evolutionary histories, but molecular data sometimes contradict morphological avian subspecies. A recent genetic survey of the Yellow-throated warbler (*Dendroica dominica*) found that none of its three continental subspecies qualified as evolutionary significant units. Therefore, these subspecies either do not correspond to biological entities or the morphological differences between them have evolved rapidly. Since there has been no range-wide quantitative assessment of Yellow-throated warbler subspecies, it has been impossible to gauge the amount of rapid evolution that has occurred if these subspecies are products of recent differentiation or to test whether morphological differences are clinal, which might suggest phenotypes are influenced by the rearing environmental. Here I perform a range-wide morphological reassessment of the continental Yellow-throated warbler subspecies in an effort to quantify their differences and examine if and how differences relate to geography. Results indicate much overlap in the morphological characters most important in diagnosing subspecies: bill length and proportion of yellow in lore, and discriminant function analysis fails to correctly assign most individuals especially those collected near the subspecies' border. There is a strong west to east clinal change in bill length and proportion of yellow in lore and no evidence of discrete morphological groups. I recommend eliminating the subspecies *D. d. albilora* and *D. d. stoddardi* because they can not be reliably diagnosed by morphology or mtDNA.



## INTRODUCTION

The rise of the biological species concept during the mid-twentieth century fostered an explosion in the number of described avian subspecies, and, though the utility of the subspecies rank was questioned (Wilson and Brown 1953), it was believed that variation within species represented local adaptations of evolutionary significance (Mayr 1982). The assumption that subspecies have unique evolutionary histories has subsequently led to the use of subspecies in roles that require their evolutionary independence, such as taxonomy, evolution studies, and conservation plans (Zink 2004). Many of the morphological traits used to designate subspecies, however, can be directly affected by the rearing environment (James 1983), and when morphological subspecies are subjected to independent tests of evolutionary isolation (i.e. neutral molecular markers) they often fail to meet the requirements of evolutionary significant units (Ball and Avise 1992; Zink 2004).

The above situation is exemplified by the Yellow-throated Warbler (*Dendroica dominica*). The Yellow-throated Warbler is mid-sized warbler with a black face, white supercilium, and bold yellow throat patch. It is a neotropical migrant that breeds in the southeastern United States and is divided into three continental subspecies (a fourth subspecies is confined to the Bahamas and will not be considered here)(Hall 1996). These subspecies were not named following any rule, and their description is based on approximately 15 to 20 individuals per subspecies (Ridgway 1902; Sutton 1951). Also, the specimens used to describe *dominica* and *albilora* were taken from the extreme eastern or western portion of their ranges (Ridgway 1902). The subspecies differ in morphology, ecology, and plumage. The eastern *D. d. dominica* is reported to have a long bill (12.4-15.0 mm, sexes combined; Curson et al. 1994), have a yellow lore, breed in mixed forests or

cypress swamps, and winter mainly in the Caribbean (Curson et al. 1994; Hall 1996). The western *D. d. albilora* is reported to be larger than *dominica* (Ridgway 1902), have a short bill (10.9-12.7mm, sexes combined; Curson et al. 1994), have a white lore, breed in sycamore bottomlands or cypress swamps, and to winter mainly in Central America (Hall 1996). *D. d. stoddardi* is confined to coastal Alabama and the panhandle of Florida where it may be resident (Hall 1996). It is reported as being indistinguishable from *dominica* (Curson et al. 1994) except in having a longer and more slender bill (14.0-17.0 mm, sexes combined; Sutton 1951). There is also a migratory long-billed form on the Delaware-Maryland-Virginia (Delmarva) peninsula (Ficken et al. 1968) that is reported to be indistinguishable from *stoddardi* in appearance (Stevenson and Anderson 1994) from which it is separated by over 1400 km.

Distributions of eastern and western morphological subspecies of the Yellow-throated Warbler correspond spatially to a phylogeographic break reported for several vertebrate species including the Carolina Chickadee (*Poecile carolinensis*)(Chapter 1). Thus, in a recent evolutionary study of the Yellow-throated Warbler, a genetic partition was assumed *a priori* to correspond to the observed morphological partition, and it was hypothesized that the Yellow-throated Warbler fit temporally into this comparative phylogeographic framework (Chapter 1). Surprisingly, however, mitochondrial DNA (mtDNA) control region sequences indicated a pronounced lack of differentiation between Yellow-throated Warbler subspecies (Chapter 1). Potential causes for this discrepancy are that morphological traits have evolved faster than mtDNA or that morphological subspecies in the Yellow-throated Warbler do not correspond to evolutionary lineages. As there has been no range-wide quantitative assessment of phenotypic variation in the Yellow-throated Warbler, it is unknown whether

variation in extent of yellow coloration in the face and variation in bill properties vary discretely or gradually across the species' range. Here I reassess the morphological subspecies of the Yellow-throated Warbler in an effort to quantify their differences and examine if and how differences relate to geography.

## **METHODS**

### *Samples and measurements*

A total of 89 specimens from 10 populations located across the Yellow-throated Warbler's breeding range were collected during the breeding season (April and May) of 2006 (Table 1; Fig. 1). All of these specimens were also included in the phylogeographic study of this species (Chapter 1). Seven morphological characters (6 body and 1 plumage), including all of those used to differentiate subspecies (i.e. bill length, bill width, and proportion of yellow in lore), were measured. These characters were: bill length, bill width, bill depth (all at the anterior edge of the nares), tarsus length, wing cord, tail length (at center rectrix), and proportion of yellow area of lore, hereafter "proportion of yellow in lore" (see below). Bill measurements and tarsus length were measured to the nearest 0.01 mm using a digital caliper and wing cord and tail length were measured to the nearest 0.5 mm using a wing rule. Proportion of yellow in lore was quantified as follows. Close-up photographs of the lore of each Yellow-throated Warbler specimen were loaded into the program ImageJ for Windows<sup>TM</sup> (available at <http://rsb.info.nih.gov/ij/>) and the area of the total lore, considered the white or yellow area from the posterior end of the bill to the most anterior point of the eye, was measured. Using the same procedure, I then measured the area of the yellow within the lore. The yellow area of the lore was divided by the total lore area to estimate the proportion of yellow within the lore. Due to damage sustained during collection, 14% of the

specimens had some missing data, but this accounted for no more than 6% of missing data for any variable. In addition, bill measurements (length, width, and depth) from 52 male museum specimens collected between 15 March and 30 June were included in subspecies' level analyses, but were not assigned a population. In all analyses, only males were used. All measurements were made by myself; for tarsus length and all bill characters, the mean of three measurements was used.

### *Statistical analyses*

SPSS 12.0 for Windows™ (SPSS Inc. 2003) was used for all statistical analyses, and a p-value  $\leq 0.05$  was considered statistically significant. I tested each morphological character for departures from normality. I then determined two-tailed Pearson correlation coefficients for all pairwise comparisons between body characters. Next, I compared the means of each character using an analysis of variance (ANOVA). Two ANOVAs were run: the first with the samples grouped by population and the second with the samples grouped by subspecies (*dominica*, *albilora*, or *stoddardi*). A Tukey HSD post hoc test was used to determine maximum homogeneous groupings of populations and of subspecies for each character. The two characters most diagnostic of subspecies, bill length and proportion of yellow in lore, were plotted to determine whether they formed discrete clusters.

To further evaluate the distinctiveness of each subspecies, I grouped specimens by subspecies and applied a stepwise discriminant function analysis (DFA) using all seven morphological characters. Prior probabilities were computed from group sizes, and missing values were replaced with the mean for that character. The leave-one-out method was used to cross-validate the accuracy of the group assignments. Finally, to check for patterns between characters and geography, I computed two-tailed Pearson correlation coefficients for all

pairwise comparisons between both latitude and longitude for all characters.

## RESULTS

All data conformed to normality. Two sets of size measurements, among the six size variables measured, were significantly correlated: bill width and depth ( $r = -0.60$ ;  $n = 133$ ;  $p < 0.001$ ) and wing cord and tail length ( $r = 0.36$ ;  $n = 83$ ;  $p < 0.001$ ). ANOVA indicated the following five characters differed significantly between populations (Table 2): bill length ( $F = 16.8$ ;  $df = 85$ ;  $p < 0.001$ ), bill width ( $F = 12.9$ ;  $df = 85$ ;  $p < 0.001$ ), bill depth ( $F = 8.1$ ;  $df = 80$ ;  $p < 0.001$ ), tail length ( $F = 2.1$ ;  $df = 87$ ;  $p < 0.041$ ), and proportion of yellow in lore ( $F = 18.5$ ;  $df = 87$ ;  $p < 0.001$ ), and the following four characters differed significantly between subspecies: bill length ( $F = 12.9$ ;  $df = 137$ ;  $p < 0.001$ ), bill width ( $F = 7.0$ ;  $df = 137$ ;  $p < 0.001$ ), wing cord ( $F = 3.4$ ;  $df = 82$ ;  $p < 0.04$ ) and proportion of yellow in lore ( $F = 25.7$ ;  $df = 87$ ;  $p < 0.001$ ). The Tukey HSD test revealed overlap between all homogeneous groups in all characters differing significantly between both populations and subspecies, and, thus, no exclusive groups were identified. Bill length plotted against proportion of yellow in lore resulted in overlap between all subspecies (Fig. 3).

Discriminant function analysis produced a final model with one function (eigenvalue = 0.64) and, of the seven characters, included only proportion of yellow in lore. The overall Wilk's lambda was significant ( $\lambda = 0.61$ ,  $\chi^2 = 31.0$ ,  $n = 89$ ,  $p < 0.001$ ). Classification and cross-validation both indicated that 66% of all individuals were assigned to the correct subspecies (Table 3). Removing *stoddardi* did not affect the classification results of *dominica* or *albilora*. The majority (73%) of incorrectly assigned individuals were collected near the *dominica-albilora* border in Alabama, Ohio, and North Carolina.

Four characters were significantly correlated with geography. Bill length ( $r = -0.41$ ;  $n$

= 138;  $p < 0.001$ ; Fig. 4a) and proportion of yellow in lore ( $r = -0.57$ ;  $n = 87$ ;  $p < 0.001$ ; Fig. 4b) were both negatively correlated with longitude. Tail length ( $r = 0.37$ ;  $n = 88$ ;  $p < 0.001$ ; Fig. 4c) and bill depth ( $r = 0.22$ ;  $n = 133$ ;  $p < 0.01$ ; Fig. 4d) were both positively correlated with latitude, and proportion of yellow in lore ( $r = -0.32$ ;  $n = 87$ ;  $p < 0.005$ ) was negatively correlated with latitude.

## DISCUSSION

Results from ANOVA indicate that there are significant differences in bill length, bill width, wing cord, and proportion of yellow in lore when samples are grouped by subspecies. There are also significant differences in several characters when samples are grouped by population. Post hoc tests, however, do not place populations into exclusive groups that correspond to subspecies and, instead, indicate that populations are more similar to their nearest geographical neighbor than they are to their subspecies. A most common, albeit arbitrary, cutoff point used to define subspecies in ornithology is 75% diagnosability (Amadon 1949), and discriminant function analysis fails to assign at least 75% of either *albilora* or *stoddardi* individuals to the correct subspecies, suggesting there is only one continental Yellow-throated Warbler subspecies. The DFA correctly groups individuals from extreme eastern or western populations into subspecies but fails to correctly assign most *albilora* individuals from the more central Ohio and western Alabama populations or some *dominica* individuals from the more central North Carolina and eastern Alabama populations. Bill length and proportion of yellow in lore both gradually increase from west to east and do not show a sharp break, which would be indicative of discrete groups. Plotting bill length and proportion of yellow in lore together results in an undifferentiated cluster of points.

Though *stoddardi* birds have longer bills on average than *dominica* birds, DFA fails

to distinguish *stoddardi* and assigns all of its individuals to *dominica*. The longer-billed birds on the Delmarva peninsula do not differ significantly from the nearby population on the western shore of Maryland. Thus, the three longest billed populations that I sampled were also the three populations within 30 km of the coast. This supports a previous study that suggested shorter-billed Yellow-throated Warbler populations were more prevalent inland where they may be more associated with deciduous forests and longer-billed forms are prevalent in coastal areas with long-coned pine forests (Ficken et al. 1968). This suggests *stoddardi* may be an isolated example of what is a common form along the Atlantic coast. This may be, as suggested by Ficken et al. (1968), an adaptive response to more specific coastal habitat perhaps in part due to competition with pine Warblers (*Dendroica pinus*).

Bill shape differences between avian subspecies can be highly heritable (e.g. Schluter and Smith 1986) or greatly influenced by the rearing environment (e.g. James 1983). Transplant experiments with the Yellow-throated Warbler, such as those performed by James (1983) with Red-winged Blackbirds (*Agelaius phoeniceus*), could detect phenotypic plasticity in Yellow-throated Warbler bill shape. Lore color might also be heavily influenced by the environment. Carotenoids are probably responsible for the Yellow-throated Warbler's yellow throat and lore, and increased carotenoid consumption can cause yellows and reds to bleed into other parts of a bird's plumage (Hill 2002). Because eastern and western Yellow-throated Warblers differ in their primary breeding habitat, it is likely they ingest different levels of carotenoids. A higher carotenoid diet in *dominica* may make it more likely to allocate carotenoids to the lore. Blue tits (*Cyanistes caeruleus*) obtain more carotenoids in a deciduous versus a coniferous forest, which is the opposite of what would be expected in the Yellow-throated Warbler (Partali et al. 1987). Because it is unknown exactly how the

Yellow-throated Warbler's diet differs between eastern and western subspecies, it remains possible that the observed variation in lore color results from different diets.

Overall, my observations indicate that there is much overlap in the morphological characters used to distinguish Yellow-throated Warbler subspecies. Average differences can distinguish subspecies when individuals from across the subspecies' range are included, but average differences have been argued to be insufficient for diagnosing subspecies (Patten and Unitt 2002). The clinal change in both bill length and proportion of yellow in lore is consistent with an environmental component to these characters though more study is needed. The failure of these analyses in identifying discrete groups within the Yellow-throated Warbler corroborates earlier mtDNA surveys that indicated Yellow-throated Warbler subspecies were not evolutionary significant units (*sensu* Moritz 1994). While there is an interesting pattern of phenotypic variation within the Yellow-throated Warbler, there is probably little value in subdividing clinal continuums into different subspecies (Rising 2007). Therefore, I recommend eliminating the *albilora* subspecies. It is also clear that some avian subspecies were described with insufficient evidence and do not correspond to evolutionary lineages (e.g. Pruett et al. 2004). This seems to be the case with *stoddardi* as it can not be reliably diagnosed and there is no evidence that it is different from other long-billed forms common along the Atlantic coast. Therefore, I also recommend eliminating the subspecies *stoddardi*.

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**Table 1.** Numbers of male Yellow-throated Warblers examined in different parts of the species' range. Map numbers refer to numbers plotted on the map in Fig. 1.

Map No.	Population	State	Subspecies	<i>n</i>	Locality
1	LA	Louisiana	<i>albilora</i>	4	Pointe Coupee Parish
2	MO	Missouri	<i>albilora</i>	11	Oregon, Ripley Co.
3	AL-West	Alabama	<i>albilora</i>	8	Lawrence Co.
4	AL-East	Alabama	<i>dominica</i>	12	Conecuh, Macon, Talladega Co.
5	FL-West	Florida	<i>stoddardi</i>	9	Wakulla Co.
6	OH	Ohio	<i>albilora</i>	12	Lawrence Co.
7	NC	North Carolina	<i>dominica</i>	9	Graham Co.
8	FL-East	Florida	<i>dominica</i>	9	Marion Co.
9	MD-West Coast	Maryland	<i>dominica</i>	7	Charles, Prince George, St. Mary's Co.
10	MD-Delmarva	Maryland	<i>dominica</i>	8	Worcester Co.

**Table 2.** Morphological and plumage character measurements (mean  $\pm$  SD) and ANOVA results for ten populations of Yellow-throated Warbler. See Table 1 for detailed population information.

Character	LA	MO	AL-West	AL-East	FL-West	OH	NC	FL-East	MD-West	MD-Delmarva	ANOVA F-value
Bill length (mm)	9.16 $\pm$ 0.40	9.04 $\pm$ 0.56	9.57 $\pm$ 0.31	9.35 $\pm$ 0.35	10.07 $\pm$ 0.44	9.17 $\pm$ 0.41	9.26 $\pm$ 0.30	9.45 $\pm$ 0.32	10.45 $\pm$ 0.68	10.91 $\pm$ 0.4	16.8**
Bill width (mm)	2.82 $\pm$ 0.13	3.47 $\pm$ 0.12	2.86 $\pm$ 0.13	3.44 $\pm$ 0.54	3.35 $\pm$ 0.27	2.84 $\pm$ 0.14	2.86 $\pm$ 0.11	2.78 $\pm$ 0.22	2.82 $\pm$ 0.15	2.82 $\pm$ 0.01	12.9**
Bill depth (mm)	3.15 $\pm$ 0.09	3.57 $\pm$ 0.16	3.23 $\pm$ 0.11	3.36 $\pm$ 0.19	3.39 $\pm$ 0.14	3.18 $\pm$ 0.15	3.20 $\pm$ 0.01	3.16 $\pm$ 0.11	3.24 $\pm$ 0.08	3.27 $\pm$ 0.15	8.1**
Tarsus length (mm)	17.3 $\pm$ 0.4	16.7 $\pm$ 0.6	16.8 $\pm$ 0.3	16.9 $\pm$ 0.8	16.7 $\pm$ 0.3	16.6 $\pm$ 0.3	16.6 $\pm$ 0.5	16.8 $\pm$ 0.4	16.9 $\pm$ 0.6	17.2 $\pm$ 0.4	1.7
Wing cord (mm)	67.4 $\pm$ 2.3	67.8 $\pm$ 2.1	67.1 $\pm$ 1.2	68.2 $\pm$ 0.6	66.2 $\pm$ 1.4	67.0 $\pm$ 1.8	67.4 $\pm$ 1.5	66.8 $\pm$ 1.2	67.7 $\pm$ 0.8	67.8 $\pm$ 1.9	1.4
Tail length (mm)	48.4 $\pm$ 1.8	49.9 $\pm$ 1.5	50.0 $\pm$ 1.9	48.9 $\pm$ 1.1	48.4 $\pm$ 1.7	49.8 $\pm$ 1.5	49.1 $\pm$ 0.8	48.8 $\pm$ 1.1	49.7 $\pm$ 0.5	50.4 $\pm$ 1.3	2.1*
Proportion of yellow in lore	0.26 $\pm$ 0.45	0.11 $\pm$ 0.05	0.65 $\pm$ 0.12	0.65 $\pm$ 0.18	0.71 $\pm$ 0.07	0.38 $\pm$ 0.27	0.55 $\pm$ 0.10	0.84 $\pm$ 0.09	0.72 $\pm$ 0.10	0.76 $\pm$ 0.12	18.5**

\* P<0.05; \*\* P<0.001

**Table 3.** Predicted classification of Yellow-throated Warbler subspecies based on stepwise discriminant function analysis of seven morphological characters.

	Predicted: <i>dominica</i>	Predicted: <i>albilora</i>	Predicted: <i>stoddardi</i>	Correct classification (%)
<i>dominica</i>	40	5	0	89
<i>albilora</i>	16	19	0	54
<i>stoddardi</i>	9	0	0	0

## FIGURE CAPTIONS

**Figure 1.** Breeding range of the Yellow-throated Warbler (shaded area; adapted from Dunn and Garrett 1997) and geographic locations of sampled population in this study (circles). The pie chart represents the average proportion of yellow in lore for that population. The size of the circles is proportional to average bill size for that population. Numbers correspond to populations in Table 1. The light gray area represents the range of *albilora*. Light blue area represents the range of *dominica*. Dark gray area represents the range of *stoddardi*. Numbers along the x-axis are longitude; numbers along the y-axis are latitude.

**Figure 2.** Boxplots comparing significantly different (A) bill lengths and (B) proportion of yellow in lore between ten populations of the Yellow-throated Warbler. Horizontal lines in box plots show 10<sup>th</sup>, 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles. See Table 1 for detailed population information.

**Figure 3.** Graph of the two most discriminating characters of Yellow-throated Warbler subspecies: bill length and proportion of yellow in lore. Open circles represent *dominica*, closed squares represent *albilora*, and stars represent *stoddardi* subspecies.

**Figure 4.** Some significant correlations between morphological characters of the Yellow-throated Warbler and geography. Open circles represent *dominica*, closed squares represent *albilora*, and stars represent *stoddardi* subspecies.



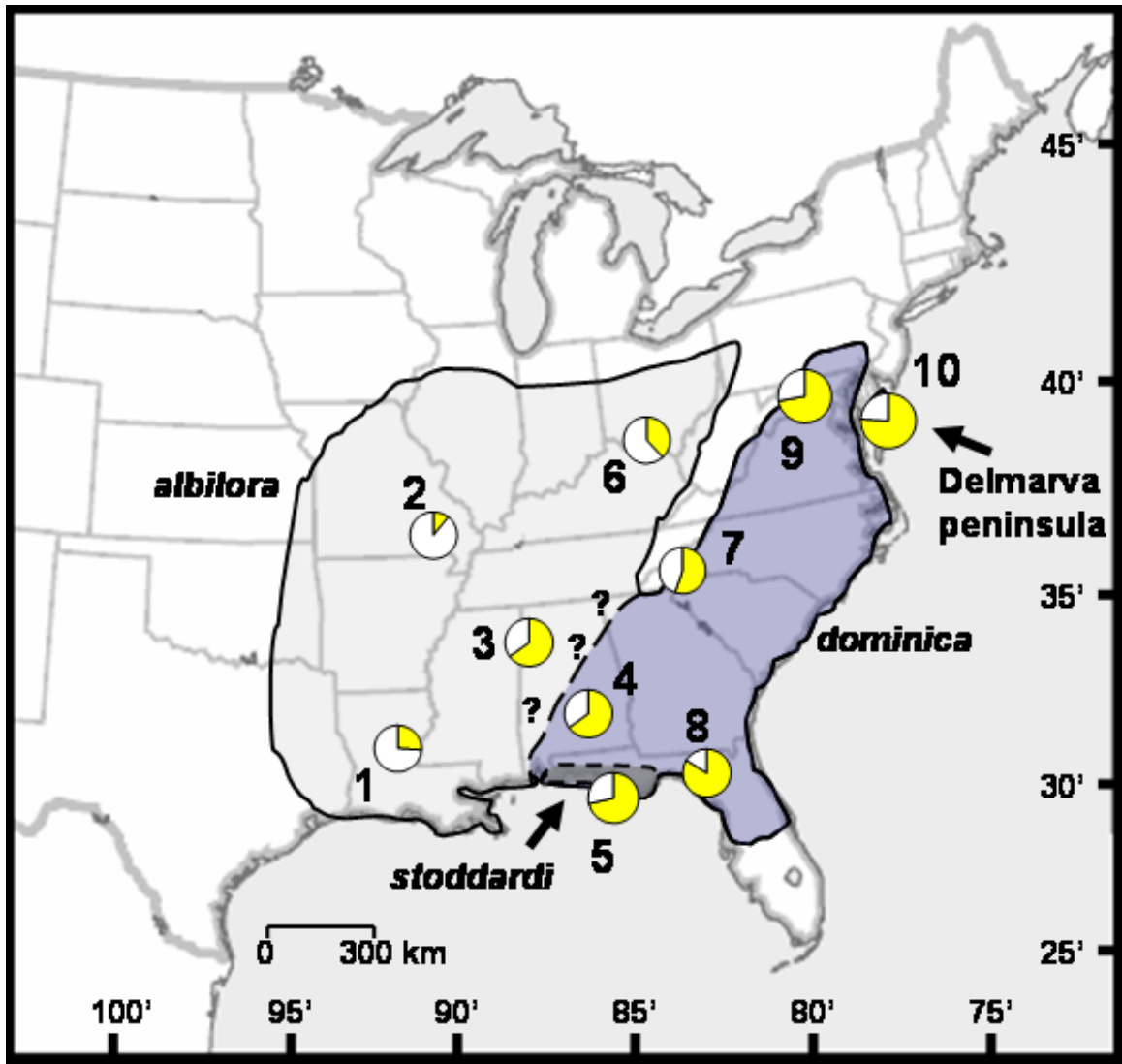


Figure 1.

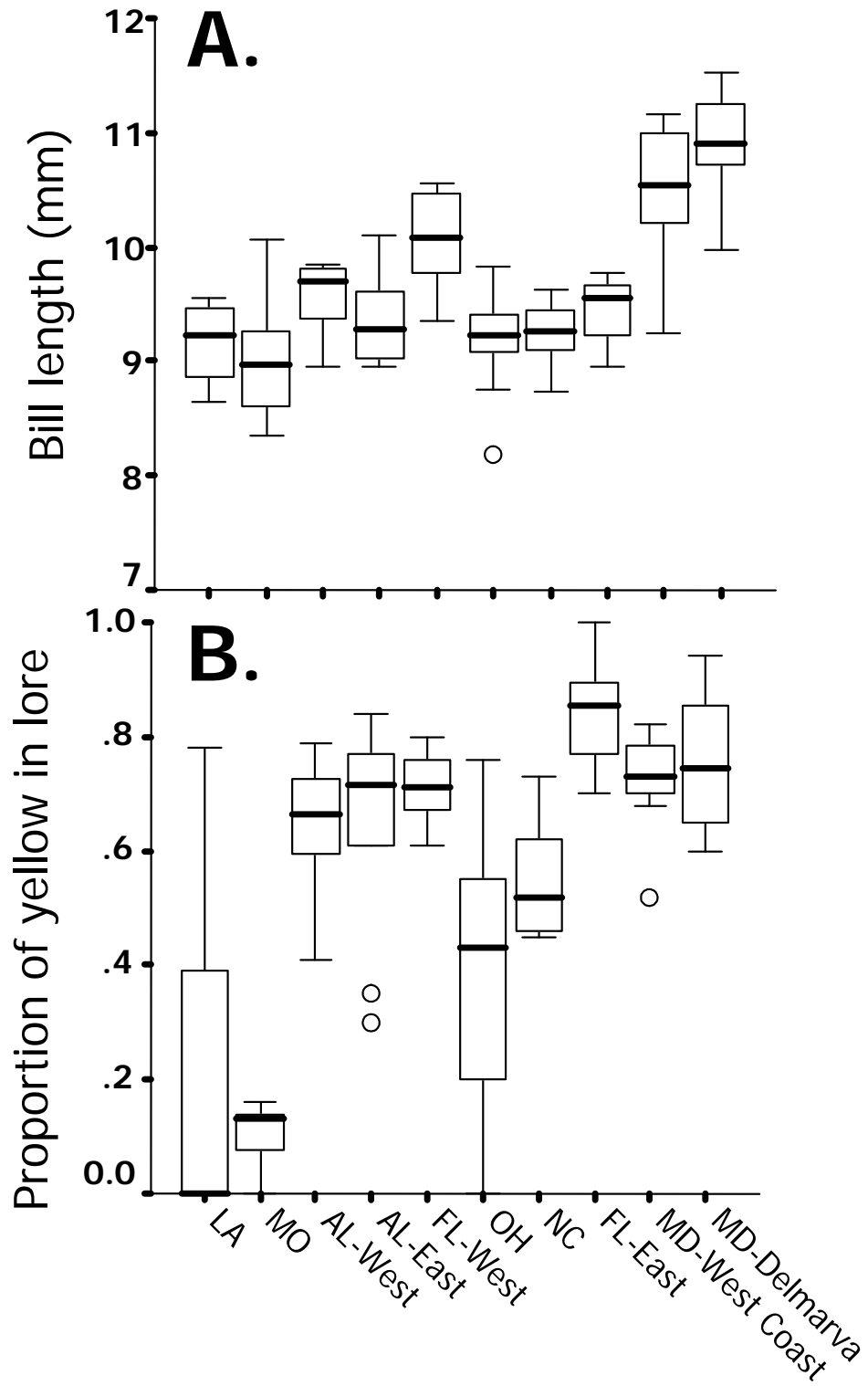


Figure 2.

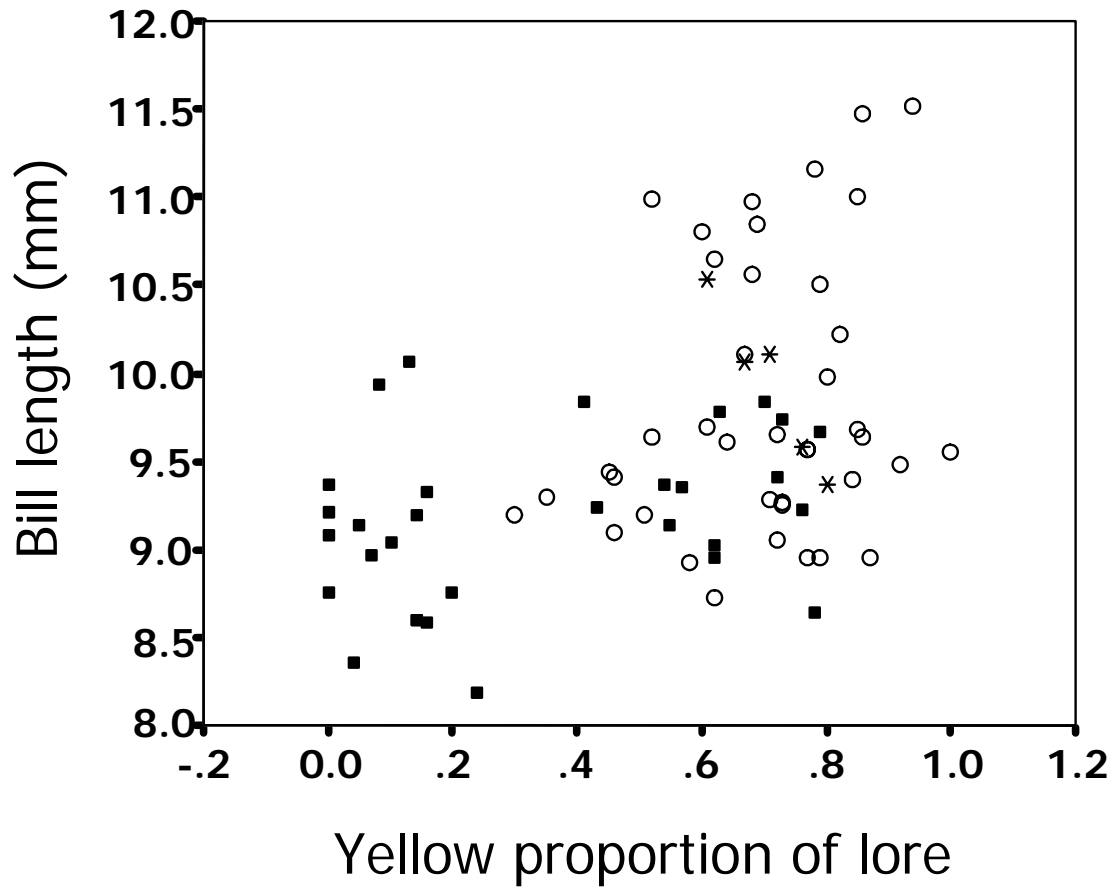


Figure 3.

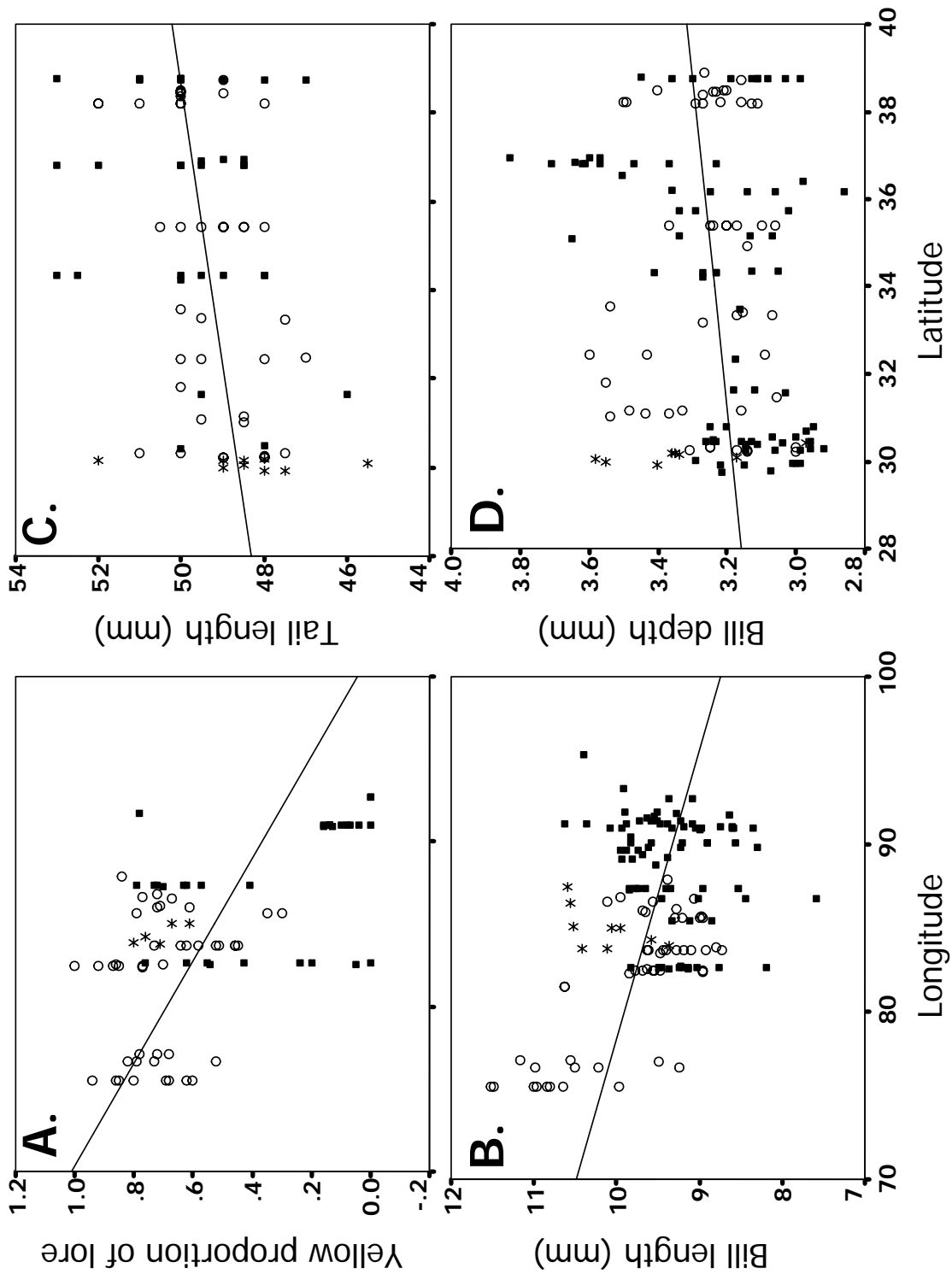


Figure 4.