ALLOZYMES AND GENETIC SIMILARITY OF BLUE-WINGED AND GOLDEN-WINGED WARBLERS

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ABSTRACT.—I studied variation in plumage color and allozymes in Golden-winged Warblers (*Vermivora chrysoptera*) from Minnesota and in Blue-winged Warblers (*V. pinus*) from Missouri and from eastern Pennsylvania. Color character scores were less than 2 in Missouri *pinus* and greater than 26 in Minnesota *chrysoptera*. The level of genetic divergence ($D = 0.001$) is comparable to that of conspecific populations of other passerine birds. I found no fixed alleles that could serve as genetic markers for studies of the evolutionary genetics of these warblers.
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HYBRIDIZATION between Blue-winged Warblers (*Vermivora pinus*) and Golden-winged Warblers (*V. chrysoptera*) has interested evolutionary biologists for 100 years, first as a perplexing taxonomic problem and an exercise in Mendelian genetics (Sage 1889, Faxon 1913, Parkes 1951), then as testimony to the process of allopatric speciation (Mayr 1942, 1963; Short 1963; Gill and Murray 1972a), and recently as a problem of behavior and ecology (Ficken and Ficken 1967, 1968a–d, 1969; Gill and Murray 1972a, b; Murray and Gill 1976; Gill 1980; Confer and Knapp 1981; Will 1986). The two warblers hybridize wherever *pinus*, which is expanding its range northward, encounters *chrysoptera*, a northern probable sister species (Short 1963, Gill 1980). The composition of local populations changes from pure *chrysoptera* initially, to mixtures of both species with a variety of hybrid and backcross phenotypes, and then to introgressed *pinus*. Despite past ornithological interest in these warblers, the genetic consequences of hybridization remain to be explored.

To establish a baseline of information on introgression, I surveyed morphological and allozyme variability in allopatric populations of these species. I used *chrysoptera* from north-central Minnesota and *pinus* from Missouri and from southeastern Pennsylvania.

METHODS

Warblers were collected in Minnesota ($n = 29$), Missouri ($n = 20$), and Pennsylvania ($n = 28$). Most were breeding males; a few were breeding females or young fledglings. The specimens of *chrysoptera* from two localities 150 km apart in north-central Minnesota (Hubbard Co. near Itasca State Park and Kanebac Co., Mille Lacs Wildlife Area) were combined into a single sample. The specimens from Itasca State Park were from the northwest corner of the range of this species and 300 km northwest of the nearest known *pinus* population in Minnesota (B. A. Fall pers. comm., Parmelee 1977). The specimens from Mille Lacs Wildlife Area were from a population located 80 km north of the nearest known breeding *pinus* (Fall pers. comm.).

Missouri *pinus* were collected in the eastern Ozark Mountains in Shannon and Oregon counties. Missouri was part of the original range of *pinus* (Short 1963, Gill 1980), and the Ozark Mountains of southern Missouri and northern Arkansas, in particular, may be the ancestral home of this warbler. To the best of our knowledge, *chrysoptera* has never bred in this region (Widmann 1907, M. B. Robbins pers. comm.). Pennsylvania *pinus* were collected in extreme northeastern Lancaster Co. in the southeastern part of the state. *Vermivora pinus* has been present as a breeding species in southeastern Pennsylvania for over a century (Stone 1894, Gill 1980), whereas *chrysoptera* is a migratory transient.

All specimens were frozen on dry ice or liquid nitrogen (Missouri) within 1 h of collection. The frozen specimens were transferred to storage at −70°C before extraction of tissues and preparation as study skins, which are deposited in the collections of The Academy of Natural Sciences of Philadelphia and the Bell Museum of Natural History of the University of Minnesota. I analyzed color characters of adult male specimens following established procedures (Gill and Murray 1972a, Gill 1980). The series of skins of *chrysoptera* taken in Hubbard Co., Minnesota, in 1984 and Clearwater Co., Minnesota, in 1986 was supplemented with 14 specimens from the Bell Museum of Natural History.

Electrophoretic analyses of enzymes present in the tissues of warblers collected in 1984 and 1985 were conducted at the Evolutionary Genetics Laboratory of

Cornell University (CLEEG). Horizontal starch-gel electrophoresis and genic nomenclature followed the techniques of May et al. (1979). An electric potential of 200–250 V (less than 90 mA) was applied until a dye marker had migrated 3–8 cm from the origin; optimal migration distances for a given buffer system varied among the proteins. Before the actual survey of allozyme variability, 48 enzymes from muscle, heart, liver, and brain tissues were screened using four standard buffers (Appendix). Allelic compositions were determined at 40 presumptive loci (Appendix). In addition to the two species of *Vermivora*, enzyme activity at the same loci in 3 specimens of Yellow-rumped Warblers (*Dendroica coronata coronata*) served as an outgroup.

I used the computer package BIOSYS-1 to calculate indices of genetic variability, conformance to Hardy-Weinberg expectations, *F* statistics of Wright (1978), and genetic distance estimates of Nei (1978).

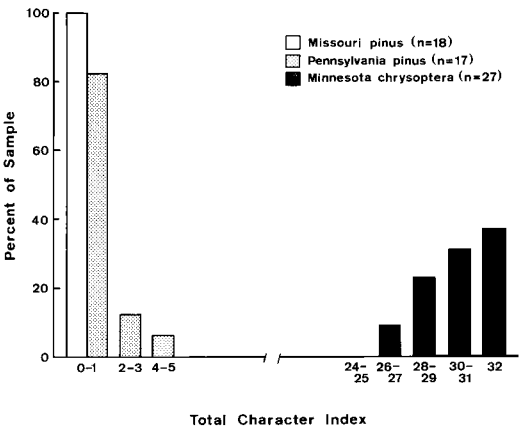

Fig. 1. Color variation in samples of *Vermivora pinus* from Missouri and Pennsylvania and *V. chrysoptera* from northern Minnesota. All *chrysoptera* specimens had black throats; all *pinus* had plain-colored throats.

Results

Morphological comparisons.—All specimens in the three samples conformed to typical *pinus* or typical *chrysoptera* phenotypes. Total color character scores were either less than 5 at the *pinus* end of the character index spectrum or greater than 26 at the *chrysoptera* end (Fig. 1). Departures from extreme character states, therefore, were subtle ones, such as traces of yellow evident only upon close examination and comparison.

Minnesota *chrysoptera* exhibited some variation in plumage coloration. Total color character scores ranged from 27 to 32, reflecting traces of yellow pigmentation, primarily in the color of the upperparts, less often in the color of the underparts, and in a few individuals, reflecting slight separation of wing bars or pale wing-bar coloration. Seven individuals had some (6) or much white (1) on the chin.

Missouri *pinus* scored 0 or "pure" *pinus* in all color characters except wing-bar color, in which half of the specimens had small amounts of yellow (character state 1). Thus, total color character scores were divided equally between 0 and 1. Most (11/17) Pennsylvania *pinus* had slight yellow in the wing bars, and a few (3) individuals had broadened wing bars or gray in the rump feather coloration. Total character scores ranged from 0 to 4. One individual had some black pigment in the auriculars, which suggested the black ear patch of *chrysoptera*. There also was a more subtle color difference between the two *pinus* populations. Missouri specimens were more brightly colored than Pennsylvania specimens. This difference was more apparent in the intensity of the bright yellow underparts than in the shade of the yellow-green upperparts.

Allelic variation within populations.—Over all samples, 16 of the 40 loci (40%) surveyed were polymorphic. At 8 of these 16 loci, alternative alleles constituted less than 5% of the alleles in the population. Polymorphic loci constituted 25–35% of the loci within populations (Table 1). Missouri *pinus*, the population with the most polymorphic loci (35%), had rare alleles at 4 loci that were monomorphic in Pennsylvania *pinus*. All loci that were polymorphic in Pennsylvania *pinus* also were polymorphic in Missouri *pinus*. The levels of polymorphism in these *Vermivora* warblers are the same as those observed in passerine birds generally as well as in other wood warbler species (Barrowclough and Corbin 1978). Values of mean heterozygosity, 0.05–0.06, also conform to the average for birds (0.063 ± 0.032 SD; Barrowclough et al. 1985).

Allelic frequencies at variable loci conformed to Hardy-Weinberg equilibria with only one minor exception, a slight deficiency of heterozygotes at the Pgd locus in *chrysoptera* (exact probability = 0.026). Only striking departures would achieve significance in the Chi-square test with moderate-size samples. Wright's (1978) *F* statistics, specifically F_{is} (an index to the fix-

TABLE 1. Genetic variability at 40 loci in populations of *Vermivora chrysoptera* and *V. pinus*. Standard errors are given in parentheses.

Population	Mean sample size/locus	Mean no. of alleles/locus	% loci polymorphic[a]	Mean heterozygosity	
				Observed	Expected[b]
chrysoptera	20.0	1.4	27.5	0.052	0.058
	(0.0)	(0.1)		(0.017)	(0.020)
pinus					
Missouri	19.9	1.5	35.0	0.065	0.071
	(0.1)	(0.1)		(0.020)	(0.022)
Pennsylvania	20.9	1.3	25.0	0.059	0.060
	(0.1)	(0.1)		(0.020)	(0.021)

[a] A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.99.
[b] Unbiased estimate in Hardy-Weinberg equilibrium (see Nei 1978).

ation of alleles in individuals relative to the rest of its subpopulation, or departures from local panmixia), complement the tests of conformity to Hardy-Weinberg equilibria. Values of F_{is} for variable loci were low. The mean value of 0.002 + 0.028 (SE) was not significantly different from 0. I conclude that the warbler populations sampled were natural panmictic units without broad distortions of allelic frequencies.

Allelic variation between populations.—Alleles fixed or common in one species also were fixed or common in the other species. I found no electrophoretic markers for detailed studies of genetic introgression. Some distinguishing rare alleles were present at 9 loci in *pinus* (Pep-1, Pep-2, Ck2, Ada, Mpi, Tpi, Gdh, Gapdh, Pep-1), and at 3 loci in *chrysoptera* (Idh2, Pgm, Ada). These alleles, however, are too subject to sampling errors to be useful as markers.

Nei's (1978) D estimates the differentiation of populations or taxa in terms of the number of allelic substitutions since they had a common ancestor. The low value of $D = 0.001$ indicates *pinus* and *chrysoptera* are less distinct than conspecific populations of passerine birds on average ($D = 0.0024$; Corbin 1983). Comparison of *pinus* and *chrysoptera* with an outgroup species, the Yellow-rumped Warbler, yielded values of $D = 0.15$ (*pinus* vs. *coronata*) and $D = 0.16$ (*chrysoptera* vs. *coronata*), which falls within the range (0.13–0.16) obtained by Barrowclough and Corbin (1978) for several other species of *Vermivora* vs. *Dendroica coronata*.

Wright's statistic F_{st} provides a convenient measure of genetic differentiation among populations in terms of the progress toward fixation of alternative alleles in different populations (Barrowclough 1980, 1983). When *pinus* (Missouri, Pennsylvania) and *chysoptera* were treated as three populations of the same species, the mean F_{st} was 0.011 ± 0.0025 (SE), which indicates that only about 1% of the allelic variation is distributed among populations. The remainder resides within populations. This value is well within the range expected from intraspecific comparisons within passerines (Corbin 1983, Barrowclough 1983).

Missouri *pinus* and Pennsylvania *pinus* exhibited practically no genetic differentiation at the loci I examined ($D = 0.000$). Allelic frequencies at each locus were extremely similar, with only one slight disparity. The principal differences between these two populations reside in the presence or absence of rare alleles.

DISCUSSION

Northern Minnesota remains a home of "pure" *chrysoptera* north of expanding *pinus* populations. Minnesota *chrysoptera* exhibit some variation in the amount of yellow present in the plumage. Not all have pure gray upperparts or pure white underparts. The color and width of the wing bars vary slightly. Possibly, such variation is due to past hybridization with unnoticed pioneering *pinus*, but, with one exception, *pinus* is unknown at this locality. A female *pinus* tried but failed to pair with a male *chrysoptera* at Itasca in 1986 (T. Highsmith pers. comm.). Also, no "Brewster's Warbler" hybrid phenotypes, the first indicators of hybridization, have been recorded in these well-known populations. I conclude that the variation in plumage coloration evident in my sample is inherent in "pure" *chrysoptera*. I suspect that in-

dividuals with traces of yellow in the body plumage are first-year males, but studies of known-age birds are required to be certain.

Missouri *pinus* represent pure, and perhaps ancestral, phenotypes and genotypes. Traces of yellow in the wing bars evident in half of the specimens apparently reflect natural variation rather than introgression of *chrysoptera* genes (Short 1963, Gill and Murray 1972a). The status of the Pennsylvania *pinus* specimens examined is less certain than that of Missouri *pinus*. The variability in wing-bar color and width and particularly the presence of black in the auriculars of one specimen suggest some introgression of *chrysoptera* genes. The size characteristics of this population also were closer to Minnesota *chrysoptera* than to Missouri *pinus* (Gill unpubl. data). This could reflect unstudied geographical size variation in *pinus*.

Despite striking differences in color and song, *pinus* and *chrysoptera* are no more different in their level of enzyme differentiation than are morphologically indistinguishable populations of passerine birds. Additional efforts to locate diagnostic allozyme markers for studies of the evolutionary genetics of introgression in the *pinus* × *chrysoptera* complex are not likely to be rewarding, but alternative approaches, such as restriction enzyme analysis of mitochondrial DNA, may be productive (Mack et al. 1986).

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APPENDIX. Allozymic frequencies in Blue-winged and Golden-winged warblers. EC numbers are given in brackets.

Locus	*pinus*[a] PA	*pinus*[a] MO	*chrysoptera*	Tissue[b]	Buffer[c]
A. Polymorphic loci					
Adenosine deaminase (Ada) [3.5.4.4]					
1	0.690	0.575	0.800	M	S4
2	0.286	0.400	0.150		
3	0.0	0.0	0.05		
4	0.024	0.0	0.0		
5	0.0	0.025	0.0		
Creatine kinase-2 (Ck2) [2.7.3.2]					
1	1.000	0.975	1.000	H	CT
2	0.0	0.025	0.0		
Glucosephosphate isomerase (Gpi) [5.3.1.9]					
1	0.952	0.950	0.975	M	S4
2	0.024	0.0	0.025		
3	0.024	0.050	0.0		
Glutamate dehydrogenase (Gdh) [1.4.1.2–4]					
1	1.000	0.975	1.000	H	CT
2	0.0	0.025	0.0		
Glutathione reductase-1 (Gr1) [1.6.4.2]					
1	0.917	0.944	0.895	H	R
2	0.083	0.028	0.026		
3	0.00	0.028	0.079		
Glyceraldehyde-3-phosphate dehydrogenase-1 (Gapdh-1) [1.2.1.12]					
1	0.810	0.875	0.850	L	R
2	0.190	0.125	0.150		
Glyceraldehyde-3-phosphate dehydrogenase (Gapdh-2) [1.2.1.12]					
1	1.000	0.975	1.000	M	CT cath
2	0.0	0.025	0.0		
Isocitrate dehydrogenase-2 (Idh2) [1.1.1.42]					
1	1.000	1.000	0.975	L	CT
2	0.0	0.0	0.025		
Mannosephosphate isomerase (Mpi) [5.3.1.8]					
1	0.952	0.950	0.975	M	M
2	0.024	0.0	0.025		
3	0.024	0.050	0.0		
Nucleoside phosphorylase (Np) [2.4.2.1]					
1	0.357	0.425	0.525	L	M
2	0.643	0.550	0.475		
3	0.0	0.025	0.0		
Peptidase with glycyl-leucine (Pep-gl-1) [3.4.13.1]					
1	0.952	0.925	0.925	M	R
2	0.0	0.0	0.075		
3	0.048	0.075	0.0		
Peptidase with glycyl-leucine (Pep-gl-2) [3.4.13.1]					
1	0.952	0.950	1.000	M	R
2	0.048	0.050	0.0		
Peptidase with phenyl-alanyl-proline (Pep-Pap-1) [3.4.13.9]					
1	0.905	0.800	0.875	M	S4
2	0.071	0.100	0.075		
3	0.024	0.075	0.050		
4	0.0	0.025	0.0		
Phosphoglucomutase (Pgm) [2.7.5.1]					
1	1.000	1.000	0.975	M	R
2	0.0	0.0	0.025		
Phosphogluconate dehydrogenase-2 (Pgd) [1.1.1.46]					
1	0.690	0.775	0.700	H	CT
2	0.286	0.200	0.275		
3	0.024	0.025	0.025		

APPENDIX. Continued.

Locus	*pinus*[a] PA	*pinus*[a] MO	*chrysoptera*	Tissue[b]	Buffer[c]
Triosephosphate isomerase (Tpi) [5.3.1.1]					
1	1.000	0.975	1.000	H	CT cath
2	0.0	0.025	0.0		
B. Monomorphic loci					
α-mannisidase (α-man) [3.2.1.24]				L	M
Adenylate kinate-1,2 (Ak-1,2) [2.7.1.20]				M	CT
Aspartate aminotransferase (Aat) [2.6.1.1]				M	CT cath
Creatine kinase-1 (Ck-1) [2.7.3.2]				M	CT cath
Diaphorase-1 (Dia) [1.6.4.3]				L	S4 origin
Esterase with methylumbelliferyl butyrate (Est-b-3) [3.1.1.1]				L	R
General protein-7 (Pro-7)				M	R
General protein-8 (Pro-8)				M, H, L	CT, S4
Glutamate pyruvate transaminase-1 (Gpt-1) [2.6.1.2]				L	S4 cath
Glycerol-3-phosphate dehydrogenase-2 (G3p-2) [1.1.1.8]				H	R
Guanine deaminase (Gda) [3.5.4.3]				L	S4, M
Isocitrate dehydrogenase (Idh-1) [1.1.1.42]				L	CT
Lactate dehydrogenase-1,2 (Ldh-1,2) [1.1.1.27]				M	S4
Malate dehydrogenase-1,2 (Mdh-1,2) [1.1.1.37]				M	S4
Malic enzyme (Me) [1.1.1.40]				L	CT cath
Methylumbellifferyl phosphatase-1,2 (Mup-1,2) [3.1.3.-]				L	M
Peptidase with phenyl-alanyl-proline-2 (Pep-Pap-2) [3.4.13.1]				M	S4
Phosphoglucokinase (Pgk) [2.7.2.3]				M	S4
Phosphogluconate dehydrogenase (Pgd) [1.1.1.46]				M	S4
Sorbitol dehydrogenase (Sdh) [1.1.1.14]				L	S4
Superoxide dismutase-1 (Sod-1) [1.15.1.1]				M	R cath

[a] PA = Pennsylvania, MO = Missouri.

[b] M = pectoral muscle, L = liver, H = heart.

[c] R = pH 8.1, Ridgeway et al. (1970); CT = pH 6.1, May et al. (1979); M = pH 8.7, Markert and Faulhaber (1965); S4 = pH 6.3, adjusted from Selander et al. (1971).