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# Novel statistical methods for integrating genetic and stable isotope data to infer individual-level migratory connectivity

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## Abstract

**Methods for determining patterns of migratory connectivity in animal ecology have historically been limited due to logistical challenges. Recent progress in studying migratory bird connectivity has been made using genetic and stable-isotope markers to assign migratory individuals to their breeding grounds. Here, we present a novel Bayesian approach to jointly leverage genetic and isotopic markers and we test its utility on two migratory passerine bird species. Our approach represents a principled model-based combination of genetic and isotope data from samples collected on the breeding grounds and is able to achieve levels of assignment accuracy that exceed those of either method alone. When applied at large scale the method can reveal specific migratory connectivity patterns. In Wilson's warblers (*Wilsonia pusilla*), we detect a subgroup of birds wintering in Baja that uniquely migrate preferentially from the coastal Pacific Northwest. Our approach is implemented in a way that is easily extended to accommodate additional sources of information (e.g. bi-allelic markers, species distribution models, etc.) or adapted to other species or assignment problems.**

*Keywords*: isoscape, microsatellite, migratory connectivity, spatial model, stable isotope

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## Introduction

Almost 20% of the worlds 10 000 bird species undertake long distance migrations (Sekercioglu 2007), but migratory connectivity – the geographic link between individuals or populations at different stages of their annual cycle, such as between breeding and wintering areas of migratory birds – has been described in relatively few cases and so remains poorly understood. Eliciting patterns in connectivity is useful for understanding the ecology and evolutionary dynamics of species, such as how demographic events across the annual cycle influence one another (Marra *et al.* 1998; Webster *et al.* 2002), and is increasingly important for understanding disease transmission routes and for making informed conservation decisions (Marra *et al.* 2011; Fuller *et al.* 2012). For example, pathogens carried by migratory birds, such as Influenza, West Nile and Salmonella, significantly

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impact domestic animal and human health and there is an urgent need to understand how spillover of pathogens from local hosts and migratory birds may change under climate warming (Fuller *et al.* 2012). Further, many songbird populations are declining, but it has often proven difficult to link breeding and wintering populations at fine enough geographic scales to determine the causes of declines in particular populations of migratory birds (Lovette *et al.* 2004; Faaborg *et al.* 2010).

While there is a great deal of valuable information available on the breeding and wintering localities of North American birds that has been painstakingly collected (Jones & Donovan 1996; Ammon & Gilbert 1999), additional information on the connectivity of individual populations is nevertheless needed for making better informed conservation decisions. Efforts to correlate breeding and wintering populations of migratory birds frequently employ extrinsic markers such as individual leg bands and tracking devices. However, return rates of banded migratory songbirds are typically very low (Webster *et al.* 2002) and tracking devices, while having improved substantially in recent years (Robinson *et al.* 2009; Bridge *et al.* 2011) are still expensive, are time consuming to employ, cannot be used effectively on small birds (<15 g), and potentially affect behavior (Saraux *et al.* 2011). Alternatively, intrinsic markers do not require recapture of individuals and thus can be a time- and cost-effective alternative to extrinsic markers when employed on a broad scale. Intrinsic markers involve sampling biological material that can be used to assign an individual of unknown origin to a population (i.e. assignment methods). Previous intrinsic methods have focused on the use of genetic markers (DNA from blood, muscle or feathers) or stable-isotope ratios (from feathers, blood, muscle or claws) (Hobson & Wassenaar 2008). Thus far, however, the spatial scale of population assignments from these intrinsic markers has been too coarse to be informative for making informed conservation decisions (Clegg *et al.* 2003; Irwin *et al.* 2011).

Genetic assignment methods generally contrast the observed genotype of an individual of unknown origin to the allele frequencies in potential source populations. Most methods only allow for the assignment of individuals to one of a small number of source populations (Rannala & Mountain 1997; Paetkau *et al.* 2004; Piry *et al.* 2004). However, more recent spatially continuous assignment models (SCAT; Wasser *et al.* 2004) allow assignments to locations over a broad geographic area, despite limited sampling. Isotope-based methods mirror the approaches for genetic data; most applications compare an observed isotope value for an animal to a set of known isotope distributions from a small set of potential source locations (Wunder *et al.* 2005; reviewed in Wunder & Norris 2008). In contrast with genetic assignment methods, spatially continuous isotopic methods usually overcome sparse geographic sampling by comparing stable-isotope ratios in tissue samples to modeled background abundances for potential source locations or populations (Hobson & Wassenaar 2008; Hobson *et al.* 2012). Studies have rarely been based models directly on samples of animal tissue (Wassenaar & Hobson 1998). Studies of bird migration often use $\delta^2$H (the ratio of $^2$H to $^1$H isotopes) observed in feathers, as feathers can be sampled non-invasively and geographic variation in $\delta^2$H (the $\delta^2$H isoscape) is known to vary latitudinally in North America, making it particularly useful for characterizing the breeding range of Neotropical migrants (Kelly *et al.* 2002; Smith *et al.* 2005; Hobson & Wassenaar 2008).

Many authors have advocated integrating isotopic and genetic approaches (Clegg *et al.* 2003; Hobson 2005; Kelly *et al.* 2005; Smith *et al.* 2005; Gómez-Díaz & González-Solis 2007; Chabot *et al.* 2012; Guillot *et al.* 2012), but doing so requires the development of new, statistically rigorous methods. Here we describe a novel population assignment method that combines genetic and isotopic assignment models under a common unified Bayesian framework, providing greater spatial resolution than would be possible from either model alone. For the genetic assignment method, our efforts build on the SCAT method of Wasser *et al.* (2004) and provide an alternative implementation of the spatial assignment model with improvements in model fitting efficiency and characterization of model uncertainty. For the isotopic assignment method, we have adopted the methods developed by Wunder (2007, 2010) where an isoscape describing $\delta^2$H variation across space, constructed using precipitation and other environmental data, is mapped to observed tissue isotope ratios. We demonstrate the utility of our methodology using genetic and isotopic data sets for hermit thrush (*Catharus guttatus*) and Wilson's warbler (*Wilsonia pusilla*). Our approach shares a Bayesian perspective with that of Chabot *et al.* (2012) but differs in implementation. Our model is available as an R package (isoscatR) that will enable other researchers to adopt this method for similar assignment tasks.

## Methods

### *Data*

The data used are a sub-sample of the overall blood and feather samples collected as part of the UCLA Center for Tropical Research (CTR) ongoing project on the population structure and conservation of Neotropical migratory birds. Blood and feathers samples were collected by CTR personnel in North America and Mexico over many years of sampling (Clegg *et al.* 2003; Smith

*et al.* 2005; Alvarado 2011). In addition samples were collected at Monitoring Avian Productivity and Survivorship (MAPS) bird banding stations between the end of May and August (for all breeding samples), and at Monitoreo de Sobrevivencia Invernal (MoSI) bird banding stations between late November and early March for wintering samples (Saracco *et al.* 2008; Saracco & DeSante 2009).

From these samples, 138 hermit thrush individuals collected between 2000 and 2008 from 14 breeding locations (Fig. 1) were sequenced at six microsatellite loci with a subset of 111 individuals also having measured $\delta^2$H. For Wilson's warbler, 163 individuals collected between 1996 and 1998 from eight breeding locations (Fig. 1) were sequenced at nine microsatellite loci, only 75 of these individuals, from five of the sampling locations, also had measured $\delta^2$H. An additional 128 overwintering Wilson's warblers collected in 1999 from eight locations in Central America were sampled and analyzed. These data included microsatellite data for four of the nine microsatellite loci from the breeding locations as well as measured $\delta^2$H. More specific details of sampling, collection and microsatellite screening protocols are included in Clegg *et al.* (2003) and Alvarado (2011) for hermit thrush and Wilson's warbler respectively. In short, all six hermit thrush microsatellite loci (Cu$\mu$02, Cu$\mu$04, Cu$\mu$10, Cu$\mu$28, Cu$\mu$32, WpD30) are based on loci developed in Gibbs *et al.* (1999) from the Swainsons thrush (*Catharus ustulatus*). Of the nine microsatellite loci developed for the Wilson's warbler, five loci (WpC6, WpC25, WpD23, WpD30, WpD4) were developed from commercially available Wilson's warbler microsatellite-enriched libraries (Genetic Identification Services, CA) and four loci (Dp$\mu$01, Dp$\mu$03, Dp$\mu$05, Dp$\mu$16) were developed in Dawson *et al.* (1997) from a Yellow warbler (*Dendroica petechia*) library.

Feather samples used for isotopic assignment were prepared following the methods of Clegg *et al.* (2003). Feathers were collected from individuals on the breeding grounds between mid-May and mid-July to ensure that they were locally breeding birds rather than passage migrants. Feathers collected from individuals on the wintering grounds should reflect the isotope signatures from feathers grown on their respective breeding grounds. Both hermit thrushes and Wilson's warblers undergo a prebasic molt on the summer grounds in which all feathers including rectrices are replaced, neither species replaces rectrices on molt-migration locations or on the winter grounds (Pyle 1997; Rohwer *et al.* 2005; Pyle *et al.* 2009). Thus, rectrices sampled any time during the year, including on the winter grounds, will reflect isotopic signals from the breeding grounds.

We express the ratio of stable hydrogen isotopes using standard delta notation ($\delta^2$H) which reflects the ratio of heavy and light isotopes in a sample as the parts per thousand (%) deviation from standard mean ocean water (vSMOW = 0%). These values are calculated as $\delta^2\mathrm{H} = (R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1) \times 1000$, where $R = {}^2H/{}^1H$. Larger values of $\delta^2$H correspond with an increase in heavier isotope abundance.

### *Genetic assignment methods*

Our genetic assignment model is based on the Gaussian process (GP) model introduced in the Smoothed and Continuous Assignment Test (SCAT) method presented in Wasser *et al.* (2004). Our implementation takes a two-step approach: first we formulate a Gaussian process model for allele frequency and use MCMC to sample from the posterior distribution of these allele frequencies at unobserved locations conditional on the allele counts at observed locations. Second, the posterior probability of an unknown sample conditional on its genotype is computed across a fine-scale grid using the allele frequencies sampled in the first step. In total, this produces the posterior probability surface of a samples' origin given its genotype and the information in the reference samples. Our approach differs from that of Wasser *et al.* in that we: (i) Use a nugget effect in the spatial covariance model to model unaccounted sources of variation and have altered the parameterization

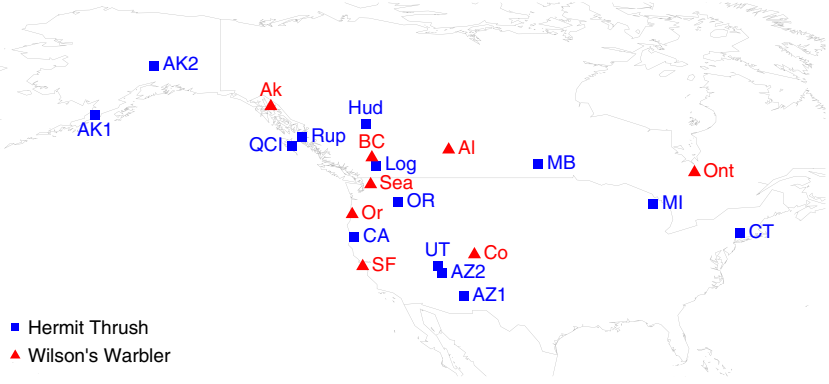


**Fig. 1.** Map of North America showing breeding area sampling locations for hermit thrush (blue) and Wilson's warbler (red).

slightly to improve mixing; (ii) Do not fix the unknown spatial covariance parameters after an initial MCMC run, but let them vary across our MCMC; (iii) Compute the posterior for the origin across a grid, rather than drawing realizations from the posterior as in Wasser *et al.*, which enables us to combine the genetic and isotope-based posteriors in a straightforward way.

### *Sampling allele frequencies from the posterior*

For a location $k$ and locus $l$, the allele count is assumed to follow a multinomial distribution. As such, the probability of the observed allele counts, $\boldsymbol{y}_{l\cdot k}$, at locus $l$ in location $k$ given an allele frequency $f_{lik}$ is

$$P(y_{l\cdot k}|f_{l\cdot k}) = \frac{s_{lk}!}{\prod_i y_{lik}!}\prod_i {f_{lik}}^{y_{lik}} \quad (1)$$

where $s_{lk} = \sum_i y_{lik}$, the total number of alleles from locus l found at location k. Allele frequencies $f_{lik}$ are modeled as a transformation of underlying, unobserved $\Theta_{lik}$ variables that are generated from a Gaussian Process:

$$f_{lik} = \frac{\exp(\Theta_{lik})}{\sum_j \exp(\Theta_{ljk})} \quad \boldsymbol{\Theta}_{li} \sim \mathrm{MVN}(\boldsymbol{\mu}_{li}, \boldsymbol{\Sigma}) \quad (2)$$

where $\mu_{li}$ is a $r \times 1$ mean column vector and $\Sigma$ is a $r \times r$ covariance matrix, where $r$ is the number of sampling locations.

The mean vector of the Gaussian process ($\mu_{li}$) determines the mean allele frequency across all locations and is parameterized as, $\mu_{li} = \xi_l \eta_{li} 1_{[r,1]}$. The parameter $\xi$ is not included in the Wasser *et al.* (2004) model and has been added as this over-parameterization has been shown to improve MCMC convergence in these settings (Liu *et al.* 1998; Liu & Wu 1999; Van Dyk & Meng 2001).

The covariance structure of the model $\Sigma$ determines how allele frequencies covary across geography. Elements of the covariance matrix are defined in terms of a spatial covariance function, $\{\Sigma\}_{j,k} = \sigma(d_{j,k}|\alpha)$ where $d_{j,k}$ is the distance between locations $j$ and $k$. Similar to Wasser *et al.* (2004) we assume the spatial process is both stationary and isotropic, and follows a powered exponential covariance function,

$$\sigma(d|\boldsymbol{\alpha}) = \alpha_0 \exp[-(d/\alpha_1)^{\alpha_2}] + \alpha_3 I_{d=0}; \quad (3)$$

however our approach differs from Wasser *et al.* in the inclusion of a nugget effect ($\alpha_3$). Adding a nugget should aid in accounting for unmodeled additional sources of variation (e.g. inadequacies of the GP model to account for subtle structure within a sampling location). The Matérn covariance function was also considered and tested, but did not produce significantly different results (data not shown). The $\alpha$ parameters are assumed to be shared across all alleles and loci.

Using this model, and genotypes from a set of reference genotypes $G_{ref}$ of known location, we sample posterior allele frequencies ($f$) using a Markov Chain Monte Carlo algorithm with priors on model parameters that were chosen to be uninformative (see supplemental material for additional detail). The MCMC was run using 300 000 total iterations, with the first 200 000 discarded as burn-in iterations. The remaining 100 000 iterations were further thinned, to reduce autocorrelation, down to 1000 posterior samples ($\boldsymbol{f}^{(1)}, \ldots, \boldsymbol{f}^{(1000)}$). For each realization of $f$ we sample allele frequencies from the posterior predictive distribution of $\tilde{\boldsymbol{f}}^{(m)}|\boldsymbol{f}^{(m)}, \boldsymbol{\alpha}, \boldsymbol{\mu}$ for a regular grid of prediction locations across North America. The posterior samples are then used in our next stage to calculate posterior probabilities for each test individual's origin.

The grid of prediction locations for a given species is defined by placing points every 1° of latitude and longitude within a bounding box that expands the east–west or north–south range of sampling locations by 20% in each direction. Non-terrestrial locations are masked using a simple polygon to approximate the North American landmass with a slight buffer so that coastal islands are included. The range and resolution of the grid is visible in the posterior surfaces shown in Fig. 2.

As the number of prediction locations is large, 2943 for hermit thrush and 1691 for Wilson's warbler respectively, sampling each $\tilde{\boldsymbol{f}}^{(m)}$ is computationally intensive. To improve performance, we extended our code to run on a graphics processor unit (GPU) and by doing so achieved approximately a 5x speedup. Additional technical details on our implementation are included in the supplementary materials.

### *Computing the posterior on individual origins*

We assume Hardy-Weinberg equilibrium and linkage equilibrium such that given the allele frequency surfaces ($\tilde{f}$), the likelihood describing the probability of the genetic sample data $S_G$ coming from location $k$ with alleles $i_l$ and $j_l$ at locus $l$, is computed by:

$$P(S_G|\tilde{\boldsymbol{f}}, k) = \prod_l P(i_l, j_l|\tilde{\boldsymbol{f}}, k) \quad (4)$$

$$P(i_l, j_l|\tilde{\boldsymbol{f}}, k) = \begin{cases} \gamma P(i_l|\tilde{\boldsymbol{f}}, k) + (1-\gamma)P(i_l|\tilde{\boldsymbol{f}}, k)^2 & \text{if} \quad i = j \\ (1-\gamma)P(i_l|\tilde{\boldsymbol{f}}, k)P(j_l|\tilde{\boldsymbol{f}}, k) & \text{if} \quad i \neq j \end{cases} \quad (5)$$

$$P(i_k|\tilde{\boldsymbol{f}}, k) = (1-\delta)\tilde{f}_{lik} + \delta/m_l \quad (6)$$

where $\delta$ is the genotyping error probability, $\gamma$ is probability of amplifying only one allele, and $m_l$ is the number of alleles at locus $l$. Missing alleles are handled by setting $P(i_l|\tilde{\boldsymbol{f}}, k) = 1$. For our analyses we fixed

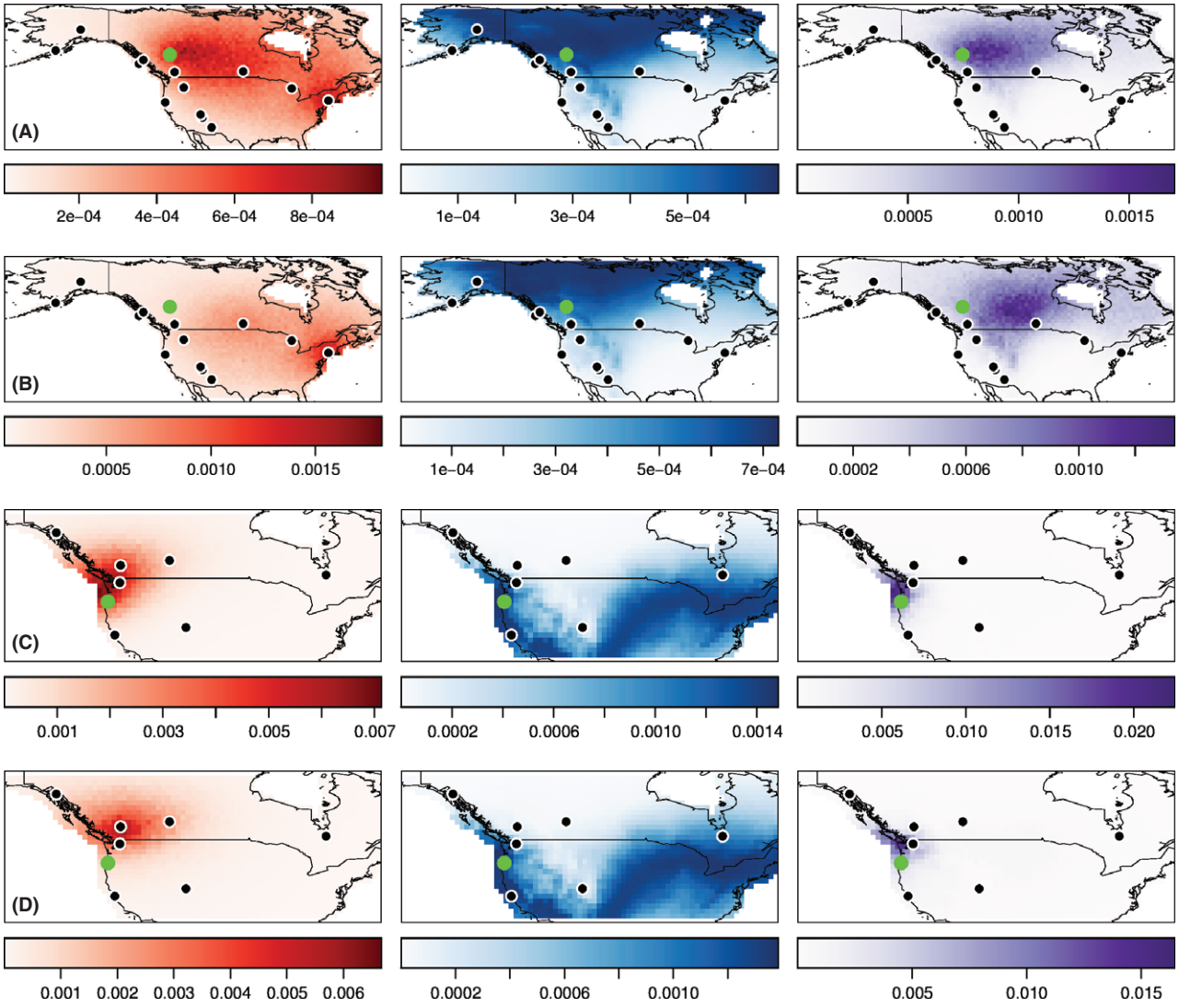


**Fig. 2.** Posterior assignment probability maps, from left to right, of the genetic, isotopic and combined assignment model output. Rows A and B reflect the results for the same hermit thrush test sample, and C and D of the same Wilson's warbler test sample. These pairs reflect the result of cross-validation by individual and cross-validation by location respectively. These cross-validation schemes involve the exclusion of an individual or a sampling location before fitting the model to the remainder of the data. The fitted model is then used to predict the origin of the excluded individuals. The ● indicates the true origin of the sample and ● indicate all other sampling locations.

$\delta = 0.05$ and $\gamma = 0.01$ based on Wasser *et al.* (2004), and we found that in practice reasonable changes to these values have little impact on the results.

To compute the likelihood of an assignment location $k$, we can integrate over the unobserved allele frequency surfaces ($\tilde{f}$) using the following Monte Carlo approximation:

$$P(S_G|k, \mathbf{G}_{ref}) \approx \frac{1}{M}\sum_{m=1}^{M} P(S_G|k, \tilde{f}^{(m)}) \qquad (7)$$

In this approximation, the $\tilde{f}^{(\cdot)}$ need to be realizations from the posterior predictive distribution of $\tilde{f}$ given the reference genotypes. We use posterior realizations from our first stage ($\tilde{f}^{(i)}$, $i = 1,\ldots,1000$, i.e. $M = 1000$). Also, we opted to use the median rather than the mean prescribed by equation 7, as we found the distribution $P(S_G|\tilde{f}^{(m)}, k)$ to be highly right skewed making the mean estimate unstable. As further validation, we found the median to display superior assignment performance to mean, as assessed by AUC.

To derive the posterior assignment probability surface for a given genetic sample ($P(k|S_G, \mathbf{G}_{ref})$) using Eq. 7 we multiply by a spatial prior ($\pi(k)$) and normalize over the grid of prediction locations to obtain a proper probability,

$$P(k|S_G, \boldsymbol{G}_{ref}) = \frac{P(S_G|k, \boldsymbol{G}_{ref})\pi(k)}{\sum_{k'} P(S_G|k', \boldsymbol{G}_{ref})\pi(k')}. \quad (8)$$

Here, $k'$ represents the set of all prediction locations for $\tilde{f}$ as described above. For all analyses present in this paper a flat prior covering North America was used for $\pi(k)$.

### *Isotope assignment*

Natural abundances of stable isotopes vary systematically across environmental and biological gradients. The isotopic assignment method we employ is based on the work of Wunder (2007, 2010) which constructs assignment models for the probability of observing a particular isotope value in animal tissues for a location, given a set of known-origin stable-isotope values. Known-origin isotope values can be based in animal tissues (e.g. feathers sampled from across a range), or from environmental samples (e.g. $\delta^2$H in rain water collected from across a range). The assignment model we use here is based on a linear calibration that relates expected $\delta^2$H values for precipitation (cf. Bowen *et al.* 2005) to $\delta^2$H values in bird feathers that were of known geographic origin. We have used this precipitation-based model as the sampling of bird feathers with measured $\delta^2$H is not as geographically extensive as precipitation $\delta^2$H data collected by the Global Network of Isotopes in Precipitation (GNIP; IAEA, WMO, 2006).

Since precipitation $\delta^2$H is measured at discrete locations that do not coincide with the feather sampling locations, it is necessary to build a spatial model of the precipitation $\delta^2$H (an 'isoscape'). This is done by fitting a Bayesian spatial linear model using a Matérn covariance to aggregated Global Network of Isotopes in Precipitation (GNIP) data ($\boldsymbol{I}_{ref}$) (IAEA, WMO, 2006). GNIP data was aggregated by calculating the average observed $\delta^2$H and other covariates during the breeding season (May through July) for each sampling station over its entire period of operation. Model predictors included altitude and precipitation as linear terms and max temperature, min temperature, average temperature, latitude, and longitude as quadratic terms based on similar isoscape models used by Bowen *et al.* (2012).

The model was fit using the spBayes package (Finley *et al.* 2012) in R (R Core Team 2012) over 15 000 MCMC iterations. After discarding the first 5000 as burnin the remaining 10 000 iterations were thinned to 1000 posterior samples. Posterior predictive samples of the precipitation isoscape ($\tilde{p}_k^{(\cdot)}$) were made using average environmental covariates for the breeding seasons between 2000 and 2009 across North America using climate data from CRU TS 3.10 (Harris *et al.* 2012) and elevation data from the ETOPO1 Global Relief Model (Amante & Eakins 2009), predictions were made for a grid of 1° × 1° latitude longitude cells across North America constructed to be equivalent to the prediction locations of the genetic assignment model.

To account for the fractionation that occurs as the environmental isotopes are integrated into the feather and that the isoscape is a model based on temporally averaged environmental observations, we model the isotope ratio for a feather sampled at location $k$ as a linear function of the precipitation isotope ratio:

$$S_I|k, \tilde{\boldsymbol{p}}, \omega, \rho, \tau^2 \sim \mathrm{N}(\omega + \rho\tilde{p}_k, \tau^2) \quad (9)$$

where $S_I$ is the observed isotope ratio of a feather, $\tilde{p}_k$ is isotope ratio based on the precipitation isoscape at location $k$, and $\omega$, $\rho$, and $\tau^2$ are parameters describing the calibration between precipitation and feather isotope ratios. We estimate $\omega$, $\rho$, and $\tau^2$ using standard least squares regression for each posterior predictive sample of $\tilde{p}_k^{(\cdot)}$ from the MCMC described above (and include a superscript to indicate posterior sample iteration in our notation below). Examples of the fitted calibration function for both species are included in Figure S2 in the supplementary materials. Next, as with the genetic data, we compute the likelihood $P(S_I|k)$ by Monte Carlo approximation using posterior samples:

$$P(S_I|k, \boldsymbol{I}_{ref}) \approx \frac{1}{M}\sum_{m=1}^{M} P(S_I|k, \tilde{\boldsymbol{p}}^{(m)}, \omega^{(m)}, \rho^{(m)}, \tau^{2(m)}) \quad (10)$$

As with the genetic assignment model, we use a median in place of the mean implied by Equation 10 and we derive a posterior assignment probability surface across the prediction grid using the approach presented in Eq. 8 for the genetic assignment method.

### *Combined assignment*

Bayesian methodologies provide a simple framework for combining the two assignment methods. For a given feather sample $S$, there are observed genetic and isotopic values $S_G$ and $S_I$ for each test individual, and genetic and isotopic reference data ($\boldsymbol{G}_{ref}, \boldsymbol{I}_{ref}$). We assume these sample values are independent conditional on the location. Consequently, we can write down the probability of observing a sample $S = (S_G, S_I)$ for any location $k$ using Bayes rule:

$$P(k|S, \boldsymbol{G}_{ref}, \boldsymbol{I}_{ref}) \propto P(S|k, \boldsymbol{G}_{ref}, \boldsymbol{I}_{ref})\pi(k) \quad (11)$$

$$\propto P(S_G|k, \boldsymbol{G}_{ref})P(S_I|k, \boldsymbol{I}_{ref})\pi(k) \quad (12)$$

Where $P(S_G|k, \boldsymbol{G}_{ref})$ and $P(S_I|k, \boldsymbol{I}_{ref})$ are given by Eqs. 4 and 9 respectively. As with Eq. 8, proper probabilities are achieved by normalization by dividing each location's probability by the sum of probabilities for all prediction locations.

One problem with a straightforward combination, as just described, is that if the genetic or isotopic models

over- or under-represent the certainty in the marginal assignment the inference may inappropriately rely on one source of data over the other. In order to ameliorate this problem, we have undertaken what we term cross-validation calibrated combined (CVCC) model tuning, whereby we attempt to optimize the combined models' performance by independently flattening or sharpening the genetic and isotopic models before constructing the final combined model. This optimization is accomplished by raising $P(S_G|k, \boldsymbol{G}_{ref})$ and $P(S_I|k, \boldsymbol{I}_{ref})$, from Eq. 12, to the power $a$ and $b$ respectively before calculating the combined assignment probability. Powers >1 result in a sharpening and powers <1 result in a flattening. In order to choose the optimal values of $a$ and $b$ used we perform a simple grid search of values for $a$ and $b$ from $10^{-1}$ to 10 and assess the performance by cross-validation using the AUC metric described below.

### *Assignment performance metrics*

As our ultimate goal is to identify probable breeding locations for birds given their genetic and isotopic characteristics, we assess our models' ability to correctly assign samples with known origins. We have opted for two separate but complementary approaches for assessment: a distance-based approach and a receiver operating characteristic (ROC) curve based approach.

Our distance-based assessment calculates the great circle distance between the known origin of a sample and the origin location with the maximum a posteriori (MAP) probability. This is useful for characterizing the performance of the MAP estimates in an interpretable way but is somewhat limited as it lacks the ability to distinguish between sharply peaked and flat posterior assignment maps.

In order to address this limitation we have also considered the assignments as a binary classification problem (classifying locations as true origins or non-origins) and assess the classification power of our model using ROC curves (see supplementary materials for additional background and implementation details) (Hilden 1991; Krzanowski & Hand 2009). We also compute the area under the ROC curve (AUC), which is an estimate of the probability that a randomly selected true origin location will have an assignment probability that is greater than the assignment probability of a randomly selected non-origin location. This statistic is particular useful as it can be used to compare the assignment accuracy and precision of different models.

### *Cross-validation procedures*

To evaluate the accuracy of our assignment method, we follow Wasser *et al.* (2004) and have employed two modes of cross-validation, cross-validation by individual and cross-validation by location. For the individual cross-validation, a single individual is excluded from the data and both genetic and isotopic models are fit and used to predict the spatial origin of the excluded individual. Location based cross-validation is similar except that all individuals from a sampling location are excluded before fitting the models. The accuracy of the assignment models are assessed by ROC and AUC.

It is important to consider both approaches, as methods that are only evaluated at locations where other individuals have been sampled (cf. Chabot *et al.* 2012) tend to produce overly optimistic results. It is exceedingly unlikely that an unknown sample will exactly coincide with one of the small number of sampling locations. As such, under our scheme cross-validation by individual reflects an ideal scenario with the true origin of the test individual being one of the sampling locations. Conversely, cross-validation by location represents a scenario where the inference will perform well only insofar as the test individual's true location is near other sampling locations. Results for both approaches are presented in Fig. 3 and Table 1.

To further explore decreased performance under cross-validation by location we have also explored whether the loss of predictive accuracy in the models under cross-validation by location occurs because: (i) the model is sensitive to the loss of sample size (removing a location can remove upwards of 20% of samples from the dataset), or (ii) the model is sensitive to the loss of unique spatial information at that sampling location. These alternatives are not mutually exclusive and we explored this effect by performing what we term size-adjusted individual cross-validation, where the location label for each individual is randomized before performing cross-validation by location. This approach is equivalent to cross-validation by location in terms of the decrease in sample size but does not remove all individuals from a sampling location.

## Results

Our results clearly indicate that for both species and all cross-validation regimes the combined assignment model outperforms the genetic or isotopic assignment models alone. In reviewing the assignment maps (representative examples presented in Fig. 2, with additional examples in Figs. S3–6) the common pattern appears to be that the genetic model tends to make very compact and specific predictions (strongly peaked posteriors), while the isotopic model makes less specific predictions (wide and flat posteriors). In practice, the higher specificity of the genetic model tends to sharpen the joint posterior when both models agree on the origin of the

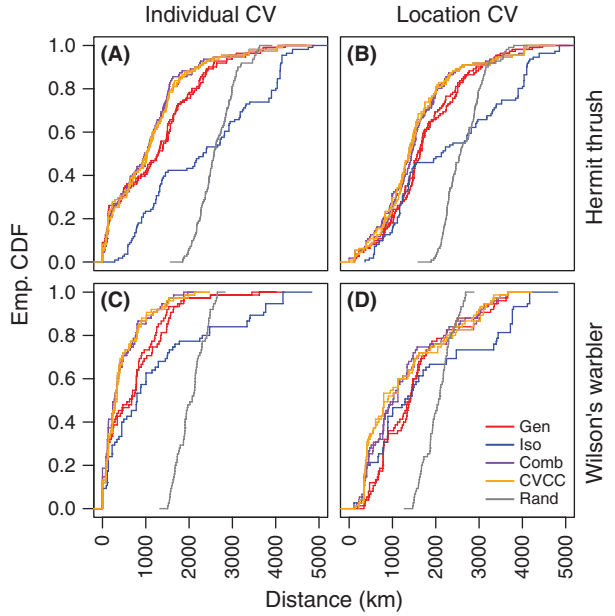


**Fig. 3.** Empirical cumulative density functions of error distances for the maximum a posteriori estimate of location. We show results for Hermit Thrush (A–B) and Wilson's Warbler (C–D) based on individual (A and B) and location (C and D) based cross-validation. The grey line represents the error expected from random assignment within the approximate breeding range of each species. As shown, the combined genetic and isotopic method has lower median error distance than either method alone. The CVCC method (a variation on the combined method) provides a small improvement over the simple combination method. Identically colored lines reflect the result of independent MCMC chains. The overlap of these lines suggests the chains have converged.

**Table 1.** Shown are the average of independent MCMC chains of the area under the ROC curves (AUCs) for each species and cross-validation method. These AUCs are the probability of randomly selected true origin location having a assignment probability that is larger than a randomly selected non-origin location. Using these metrics, a perfect classifier will have an AUC of 1 while random assignment will have an AUC of 0.5

| | | CV AUC | | |
|---|---|---|---|---|
| | Model | Ind | Loc | SA Ind |
| Hermit Thrush | Genetic | 0.843 | 0.714 | 0.836 |
| | Isotopic | 0.722 | 0.696 | 0.722 |
| | Combined | 0.890 | 0.782 | 0.884 |
| | CVCC | 0.884 | 0.794 | 0.879 |
| Wilson's Warbler | Genetic | 0.912 | 0.659 | 0.909 |
| | Isotopic | 0.826 | 0.819 | 0.825 |
| | Combined | 0.959 | 0.830 | 0.960 |
| | CVCC | 0.962 | 0.862 | 0.963 |

individual (Fig. 2C,D), whereas the isotopic model tends to flatten the posterior when the two models disagree (Fig. 2B).

The cross-validation results show that the genetic model by itself performs better than the isotopic model under cross-validation by individual, and approximately the same or worse than the isotopic model alone under cross-validation by location. Between the two species and two cross-validation schemes it is clear that the performance of the genetic model is more sensitive to the spatial structure of the sampling design than the isotope model. This pattern is consistent with the more limited genetic reference data. As the genetic model infers allele frequencies based on neighboring sampling locations the removal of a single sampling location removes useful information. This becomes more pronounced for sampling locations that are more spatially isolated. Conversely, the isotopic model fits a global environmental process model via a calibration curve, which, as a linear least squares model, is far more robust to the removal of a single sampling location.

To investigate this in more detail, we stratify assignment accuracy by sampling location (Tables S1 and S2, Supporting information) and find that losses in accuracy under cross-validation by location are most extreme for sampling locations that are the most isolated. For example, the hermit thrush sampling locations MI, CT, and MB performed relatively well under cross-validation by individual but show a large loss in prediction accuracy under cross-validation by location, whereas sampling locations AZ2 and UT, which have several close neighbors, show a relatively smaller loss in prediction accuracy. Similarly, the same pattern is observed with Wilson's warbler as sampling locations Co and Ont both show large losses in accuracy while Al, Or, and SF suffer relatively smaller losses. This seems to strongly indicate that coverage in sampling locations plays a major role in the overall performance of the genetic and, hence, the combined model.

This is further supported by our results from the size adjusted individual cross-validation approach. Our results (Fig. 3, Fig. S1C, F, Supporting information) show that this cross-validation method has accuracy that is almost identical to cross-validation by individual and not location. As such, the loss in predictive accuracy for location-based cross validation is driven almost entirely by the loss of information present at a spatial location and not the loss of sample size. This finding emphasizes the importance of sampling coverage relative to sampling depth for performance.

### *Performance of combined assignment*

Results of the cross-validation calibrated combined model (CVCC) grid search are shown in Fig. 4 which displays a raster of the resulting AUCs with the optimal

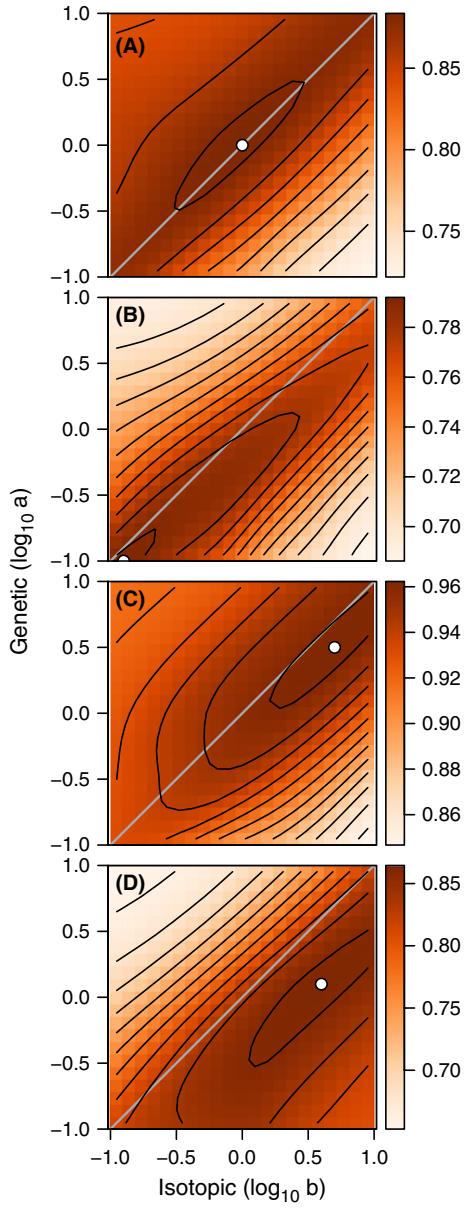


**Fig. 4.** Heat maps of the area under the ROC curves (AUCs) resulting from different parameter choices in the cross-validation calibrated combined (CVCC) model for hermit thrush (A and B) and Wilson's warbler (C and D) under cross-validation by individual and cross-validation by location respectively. The plots show the result of empirically tuning the combined model by sharpening or flatten the genetic and isotopic models. This is achieved by raising the models to the power $a$ and $b$ respectively, with values above 1 resulting in a sharpening of the model and values below 1 flatten the model. To choose parameter values we conduct a grid search of values between -10 and 10. Here ◦ indicates the value of $a$ and $b$ resulting in maximal AUC and that are used for the results in Fig. 3.

model indicated by the white dot (the empirical CDFs and ROC curve for the optimal CVCC are included in Fig. 3 and Fig. S1 respectively).

As shown in Fig. 3, the CVCC approach shows minor improvements over the naive combination ($a = b = 1$). In the case of cross-validation by individual, CVCC exhibits no meaningful improvement, based on the AUC criterion. The very small improvement in the Wilson's warbler model appears to occur due to a general sharpening of the combined model and slightly smaller sharpening of the isotopic model ($\log_{10}a = 0.5$, $\log_{10}b = 0.7$). This pattern is suggestive of a small amount of under-fitting by both models. Additionally, the rasters reemphasize the relative advantages of the genetic model over the isotopic model under cross-validation by individual, as sharpening the isotopic model while flattening the genetic model (i.e. the region below the 1:1 line in Fig. 4A, C) results in rapidly declining AUC, whereas AUC is relatively constant when the model tuning is the other way around.

The results for cross-validation by location are more interesting, as the CVCC tuning exhibits small but more meaningful improvements in model performance. In the case of the hermit thrush, we see a general flattening of the combined model with slightly more flattening of the genetic model ($\log_{10}a = -1$, $\log_{10}b = -0.9$), indicating potential overfitting. From the way that the calibration alters the shape of the ROC curve, it is evident that model improvement occurs by trading a small amount of precision from locations at which the model was performing very well in order to significantly improve at more middling locations where the false positive rate between is between 0.15 and 0.45. We observe a different pattern with the Wilson's Warber, as the calibration appears to almost exclusively favor sharpening ($\log_{10}a = 0.1$, $\log_{10}b = 0.6$). Given the poor performance of the genetic model under cross-validation by location for this species, we might expect the calibration to flatten the genetic model contribution as much as possible. However, these results seem to indicate that there is still useful information that is unique to the genetic model. Overall, the different qualitative outcomes in model tuning from the hermit thrush and Wilson's warbler make clear that the appropriate weighting of isotopes and genetics will be dataset dependent.

### *Application: assignment of breeding locations for Wilson's warblers wintering in Central America*

The ultimate goal of these methods is to use the fitted models from birds on their breeding range to predict the geographic origin of individuals encountered elsewhere during a different phase in their life-cycle. Using preliminary data available for Wilson's warblers on their wintering grounds (126 individuals from eight locations in Central America), we predict breeding locations. For these wintering samples only a subset, four of

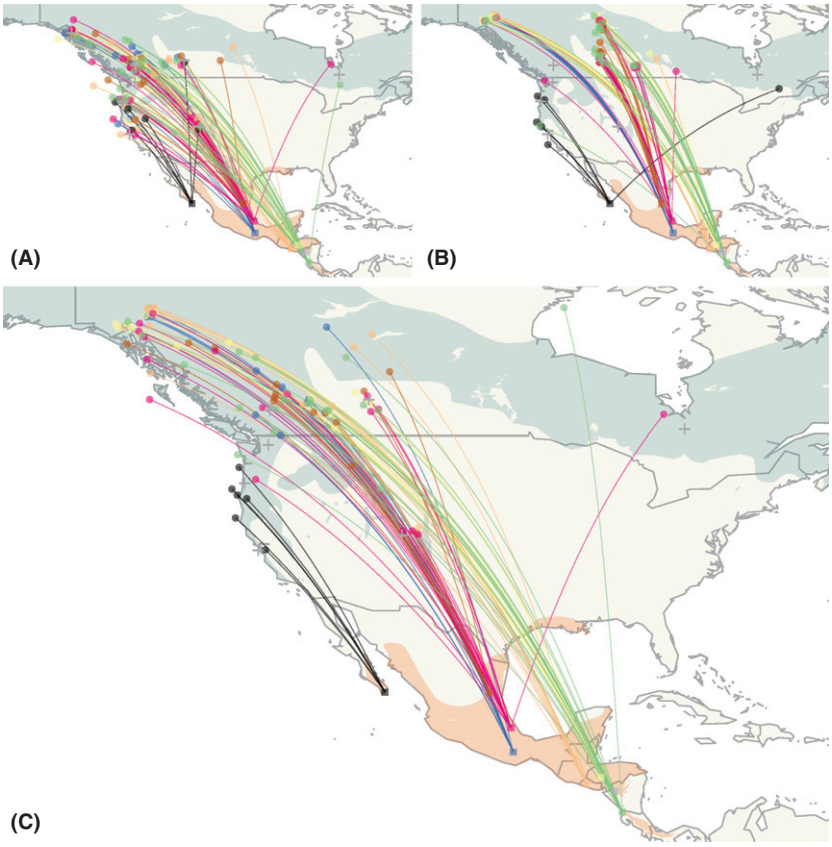


**Fig. 5.** Maps showing connectivity between sampling locations of wintering Wilson's warblers and maximum a posteriori (MAP) estimates of breeding season origin using genetic (A), isotopic (B) or combined (C) models. Connections are indicated using great circle arcs and are colored according to wintering location. Breeding and wintering range maps for Wilson's warbler are indicated in orange and blue respectively (Ridgely *et al.* 2007). Each assigned location is a point estimate with associated uncertainty, but the collective distribution of assigned origins is revealing of migratory connectivity between regions of mainland Mexico and locations in Western North America and Baja and the coastal Pacific Northwest.

nine, microsatellite loci present in the breeding data were available. While this data are sufficient to generate spatial predictions, the results are necessarily more uncertain than predictions made for individuals with all 9 loci available. Figure 5 presents the results combining these arcs for all individuals, where the color-coding reflects the wintering sampling location, to depict the patterns of migratory connectivity between the wintering and breeding ranges. While the majority of the individuals are localized to breeding locations in western Canada, the Wilson's warblers wintering on the tip of Baja uniquely cluster to breeding locations along the coast of California and Oregon, suggesting a potential subpopulation with distinct migratory behavior. Additionally, only two birds localize to breeding grounds in the eastern half of North America, but data are insufficient to determine if this a failure of the assignment model or a true signal.

## Discussion

We have presented a novel methodology for the assignment of migratory birds to breeding populations that combines genetic and isotopic methods, and by doing so improves upon the resolution of either method alone.

One explanation for why our combined approach is more effective then either marker alone is because the geographic patterns of variation for the two data sources are relatively orthogonal. In North America, there is a continent-wide latitudinal pattern in stable hydrogen isotope ratios ($\delta^2$H) resulting from patterns of average precipitation during the growing season that are transmitted through food webs (Hobson & Wassenaar 2008). In contrast, genetic variation in the two species we considered is typically distributed longitudinally, with eastern and western populations more genetically differentiated than populations to the north and south (Clegg *et al.* 2003; Smith *et al.* 2005). The east–west divide in many species that breed in North America often reflects historical patterns of diversification and gene flow. Thus, combining information from both markers allows for greater resolution of populations because it leverages the orthogonal nature of the variation in each of the two markers. Indeed, the respective advantages of each marker have been known for some time and have been used together to examine patterns of connectivity (Clegg *et al.* 2003; Kelly *et al.* 2005; Boulet & Norris 2006). However, our approach provides a rigorous statistical framework that combines information from both types of markers into a single analytical framework.

Recently, a number of modeling frameworks for using stable isotopes to assign origins of migratory animals have been proposed, a review of the most common frameworks can be found in Wunder (2012). Each of these frameworks seeks to improve assignment performance by integrating additional information sources. For example, Royle & Rubenstein (2004) used estimated abundance, Wunder *et al.* (2005) used sample size, and Kelly *et al.* (2005) used haplotype as priors for otherwise isotope-based nominal assignment models. Likewise, Hobson *et al.* (2009) used geographically binned band recovery data for constraining an otherwise isotope-based continuous assignment model. van Wilgenburg & Hobson (2011) use band recovery data to infer direction of migration and this was used to further refine an isotope-based continuous assignment model. Our model differs from these examples in that it combines a model based on genetics with a model based on stable isotopes in a single theoretically cohesive model framework. We have focused on how best to combine these methods and demonstrate the improvement that arises from combining these two sources of information.

Adding in yet additional information will only improve the assignments further. Indeed, technological advancements in molecular methods and tracking devices are providing a wealth of alternative methods for determining patterns of migratory connectivity, each with its own set of advantages and limitations. One advantage of the method described here is that it is inherently flexible and can easily be extended to include other sources of data. Thus, the overall efficacy of the joint assignment method that we describe would be expected to increase with addition of other data sources; this might include additional genetic data [e.g. genetic (Single Nucleotide Polymorphisms, mitochondrial haplotypes), biogeochemical (alternative isotopes and trace elements), or morphometric data, provided that each additional dataset can be formulated as a principled probabilistic model ($P(S \mid k,M)$, the probability of some sample data $S$ given a location $k$ and model $M$]. Furthermore, there is also the possibility of incorporating informative spatial priors such as species distribution models, and the possibility for integrating data from extrinsic markers including band returns (cf. Hobson *et al.* 2009) and tracking devices (e.g. geolocators, satellite tracking, etc.), all of which would be expected to further improve assignment reliability. However, a limitation with the intrinsic methods that we describe is that patterns of connectivity are not revealed unless samples are collected from across the species range and from across the full isotopic gradient, including both wintering and breeding areas. As a result, unraveling patterns of migratory connectivity will depend upon large research networks dedicated to sample collection, data generation and analysis (Saracco *et al.* 2008; Saracco & DeSante 2009).

Our assignments of wintering Wilson's warblers to breeding populations demonstrate the potential utility of using intrinsic markers to identify patterns of migratory connectivity on a broad scale. The genetic data used to assign wintering individuals to breeding populations was comparatively incomplete: the data set was restricted to four of the nine microsatellite loci used to train and cross-validate our model. Nonetheless, some broad scale patterns of connectivity emerged. Most notably, we detect an apparent strong pattern of connectivity between Wilson's warblers breeding along the Pacific coast and wintering in southern Baja. If this pattern of connectivity holds up to further sampling, then the population dynamics of Wilson warblers breeding in the Pacific Coast region of California through southern British Columbia may be significantly affected by habitat alteration on the Baja Peninsula. It will be worth assessing further if this structured connectivity has relationships to the distribution of Wilson's warbler subspecies (e.g. the chryseola subspecies, Ammon & Gilbert 1999). Further, only two birds were assigned to breeding areas in the eastern half of North America. Previous work by Kimura *et al.* (2002) indicated that Wilson's warblers breeding in the eastern part of their breeding range wintered near Guatemala and southern Mexico, locations for which genetic and isotopic data are not currently available. Increased sampling across both breeding and wintering areas will likely improve robustness of our model as well as our ability to detect patterns of migratory connectivity.

There is a wide utility for the approach described here. From a conservation standpoint, many populations of migratory birds are declining (Faaborg *et al.* 2010) and developing appropriate management and conservation approaches requires knowledge of full life cycle biology. In many cases it is not known whether population declines result from issues on the breeding grounds, on the wintering grounds, or along migration corridors. Linking populations along each part of the annual cycle is often necessary for revealing the causes of declines. Another important reason for understanding connectivity is to understand patterns of disease transmission. Many diseases important for human health are transported by migrating wild birds and understanding movement patterns of birds is essential to understanding possible outbreak patterns (Fuller *et al.* 2012). Finally, there is much that can be learned about the ecology and evolution of birds from a more through understanding of migratory connectivity. For example, efforts to understand population expansions over past glacial periods may be revealed by analyzing migratory pathways, and could offer new insights into

the mechanisms of speciation (Ruegg & Smith 2002; Milá *et al.* 2007).

In sum, we present a new method that combines genetic and isotopic assignment under a common unified framework that describes where migratory birds winter and breed at a finer spatial resolution than was previously possible. This methodology and supporting tools show considerable potential for describing patterns of connectivity and should assist in studies investigating population declines in migratory birds, the spatial and temporal transmission dynamics of avian-born diseases and many basic questions regarding ecology and the evolution of migration.

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C.R. was responsible for implementation and execution of the analyses and wrote the body of the paper. M.W. and A.S. aided in design of the isotope analysis and contributed directly with preliminary analyses. A.A., K.R. and R.H. provided genetic data. J.K. provided isotopic data. R.S. and D.S. provided field samples. T.S. organized the collaboration and helped write the paper. J.N. guided the design and execution of the analyses and helped write the paper. T.S. and J.N. jointly conceived of the study. All authors contributed commentary and editing to the final paper.

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## Data accessibility

All genetic and isotopic data will be made available as part of the the paper's supplementary material.

Software necessary for these analyses and related tasks, such as diagnostics, are implemented as part of the R package, isoscatR and will be made available on CRAN. Development versions of the package are currently available at the following URL https://github.com/rundel/isoscatR.

Supplementary R scripts used to run the analyses and generate figures will be published and maintained on the author's github account (https://github.com/rundel).

## Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1** Hermit thrush – Table shows percentiles of the distribution of great circle distances (in km) between the center of the grid cell of known origin to the center of the grid cell with maximum median posterior probability for all samples at the given location.

**Table S2** Wilson's warbler – Table shows percentiles of the distribution of great circle distances (in km) between the center of the grid cell of known origin to the center of the grid cell with maximum median posterior probability for all samples at the given location.

**Fig. S1** ROC curves for Hermit Thrush (A–C) and Wilson's Warbler (D–F) under individual (A and B) and location (B and E) based cross-validation.

**Fig. S2** Isotope model linear calibration function fits for hermit thrush and Wilson's warbler.

**Fig. S3** Hermit thrush posterior assignment probability maps of the genetic, isotopic and combined assignment model output under cross-validation by location. Each row reflects the assignment probability surfaces for a different individual, with the first three reflecting the best and last three reflecting the worst assignment outcomes as assessed by distance from true origin to the location of maximum posterior assignment probability.

**Fig. S4** Hermit thrush posterior assignment probability maps of the genetic, isotopic and combined assignment model output under cross-validation by location. Each row reflects the assignment probability surfaces for a different randomly selected individual.

**Fig. S5** Wilson's warbler posterior assignment probability maps of the genetic, isotopic and combined assignment model output under cross-validation by location. Each row reflects the assignment probability surfaces for a different individual, with the first three reflecting the best and last three reflecting the worst assignment outcomes as assessed by distance from true origin to the location of maximum posterior assignment probability.

**Fig. S6** Wilson's warbler posterior assignment probability maps of the genetic, isotopic and combined assignment model output under cross-validation by location. Each row reflects the assignment probability surfaces for a different randomly selected individual.