

# Geographic Variability in Mitochondrial Introgression Among Hybridizing Populations of Golden-winged and Blue-winged Warblers

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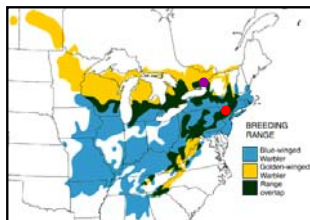
## ABSTRACT

The Golden-winged Warbler (*Vermivora chrysoptera*) is of conservation concern because of its current rapid population decline throughout the northeastern U.S. The advancement of Blue-winged Warblers (*V. pinus*) into historically Golden-winged habitats and the hybridization of the two species in areas of sympatry may play a role in the decline of Golden-winged populations. In this study, we obtained mitochondrial DNA (mtDNA) sequences from 104 individuals of known phenotype from two study sites that have different histories of contact. We surveyed the relationships between phenotype and mtDNA haplotype in individuals of each population and found that there is variation between sites in terms of the extent of introgression. This comparison suggests that sites with different histories of contact may experience differing levels and patterns of hybridization and subsequent introgression.

## INTRODUCTION

The Golden-winged Warbler (*Vermivora chrysoptera*) has declined throughout the northeastern U.S. for more than 30 years and is of high conservation concern as a result. The breeding range of the Golden-winged Warbler has expanded to the northwest and contracted along its southern and northeastern limits. Its sister species, the Blue-winged Warbler (*V. pinus*), has also expanded to the north and northeast and often encroaches on the southern limit of the Golden-winged Warbler. Where these species overlap in breeding range, they produce hybrid individuals. Sympatric populations may have 7 to 15% hybrid phenotypes including the typical "Brewster's" and the far less common "Lawrence's" hybrid types. Habitat loss may be a major cause of population decline and range shift, but hybridization may also play a role. When Blue-winged Warblers expand their range into Golden-winged populations, Golden-winged Warblers typically disappear from the area within 50 years. Previous research on hybridization in a region of Golden-winged decline revealed rapid, asymmetric introgression of mitochondrial DNA (mtDNA) from Blue-winged Warblers into phenotypic Golden-winged Warblers (Gill 1997). This study aims to expand on Gill's work by surveying mitochondrial introgression at additional sites with different histories and stages of contact between the two species.

Figure 1: Breeding ranges of Blue-winged and Golden-winged Warblers



Golden-winged Warbler Atlas Project, Cornell Lab of Ornithology  
Adapted from A Field Guide to Warblers of North America. 1997 Houghton Mifflin Co.

## METHODS

### Populations Sampled

- Sterling Forest State Park, NY (SFSP): History of coexistence; both Golden-winged and Blue-winged Warblers have coexisted here for about a century, a virtually unique circumstance. Hybrid individuals have been sighted here for over 30 years, but have probably been present since the 2 species came into contact
- Queen's University Biological Station, Ontario, Canada (QUBS): History of separation; Golden-winged Warblers arrived in the area in the mid 1900's and existed in isolation until the mid 1990's, when hybrid individuals were first sighted in the area. Blue-winged Warblers were sighted in the late 1990's in low frequency and are expected to increase in numbers in the near future
- 104 individuals total: 45 from SFSP, 59 from QUBS (70 Golden-winged Warblers, 15 Blue-winged Warblers, 14 "Brewster's" Warblers, 5 "Lawrence's" Warblers)

### Laboratory Techniques

- Extracted whole genomic DNA from blood samples collected in the field
- Performed polymerase chain reaction (PCR) to amplify NDII gene of mitochondrial genome (1041 bp)
- Generated sequence from 690 bp region of NDII gene via dideoxy-terminator cycle sequencing and electrophoresis on an ABI 3100 automated sequencer

### Introgression Analysis

- Aligned sequences using Sequencher software (Gene Codes Corp. 1998) and checked for sequencing artifacts and errors
- Estimated genetic distances between unique haplotypes in PAUP\* (Swofford 2002)
- Created a gene genealogy or network of each haplotype that summarizes the relationships between haplotypes using TCS 1.13 (Clement et al. 2000)

## RESULTS

We used sequence data to identify 28 unique NDII haplotypes among the 104 surveyed individuals.

A parsimony-based network depicting the relationships among these 28 unique haplotypes is shown in figure 2.

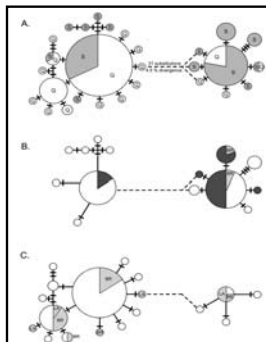
These results suggest the presence of two distinct haplotype groups: Nucleotide variation within each group is modest (1-6 nucleotide substitutions), whereas a much larger degree of mitochondrial divergence (31-37 substitutions) separates the two haplotype clusters.

We presume that these clusters represent the ancestral Golden-winged (AGW) and ancestral Blue-winged (ABW) haplotype groups for several lines of reasoning:

- The magnitude of divergence between the two groups is similar to the findings of Gill's RFLP-based survey of Blue-winged and Golden-winged populations
- Each haplotype cluster had a predominant phenotype that was geographically consistent with the respective distributions of the two species
- The site that has historically been isolated from Blue-Winged populations shows high correlation between Golden-Winged phenotype and the haplotype group we have inferred to be the AGW group.

Table 1 summarizes the phenotype-haplotype relationships at each site.

Figure 2. mtDNA haplotype networks for two mixed Blue-winged/Golden-winged Warbler populations.



Each unique haplotype is indicated by a circle with an area proportional to the number of sampled individuals with that haplotype. Hatch marks along branches represent single nucleotide substitutions. The left-hand haplotype cluster represents the assumed ancestral Golden-winged group (AGW), and the right-hand cluster the assumed ancestral Blue-winged group (ABW). A. Pooled samples from both study sites: shaded regions represent individuals from Sterling Forest State Park (SFSP) and white regions represent individuals sampled at Queen's University Biological Station (QUBS). B. Subset of haplotypes found at SFSP. C. Subset of haplotypes found at QUBS. In parts B and C, haplotype positions correspond to the pooled sample in A, and white areas represent phenotypic Golden-winged Warblers, black areas represent phenotypic Blue-winged Warblers, and shaded areas represent hybrid phenotypes (BR indicates Brewster's hybrid phenotype and LA Lawrence's phenotype).

Table 1. Relationships between phenotype and mtDNA haplotype among individuals sampled at Sterling Forest State Park, NY and Queen's University Biological Station, Ontario, Canada. Figures in each column represent number of individuals.

Phenotype	Haplotype Group			
	Sterling Forest State Park		Queen's University Biological Station	
	ABW*	AGW**	ABW	AGW
Blue-winged	13	2	—	—
Golden-winged	12	16	4	38
Brewster's hybrid	2	0	1	11
Lawrence's hybrid	—	—	2	3
*Ancestral Blue-winged haplotype group				
**Ancestral Golden-winged haplotype group				

## DISCUSSION

Different populations of Golden-winged and Blue-winged Warblers show different patterns of mitochondrial introgression. This is most likely due to different stages of contact between the two species and intrinsic differences between sites. While Gill (1997) observed near-total introgression of Blue-winged mtDNA into Golden-winged phenotypes, our results indicate that this phenomenon is not found at all sites.

There is evidence of bi-directional introgression of mtDNA, which could indicate that Blue-winged Warblers may not always genetically displace Golden-winged Warblers as previous research suggested.

SFSP has had a mixed population of both Blue-winged and Golden-winged Warblers for a century, and there are still two distinct haplotype groups present at this site. Thus, genetic segregation has been fairly strong. However, there is still the overall tendency for genetic loss of Golden-winged mtDNA. We do not know if the current level of introgression is stable or still increasing. These findings have conservation implications for both SFSP and Golden-winged and Blue-winged Warblers in general.

## REFERENCES

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