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RESEARCH ARTICLE

GPS tracking and population genomics suggest itinerant breeding across drastically different habitats in the Phainopepla

Daniel T. Baldassarre,[1,a] Leonardo Campagna,[2,3] Henri A. Thomassen,[4] Jonathan W. Atwell,[5] Miyoko Chu,[6] Lisa H. Crampton,[7] Robert C. Fleischer,[8] and Christina Riehl[1]

[1] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, USA
[2] Fuller Evolutionary Biology Program, Cornell Laboratory of Ornithology, Cornell University, Ithaca, New York, USA
[3] Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York, USA
[4] Institute for Evolution and Ecology, University of Tübingen, Tübingen, Germany
[5] Department of Biology, Indiana University, Bloomington, Indiana, USA
[6] Communications Program, Cornell Laboratory of Ornithology, Ithaca, New York, USA
[7] Kaua'i Forest Bird Recovery Project, University of Hawaii at Manoa, Hanapepe, Hawaii, USA
[8] Center for Conservation Genomics, Smithsonian Conservation Biology Institute, Washington, D.C., USA
[a] Current address: Department of Biological Sciences, SUNY Oswego, Oswego, New York, USA
* Corresponding author: daniel.baldassarre@oswego.edu

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ABSTRACT

Migratory birds generally divide the annual cycle between discrete breeding and nonbreeding ranges. Itinerant breeders, however, reproduce twice at different geographic locations, migrating between them. This unusual flexibility in movement ecology and breeding biology suggests that some species can rapidly modulate the conflicting physiological and behavioral traits required for migration and reproduction. The Phainopepla (*Phainopepla nitens*), a songbird of the southwestern USA, has long been suspected to breed first in desert habitats in spring, then migrate to woodland habitats to breed again in summer. However, direct evaluation of movement and gene flow among individuals breeding in different locations has previously been logistically intractable. We deployed GPS tags on free-flying Phainopeplas in southern California, all of which migrated to hypothesized woodland breeding habitats after desert breeding (an average distance of 232 km). GPS data also revealed previously unknown fall and spring stopover sites. Population genomic analyses revealed no genetic differentiation among desert and woodland breeding populations, indicating significant movement and gene flow across the region. Finally, we used random forest analyses to quantify substantial environmental differences among temporal stages. Our results provide direct evidence that individual Phainopeplas do indeed move between 2 drastically different breeding habitats in the same year, representing a rare and extreme example of life-history flexibility.

Keywords: GPS tracking, itinerant breeding, life history, Phainopepla, population genomics, random forest

Análisis de rastreo por GPS y genómica poblacional sugieren que el Capulinero negro (*Phainopepla nitens*) se reproduce de manera itinerante en habitats drásticamente diferentes

RESUMEN

Las aves migratorias generalmente dividen su ciclo anual entre dos rangos geográficos discretos: el rango reproductivo y el no reproductivo. Las aves denominadas reproductores itinerantes, sin embargo, se reproducen dos veces en diferentes ubicaciones geográficas, migrando entre ellas. Esta flexibilidad inusual en aspectos contradictorios de la ecología del movimiento y la biología reproductiva, sugiere que algunas especies pueden modular rápidamente los rasgos fisiológicos y comportamentales que se requieren para la migración y la reproducción. *Phainopepla nitens*, un ave Passeriforme del sudoeste de EEUU, ha sido sospechada por mucho tiempo de reproducirse primero en ambientes desérticos en primavera y luego migrar a ambientes boscosos para reproducirse nuevamente en verano. Sin embargo, hasta ahora no ha sido posible logísticamente evaluar de manera directa el movimiento y el flujo génico entre individuos reproduciéndose en diferentes lugares. En este estudio, colocamos marcadores de GPS en individuos libres de *P. nitens* en el sur de California, los cuales migraron a los hipotéticos ambientes reproductivos boscosos luego de reproducirse en el desierto (una distancia promedio de 232 km). Los datos de GPS también revelaron sitios de parada de otoño y primavera previamente desconocidos. Los análisis de genómica poblacional revelaron que no existe diferenciación genética entre las poblaciones reproductivas del desierto y del bosque, indicando movimientos y posible flujo génico a través de la región. Finalmente, usamos análisis de bosques aleatorios para cuantificar diferencias ambientales entre los sitios ocupados por las aves en los diferentes períodos temporales. Nuestros resultados brindan evidencia directa de que los individuos de *P. nitens* efectivamente se mueven entre dos ambientes reproductivos drásticamente diferentes en el mismo año, representando un ejemplo raro y extremo de flexibilidad en la historia de vida.

Palabras clave: bosques aleatorios, genómica poblacional, historia de vida, Phainopepla, rastreo por GPS, reproducción itinerante

INTRODUCTION

Life-history theory posits that traits such as reproductive rate, development time, and longevity vary along a limited number of axes due to physiological constraints (Ricklefs and Wikelski 2002). Certain life-history components may be fundamentally at odds, reducing the flexibility and complexity of life histories observed in nature. In birds, reproduction and migration are 2 of the most energetically demanding events in the annual cycle, typically occurring at different times of the year with little or no overlap (Newton 2008, 2011). The risks and costs associated with migration impose severe constraints on reproduction, curtailing the time available for breeding (Newton 2008) and requiring rapid physiological shifts between breeding and nonbreeding states (Wingfield 2005).

The vast majority of birds, therefore, partition the annual cycle into 2 distinct breeding and nonbreeding periods (Newton 2008). A few species, however, are thought to breed in 2 locations in the same year and migrate between them (Moreau 1951, Ward 1971, Bucher 1982, Jaeger et al. 1986, Hamilton III 1998, Wilson et al. 2016). These species, known as itinerant breeders, are commonly characterized by reliance on a regionally shifting food supply, rapid breeding bouts, and high rates of nest failure. Itinerant breeders pose a challenge to the common assumption that migrants are limited in their ability to breed opportunistically, either by the time costs associated with switching between physiological states, or by inherent conflicts between the endocrine mechanisms underlying those states (Ricklefs and Wikelski 2002, Wingfield 2005). Direct evidence for itinerant breeding, though, is rare: individual birds have been conclusively documented moving between different breeding populations in only the Red-billed Quelea (*Quelea quelea*; Jaeger et al. 1986) and the Tricolored Blackbird (*Agelaius tricolor*; Wilson et al. 2016). Several other cases of itinerant breeding are suspected based on observations of aberrant individuals (Osborn 2000) or population-level patterns (Rohwer et al. 2009, Newton 2011), but these inferences have been largely inconclusive (Rohwer et al. 2012). Careful study of itinerant breeders can thus provide insights into the ecology and evolution of migratory and reproductive diversity.

The Phainopepla (*Phainopepla nitens*), a songbird of the southwestern USA and northwestern Mexico, has been suspected to be an itinerant breeder for over a century (Gilman 1903). Historically, authors have disagreed over the idea that the same individuals move between habitats and breed twice (Gilman 1903, Dawson 1923, Crouch 1943, Walsberg 1977, Chu 1999), but several lines of evidence suggest that southern California Phainopeplas breed first in Mojave and Sonoran desert habitats in spring, then migrate to coastal oak woodland habitats to breed again in summer. First, the timing of egg laying is bimodal, with a March peak in the desert followed by a June peak in coastal woodlands (Walsberg 1977, Chu 1999). Second, migratory flocks have been observed in spring and fall (Walsberg 1977, Chu 1999). Third, birds exhibiting postbreeding molt have only been observed following the summer woodland breeding period (Chu 1999). Fourth, there are no significant differences in external morphology between birds captured in desert and woodland habitats (Chu 1999). Finally, Phainopeplas are vocal mimics, and individual birds in desert habitats were observed mimicking species found in woodland habitats and vice versa (Chu 2001). This change in physical environment is also accompanied by a shift from territoriality in the desert to loose coloniality in woodlands (Walsberg 1977, Chu 1999), meaning that Phainopeplas exhibit remarkably flexible social behavior if the same individuals do indeed breed under both conditions. To date, it has not been possible to rule out the alternative hypotheses that desert- and woodland-breeding populations are either completely spatially separate, or have distinct phenologies, with individuals breeding in only one habitat type per year and forming nonbreeding groups the rest of the year (Gilman 1903, Dawson 1923, Crouch 1943).

Our goals were to determine whether individual Phainopeplas migrate between and breed in desert and woodland habitats, and to quantify the variation in environmental conditions experienced during the phases of their annual cycle. We first deployed miniaturized GPS loggers on desert-breeding individuals to precisely track their migration. We also performed population genomic analyses of breeding individuals across 5 desert and 4 woodland breeding sites throughout southern California and Nevada. If the same individuals breed in desert and woodland habitats, we expect to find no genetic differentiation among habitat types. Finally, we used a combination of land cover and climate variables to quantify differences in the physical environment at each temporal stage. Combined, these analyses characterize the spatial and temporal dynamics of the Phainopepla's migratory and breeding strategies throughout the annual cycle, providing evidence to evaluate the itinerant breeding hypothesis.

METHODS

General Field Methods

We captured adult Phainopeplas using mist nets. At a Mojave Desert population in Afton Canyon, California (35.04°N, 116.38°W), where we banded and monitored the entire breeding population, we deployed miniaturized (1 g) PinPoint-10 archival GPS loggers (hereafter "tags", Lotek Wireless, Newmarket, Ontario, Canada) on 24 adults (14 males, 10 females) that bred in this desert habitat in March and April, 2017. We intensively monitored

the population to confirm that all tagged individuals attempted breeding, although none successfully fledged young due either to predation or destruction of nests by a severe windstorm. Tags small enough for passerines have only recently been developed (Hallworth and Marra 2015, Siegel et al. 2016, Fraser et al. 2017), and have distinct advantages over light-level geolocators that are inherently inaccurate over small latitudinal changes (Lisovski et al. 2018). The tags were 4% of the average bird's weight (24.8 g, range: 21.25–31.75 g), and were attached using a modified leg-loop harness (Rappole and Tipton 1991). The programmed schedule of GPS signal acquisitions varied among individuals and over the course of deployment but averaged 1 location per 4.5 days (range: 3–10 days). GPS points were recorded during daylight hours to avoid recording roosting locations. We revisited this site in October and November, recaptured tagged birds returning for the winter, removed the tag, and retrieved the data. For population genomic analyses, we captured breeding birds at 5 desert and 4 woodland sampling locations throughout southern California and Nevada (Table 1). Breeding was confirmed by direct field observations of nesting activity (Hogback Creek, California: 12 breeding pairs; Tejon Ranch Conservancy, California: 23 breeding pairs; King Gillette Ranch, California: 18 breeding pairs; Starr Ranch, California: 22 breeding pairs; unpublished data). We intentionally sampled breeding individuals in each location, so as to avoid quantifying gene flow from birds migrating but not breeding in a given location. We collected ~40 μL of blood from the brachial vein and stored it in lysis buffer for DNA extraction.

GPS Tracking

We plotted the GPS tracks using the R (R Core Team 2018) package *ggmap* (Kahle and Wickham 2013). Each individual's GPS track was characterized by a general pattern of 3 or 4 relatively stationary periods interspersed by long flights. To objectively delineate these stationary periods for spatial analyses, we first calculated the shortest great-circle distance between consecutive points using the Haversine method in the R package *geosphere* (Hijmans 2017). We then plotted these flight distances as a function of date and considered a stationary period to have begun when the individual made 2 consecutive flights of decreasing distance (i.e. the distance between points C and D < between B and C < between A and B), and to have ended when the individual made 2 consecutive flights of increasing distance (i.e. the distance between points C and D > between B and C > between A and B). In rare instances where an individual made only 1 long flight between obviously stationary periods, we made a qualitative assessment. This approach allowed us to delineate the start and end dates for every stationary period, and to compile a set of GPS points within each of these stages.

Laboratory Methods

We extracted genomic DNA from 96 individuals using the DNeasy Blood and Tissue Kit (Qiagen, California, USA) and quantified concentration with a Qubit fluorometer (Invitrogen, California, USA). We generated ddRADseq markers following the protocol described in (Peterson et al. 2012) and modified by Thrasher et al. (2018). Briefly, we digested the DNA samples with *EcoRI* and *MspI*, ligated adapters, ran low-cycle PCR to add index primers, selected fragments in the range of 300–600 base pairs (bp) using BluePippin (Sage Science, Beverly, Massachusetts, USA), and pooled index libraries together. We sequenced the pooled fragments on an Illumina HiSeq 2500 machine at the Princeton University Lewis-Sigler Institute for Integrative Genomics that produced 150 bp single-end reads.

RAD Loci Assembly and Single Nucleotide Polymorphism Calling

We obtained a total of 188.3 million raw reads across all individuals (23.5 ± 1.8 million per index group). We trimmed all sequences to 145 bp with FASTX Trimmer (http://hannonlab.cshl.edu/fastx_toolkit), filtered reads using FASTX Quality Filter, and demultiplexed the reads using the *process_radtags* module from STACKS 1.48 (Catchen et al. 2011). After demultiplexing, we retained 1.19 ± 0.86 million sequences per individual, all of which were 140 bp after barcode removal.

TABLE 1. Sampling locations for population genomic analyses. Site codes are used in Figure 3.

Sampling location	Habitat	Site code	Latitude	Longitude	*n*
Afton Canyon, CA	Desert	AC (D)	35.04	−116.38	13
Granite Mountains, CA	Desert	GM (D)	34.78	−115.65	5
Big Bend of Colorado, NV	Desert	BBS (D)	35.09	−114.64	9
Las Vegas, NV	Desert	LVS (D)	36.17	−115.19	4
Deep Canyon, CA	Desert	DC (D)	33.66	−116.37	10
Hogback Creek, CA	Woodland	HC (W)	36.66	−118.14	10
Tejon Ranch Conservancy, CA	Woodland	TRC (W)	35.07	−118.74	5
King Gillette Ranch, CA	Woodland	KGR (W)	34.10	−118.70	6
Starr Ranch, CA	Woodland	SR (W)	33.63	−117.56	10

We assembled RAD loci de novo in STACKS by running *ustacks/cstacks/sstacks* controlled by the *denovo_map* program. This de novo pipeline requires the user to set thresholds for different assembly parameters (e.g., m = minimum depth of coverage, M = number of mismatches between sequences, and n = number of mismatches between individuals). To explore the sensitivity of the results to the parameters used for the de novo assembly, we created 2 different assemblies with different combinations of parameters: $m = 10, M = 2, n = 2$ (hereafter m10M2n2); and $m = 20, M = 5, n = 5$ (hereafter m20M5n5). Higher M and n thresholds can tend to lump loci, whereas lower values may over-split loci. A lower threshold for the minimum depth of coverage parameter, m, should allow more loci to be retained. We therefore generated an assembly with low parameter values and one with higher ones, yet obtained similar results (see below). The catalog for m10M2n2 contained 72,848 RAD loci and the catalog for m20M5n5 contained 68,435 loci.

We exported single nucleotide polymorphisms (SNPs) from these catalogs using the *populations* module from STACKS. We discarded 24 samples with either a low number of total reads and/or high levels of missing data as a tradeoff to retain a larger number of SNPs. We only allowed 1 SNP per RAD locus to avoid using tightly linked SNPs, required a minimum coverage of 5x, and applied a 2% minor allele frequency filter. We explored the effect of retaining more loci at the expense of allowing for more missing data by using 2 missing data thresholds: requiring 50% or 70% of samples to be genotyped to export a SNP. These criteria produced 4 different datasets: 581 SNPs (m10M2n2 assembly, 30% missing data), 544 SNPs (m20M5n5 assembly, 30% missing data), 4,140 SNPs (m10M2n2 assembly, 50% missing data), and 4,307 SNPs (m20M5n5 assembly, 50% missing data). The 4 datasets produced concordant results, but we present those from m10M2n2 with 30% missing data in the main text.

Population Genomic Analyses

We searched for population structure in our dataset using a variety of complementary methods which extract information either from SNPs or entire haplotypes. We first input the SNPs into a discriminant analysis of principal components (DAPC; Jombart et al. 2010) using the R package *adegenet* (Jombart 2008) to visualize genetic clustering. We then calculated the overall level of divergence between desert and woodland habitats and among all sampling locations by performing an analysis of molecular variance (AMOVA) in the R package *poppr* (Kamvar et al. 2014), testing significance by comparing with 10,000 random permutations. We also calculated fixation indices (F_{ST} values) at these same hierarchical levels using the R package *hierfstat* (Goudet 2005). To incorporate a model-based approach with a different statistical framework, we used the genetic clustering method *snapclust* (Beugin et al. 2018) within *adegenet* to implement a maximum likelihood-based assignment of individuals to genetic clusters (k). We ran *snapclust* for $k = 1–9$ and compared model fits using Bayesian information criterion (BIC). Finally, we utilized haplotype information in the program *fineRADstructure* (Malinsky et al. 2018) to estimate shared ancestry and derive a coancestry matrix among individuals. We used the haplotype data from the m10M2n2 assembly with 30% missing data, allowing up to 10 SNPs per locus, and default values for the remaining parameters. Analyses excluding individuals with different levels of missing data (30, 50, and 70%) were consistent, so we present results with all individuals included, derived from 1,263 RAD loci. This dataset was not obtained using a minor allele frequency filter and also contains invariant loci.

Random Forest Analysis of Environmental Variables

To characterize the environmental conditions during the time of year that birds were present in a given area, we used time series of vegetation and climate data (Supplementary Material Table S1). Vegetation data consisted of the Normalized Difference Vegetation Index (NDVI) at 250-m spatial resolution and 16-day temporal resolution for 2000–2018 obtained from the Global Land Cover facility database. We chose this particular NDVI dataset because it consists of high-quality data checked for quality and corrected for anomalies from sources such as cloud cover and instrument errors. Climate data consisted of 7 monthly variables: mean temperature, minimum and maximum temperature, mean precipitation, mean solar radiation, mean wind speed, and mean water vapor pressure at 30 arcsec (1 km × 1 km) spatial resolution from 1970–2000 (Fick and Hijmans 2017), available at www.worldclim.org. Because we analyzed differences between seasons, and solar radiation partially depends on the time of the year (i.e. the hours of daylight), we computed the solar radiation in kJ/m^2/hour of daylight. We identified the periods defining each temporal stage (see movement criteria above) and selected 16-day NDVI data and monthly climate data within these timeframes, or with the best possible match. We processed geographic information system (GIS) layers in QGIS 2.18.14 with GRASS 7.2.2 (Team 2016). Importantly, although the environmental data we included in our analyses come from years prior to when the GPS data were collected, we are still able to quantify general environmental differences among the different regions, independent of weather anomalies that can bias analyses that attempt to extract environmental data from the exact time periods when birds are present. In general, our aim was to explore the possibility that birds are moving over considerable distances in a multi-step migration, across quantitatively different types of landscapes.

For this reason, we did not restrict our vegetation or climate dataset to the precise dates when birds were present. Movement decisions of individuals as a function of current, local conditions may be relevant to this species, but is not the focus of this study.

To test whether the different temporal stages were characterized by different environmental conditions, we ran random forest models (Liaw and Wiener 2002) in R. No a priori assumptions are made about the relationship between predictor and response variables, allowing for the possibility of nonlinear relationships with complex interactions. This approach has the benefit of allowing the model to determine what environmental variables distinguish different sites without making assumptions about which are biologically meaningful to the species in question. The iterative nature of these models provides statistically rigorous statements about the relationships between predictor and response variables, as measured by the percent of variation explained by the full forest, and by measures of individual variable importance (Breiman et al. 1984), and results have been shown to outperform traditional regression techniques (Breiman 2001, Prasad et al. 2006). Using the movement criteria described above, we compiled a list of GPS points within each temporal stage. Most of these points came directly from the tags, but we also included several GPS points from field surveys during the same time periods (see raw data in Dryad). We subsampled this list of GPS points by removing close points until there was only a single point in any 1-km grid cell, which represented the coarsest resolution of the environmental data. Because our dataset contained many more sites for the woodland breeding stage (n = 53) than for the other stages (desert breeding: n = 12, spring stopover: n = 8, fall stopover: n = 17), we explored the influence of the imbalance in group sizes. To do so, we iteratively subsampled the woodland breeding sites down to n = 15 and reran a random forest model 1,000 times.

RESULTS

GPS Tracking

Return rates were similar between tagged (6/24, 25%) and untagged (12/42, 29%) birds. There was no difference in bird weight before tag deployment and after removal (paired t-test: t = −1.18, df = 4, P = 0.3). One tag was lost, so we analyzed GPS data from 5 individuals (4 males and 1 female), with an average of 51 GPS points (range: 45–58) over 223 days (range: 211–235 days).

All 5 individuals departed Afton Canyon shortly after tag deployment (mean: April 5, range: March 25 to April 19), and migrated to woodland habitats along the California coast an average of 232 km away (range: 153–395 km; Figures 1 and 2). Four individuals spent time (mean: 17 days, range: 5–29 days) at a spring stopover site between the desert and woodland breeding sites (Figures 1B–E and 2A–D), with the fifth individual migrating directly to woodland habitat (Figures 1F and 2E). Arrival in coastal woodland habitats was highly synchronous (mean: May 22, range: May 19 to May 29), and coincided with the onset of woodland breeding known from previous studies (Walsberg 1977, Chu 1999; Figure 2). Departure date from potential woodland breeding sites was more variable than arrival date (mean: August 26, range: August 5 to September 26), but also matched the known phenology. After departing from the woodland habitat, 4 individuals spent time (mean: 35 days, range: 9–64 days) at a fall stopover site before returning to the original desert site (Figures 1B, 1C, 1E, 1F, and 2A, 2B, 2D, 2E), including 2 that spent this time in northern Mexico (Figures 1E, 1F), with the fifth individual migrating directly back to the desert breeding site (Figures 1D, 2C). There was substantial variation in the total distance traveled among individuals (mean: 1,355 km, range: 871–1,991 km), with an average rate of 6 km/day (range: 4–9 km day^{-1}). Four discrete temporal stages emerged from the GPS data: desert breeding, spring stopover, woodland breeding, and fall stopover (Figure 2F).

Population Genomic Analyses

Analyses using the 581-SNP dataset revealed no genetic differentiation between individuals breeding in desert and woodland habitats or among individual sampling locations. First, all sampling locations clustered together according to DAPC plotted on 2 discriminant factors (Figure 3). Second, 99% of the genetic variation in the dataset was due to within-individual variation, with nonsignificant fixation index values (Φ) between woodland and desert habitats and among sampling locations (Table 2). Similarly, F_{ST} was low between woodland and desert habitats (0.006) and among pairwise sampling locations (mean: 0.041, range: 0.024–0.077; Supplementary Material Table S2). The likelihood-based method *snapclust* assigned individuals to one genetic cluster, which produced the best model fit compared with k = 2–9 (ΔBIC range: 3,340–16,828; Supplementary Material Figure S1). These results were qualitatively similar to those obtained with the 3 other SNP datasets (Supplementary Material Tables S2 and S3, Supplementary Material Figures S1 and S2). Similarly, using haplotype information, *fineRADstructure* revealed no clusters of coancestry between habitats or sampling locations, consistent with analyses using 1 SNP per RAD locus (Supplementary Material Figure S3).

Random Forest Analysis of Environmental Variables

Our random forest classification model performed well, with an error rate of 3.33%. Iterative subsampling of the woodland sites suggested little influence of the imbalance

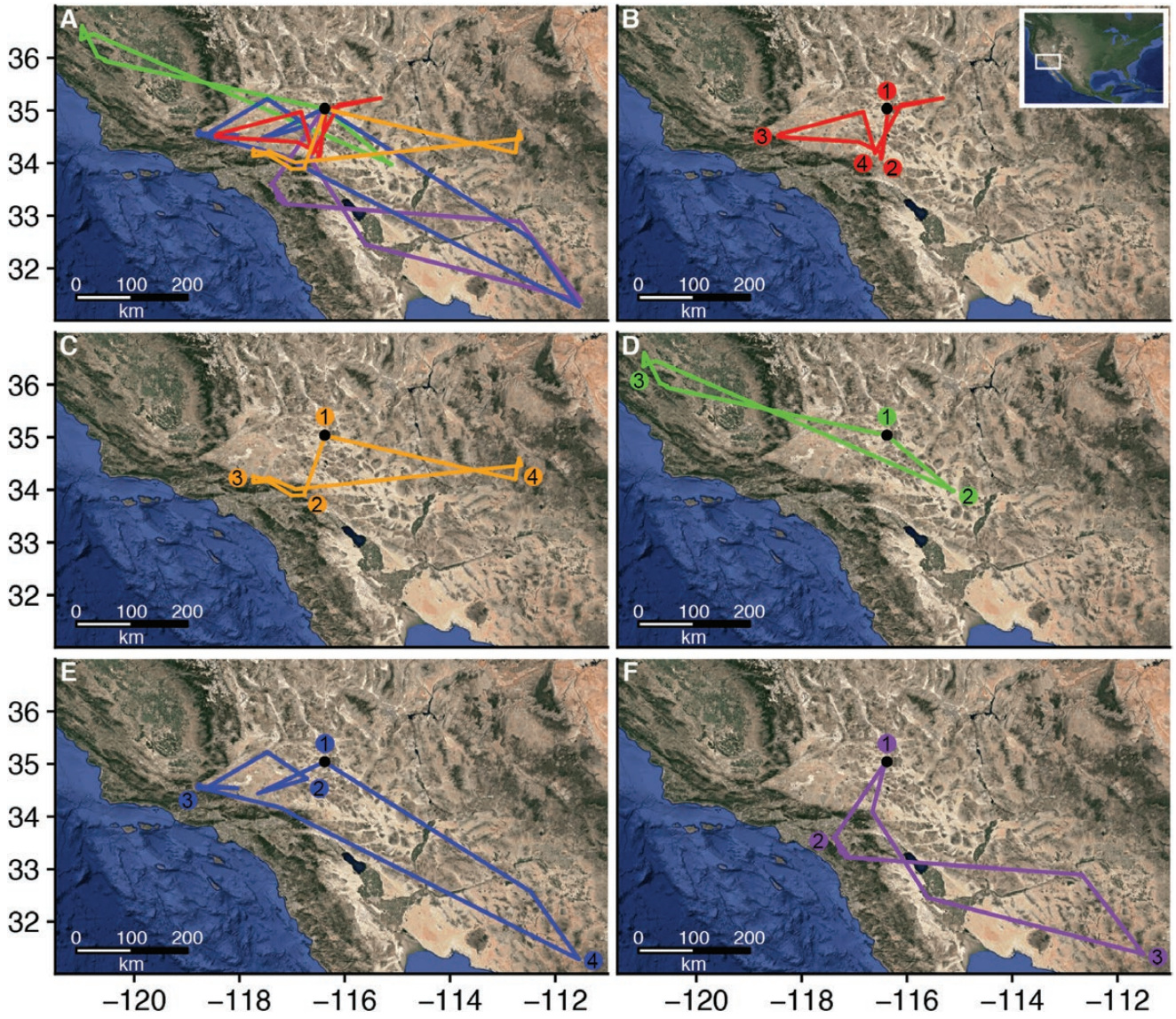

FIGURE 1. GPS tracks for 5 individuals (**A**) tagged after desert breeding at Afton Canyon, CA (black dot). Lines connect locations where GPS fixes were taken, but may not represent the precise route traveled by individuals. Tracks are isolated per individual in panels **B–F**. Numbers along the tracks indicate temporal stages in chronological order. Individuals in panels **B–E** are males, while the individual in panel **F** is a female.

in group sizes (mean error rate: 5.38% ± 0.78%). The model grouped 1/12 desert breeding sites with the spring stopover sites, 1/8 spring stopover sites with the woodland breeding sites, and 1/17 fall stopover sites with the woodland breeding sites (Table 3). The most important variable in distinguishing between stages was solar radiation per hour of daylight, closely followed by precipitation (Figure 4A), and ranking did not change in the 1,000 permutations of woodland breeding sites. Nonparametric Kruskal-Wallis tests with post-hoc Mann-Whitney U tests revealed that solar radiation per hour of daylight was highly significantly different among stages (Kruskal-Wallis $\chi^2 = 68.7$, df = 3, $P < 0.001$; Mann-Whitney U P-values: desert breeding-spring stopover = 0.001, all others <0.001; Figure 4B), as was precipitation, except for no difference between desert breeding and spring stopover (Kruskal-Wallis $\chi^2 = 67.8$, df = 3, $P < 0.001$; Mann-Whitney U P-values: desert breeding-spring stopover = 1.0, all others <0.001; Figure 4C). In fact, we found significant differences among stages in all seasonal environmental variables, with significant post-hoc differences between at least 4/6 pairs of stages in all variables (Supplementary Material Table S4 and Supplementary Material Figure S4).

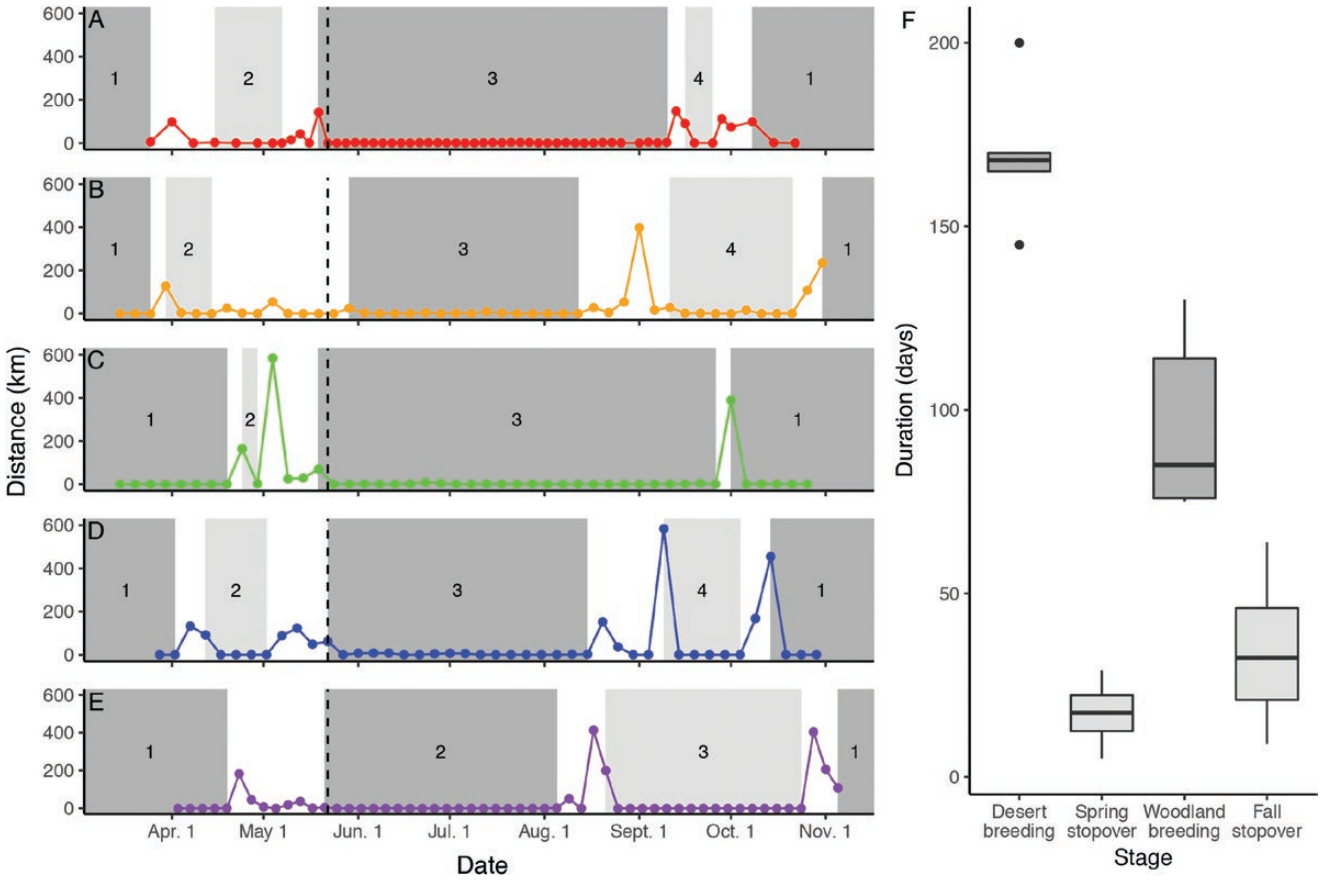

FIGURE 2. Distance traveled as a function of date for each tagged individual (**A–E**). Shaded sections represent temporal stages (dark gray: breeding, light gray: stopover). Each individual's track begins near the end of the desert breeding stage and ends upon return to that same desert breeding site. Numbers denote consecutive stages for each individual, corresponding to the numbers in Figure 1, but are not comparable among individuals, as some did not experience every stage. The vertical dashed line indicates the average date of arrival to the woodland breeding habitat. Boxplots representing medians and quartiles of the amount of time tagged individuals spent in each stage (**F**).

DISCUSSION

Itinerant Breeding in Desert and Woodland Habitats

Multiple lines of evidence suggest that the Phainopepla is an itinerant breeder, with the same individual birds potentially breeding in desert habitat in spring, and again in coastal woodland habitat in summer. Most other proposed examples of itinerant breeding in birds have relied on population-level observations (Bucher 1982, Hamilton III 1998, Rohwer et al. 2009) or were limited to one aberrant individual (Osborn 2000). Studies of the Tricolored Blackbird (Wilson et al. 2016) and Red-billed Quelea (Jaeger et al. 1986) have also documented individual birds moving between and breeding in multiple locations, but the present study is the first to also quantify lack of genetic structure among potentially itinerant breeding populations and to quantify environmental variation.

While we recognize the limitations of overinterpreting movement data from 5 individuals, GPS data clearly revealed that all birds tagged after desert breeding migrated to coastal woodland habitats during summer (Figure 1). Arrival to the woodland habitats was highly synchronous compared with temporal patterns during other stages, consistent with the beginning of a distinct woodland breeding phase (Figure 2). All 5 individuals arrived in woodland habitats within 10 days of each other, which is particularly remarkable considering they went to 5 separate locations ranging from Monterey to Riverside counties (~400 km apart). Furthermore, the timing of arrival and departure was highly consistent with the previously established phenology of the woodland breeding phase (Walsberg 1977, Chu 1999). Although tagged birds were not observed during surveys in woodland habitats, other Phainopeplas were seen breeding in woodland habitat in summer (D.T.B., J.W.A., and M.C. personal observation). Thus, the GPS data revealed that desert-breeding individuals migrated to habitats when and where Phainopeplas are known to breed in summer. Importantly, these movement patterns cannot rule out the possibility that an individual would only breed in the woodland habitat after having failed to breed in the

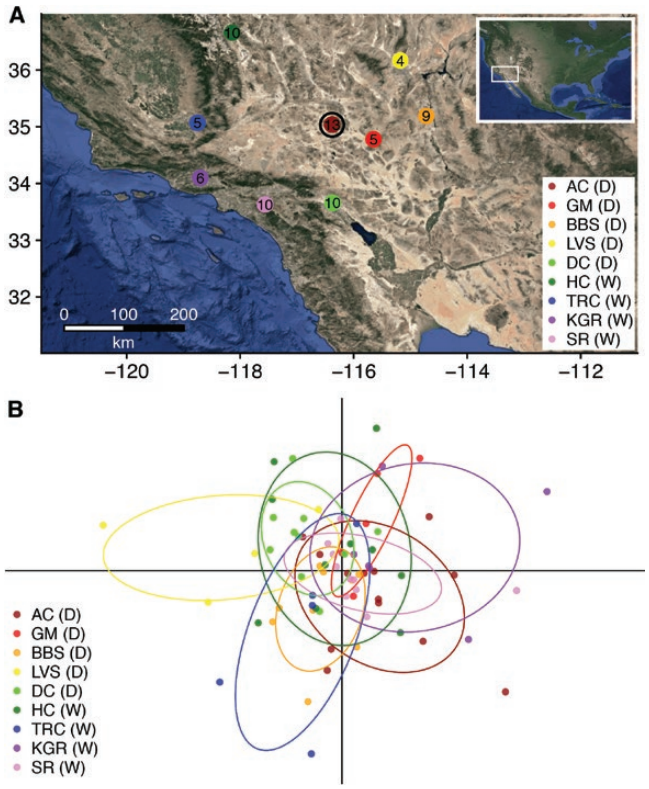

FIGURE 3. Sampling locations and samples sizes used in population genomic analyses (**A**). The site where GPS tags were deployed is enclosed with a black circle. Results of DAPC analysis of multivariate genetic clustering where the axes represent the first and second discriminant factors (**B**). Sampling locations are color-coded with individual points and 95% inertia ellipses, with colors matching the locations from **A**. In both **A** and **B**, D = desert breeding habitat and W = woodland breeding habitat. Site codes are provided in Table 1.

desert habitat, or that successful desert breeders migrate to woodland habitat but spend the summer as nonbreeders alongside breeding individuals.

There was wide individual variation in the distance traveled and area covered during the hypothesized woodland breeding period (Supplementary Material Figure S5). A closer analysis of movement patterns during this period suggests that some individuals may have attempted to breed, while others did not. Some individuals had restricted movement patterns highly consistent with attending to a nest (Supplementary Material Figures S5A and S5E), while others likely did not remain in one area long enough to raise a clutch (Supplementary Material Figures S5B–D). However, we are again hesitant to overinterpret these movement data due to the relatively coarse temporal scale. Scheduling more GPS fixes during the woodland breeding stage could alleviate this issue, and field surveys of individually marked birds in both habitats are necessary.

While movement data alone cannot confirm breeding in the woodland habitat, genomic analyses of population structure also support the itinerant breeding hypothesis. We found no evidence of genetic structure between populations of breeding individuals in desert and woodland habitats, or among any individual sampling locations (Table 2 and Figure 3, Supplementary Material Tables S2 and S3, Supplementary Material Figures S1–S3). The lack of genetic differentiation among groups of individuals breeding in both habitat types suggests 1 panmictic breeding population, although we cannot definitively ascertain whether individual birds bred at 2 locations in the same year. Importantly, our geographic sampling scheme, although not comprehensive relative to the species range, covered a spatial area similar to that traversed by GPS-tracked individuals. We can thus directly infer a link between physical movement among desert and woodland sites and gene flow significant enough to prevent genetic differentiation. However, more widespread geographic sampling could reveal broader population structure not evident in this study, as the species range also includes 5 other southwestern states and northwestern Mexico.

A key to the movement ecology of the Phainopepla may be its reliance on an ephemeral and patchily distributed food resource. In desert habitat, they eat primarily Desert Mistletoe (*Phoradendron californicum*) berries (Walsberg 1975, Crampton et al. 2011, Crampton and Sedinger 2011), which peak in winter and can be sufficient to sustain a clutch in spring. However, berries are scarce outside of this period, which likely spurs migration to woodland habitat where several other berry-producing species and insect prey are available (Anderson and Ohmart 1978). When mistletoe is abundant, birds can raise at least one clutch in the desert before migrating, but this does not always happen (Walsberg 1977). Widespread mistletoe crop failure is common, and the entire population may refrain from breeding in response (Walsberg 1977, Chu 1999).

TABLE 2. AMOVA results partitioning genomic variance using 581 SNPs.

Source	df	Sum square	Mean square	σ	% var	Φ	*P*
Desert–woodland	1	98.07	98.07	−0.8	−0.09	−0.0009	0.6
Among sites	7	714.92	102.13	0.79	0.88	0.009	0.09
Among individuals	63	5665.7	89.93	0.53	0.59	0.006	0.4
Within individuals	72	6399.2	88.88	88.88	98.63	0.014	0.28

df = degrees of freedom, σ = variance, % var = percent of the total variance explained, Φ = fixation index.

TABLE 3. Confusion matrix of random forest classifications indicating the number of sites grouped within their own or other stages, and the total classification error for each stage. The expected classification error for a 4-group model that is performing no better than random is 0.75.

Temporal stage	Desert breeding	Spring stopover	Woodland breeding	Fall stopover	Classification error
Desert breeding	11	1	0	0	0.083
Spring stopover	0	7	1	0	0.125
Woodland breeding	0	0	53	0	0
Fall stopover	0	0	1	16	0.059

FIGURE 4. Variable importance from a random forest model distinguishing among temporal stages based on the seasonal conditions when Phainopeplas were present (**A**). See Supplementary Material Table S1 for definitions of each variable. Bar length is proportional to variable importance. Boxplots representing medians and quartiles of the top 2 important variables, solar radiation per hour (**B**) and precipitation (**C**), compared across temporal stages.

These patterns suggest that the Phainopepla has evolved to breed opportunistically in the desert in response to erratic abundance of food resources, before migrating to take advantage of new resources in woodland habitats in summer where they possibly breed again. Previous work demonstrating a seasonal increase in gizzard size as birds incorporate more diverse prey in woodland habitats supports the notion of dietary flexibility (Walsberg and Thompson 1990). Reliance on ephemeral food resources characterizes other itinerant breeders, such as the Red-billed Quelea, which tracks unpredictable rainfall and corresponding grass seeding across the Ethiopian Rift Valley (Ward 1971, Jaeger et al. 1986). Importantly, it is unclear how much annual variation there may be in the Phainopepla's migration ecology, especially resulting from weather anomalies. We present here GPS tracking information from one season when movement decisions may have been influenced by a recent El Niño event (Jacox et al. 2016). Indeed, we might expect substantial temporal variation to be a hallmark of itinerant breeders.

Random forest analyses allowed us to quantify significant differences between desert and woodland breeding sites in a suite of environmental variables. In fact, using seasonal data representative of conditions at times of the year when birds were actually present (Supplementary Material Table S2), we were able to distinguish all 4 stages as having unique environmental characteristics (Table 3 and Supplementary Material Table S4). Solar radiation per hour of daylight and precipitation were particularly important contributors to these models (Figure 4), but we also statistically confirmed differences between desert and woodland breeding habitats in vegetation and tree cover among other variables (Supplementary Material Table S4 and Supplementary Material Figure S4). It is difficult to infer causation between retention of a particular environmental variable in the model and the biological impact on individual breeding or migratory decisions. Measured environmental variables may be correlated with an unknown variable that has a more direct influence. Nonetheless, these results highlight a unique aspect of the Phainopepla system compared with other itinerant breeders, as they are capable of breeding in 2 significantly different physical environments, whereas other species move geographically within a relatively homogenous environment, utilizing the same food resources throughout. For the Phainopepla, this change entails a shift not only in climatic conditions, but in diet, habitat, nesting substrate, predator community, and social system as well (Walsberg 1977, Chu 1999), indicative of an unusually high degree of behavioral and physiological plasticity.

Use of Stopover Sites Between Desert and Woodland Breeding

Migration between desert and woodland sites has long been hypothesized, but the existence of distinct intervening spring and fall stopover stages was unanticipated (Figures 1 and 2). Although we use the term "stopover" here as a

matter of convention, it is important to note that the spatiotemporal stability and function of Phainopepla stopover sites may differ from those traditionally defined in long-distance temperate zone migrants (e.g., quickly refueling during migration). Four individuals spent time in a spring stopover location in the southern Mojave Desert on route to woodland breeding habitat. Afton Canyon, the desert breeding site where birds were tagged, is near the northern end of the Phainopepla's desert breeding range, which may predispose spring migrants to head south initially before migrating to the coast. In addition, these individuals may have moved south in preparation to use the San Gorgonio Pass as a conduit west to coastal woodlands. The temporal resolution of the GPS data makes precise routes difficult to determine, but at least one individual clearly traveled through the San Gorgonio Pass in spring (between points 2 and 3 in Figure 1C). Random forest analyses indicated that spring stopover sites were environmentally distinct from those occupied during other stages (Table 3), suggesting they may provide unique resources to birds during this time rather than simply representing a geographic intermediary between the desert and woodland breeding stages.

After potentially breeding in woodland habitat, 4 individuals spent a considerable amount of time at a fall stopover site before returning to Afton Canyon. One individual's fall stopover site was located near its spring stopover site (Figure 1B), but 3 others traveled considerable distances east/southeast to the eastern edge of the Sonoran Desert (Figures 1C, 1E, 1F). One possibility is that Phainopeplas use this stationary period to undergo prebasic molt, which can involve replacing all flight feathers, and is known to occur around this time (Miller 1933). Extensive molt is energetically expensive for birds, which may impose selection to molt during a relatively sedentary period after breeding (Holmgren and Hedenström 1995, Pyle et al. 2018). In addition, birds may be taking advantage of yet more ephemeral food resources during the fall stopover period. Late summer monsoon rains in this area cause shrubs such as Wolfberry (*Lycium berlandieri*) and Blue Elderberry (*Sambucus nigra*) to fruit in September and October, when tagged individuals were present (Chiang and Landrum 2009, USDA NRCS 2019). Random forest analyses support this idea, with significantly higher mean monthly precipitation characterizing the fall stopover stage compared with other stages, despite high variability (Figure 4C). A similar phenomenon has been documented in the Painted Bunting (*Passerina ciris*), which has a fall migration phenology concurrent with monsoon productivity in this same region (Bridge et al. 2016). This pattern bolsters the notion that unpredictable food resources dictate the Phainopepla's movement ecology, but direct observations of foraging and molting during the fall stopover stage are necessary to confirm this hypothesis.

Conclusions

The Phainopepla and other, more definitively identified itinerant breeders suggest that there may be unrecognized plasticity in avian life histories typically regarded as inflexible. In highly mobile species that are dependent on food supplies that shift seasonally over a regional scale, itinerant breeding may evolve as a response to this temporal and geographic variation. The Phainopepla is particularly unusual in that it is capable of breeding in habitats that vary not only in the physical environment, but in the social environment as well (Walsberg 1977, Chu 1999). This shift likely requires extensive behavioral and physiological plasticity that deserves further study (Chu et al. 2002). This phenomenon challenges the notion that breeding and migration are 2 discrete, rigid, and conflicting components of the annual cycle. With increasing ability to study the migration ecology of a variety of species in greater detail, it is possible that additional taxa will be found capable of modulating their life history in this way.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *The Auk: Ornithological Advances* online.

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