18

THOMAS B. SMITH, SONYA M. CLEGG,
MARI KIMURA, KRISTEN C. RUEGG,
BORJA MILÁ, AND IRBY J. LOVETTE

Molecular Genetic Approaches to Linking Breeding and Overwintering Areas in Five Neotropical Migrant Passerines

DEMOGRAPHIC STUDIES OF NEOTROPICAL MIGRANT songbirds have been limited by the difficulty of following them through a complete annual cycle. As population regulation conceivably may occur in either the breeding or wintering areas, or on migration routes, determining levels of connectivity between breeding and wintering areas is fundamental to understanding the dynamics of Neotropical migrant populations. Recent studies have explored the potential for genetic markers to determine the breeding origin of migrating and overwintering birds. The utility of this method is dependent upon the level of geographic structure in a particular species. The finer the scale of geographic structure resolved by a particular genetic marker, the more useful it is in resolving breeding origins. We assessed the utility of mitochondrial DNA (mtDNA) markers in determining breeding origins of five long-distance Neotropical migrants: the Yellow-breasted Chat (*Icteria virens*), Nashville Warbler (*Vermivora ruficapilla*), Common Yellowthroat (*Geothlypis trichas*), Wilson's Warbler (*Wilsonia pusilla*), and Swainson's Thrush (*Catharus ustulatus*) and used and contrasted both mtDNA and microsatellite analyses in Wilson's Warbler. We assessed the extent of mtDNA phylogeographic structure and used these data to assign individuals captured on wintering sites in Mexico and Central and South America to their respective breeding areas. Genetic structure on the breeding grounds was found on a broad continent-wide scale for all five of these species, thus enabling the assignment of overwintering individuals to either eastern or western breeding lineages. Patterns of genetic divergence were not always in concordance with morphological subspecies definitions. The degree of admixture

of genetic lineages on overwintering grounds varied for each species, with high geographic segregation for the Yellow-breasted Chat and Swainson's Thrush and more geographic mixing of lineages for the Common Yellowthroat, Nashville Warbler, and Wilson's Warbler. The suggested distribution of morphological subspecies on wintering grounds was not always supported by the genetic analysis. The methods used here allowed associations of breeding and wintering grounds at a broad scale. The ability to link populations at a finer geographic scale may be possible when molecular genetic techniques are combined with other sources of information on geographic origin.

INTRODUCTION

Characterizing levels of population connectivity between breeding and overwintering areas has proven to be a challenge for the majority of migratory songbirds. The lack of specific information on levels of connectivity has limited integrated studies of life history and population regulation in migrant species (Webster et al. 2002). The few studies that have been able to examine demographic processes on both breeding and wintering areas have revealed previously unappreciated interactions and relationships (e.g., Marra et al. 1998; Sillett et al. 2000; Gill et al. 2001). Documenting levels of connectivity has the potential to aid in understanding the relationship between migratory behavior and gene flow (e.g., Arguedas and Parker 2000) and its theorized importance in speciation (Winker 2000). In a conservation context, if links between breeding and wintering areas could be resolved to a fine enough geographic scale, demographic trends and land use changes could be related, thereby informing management decisions. Finally, because migratory birds may act as intermediate hosts in some human diseases, studies of connectivity may aid in understanding the epidemiology of diseases (Rappole et al. 2000; Alekseev et al. 2001).

The identification of markers that reveal the origin of an individual or link populations at different stages of the annual cycle is an essential first step in studies of population connectivity (Wenink and Baker 1996; Haig et al. 1997; Webster et al. 2002). Although traditional banding studies are useful for some avian taxa, particularly shorebirds and waterfowl, they have typically been of limited utility for migrant songbirds, for which return rates are often extremely low (Berthold 1993; Webster et al. 2002). For example, of the more than 140,000 individuals of Wilson's Warbler (*Wilsonia pusilla*) banded in the United States and Canada to date, only three have been recovered on their wintering areas in Latin America, yielding a dismal return rate of 0.002% (Bird Banding Laboratory, Laurel, Maryland). Similarly, although radio and satellite telemetry are valuable for determining movements of large-bodied migrants capable of carrying heavy transmitters (Ristow et al. 2000), most passerines are too small to carry the necessary transmitter and battery to be tracked efficiently over large distances. An alternative to marking and tracking individuals is to use population-specific genetic markers. A major advantage of this approach is that it relies on the association among individuals in a population, and therefore a particular individual does not have to be recaptured or followed.

A population-based molecular approach is a potentially powerful tool for assessing levels of connectivity between breeding and overwintering sites (Webster et al. 2002). For example, molecular genetic markers have been used successfully to examine connectivity in shorebirds (Wenink and Baker 1996; Haig et al. 1997) and more recently in some small passerines (Buerkle 1999; Milot et al. 2000; Kimura et al. 2002; Ruegg and Smith 2002; Clegg et al. 2003; Lovette et al. 2004). To apply molecular methodology effectively to the question of connectivity, genetic variation in populations needs to be geographically structured and the chosen molecular marker must be sensitive enough to detect existing structure. Molecular markers vary widely in their capacity to detect variation at a given phylogenetic level (i.e., species, subspecies, or populations); thus, considerable care must be taken when selecting markers for studying connectivity.

The main classes of genetic marker that have been used to study connectivity in birds include allozymes, random amplified polymorphic DNA (RAPD), mitochondrial DNA (mtDNA) and, more recently, microsatellites (Haig et al. 1997; Webster et al. 2002; Clegg et al. 2003). These markers evolve at different rates (in general, lowest for allozymes and highest for microsatellites), and therefore have the potential to provide different levels of geographically structured genetic variation (Avise 1994). Studies to date using the various classes of markers indicate that levels of genetic variation are generally low to negligible in Neotropical migrant species, especially those with geographic distributions in formerly glaciated areas (Ball and Avise 1992; Seutin et al. 1995; Buerkle 1999; Arguedas and Parker 2000; Gibbs et al. 2000; Milá et al. 2000; Winker 2000; Lovette and Bermingham 2001; Kimura et al. 2002). Several studies have contrasted migratory and sedentary species, documenting higher magnitudes of phylogeographic variation in the latter (Gill et al. 1993; Klein 1994; Zink 1997; Lovette et al. 1998). This difference has been attributed to higher gene flow in migrants (e.g., Arguedas and Parker 2000) and the genetic effects of rapid demographic expansions following recent glaciation events (e.g., Milá et al. 2000). The application of molecular markers to studies of connectivity in migrant songbirds might be affected by these factors, and further comparative studies are needed to make generalizations regarding the amount of structure expected in this group. Additionally, it is important to explore the potential of new molecular techniques, such as using amplified fragment length polymorphisms (AFLP) to find informative single-nucleotide polymorphisms (SNP) (Bensch et al. 2002), and of combining molecular data with other sources of information such as data from chemical isotopes (Hobson, Chap. 19, this volume), banding data (Ruegg and Smith 2002), remote sensing (Szép and Møller, Chap. 29, this vol-

ume), and disease information (Rintamaki et al. 2000; Ricklefs et al., Chap. 17, this volume), and how combined approaches could augment studies of connectivity.

Here, we summarize results on five species of Neotropical migrants. Our specific objectives are to: (1) describe the population structure on breeding grounds by using genetic markers, particularly mtDNA, and in one species also microsatellites; (2) assess the scale at which these markers can be successfully used to study connectivity between breeding and overwintering areas; (3) discuss how these data can contribute to life history and demographic studies; and (4) discuss and illustrate how molecular genetic data might be integrated with other sources of data to better assess connectivity.

METHODS

Study Species

Two main criteria were used in choosing the species we examined. First, to maximize the scope of geographic coverage and the potential for detecting structure if it existed, we chose North American songbird species with subspecific variation and broad continental breeding ranges. Secondly, we selected species for which sample sizes were sufficient and distributed widely across both breeding and wintering areas.

The Yellow-breasted Chat (*Icteria virens*) is found throughout eastern deciduous forests and western riparian habitats. The two recognized subspecies differ subtly in size, plumage, and song characteristics (Eckerle and Thompson 2001). The eastern subspecies, *I. v. virens,* breeds from the eastern Great Plains to the eastern United States and is thought to winter from eastern Mexico to Central America (Eckerle and Thompson 2001). The western subspecies, *I. v. auricollis,* has a more fragmented distribution, breeding from the western Great Plains westward and is thought to winter from western Mexico to central Guatemala (Eckerle and Thompson 2001).

The Nashville Warbler (*Vermivora ruficapilla*) has a disjunct breeding range in North America. The two recognized subspecies differ in morphology and plumage; the western race is brighter in plumage and has a longer tail (Williams 1996). The eastern subspecies, *V. r. ruficapilla,* breeds from the northern hardwood and boreal forest of the eastern United States, central Quebec, and westward to parts of central Manitoba. The suggested overwintering range is in eastern Mexico and Guatemala (Williams 1996). The western subspecies, *V. r. ridgwayi,* has a patchy distribution from southern British Columbia south to parts of the western United States. The suggested overwintering range of the western subspecies is California and western Mexico (Williams 1996).

The Common Yellowthroat (*Geothlypis trichas*) is the most widespread wood-warbler in North America. This species shows a complex pattern of subspecific variation, with some authors recognizing as many as 13 subspecies and considerable clinal variation (Lowery and Monroe 1968). The breeding range of this species spans most of continental North America. Both wintering and breeding populations occur in the southern United States and parts of central Mexico, and strictly wintering populations are found in Baja California, parts of western Mexico, eastern Mexico to Panama, and portions of the West Indies and Bermuda (Guzy and Ritchison 1999).

Wilson's Warbler (*Wilsonia pusilla*) is a common wood-warbler associated with wet habitats. The three recognized subspecies differ subtly in coloration and size (Lowery and Monroe 1968; Pyle et al. 1997). *W. p. pusilla* breeds from the boreal forests of eastern Canada west to British Columbia; *W. p. pileolata* breeds from Alaska to parts of Montana and Idaho; and *W. p. chryseola* breeds along the Pacific Coast to south-central California. The three subspecies have a wintering range extending from eastern and western Mexico (including southern Baja) and from parts of southern Louisiana and Texas to Panama (Ammon and Gilbert 1999).

Swainson's Thrush (*Catharus ustulatus*) breeds in interior forest, secondary growth, and riparian thickets. Although a number of subspecies have been described, two main groups are recognized on the basis of plumage coloration. The olive-backed group, *C. u. alame* and *C. u. swainsoni,* is found in continental regions of Canada to western Alaska, and the russet-backed group, *C. u. ustulatus* and *C. u. oedicus,* is spread along the Pacific Coast (Bond 1963). It is suggested that the olive-backed group winters primarily in South America, whereas the russet-backed group winters primarily in southern Mexico and Central America (Bond 1963; Ramos and Warner 1980).

Sampling and Molecular Approaches

Blood and feather samples were collected from adult birds mist-netted at breeding sites in Canada and the United States and at overwintering sites in Mexico, Central America, and South America. Sampling locations and sample sizes for each species are shown in table 18.1. Blood samples were obtained by sub-brachial venipuncture, and feather samples by plucking the outermost two rectrices. See Kimura et al. (2002), Ruegg and Smith (2002), and Lovette et al. (2004) for methods of DNA extraction, sequencing, and restriction enzyme digests, and Clegg et al. (2003) for methods of genotyping individuals using microsatellites in Wilson's Warblers.

For each species, we first used samples from across the breeding range to reconstruct a phylogeny based on mtDNA sequence (see above papers). We then identified restriction enzymes that were diagnostic of statistically well-supported, geographically defined lineages (table 18.2). Enzyme assays were used to screen samples from individuals captured on overwintering areas to assign them to geographically defined breeding areas.

Table 18.1 Sampling localities in breeding and overwintering areas, locality codes (as shown in figs. 18.2 and 18.3), and numbers of genetic samples obtained for the five species in the study

Breeding localities	Figure code	YBCH	NAWA	COYE	WIWA	SWTH
Juneau, Alaska	AK1	—	—	—	—	5
Denali National Park, Alaska	AK2	—	—	—	15	—
Tetlin National Wildlife Refuge, Fort Yukon, Alaska	AK3	—	—	—	—	15
Yukon Flats National Wildlife Refuge Alaska	AK4	—	—	—	—	20
Kotzebue, Alaska	AK5	—	—	—	—	7
Pitt Lake, British Columbia, CAN	BC1	—	—	2	—	—
Queen Charlotte Island, British Columbia, CAN	BC2	—	—	—	—	10
Squamish, British Columbia, CAN	BC3	—	—	—	—	11
Pemberton, British Columbia, CAN	BC4	—	—	—	—	19
Revelstoke, British Columbia, CAN	BC5	—	—	—	—	11
Quesnel, British Columbia, CAN	BC6	—	—	—	—	11
Hinton, Alberta, CAN	AB	—	—	—	14	—
Mt. Baker National Forest, Washington	WA	—	—	3	12	—
Paisley, Oregon	OR1	1	—	—	—	—
Williams, Oregon	OR2	—	4	—	—	—
Umatilla National Forest, Oregon	OR3	—	—	—	5	24
Siuslaw National Forest, Oregon	OR4	—	—	—	16	20
Boise, Idaho	ID	—	3	—	—	—
Shasta, California	CA1	4	—	—	—	—
Bolinas, California	CA2	—	—	1	—	15
Los Banos, California	CA3	—	—	3	—	—
Foresthill, California	CA4	—	2	—	—	—
Tahoe National Forest, California	CA5	—	—	—	15	—
Kings Canyon National Park, California	CA6	—	—	—	12	—
Pillar Point, California	CA7	—	—	—	17	—
Big Sur, California	CA8	—	—	—	8	—
Ruby Lake, Nevada	NV1	4	—	—	—	—
Lake Mead, Nevada	NV2	—	—	3	—	—
Holter Dam, Montana	MT1	1	—	4	—	—
Denton, Montana	MT2	1	—	—	—	—
Flathead National Forest, Montana	MT3	—	—	—	—	20
Atlantic City, Wyoming	WY	3	—	1	—	—
Manila, Utah	UT1	2	—	—	—	—
Grantsville, Utah	UT2	9	—	—	—	—
Mt. Timpanogos, Utah	UT3	—	—	—	—	11
Grand Mesa, Colorado	CO	—	—	—	14	—
Junction City, Kansas	KS	1	—	4	—	—
Fort Leonard Wood, Missouri	MO	4	—	—	—	—
Owensburg, Indiana	IN	3	—	4	—	—
Fort Knox, Kentucky	KY	3	—	—	—	—
Cuyahoga, Ohio	OH	—	—	3	—	—
Seney National Wildlife Refuge, Michigan	MI1	—	—	1	—	—
Dearborn, Michigan	MI2	—	4	—	—	—
Bristol, Tennessee	TN	4	—	—	—	—
Charleston, South Carolina	SC	2	—	—	—	—
Finland, Minnesota	MN	—	2	—	—	—
Kakabeka, Ontario, CAN	ON1	—	4	—	—	—
Hilliardton, Ontario, CAN	ON2	—	—	2	4	—
Thunder Bay, Ontario, CAN	ON3	—	—	—	—	9
Kakabeka Falls, Ontario, CAN	ON4	—	—	—	—	10
Camp Myrica, Quebec, CAN	PQ1	—	3	5	16	—
Charlevoix, Quebec City, Quebec, CAN	PQ2	—	—	—	—	2
Fredericton, New Brunswick, CAN	NB	—	1	4	4	—
Rochester, New York	NY	—	4	—	—	—
South Britain, Connecticut	CT	—	—	2	—	—
Truro, Massachusetts	MA	—	—	4	—	—
Fort Polk, Louisiana	LA	1	—	—	—	—

continued

Table 18.1 (continued)

Overwintering localities	Figure code	YBCH	NAWA	COYE	WIWA	SWTH
Los Cabos, Baja California Sur, MEX	BCS	7	—	11	7	—
Chupaderos, Sinaloa, MEX	SIN	2	12	—	—	—
Sierra de Manatlán Biosphere Reserve, Autlan, Jalisco, MEX	JAL	1	30	—	22	—
Nevado de Colima, Colima, MEX	COL	—	2	—	—	—
La Maria, Colima, MEX	COL	—	—	—	25	—
Huautla, Morelos, MEX	MOR	1	7	—	—	—
Zacualtipán, Hidalgo, MEX	HGO	—	5	—	—	—
El Cielo Biosphere Reserve, Tamaulipas, MEX	TAM	—	—	—	13	—
Coatepec, Veracruz, MEX	VER1	1	—	—	19	—
Catemaco, Veracruz, MEX	VER2	5	—	—	7	—
Chila, Oaxaca, MEX	OAX1	5	6	—	—	—
Animas de Trujano, Oaxaca, MEX	OAX2	2	23	9	15	33
Hidalgo, Oaxaca, MEX	OAX3	—	6	—	—	—
El Triunfo Reserve, Chiapas, MEX	CHS	—	—	—	—	20
El Ocote Reserve, Chiapas, MEX	CHS	7	—	—	6	18
San Ignacio, BZ	BZ	13	—	9	—	—
Cockscomb Basin, BZ	BZ	—	—	—	1	—
El Boqueron Volcano, ES	ES	2	2	—	—	2
San Salvador, ES	ES	—	—	—	15	—
Tegucigalpa, HON	HON	—	—	—	25	4
Esteli, NIC	NIC	—	—	—	9	—
Santa Elena, CR	CR1	—	—	—	10	4
San Vito, CR	CR2	—	—	—	12	5
Cerro Jefe, PN	PN	—	—	—	—	3
Mindo, EC	ECU	—	—	—	—	18
Nuevo Peru, PE	PE	—	—	—	—	6
Santa Cruz, BOL	BOL	—	—	—	—	2
Totals		*89*	*120*	*75*	*338*	*335*

Note: Country abbreviations: CAN = Canada; MX = Mexico; ES = El Salvador, BZ = Belize; HON = Honduras; NIC = Nicaragua; CR = Costa Rica; PN = Panama; EC = Ecuador; PE = Peru; BOL = Bolivia. Species abbreviated: YBCH = Yellow-breasted Chat; NAWA = Nashville Warbler; COYE = Common Yellowthroat; WIWA = Wilson's Warbler; SWTH = Swainson's Thrush.

RESULTS AND DISCUSSION

Patterns of Variation on the Breeding Grounds

Several common patterns in population genetic structure are evident among all five species (fig. 18.1A–E). The most obvious similarity is that each species is divided into two main haplotype groups associated to varying degrees with eastern and western sampling sites (fig. 18.2A–E). The level of divergence between these groups was between 0.5 and 2%, consistent with a late-Pleistocene divergence (e.g., Avise and Walker 1998; Kimura et al. 2002; Ruegg and Smith 2002; Lovette et al. 2004). Another similarity among the five species was the relative lack of structure within eastern and western haplotype groups. Low levels of variation could be due to current or historical gene flow or past demographic events (e.g., Milá et al. 2000). The high level of homogeneity across broad geographic areas was most evident in the eastern lineage of all sufficiently sampled species, suggesting that eastern and western lineages may have had different demographic histories. For example, the broad distribution of very similar haplotypes within the east could suggest that these lineages may have experienced a more rapid demographic expansion following a Pleistocene glaciation event than occurred in western regions. In general, there was a slightly higher level of phylogenetic structure within western groups (fig. 18.1), possibly stemming from a less

Table 18.2 Summary of the mtDNA regions and restriction enzymes used to discriminate among eastern and western forms of each species

Species	mtDNA region	Restriction enzyme
Yellow-breasted Chat	ATPase	East: *Rsa*I West: *Dpn*II
Common Yellowthroat	ATPase	East: *Tsp*45 West: *Bst*NI
Nashville Warbler	ATPase	East: *Hinc*II West: *Stu*I
Swainson's Thrush	Control region I	West: *Sfc*I
Wilson's Warbler	Control region I & cytochrome b	East: *Nsi*I West: *Hinc*II

Note: The eastern lineage of Swainson's Thrush was defined by the absence of the *Sfc*I site.

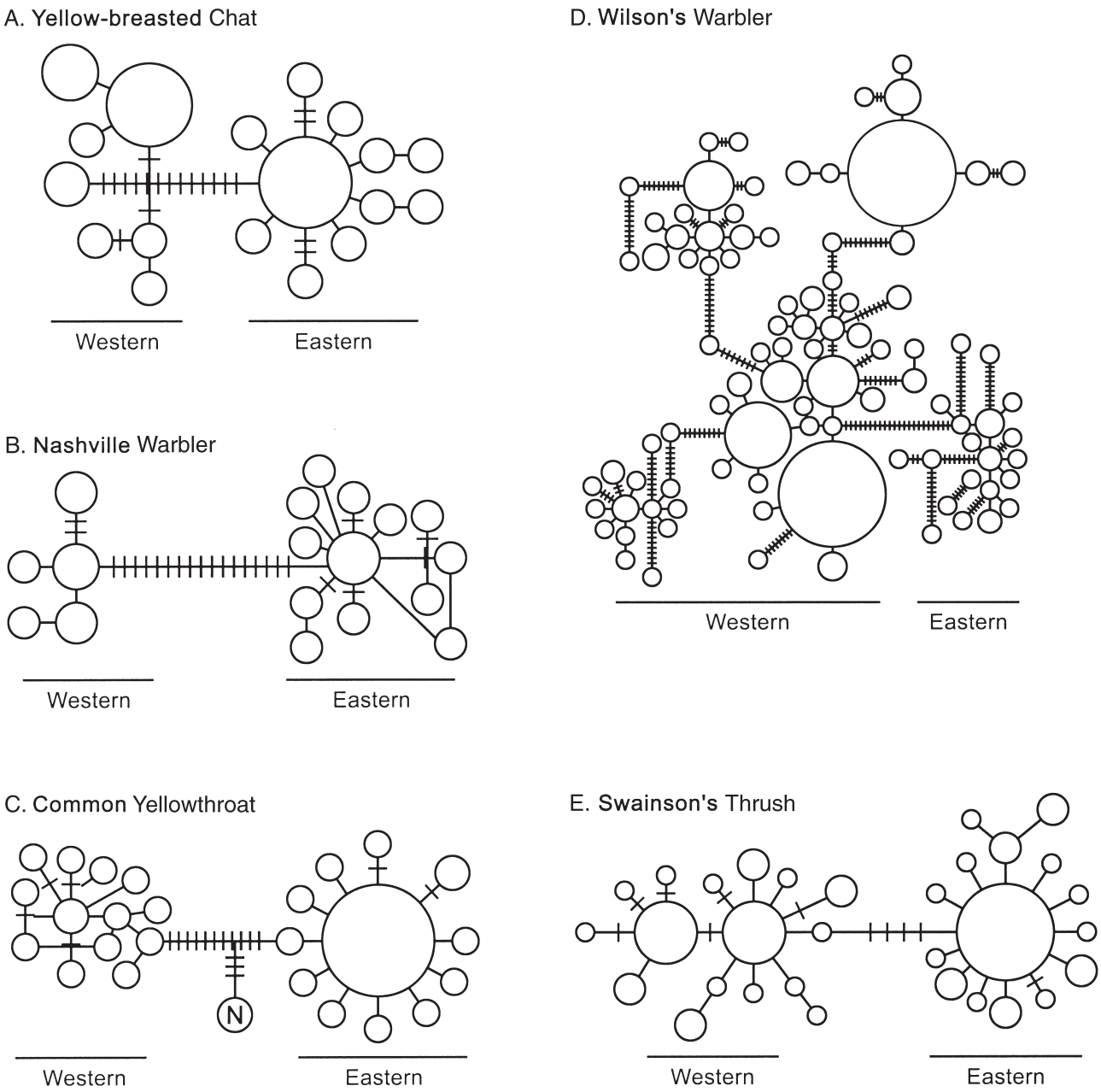

Fig. 18.1. Minimum-spanning network with each unique haplotype indicated by a circle and area proportional to the number of individuals sampled. Hatch marks along branches indicate inferred haplotype differences. Eastern and western geographic lineages are indicated below each network. (A) Yellow-breasted Chat: mtDNA ATPase sequences were obtained from 34 individuals, including 11 eastern and 7 western individuals. A total of 18 unique haplotypes with 17 nucleotide substitutions (1.8% sequence divergence) between eastern and western populations (Lovette et al. 2004). (B) Nashville Warbler: sequences obtained from 27 individuals, including 18 eastern and 9 western individuals. Eastern and western haplotypes differed by 16 to 22 substitutions, 1.7–2.3 % sequence divergence (Lovette et al. 2004). (C) Common Yellowthroat: sequences from 47 individuals with a maximum of 19 nucleotide substitutions (2%) (see Lovette et al. 2004). Divergent Nevada haplotype indicated by "N." (D) Wilson's Warbler: mtDNA control region sequences from 200 individuals. A total of 94 unique haplotypes were identified, and eastern and western haplotypes differed by a minimum of 22 substitutions (see Kimura et al. 2002). (E) Swainson's Thrush: mtDNA control region sequences from 183 individuals with a net sequence divergence between lineages of 0.69% (Ruegg and Smith 2002).

severe effect of glaciation in the west and/or the maintenance of a higher level of population subdivision over long periods.

Although these species share the general patterns described above, some clear species-specific differences in phylogeographic patterns are evident. The geographic location of the east-west split in each species differed (fig. 18.2). In the Yellow-breasted Chat, Nashville Warbler, and Wilson's Warbler, the two lineages corresponded well to sampling locations in eastern and western North America (although this is not conclusive for Wilson's Warbler because of sampling gaps) (fig. 18.2A,B,D). However, in the

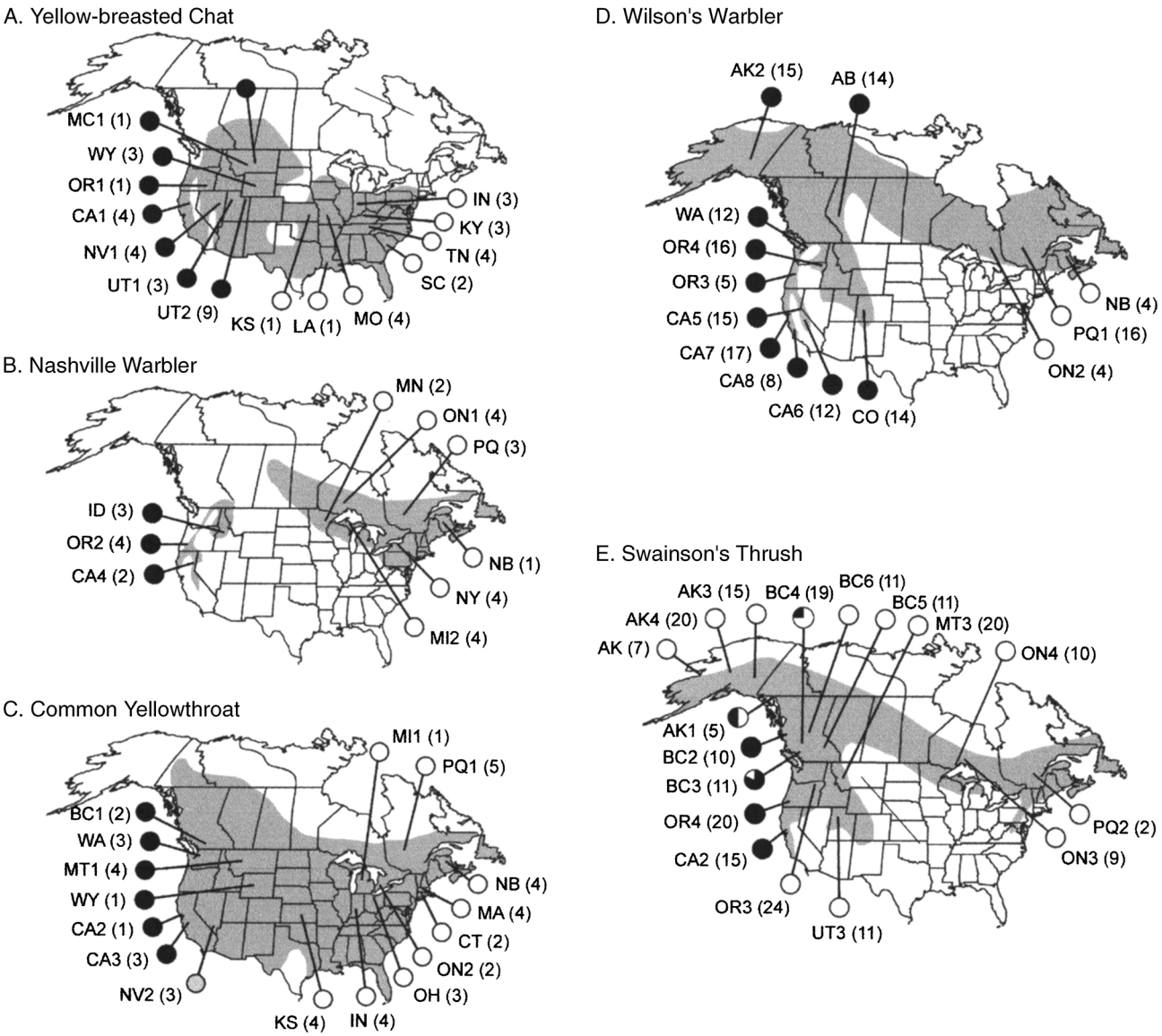

Fig. 18.2. Distribution of eastern and western haplotypes in Yellow-breasted Chat (A), Nashville Warbler (B), Common Yellowthroat (C), Wilson's Warbler (D), and Swainson's Thrush (E), superimposed on their breeding distribution (shaded area). Western and eastern haplotypes are shown in black and white, respectively. Gray circle for Nevada sample of Common Yellowthroat corresponds to haplotype N in fig. 18.1. Numbers in parentheses indicate sample sizes. See table 18.1 for location abbreviations.

Common Yellowthroat, the eastern lineage extends west to central Montana (Lovette et al. 2004) (fig. 18.2C) and in Swainson's Thrush, the eastern lineage extends to central British Columbia and western Alaska (Ruegg and Smith 2002) (fig. 18.2E). Therefore, post-glacial climate and vegetation changes may have affected current lineage ranges differently in each species.

In addition, some species showed hints of greater phylogenetic structure that are important to note. In the Common Yellowthroat, a divergent haplotype from Nevada was separated from the eastern group by seven to nine substitutions and from the western by 12–16 substitutions (fig. 18.1C). This population begs further investigation and may represent a distinct migratory population or possibly a non-migratory population that extends southward beyond where we sampled (Lovette et al. 2004). In Wilson's Warbler more structure was detected among western populations than for the other species. An analysis of molecular variance (AMOVA) revealed both significant within-population and between-population variation (Kimura et al. 2002). It is possible, however, that similar complexities could be revealed in the other species if sampling were conducted with an intensity similar to that for these western Wilson's Warbler populations. Further examination of variation in Wilson's Warbler using five microsatellite loci also showed a genetic difference between the one eastern population and all other western populations (pairwise F_{ST} values shown in table 18.3). However, despite the extra statistical power af-

Table 18.3 Pairwise F_{ST} between sampled Wilson's Warbler populations based on analysis of five microsatellite loci

	AK	BC	AB	WA	OR	CA	CO	PQ
BC	0.026	—	—	—	—	—	—	—
AB	0.020	0.002	—	—	—	—	—	—
WA	0.005	0.014	0.002	—	—	—	—	—
OR	0.018	0.035*	0.019	0.006	—	—	—	—
CA	0.012	0.013	–0.010	–0.007	0.004	—	—	—
CO	0.030*	0.026	0.017	0.007	0.007	0.017	—	—
PQ	0.134*	0.156*	0.145*	0.125*	0.130*	0.129*	0.138*	—

Note: Asterisk denotes values significantly different from zero following table-wide corrections for multiple comparisons. Abbreviations: AK = Alaska; BC = British Columbia; AB = Alberta; WA = Washington; OR = Oregon; CA = California; CO = Colorado; PQ = Quebec.

forded by multiple loci, further structure within the western portion of the species range was not detected. Population structure in terms of F_{ST} values was minimal, no isolation by distance relationship was detected, and model-based clustering methods failed to identify genetically similar groups within the western samples (Clegg et al. 2003).

Distribution of Genetic Lineages at Overwintering Sites

The distribution of eastern and western lineages on the wintering grounds differed among species (fig. 18.3A–E). These differences ranged from complete segregation to some geographic mixing of eastern and western groups at locations on the wintering grounds. In the Yellow-breasted Chat there was no evidence of mixing of eastern and western groups at wintering locations, although samples for any given site were small (fig. 18.3A). Overwintering western groups were distributed from southern Baja California to Oaxaca, Mexico. Eastern groups were found from Veracruz south through Chiapas, and at sites in Belize and El Salvador. Samples for the Common Yellowthroat were restricted to only three sites but nevertheless are informative (fig. 18.3C). Only western individuals were found in southern Baja, a mixed population was found in Oaxaca, and only eastern individuals were found in Belize. In contrast, haplotype distributions for Nashville Warblers revealed only two out of nine sites with western birds (a site in Sinaloa with nine individuals and a site in Oaxaca with one individual), whereas eastern individuals were distributed throughout the wintering range (fig. 18.3B). This discrepancy could be explained by a difference in the population sizes of the two subspecies; the western subspecies has a more restricted breeding distribution (Williams 1996) and may be less common. In Swainson's Thrush there was a nearly complete segregation of eastern and western groups on the wintering grounds (fig. 18.3E). Eastern groups were found primarily from Panama to northern South America, whereas western groups were found in southern Mexico and Central America. In the Wilson's Warbler, limited mixing of breeding lineages at overwintering sites was evident, mostly in Veracruz and Chiapas. Western haplotypes predominated throughout the wintering range (Figure 18.3D).

Morphological Subspecies and Genetic Variation

Information on morphological subspecies has been useful in the context of establishing connectivity in cases where differences between groups are clear (Webster et al. 2002). However, in many species, morphological differentiation is lacking, or subspecies differ in a very gradual and subtle manner, making it difficult to unequivocally identify a wintering-ground individual as belonging to one particular morphological subspecies or another. In the latter case, genetic information could be useful to verify identification of wintering ground individuals if the subspecies exhibit consistent genetic differences.

In the species examined in detail here, there are varying levels of concordance between the distribution of morphologically recognized subspecies and the distribution of genetic groups on breeding and wintering grounds. In Yellow-breasted Chats, our molecular results were concordant with subspecific variation and the distribution of the subspecies on both the breeding and wintering grounds (Lowery and Monroe 1968; Eckerle and Thompson 2001; Lovette et al. 2004). In the Nashville Warbler, the allopatric eastern and western subspecies (*V. r. ruficapilla* and *V. r. ridgwayi,* respectively) were genetically divergent, but these groups were not always found in their predicted overwintering sites (Williams 1996). Eastern-breeding individuals appear to utilize a broader overwintering range than previously thought, being found throughout eastern and western Mexico. We found very few western individuals in samples from western Mexico where this subspecies is thought to winter according to subspecies descriptions, despite having relatively large sample sizes.

It is possible that the lack of genetically western individuals found on the wintering grounds is due to biases in sampling if those birds occupy particular habitats that were not

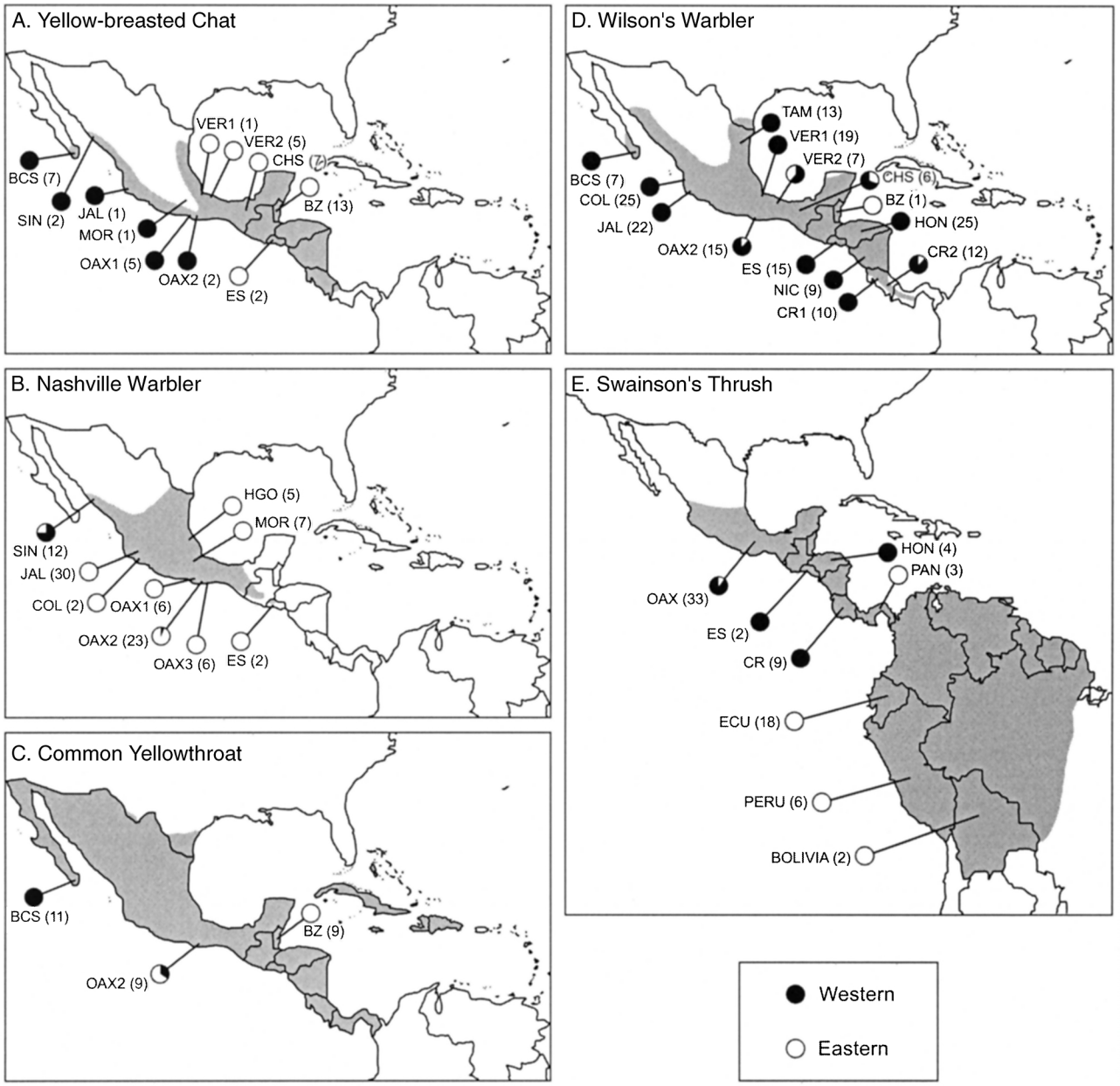

Fig. 18.3. Distribution of eastern and western haplotypes in Yellow-breasted Chat (A), Nashville Warbler (B), Common Yellowthroat (C), Wilson's Warbler (D), and Swainson's Thrush (E), superimposed on their overwintering distribution (shaded area). Western and eastern haplotypes are shown in black and white, respectively. Numbers in parentheses indicate sample sizes. See table 18.1 for location abbreviations. (Modified from Kimura et al. 2002, Ruegg and Smith 2002, and Lovette et al. 2004.)

sampled. Despite this, it seems that the morphological differences between the Nashville Warbler subspecies are too subtle to allow objective identification of the forms on the wintering grounds. In the case of the Common Yellowthroat, sampling was insufficient to comment on the concordance between morphological subspecies and genetic groups. In the Wilson's Warbler, there was partial concordance between subspecies designations and genetic groups. The eastern subspecies *W. p. pusilla* formed a well-supported lineage, but mtDNA molecular markers could not distinguish the two western subspecies (*W. p. chryseola* and *W. p. pileolata*) (Kimura et al. 2002). We are unable to comment on the concordance between morphological subspecies and genetic groups on the wintering grounds in the Wilson's Warbler, as subspecies on the wintering grounds were not previously described (Ammon and Gilbert 1999). In the Swainson's Thrush, there was genetic divergence between morphological subspecies groups (olive-backed group and russet-backed group), and these groups wintered in the locations predicted on the basis of morphology (Ramos and Warner 1980).

Overall, the use of morphological traits to identify breeding-ground origins of wintering-ground birds is species specific. For several of the species in our study, differences

among subspecies do not provide reliable markers of breeding-ground origin. There are several potential sources of error when using subspecies to link wintering and breeding populations. For example, morphological differences in measurable traits such as wing and tail length and plumage characteristics can be differentially shaped by natural selection and can swamp the effects of gene flow (Rice and Hostert 1993; Orr and Smith 1998). Thus morphological differences among subspecies may result from differential selection, even in the presence of gene flow. Here, populations would not be demographically independent despite morphological differentiation.

In contrast, consider two populations occurring in the same habitat but separated by a high mountain range. Here, one might find substantial genetic divergence in populations across the divide, with each demographically independent of the other, but an identical pattern of morphological variation owing to the similarity of selection pressures in like habitats on each side of the mountain. Under these circumstances, although it would potentially be possible to classify wintering individuals by breeding population using genetic techniques, it would not be possible using morphologic characters important in fitness. In fact, in this instance using morphology alone to link wintering individuals with breeding populations would result in combining wintering individuals from demographically independent populations from either side of the mountain. Another source of potential error could arise if particularly plastic traits are used for establishing connectivity. Some avian traits are known to be seasonally variable. For example, mandible length has been shown to change seasonally because of wear (Gosler 1986). Thus, using subspecies designations alone to study connectivity may be misleading.

Life History, Demography, and Microevolutionary Processes

The use of molecular markers offers some exciting possibilities for the study of life history, demography, and evolutionary processes. By using a simple restriction enzyme assay, we can now distinguish between eastern and western breeding lineages in several passerine species. The ability to easily distinguish between eastern- and western-origin individuals offers the possibility of studying the relationship between regional breeding origin and life history and ecology. For example, are arrival and departure times on wintering grounds similar or different for eastern- and western-breeding individuals? Do eastern and western individuals winter in different locations? In wintering locations where eastern and western individuals co-occur, are there fine-scale differences in habitat use and behavior? The use of molecular tools makes it possible to study these and other factors associated with migration—factors that may have significant fitness consequences.

Molecular techniques can also provide interesting insights into the demographic history of a species. For example, several recent studies have demonstrated how low levels of genetic differentiation in North American passerines may be due to a rapid demographic expansion following a late Pleistocene glacial event (Milá et al 2000; Ruegg and Smith 2002). In the Swainson's Thrush, the molecular data suggested that both eastern and western lineages had undergone recent demographic expansions (Ruegg and Smith 2002). Furthermore, when molecular data were combined with data on banding returns, they suggested that individuals from east of the Coast Mountains in Alaska and British Columbia may be retracing their post-glacial expansion routes during their yearly migration.

The cases where there is a lack of concordance between subspecific variation and molecular data provide an interesting avenue for investigation in itself. Because of the effects of differential selection, morphological differences among subspecies or populations may not match patterns of neutral genetic variation (Orr and Smith 1998; Smith et al. 2001). This has been observed in a number of passerine species, such as Swamp Sparrows (*Melospiza georgiana*) (Greenberg et al. 1998) and Pied Flycatchers (*Ficedula hypoleuca*) (Haavie et al. 2000).

Utility of Molecular Markers and Future Directions

Results from the five passerine species examined suggest that mitochondrial DNA variation can resolve connectivity between breeding and wintering areas only at large geographic scales. However, the very real possibility remains that the lack of genetic structure found at finer geographic scales is due to limitations of markers. For the Wilson's Warbler, the application of microsatellite loci did not improve the level of geographic resolution above that found by using mtDNA, despite having five independent markers and the opportunity to conduct frequency-based analyses (Clegg et al. 2003). Nevertheless, the use of other, more variable molecular markers or a higher number of markers may ultimately increase resolution and the ability to link populations at a finer scale. In particular, a pioneering approach by Bench et al. (2002) used the amplified fragment length polymorphism (AFLP) method (which produces dominant markers that do not lend themselves to standard population genetic analyses because homozygous individuals cannot be distinguished from heterozygous individuals) to find informative single nucleotide polymorphisms (SNP), which are codominant and thus allow populations to be analyzed with standard population genetic methods. Using this approach, Bensch et al. (2002) were successful in distinguishing migratory populations of Willow Warblers (*Phylloscopus trochilus*) that could not be differentiated by mtDNA and microsatellite markers.

Although the choice of genetic marker or suite of markers should be considered, which works best also depends on life history characteristics. For example, mtDNA markers have been quite successful in establishing connectivity in some waders (Haig et al. 1997; Wennerberg 2001), suggesting that strong philopatry along with subdivided breeding

distributions likely make some species more amenable to genetic methods than others.

Innovative new analytical approaches may also help in defining genetic structure. For example, multivariate molecular analyses developed and applied successfully to studying plant population structure show promise (Gram and Sork 2001), but to date have not been used to examine structure in vertebrates. Moreover, population assignment tests that use frequency data on multiple markers might also hold considerable promise.

Using molecular genetic markers to study population connectivity will ultimately be most successful when combined with other types of data such as banding returns, morphologically based subspecific variation, stable isotope markers, radio and satellite telemetry, and variation in disease strains. Ruegg and Smith (2002) combined molecular genetic markers, band returns, and descriptions of subspecific variation for the Swainson's Thrush to show that eastern and western groups had different migration routes and overwintering locations. The use of stable isotope ratios to link breeding and wintering ranges of some migratory songbirds at broad geographic levels has already shown promise (Hobson and Wassenaar 1997; Marra et al. 1998; Rubenstein et al. 2002; Webster et al. 2002; Hobson, Chap. 19, this volume). Stable isotope markers and molecular genetic markers may provide complementary sources of information. Molecular genetic markers are useful in distinguishing east-west patterns of variation, and some isotope ratios are useful in distinguishing north-south variation (Hobson and Wassenaar 1997). Thus, combining the two methods offers the possibility of better geographical delimitation of breeding populations. The combination of genetic and isotopic methods has been used to shed light on migratory patterns in Wilson's Warblers. First, individuals sampled from the wintering grounds could be identified by their genetic haplotypes as being of eastern or western breeding origin. Second, information from stable hydrogen isotopes could be employed to show that among the individuals from western breeding ranges, individuals from the northern part of the western range occupied the most southerly overwintering habitats, whereas individuals from more southerly breeding areas in the west occupied the northern parts of the overwintering range (Clegg et al. 2003). This demonstration of leapfrog migration using both molecular and isotopic information is an example of the power of using multiple approaches.

Radio and satellite telemetry has been a useful technique for tracking larger birds (Ristow et al. 2000). The development of smaller, lighter transmitters in the future may enable this method to be used for small-bodied passerines. Finally, using variation in disease strains to distinguish patterns of connectivity may hold promise (Ricklefs et al., Chap. 17, this volume). For example, PCR-based assays of avian blood parasites allow not only for easy and inexpensive ways to detect the presence of disease, but also for haplotypes of a given pathogen to be differentiated (Sehgal et al. 2001). Combining multiple techniques will likely be the way forward to maximize the resolution of connectivity between breeding and wintering migrant passerines and to link demographic processes across different stages of the annual cycle.

ACKNOWLEDGMENTS

We thank D. DeSante and the Institute for Bird Populations, the Monitoring Avian Productivity and Survivorship Program (MAPS) banders, the Point Reyes Bird Observatory, and the many independent banders across the hemisphere that donated genetic samples. For sharing their study sites or facilitating fieldwork, we thank Y. Aubry, P. Bichier, R. Carlson, A. Cruz, O. Cruz, R. Dickson, O. Figueroa, T. Gavin, M. Grosselet, K. Holl, L. Imbeau, O. Komar, J. Montejo, B. Murphy, A. Oliveras, E. Rodríguez, E. Ruelas, E. Santana, W. Schaldach, C. Spytz, P. Thorn, and the Belize Audubon Society. We would like to thank P. Marra, R. Greenberg, and two anonymous reviewers for comments that greatly improved the manuscript. This work was supported by grants from San Francisco State University, National Institutes of Health Office of Research on Minority Health (5P20RR11805), The Turner Foundation, the Environmental Protection Agency (R827109-01-0), and the National Science Foundation (DEB-9726425 and IRCEB-9977072) to T. Smith.

LITERATURE CITED

Alekseev, A. N., H. V. Dubinina, A. V. Semenov, and C. V. Bolshakov. 2001. Evidence of ehrlichiosis agents found in ticks (Acari: Ixodidae) collected from migratory birds. Journal of Medical Entomology 38:471–474.

Ammon, E. M., and W. M. Gilbert. 1999. Wilson's Warbler (*Wilsonia pusilla*). Pages 1–28 *in* The Birds of North America, no. 478 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia.

Arguedas, N., and P. G. Parker. 2000. Seasonal migration and genetic population structure in house wrens. Condor 102:517–528.

Avise, J. C. 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, New York.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London, Series B, Biological Sciences 265:457–463.

Ball, R. M., and J. C. Avise. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. Auk 109:626–636.

Bensch S., S. Åkesson, and D. E. Irwin. 2002. The use of AFLP to find an informative SNP: genetic differences across a migratory divide in willow warblers. Molecular Ecology 11: 2359–2366.

Berthold, P. 1993. Bird Migration: A General Survey. Oxford University Press, New York.

Bond, G. M. 1963. Geographic variation in the thrush *Hylocichla ustulata*. Proceedings of the U.S. National Museum 114: 373–387.

Buerkle, C. A. 1999. The historical pattern of gene flow among migratory and non-migratory populations of prairie warblers (Aves: Parulinae). Evolution 53:1915–1924.

Clegg, S. M., J. F. Kelly, M. Kimura, and T. B. Smith. 2003. Combining genetic markers and stable isotopes to reveal leapfrog migration in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). Molecular Ecology 12: 819–830.

Eckerle, K. P., and C. F. Thompson. 2001. Yellow-breasted chat (*Icteria virens*). Pages 1–28 *in* The Birds of North America, no. 572 (S. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia.

Gibbs, H. L., R. J. G. Dawson, and K. A. Hobson. 2000. Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? Molecular Ecology 9:2137–2147.

Gill, F. B., A. M. Mostrom, and A. L. Mack. 1993. Speciation in North American chickadees: I. Patterns of mtDNA genetic divergence. Evolution 47:195–212.

Gill, J. A., K. Norris, P. M. Potts, T. G. Gunnarsson, P. W. Atkinson, and W. J. Sutherland. 2001. The buffer effect and large-scale population regulation in migratory birds. Nature 412: 436–438.

Gosler, A. G. 1986. Pattern and process in the bill morphology of the Great Tit *Parus major.* Ibis 129:451–476.

Gram, W. K., and V. L. Sork. 2001. Association between environmental and genetic heterogeneity in forest tree populations. Ecology 82:2012–2021.

Greenberg, R., P. J. Cordero, S. Droege, and R. C. Fleischer. 1998. Morphological adaptation with no mitochondrial DNA differentiation in the coastal plain Swamp Sparrow. Auk 115: 706–712.

Guzy, M. J., and G. Ritchison. 1999. Common Yellowthroat (*Geothlypis trichas*). Pages 1–24 *in* The Birds of North America, no. 48 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia.

Haavie, J., G.-P. Sætre, and T. Moum. 2000. Discrepancies in population differentiation at microsatellites, mitochondrial DNA and plumage colour in the pied flycatcher: inferring evolutionary processes. Molecular Ecology 9:1137–1148.

Haig, S. M., C. L. Gratto-Trevor, T. D. Mullins, and M. A. Colwell. 1997. Population identification of western hemisphere shorebirds throughout the annual cycle. Molecular Ecology 6:413–427.

Hobson, K. A., and L. I. Wassenaar. 1997. Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. Oecologia 109: 142–148.

Kimura, M., S. M. Clegg, I. J. Lovette, K. R. Holder, D. J. Girman, B. Milá, P. Wade, and T. B. Smith. 2002. Phylogeographic approaches to assessing demographic connectivity between breeding and overwintering regions in a Nearctic-Neotropical warbler (*Wilsonia pusilla*). Molecular Ecology 11:1605–1616.

Klein, N. 1994. Intraspecific molecular phylogeny in the yellow warbler (*Dendroica petechia*), and implications for avian biogeography in the West Indies. Evolution 48:1914–1932.

Lovette, I. J., and E. Bermingham. 2001. Mitochondrial perspective on the phylogenetic relationships of the Parula Woodwarblers. Auk 118:211–215.

Lovette, I. J., E. Bermingham, G. Seutin, and R. E. Ricklefs. 1998. Evolutionary differentiation in three endemic West Indian warblers. Auk 115:890–903.

Lovette, I. J., S. M. Clegg, and T. B. Smith. 2004. Limited utility of mtDNA markers for determining connectivity among breeding and overwintering locations in three Neotropical migrant birds. Conservation Biology 18:156–166.

Lowery, G. H., and B. L. Monroe. 1968. Family Parulidae. Pages 5–93 *in* Checklist of Birds of the World (R. A. Paynter, ed.). American Ornithologists' Union, Cambridge, Mass.

Marra, P. P., K. A. Hobson, and R. T. Holmes. 1998. Linking winter and summer events in a migratory bird by using stable-carbon isotopes. Science 282:1884–1886.

Milá, B., D. J. Girman, M. Kimura, and T. B. Smith. 2000. Genetic evidence for the effect of a postglacial population expansion on the phylogeography of a North American songbird. Proceedings of the Royal Society of London, Series B, Biological Sciences 267:1033–1040.

Milot, E., H. L. Gibbs, and K. A. Hobson. 2000. Phylogeography and genetic structure of northern populations of the yellow warbler (*Dendroica petechia*). Molecular Ecology 9:667–681.

Orr, M. R., and T. B. Smith. 1998. Ecology and speciation. Trends in Ecology and Evolution 13:502–506.

Pyle, P. 1997. Identification Guide to North American Birds. Slate Creek Press, Bolinas, Calif.

Ramos, M. A., and D. W. Warner. 1980. Analysis of North American subspecies of migrant birds wintering in Los Tuxlas, southern Veracruz, Mexico. Pages 173–180 *in* Migrant Birds in the Neotropics: Ecology, Behavior, Distribution, and Conservation (E. A. Keast and E. S. Morton, eds.). Smithsonian Institution Press, Washington, D.C.

Rappole, J. H., S. R. Derrickson, and Z. Hubalek. 2000. Migratory birds and spread of West Nile virus in the Western Hemisphere. Emerging Infectious Diseases 6:319–328.

Rice, R. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: what have we learned in 40 years? Evolution 47:1637–1653.

Rintamaki, P. T., M. Ojanen, H. Pakkala, M. Tynjala, and A. Lundberg. 2000. Blood parasites of juvenile Willow Tits *Parus montanus* during autumn migration in northern Finland. Ornis Fennica 77:83–87.

Ristow, D., P. Berthold, D. Hashmi, and U. Querner. 2000. Satellite tracking of Cory's Shearwater migration. Condor 102: 696–699.

Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

Rubenstein, D. R., C. P. Chamberlain, R. T. Holmes, M. P. Ayres, J. R. Waldbauer, G. R. Graves, and N. C. Tuross. 2002. Linking breeding and wintering ranges of a migratory songbird using stable isotopes. Science 295:1062–1065.

Ruegg, K. C., and T. B. Smith. 2002. Not as the crow flies: an historical explanation for circuitous migration in Swainson's thrush (*Catharus ustulatus*). Proceedings of the Royal Society of London, Series B, Biological Sciences 269: 1375–1381.

Schneider, S., and L. Excoffier. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.

Sehgal, R. N. M., H. I. Jones, and T. B. Smith. 2001. Host specificity and incidence of Trypanosoma in some African rainforest birds: a molecular approach. Molecular Ecology 10: 2319–2327.

Seutin, G., L. M. Ratcliffe, and P. T. Boag. 1995. Mitochondrial DNA homogeneity in the phenotypically diverse redpoll finch complex (Aves: Carduelinae: *Carduelis flammea-hornemanni*). Evolution 49:962–973.

Sillett, T. S., R. T. Holmes, and T. W. Sherry. 2000. Impacts of a global climate cycle on population dynamics of a migratory songbird. Science 288:2040–2042.

Smith, T. B., C. J. Schneider, and K. Holder. 2001. Refugial isolation versus ecological gradients: testing alternative mechanisms of evolutionary divergence in four rainforest vertebrates. Genetica (Dordrecht) 112/113:383–398.

Webster, M. S., P. P. Marra, S. M. Haig, S. Bensch, and R. T. Holmes. 2002. Links between worlds: unraveling migratory connectivity. Trends in Ecology and Evolution 17:76–83.

Wenink, P. W., and A. J. Baker. 1996. Mitochondrial DNA lineages in composite flocks of migratory and wintering dunlins (*Calidris alpina*). Auk 113:744–756.

Wiens, J. A. 1989. The Ecology of Bird Communities. Cambridge University Press, New York.

Williams, J. M. 1996. Nashville Warbler (*Vermivora ruficapilla*). Pages 1–20 *in* The Birds of North America, no. 205 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia.

Winker, K. 2000. Migration and speciation. Nature 404:36.

Zink, R. 1997. Phylogeographic studies of North American birds. Pages 301–324 *in* Avian Molecular Evolution and Systematics (D. P. Mindell, ed.). Academic Press, San Diego.

Zink, R. M., A. E. Kessen, T. V. Line, and R. C. Blackwell-Rago. 2001. Comparative phylogeography of some aridland bird species. Condor 103:1–10.