USING INTRINSIC AND EXTRINSIC MARKERS TO LINK BIRD POPULATIONS ACROSS THE AMERICAS

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Understanding the movements of birds during all phases of their annual cycle is highly valuable for effective conservation, but difficult because many species travel at night, over mountains and oceans, and across international borders. Recent advances in the use of intrinsic and extrinsic markers are making the study of movements easier and cheaper, enabling conservation efforts to be directed where they are most needed. Many tracking methodologies can be easily incorporated into existing hemispheric bird monitoring efforts such as the Monitoring Avian Productivity and Survivorship (MAPS) and Monitoring Overwinter Survival (MoSI) programs. As an example of intrinsic markers, we describe recent project that used genetic markers to identify distinct populations of Wilson's Warbler (*Cardellina pusilla*) during multiple phases of the annual migration cycle. We identified wintering areas in the Neotropics, migration routes, and a "schedule" of migration for six distinct breeding populations. In a project using extrinsic markers, we used micro-GPS to track the year-round movements of two Black-headed Grosbeaks that nested in California and spent the late summer/early fall in northwestern Mexico, probably to molt, before migrating more than one thousand kilometers further south into Mexico, where they spent the rest of the non-breeding season. This was the first time that apparent "molt migration" between the western United States and Mexico wasdocumented using GPS technology. We discuss some of the implications and conservation benefits of detailed research into migratory connectivity.

Introduction

In the spring of 1822, a rather unusual White Stork (*Ciconia ciconia*) arrived near the German village of Klutz, northeast of Hamburg, on the Baltic Sea. White Storks were not uncommon in that part of Europe in the early 19th century, but this one was eye catching: it had a long stick protruding from its neck which, remarkably, did not appear to be hindering its flight. Out of curiosity, the stork was shot. Its death would provide an astounding piece of ornithological information: the stick was an 80 cm-long spear, of African origin (Bairlein 2008). A few early observers of the natural world, including Aristotle, understood that many birds moved south in fall and north in spring (Berthold 2001), but it was much more widely believed that animals so small and delicate were incapable of travelling long -- and certainly not inter-continental – distances. Certain species disappeared in one season, and others appeared, but nobody knew where the former went, or where the latter came from. Previous theories, amusing to consider today, included hibernation in rivers or lakes, transformation into other bird species, or even transformation into mice. The *pfeilstorch* ("arrow stork") was the first piece of documented evidence that a bird could move thousands of kilometers during one migration cycle.

Though ornithological science has made enormous advances in the last two centuries, understanding the movements of migratory birds is still a surprisingly difficult problem to untangle. Though the arrow storks (ultimately, there more than a dozen) were a uniquely Afro-European phenomenon, the questions they raise are global, and deeply relevant to the conservation of Neotropical migratory birds: Where do species birds go at different seasons? And what are the implications for full annual cycle conservation?

More than half of the birds that breed in North America migrate to Mexico, Central America, the Caribbean, or South America during the non-breeding season (U.S. Fish and Wildlife Service 2000). Many of these species are listed as Threatened or Endangered, or are categorized by the Service as a Species of Conservation Concern or listed on the International Union for the Conservation of Nature Red List of Threatened Species (IUCN 2017). Effective conservation planning and implementation depends on understanding the full annual cycle movements of migratory birds, because efforts to conserve species by improving habitat in the less limiting season will have little effect on population size in the most limiting season (Sherry and Holmes 2005). If a species is declining due to factors operating on the Neotropical wintering grounds, conservation resources will do the most good when allocated there, rather than the breeding grounds (and vice versa). Yet, many important life history aspects of these "birds of two worlds" (Greenberg and Marra 2005) remain opaque. For example, our understanding of when and how these populations are limited is relatively poor for most species.

The Institute for Bird Populations (IBP), the UCLA Center for Tropical Research, and other partners, are engaged in research that seeks to answer two fundamental questions about migratory bird movements that have significant implications for full annual cycle conservation: Can we link the breeding and non-breeding areas for specific populations of birds? And can we link specific breeding, wintering, and migration/stopover sites for individuals?

Linking Populations Across the Full Annual Cycle

Distinct populations of a species may inhabit different regions and climates, are often under widely divergent population pressures, and may require conservation strategies suited to their circumstances. For example, Wilson's Warbler (*Cardellina pusilla*), a Neotropical migrant passerine that is widely distributed across both its breeding range and wintering ranges, nests in northern forests across nearly every Canadian province and northern U.S. state. The overall population of the species declined about 1.8% per year from 1966-2015 (n=989 survey routes; Sauer et al. 2017), though the change by regional population differs markedly in the 14 regions differentiated by the analysis. For example, the Boreal Taiga Plains population (n=45 survey routes) increased at about 3.14% per year; the Coastal California population (n=75 survey routes) remained relatively flat (0.06% annual decline); and the Sierra Nevada population (n=31 survey routes) declined at 4.19% per year (Figure 1).

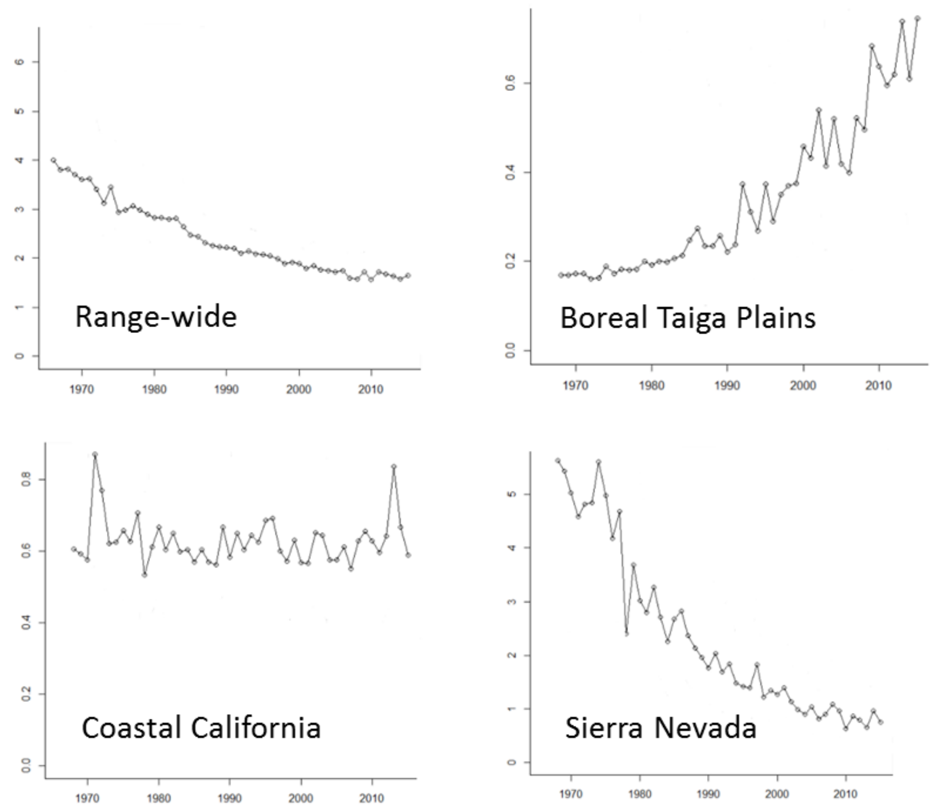

Figure 1. North American Breeding Bird Survey population trends of Wilson's Warbler, 1966-2015. The Y-axis represents an index of the mean count of Wilson's Warbler on a typical route for a given year. Though the species is declining range-wide, some regions, such as the Boreal Taiga Plains, are showing an apparent population increase; others, such as Coastal California, are maintaining relatively stable populations; and populations in some areas, such as the Sierra Nevada, are declining sharply. Data from the North American Breeding Bird Survey (Sauer 2017).

Given this information, it makes sense to focus conservation efforts on the populations most in need. However, at least one important question remains: how do we know which life history stage – breeding, migration, or wintering -- is most responsible for the decline? For the Wilson's Warbler example, how do we know if the rapidly declining Sierra Nevada population is suffering from low productivity in California, low survival on its wintering grounds, or factors during migration? And, just as importantly: Where *are* its wintering grounds and migration routes?

In 1989, The Institute for Bird Populations (IPB) initiated the Monitoring Avian Productivity and Survivorship (MAPS) Program to examine avian vital rates (especially productivity, adult apparent survival, and recruitment) that most strongly drive population change in landbirds. The MAPS Program uses a standardized system of passive mist netting, banding, and recapture to derive or model avian vital rates (DeSante et al. 2009). During nearly three decades, more than 1,300 MAPS stations have operated across North America, generating nearly two million avian capture records. In 2002, IBP initiated the Monitoreo de Sobrevivencia Invernal (MoSI) Program, which uses a similar protocol to examine the demographics of migrant and resident passerines and near-passerines in the northern Neotropics. About 60 MoSI stations are currently active in 13 countries, including Costa Rica. Analysis of MAPS capture records of Wilson's Warbler provide strong inference that annual survival of first year young – likely acting on the non-breeding grounds – has been the primary vital rate driving population change in this species (DeSante et al. 2015). Other species have shown low or declining productivity, likely with factors acting on the breeding grounds, and several species have demonstrated evidence of a combination of factors (DeSante et al. 2015).

Taking the next step – linking specific declining breeding populations with their wintering areas where poor winter survival may be responsible for the decline – often requires the use of either extrinsic markers (something attached to the outside of a bird, discussed in more detail below) or intrinsic markers (something that is physically a part of the bird). Two sets of intrinsic markers that have emerged as feasible, economical, and effective methods are the analysis of genetic markers and stable isotopes. Both use information contained within body tissues like feathers or blood. Genetic markers use various tissue samples collected from birds; isotopic analysis examines the ratio of stable isotopes present in the vane. Both methods can link an individual bird from which the feather was taken to its population of origin. For genetic analysis, feathers from the same population must be collected at both breeding and wintering areas. For isotopic analysis, if the feather was grown on the breeding grounds and collected on the wintering grounds, analysis can identify the area where the bird nested (or hatched). For this reason, understanding of the molt phenology of the study species is essential, as many species do not grow new feathers until after they leave their breeding grounds.

Some of our most recent work with genetic markers involved the delineation of breeding populations and linking breeding and wintering areas and migratory routes for Wilson's Warbler. Ruegg et al. (2014) used single nucleotide polymorphism (SNP), a DNA sequence variation occurring when a single nucleotide in the genetic code differs between individuals or homologous chromosomes. We used a 1,648 feathers and blood samples collected at 68 MAPS, MoSI, and Landbird Monitoring of North America (LaMNA) stations from across the breeding, wintering and migratory range of the species. Genetic samples, consisting of the base of one outer rectrix (or, some cases, blood collected by brachial vein puncture and preserved in lysis buffer; Seutin 1991), were purified using Qiagen DNeasy Blood and Tissue Kit and quantified using a NanoDrop™ Spectrophotometer (Thermo Scientific, Inc; Smith et al. 2003). Breeding (June 10 – July 31), spring migration (March 1 – May 31), and wintering (December 1 – February 28) samples were collected and categorized into groups based on collection date, signs of breeding (presence/size of a cloacal protuberance), signs of migration (extent of fat) and life history timetables for the Wilson's Warbler (Ammon and Gilbert 1999). The Fluidigm Corporation EP1™ Genotyping System was used to genotype 96 SNP loci using 94 individuals per run and assignments of individuals to breeding populations was done using the program GSI_Sim (Anderson 2010; Anderson et al. 2008).

We identified six distinct populations of Wilson's Warbler, roughly corresponding to existing taxonomic subspecies (Lowery and Monroe 1968), although our analysis distinguished two populations within the range of *C. p. pileolata* (Alaska, Canada, and northern Rockies); and three populations within the range of *C. p. chryseola* (Pacific coastal lowlands from southwest British Columbia through southern California and the Sierra Nevada; Figure 2). From genetic analysis of feathers from the wintering grounds, we were also able to identify the wintering areas of these populations. Most genetically distinct populations wintered in separate regions, although there was some mixing on the wintering grounds, especially for the smaller California and Northwestern U.S. populations.

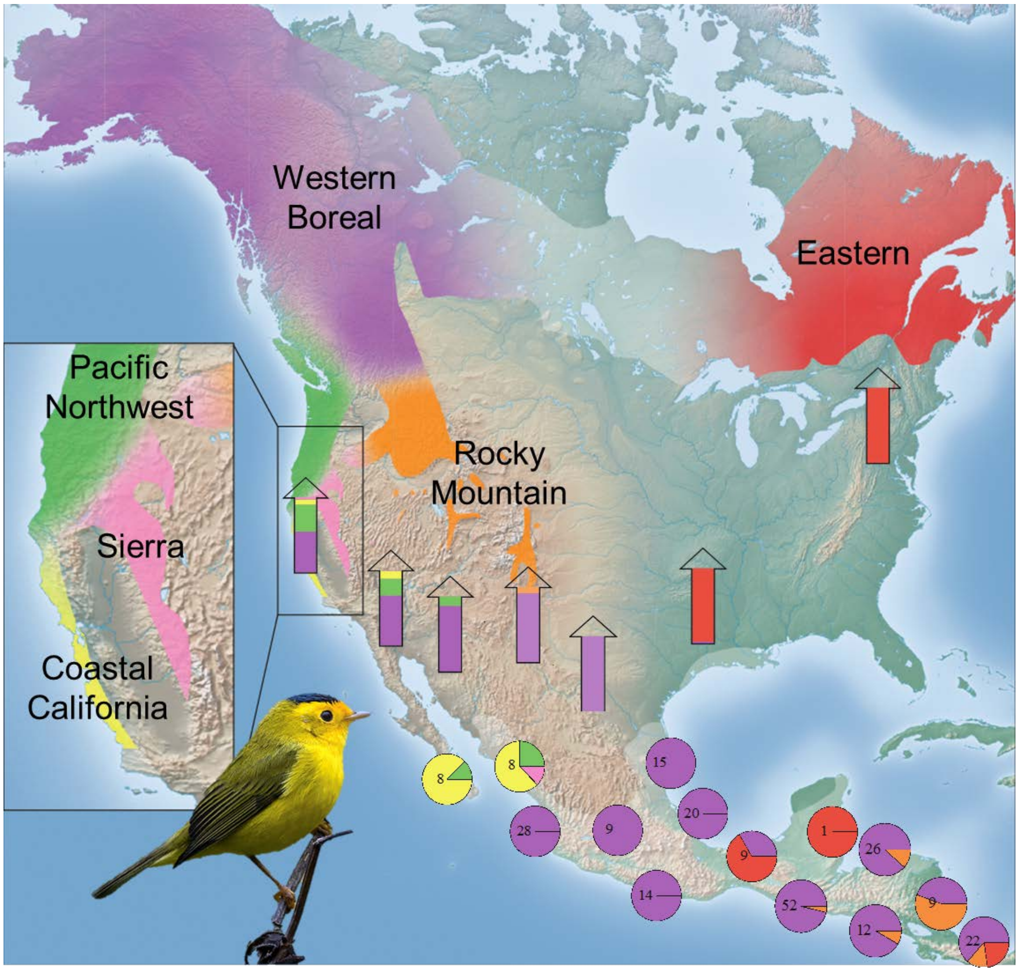

Figure 2. Migratory connections in the Wilson's warbler identified using genetic markers. The colors across the breeding represent genetically distinct populations. The pie charts over the wintering areas represent the relative proportion of individuals from those breeding ranges that were captured during the non-breeding season. Similarly, the arrows show the location of migration/stopover station locations, with the colors representing the proportion of birds from each population caught there during northward spring migration. (Figure from Ruegg et al. 2014 and used with permission.)

In addition, different populations migrated at different times. Using data and feathers collected at MAPS stations across the southern U.S. along migratory/stopover pathways, we discovered that the more southerly breeding populations generally migrated earlier than the more northerly ones (Ruegg et al. 2014).

The implications for the conservation of this and other species are potentially far reaching. For example, to improve the conservation outlook for this species, we recommend focusing attention on the non-breeding grounds (where annual survival appears to be a prominent factor in the population regulation of this species) especially in the wintering areas of the most steeply declining populations, for example west central Mexico, the wintering grounds of the Sierra Nevada population.

Linking specific breeding and wintering sites

The use of genetic markers and stable isotopes provide insights into the links between regional populations of migratory birds. In addition, several new technologies offer a more precise determination of the connections between specific breeding, stopover, and wintering sites. The miniaturization of electronics has enabled researchers to track large birds such as raptors, waterfowl, or wading birds continuously by satellite. Much smaller archival GPS units, which store data but require retrieval of the device, can track birds to within a few meters and are light enough to safely fit on birds as small as thrushes (Bridge et al. 2011) with continued miniaturization proceeding rapidly. Archival light-level geolocators, which record information on ambient light levels to estimate an individual's location, track birds with less precision, but can be affixed to even smaller birds, such as warblers (Stutchbury et al. 2009, Bridge et al. 2013).

During the 2014 North American summer (breeding season), in cooperation with biologists from Yosemite National Park, we outfitted nine adult Black-headed Grosbeaks (*Pheucticus melanocephalus*) with archival GPS tags to determine locations where the birds spent the non-breeding season. The birds were captured at a MAPS station in Yosemite National Park. In the summers of 2015 and 2016 we recaptured two of the birds and retrieved the GPS units. Data revealed that, by late summer 2014, the birds had moved approximately 1,300 km. from Yosemite National Park to Sonora, Mexico, where they remained at least two months before moving more than 1,300 km. further south into Mexico (the two individuals went to sites approximately , where they remained, apparently until beginning their northward migration (Siegel et al. 2016). (The autumn locations of the two birds, both in Sonora, were approximately 50 km apart; the winter locations, one bird near the Jalisco-Michoacan border, the other near the Sinaloa-Durango border region were about 500 km apart.) One of the most interesting aspects of the findings was the protracted stay in Sonora, which was consistent with the expected behavior of a molt-migrating bird. Molt is an energetically-taxing period of time (Murphy and King 1991, Voelker and Rohwer 1998), and may also impair flight ability (Swaddle and Witter 1997). Several species have been documented to move in search of stable new food sources during this time (Jehl 1990).

Figure 2. Recorded locations throughout the annual cycle of a Black-headed Grosbeak GPS-tagged and recaptured a year later at Hodgdon Meadow, Yosemite National Park (inset). A second Grosbeak, captured after the original publication went to press, exhibited similar movements. (Figure from Siegel et al. 2016 and used with permission.)

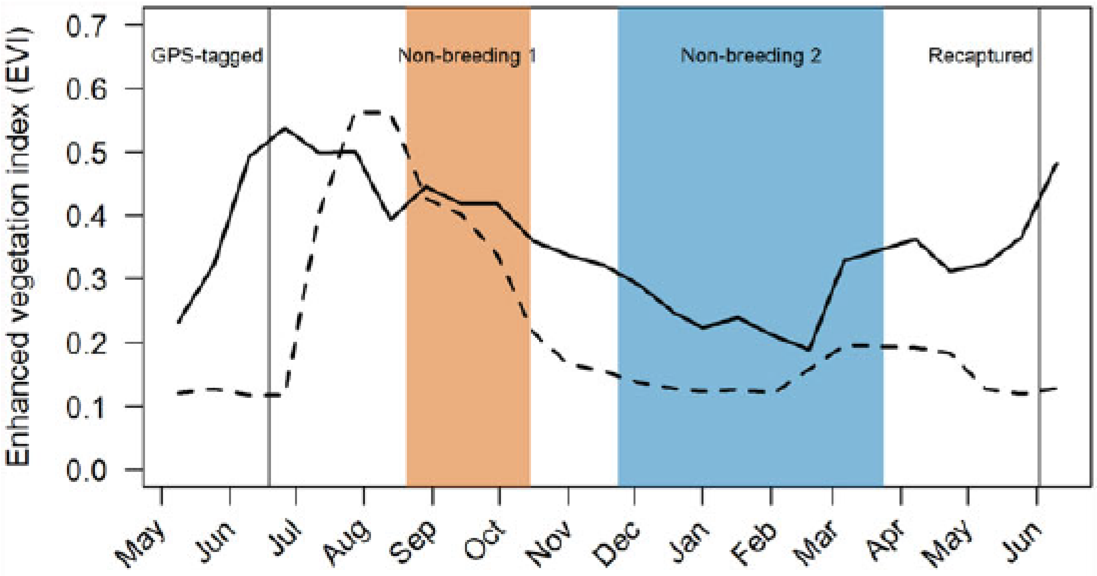

Figure 3. Enhanced vegetation index (EVI) data obtained from May 2014 to June 2015 at locations in Mexico where a male Black-headed Grosbeak captured and GPS-tagged in Yosemite National Park spent the presumed molting period (dashed line) and the remainder of the winter (solid line). Shaded area labeled Non-breeding 1 and Non-breeding 2, respectively, represent the minimum period of time the bird spent in Sonora (Non-breeding 1), and then near the border between Michoacan and Jalisco (Non-breeding 2). (Figure from Siegel et al. 2016 and used with permission.)

Climate change in the coming decades is expected to delay the annual onset of the monsoon while also accelerating the initiation of arid, summer-like conditions throughout much of western North America. If this produces a temporal mismatch between fall migration and the monsoon-driven conditions, it could have serious impacts for molt-migrating birds.

Summary

Recent advances in molecular, micro-electronic, and other technologies are being put to active use by ornithologists and conservation professionals from around the world. The results are providing new insights into full annual cycle dynamics and the needs of migratory birds at all stages of their lives. Previously undescribed ranges, habitats, and movement patterns are being delineated. More targeted conservation actions are now possible, based on this information, though caution is warranted, as the movements patterns discovered are often more complex and varied than previously surmised. The technologies described above and others, are becoming cheaper and easier to deploy, and can be used in conjunction with ongoing monitoring efforts such as the MAPS and MoSI Programs.

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