Title: Pedigree-based assessment of recent population connectivity in a threatened rattlesnake

Running Title: Contemporary pedigree-based connectivity

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Abstract:

Managing endangered species in fragmented landscapes requires estimating dispersal rates between populations over contemporary timescales. Here we develop a new method for quantifying recent dispersal using genetic pedigree data for close and distant kin. Specifically, we describe an approach that infers missing shared ancestors between pairs of kin in habitat patches across a fragmented landscape. We then apply a stepping-stone model to assign unsampled individuals in the pedigree to probable locations based on minimizing the number of movements required to produce the observed locations in sampled kin pairs. Finally, we use all pairs of reconstructed parent-offspring sets to estimate dispersal rates between habitat patches under a Bayesian model. Our approach measures connectivity over the timescale represented by the small number of generations contained within the pedigree and so is appropriate for estimating the impacts of recent habitat changes due to human activity. We used our method to estimate recent movement between newly discovered populations of threatened Eastern Massasauga Rattlesnakes (*Sistrurus catenatus*) using data from 2996 RAD-based genetic loci. Our pedigree analyses found no evidence for contemporary connectivity between five genetic groups, but, as validation of our approach, showed high dispersal rates between sample sites within a single genetic cluster. We conclude that these five genetic clusters of Eastern Massasauga Rattlesnakes have small numbers of resident snakes and are demographically isolated conservation units. More broadly, our methodology can be widely applied to determine contemporary connectivity rates, independent of bias from shared genetic similarity due to ancestry that impacts other approaches.

Key Words: Connectivity, Population Genetics, Dispersal, Pedigree, *Sistrurus catenatus*

Introduction:

Quantifying contemporary connectivity between populations is a major conservation goal for threatened species living in fragmented landscapes (Cayuela et al., 2018; ; Couvray & Coupé, 2018; Garner et al., 2016; Lowe & Allendorf, 2010). For these species, contemporary connectivity will influence demographic processes and impact the likelihood of long-term persistence or the chance of recovery (Baguette, Blanchet, Legrand, Stevens, & Turlure, 2013; Benson et al., 2016; Cushman, Landguth, & Flather, 2013). For example, if connectivity between two habitat patches is low, then a disease outbreak in one patch is unlikely to spread to individuals in the other patch which in turn reduces the chance of widespread declines (Haddad et al., 2014; Ogden, 2015). In contrast, high connectivity between several habitat patches with only a few individuals in each will help reduce the probability of inbreeding depression (Beier & Noss, 1998; Christie & Knowles, 2015; Gregory & Beier, 2014). It is important to note that definitions of connectivity can vary widely between studies. Here we focus on functional connectivity, i.e. the ability of individuals to move, survive, and potentially reproduce in new habitats (Cayuela et al., 2018)

The use of data from neutral genetic markers to quantify connectivity has become widespread as an alternative to more costly and time intensive field techniques used to directly measure individual movement (Cayuela et al., 2018; Couvray & Coupé, 2018; Fountain et al., 2018; Jaquiéry, Broquet, Hirzel, Yearsley, & Perrin, 2011; Lowe & Allendorf, 2010). For example, assignment-based tests have been a commonly used method for analyzing genetic data for measuring connectivity based on the mismatch between capture location and genetic assignment of individuals (Cayuela et al., 2018; Wilson & Rannala, 2003). Specifically, the program BayesAss has been shown to match dispersal rates generated from mark-recapture data for at least some species (Wang & Shaffer, 2017). However, BayesAss has

limitations including a “golden zone” where it can best match estimated dispersal rates to the true rates, while being less robust to low or high rates (Faubet, Waples, & Gaggiotti, 2007; Malenfant, Davis, Cullingham, & Coltman, 2016). For example, Samarasin, Shuter, Wright, and Rodd (2017) demonstrated that in situations where a species had high historical movement rates that were recently greatly reduced, most genetic methods estimate rates closer to the average dispersal. In such a scenario, then anthropogenic impacts to fragmented populations may be underestimated or missed entirely. These issues point to the need for additional ways of measuring recent connectivity between populations.

A recent alternative for determining contemporary connectivity is to analyze patterns of spatial relatedness (Escoda, Fernández-González, & Castresana, 2019; Escoda, González-Esteban, Gómez, & Castresana, 2017; Fountain et al., 2018; Wang, 2014b). Spatial relatedness is a metric that captures recent past dispersal events, with clear temporal bounds set by the genealogically oldest generation analyzed (Couvray & Coupé, 2018; Fountain et al., 2018; Vandergast, Kus, Preston, & Barr, 2019; Wang, 2014a). Generally, two broad relatedness approaches have been used: 1) Quantifying pairwise relatedness and geographic distance between closely related pairs (Aguillon et al. 2017), and 2) Reconstructing pedigrees to identify likely migrant individuals (Costello, Creel, Kalinowski, Vu, & Quigley, 2008; Kormann, Gugerli, Ray, Excoffier, & Bollmann, 2012; Vandergast et al., 2019). Methods based on pairwise relatedness often rely on binning individuals with high relatedness into pedigree classes, and then comparing geographic distances between close pedigree classes (Aguillon et al. 2017). However, pairwise estimates may be unreliable in situations with small, highly inbred populations of threatened species as even distant relatives will have a high relatedness coefficient (Pemberton, 2004; Pemberton, 2008).

In contrast, pedigree-based approaches can resolve relationships even in inbred populations and detect more distant relationships such as grandparent-grandchild pairs (Kormann et al., 2012;

Pemberton, 2004; Pemberton, 2008). One major benefit to pedigrees is that rates are specific to the timeframe of the pedigree itself which can allow focus on recent events. However, current methods either use parent-offspring pairs for determining movement rates, or only quantify distance between related individuals without estimating actual dispersal rates (Escoda et al., 2017; Fountain et al., 2018). Furthermore, even with extensive sampling, finding close kin to use for deriving rates can be challenging (Costello et al., 2008; Escoda et al., 2017).

Here, we demonstrate a novel method for quantifying contemporary connectivity that uses pedigrees based on both recent and more distant relatives. Specifically, we show that distant relatives can be used to quantify connectivity with the use of a parsimony-based stepping-stone model to estimate the location of missing individuals between distantly related ones in the dataset. Kormann et al. (2012) first proposed using parsimony modeling to incorporate full-sibling pairs into connectivity analysis. Here we expand their approach to utilize extended pedigree relations. A key advantage of our method is that it greatly increases the data available for inferring pedigrees, as parent-offspring pairs can be hard to identify in wild populations (Costello et al., 2008).

To illustrate our approach, we applied our method to estimate connectivity within the last 3 generations between several local populations of the Federally threatened Eastern Massasauga Rattlesnake (*Sistrurus catenatus*) in North East Ohio, USA. *S. catenatus* is a small rattlesnake species that was once widely distributed across eastern North America, and now only persists in small populations surrounded by unsuitable habitat (Szymanski et al., 2016). Connectivity in *S. catenatus* in NE Ohio has previously been studied using BayesAss by Chiucchi and Gibbs (2010) who found consistently low movement rates across both contemporary and historic timeframes. However, these results are suspect as recent research has found that BayesAss can be influenced by major changes in movement rates (Samarasin et al., 2017). Additionally, several new patches of occupied habitat have been discovered in

this region and may form connections with the previously studied populations. To aid conservation efforts for this species, it is critical to know if current sites represent many isolated populations, or if they form a single management unit with regular movement between populations.

The goals of our study were to: (1) Develop a novel method that uses pedigree reconstruction between distant relatives to quantify contemporary dispersal (within the last three generations for this study), and (2) Apply our methodology to measure connectivity in *S. catenatus* in NE Ohio to determine if local habitat patches are isolated or if dispersal is occurring.

Materials and Methods:

2.1 Sampling and DNA sequencing

We collected blood and scale samples from individual *S. catenatus* across 14 locations representing distinct habitat patches from 2007–2018 in Ashtabula County in Ohio (Fig. 1). Individuals were captured following standardized field surveys using coverboards and were marked via scale clips to document recaptures. For individuals over 34g (approximately 1 year or older), a PIT tag was inserted to allow for more detailed mark-recapture data to be collected. Genomic DNA was extracted from 200 ul of blood or scale clips using a phenol-chloroform protocol. Following extraction, genomic libraries were prepared from individual samples using a double-digest RAD-seq approach (Peterson, Weber, Kay, Fisher, & Hoekstra, 2012). Specifically, DNA was first digested with EcoR1 and Pst1, and then size selected to 300-600bp following the modified protocol of (DaCosta & Sorenson, 2014) and described in detail in (Sovic, Fries, Martin, & Gibbs, 2019). Our protocol followed that described in Sovic et al. (2019) except we used a 6 bp cutter enzyme (Pst1) to increase the number of loci recovered. Individual libraries were then pooled into libraries of 80-120 samples before generating SE 100 bp reads using Illumina HiSeq2500 or HiSeq4000 platforms.

2.2 Bioinformatic processing and SNP identification

Raw fastq files were demultiplexed and then aligned to a whole-genome assembly for *S. catenatus* (Mason et al., in prep.) using ipyrad (version 0.9.53). We used the following parameters in ipyrad: maximum of 5bp below a minimum phred Q-score of 33 per read, minimum coverage of 6x per base, maximum of 8 indels per read, a minimum length of 35bp post filtering, and we trimmed the ends of raw reads by 5bp after removing the adapter sequences, similar to the recommendations made by Fountain, Pauli, Reid, Palsbøll, and Peery (2016). Following alignment and preliminary filtering in ipyrad, we exported all polymorphic loci across individuals as a single VCF file. We then imported the VCF of all individuals into PLINK to perform final filtering (Purcell et al., 2007). We first filtered on a minor allele frequency of 0.01 to remove any alleles only found in a single individual, and then removed all non-biallelic SNPs. We then iteratively filtered on both missing data per individual and missing data per loci following the recommendations of (O'Leary, Puritz, Willis, Hollenbeck, & Portnoy, 2018) to optimize the total number of both individuals and loci in the final dataset.

2.3 Defining genetic clusters

Genetic data was then imported in R (version 3.5.3) via RStudio (version 1.1.463) using the package 'radiator'. We then used a two-step procedure to determine the optimal number of genetic clusters. First, we used 'adegenet' (Jombart, 2008; Jombart & Ahmed 2011) to identify genetic clusters in the data. This method is a model-free clustering algorithm that identifies the optimum number of genetic clusters in a dataset by minimizing within group genetic variation and maximizing between group variation without relying on assumptions of Hardy-Weinberg equilibrium or linkage equilibrium for individual loci. We initially ran the find.clusters model followed by a Discriminate Analysis of Principle

Components (DAPC) to identify the most likely number of genetic units (K) without including sampling locations (Jombart, 2008; Jombart & Ahmed, 2011; Jombart, Devillard, & Balloux, 2010).

Second, as an alternate approach we then took the three best K-cluster values from the find.clusters model based on the lowest Bayesian information criterion across all K values, and modelled the contributions of each group using the spatially-explicit algorithm in conStruct (Bradburd, Coop, & Ralph, 2018). Unlike the find.clusters model, conStruct includes the geographic locations of samples and uses both genetic data and location to partition variance between groups (Bradburd et al., 2018). For each possible K value, we ran conStruct's spatial model with ten independent MCMC chains with 15,000 iterations. The top chains for each K were then chosen by assessing overall fit before comparing between K values (Bradburd et al., 2018). To choose the best K-clusters, we used the layer.contributions function to quantify the amount of genetic variation each additional group supported (Bradburd et al., 2018). We then applied the cut-off recommended by Bradburd et al. (2018) to reject K-values containing groups with less than 10% of the overall variation from the dataset (Bradburd et al., 2018). We then compared the number of clusters chosen under the layer contribution cutoff to the number recommended using the cross-validation method in conStruct (Bradburd et al., 2018).

We also estimated contemporary Ne for each genetic cluster using the LDNe method (Waples and Do, 2008), as implemented in the program NeEstimator (Do et al., 2014) for genetic clusters of individuals identified using the methods described above. This method estimates Ne based on patterns of linkage disequilibrium between loci and was shown to perform well relative to other methods when calculating Ne under scenarios of low Ne and low migration rates (Gilbert & Whitlock, 2015). We used a "two allele" minimum for each locus within each population based on the recommendations of Waples and Do (2010) relative to the sample size of individuals (< 25) in almost all our populations. Confidence intervals for Ne values were estimated using a parametric approach implemented in the program.

2.4 Quantifying dispersal using pedigrees

To quantify if individual genetic clusters were isolated over contemporary timescales, we estimated per generation dispersal rates between each cluster, i.e. the probability that a given individual will move from one cluster to another over its lifespan (Cayuela et al., 2018) using individual pedigree information (Cayuela et al., 2018; Fountain et al., 2018). Here we broaden this approach developing a method that explicitly makes use of pedigree relations between more distantly related individual by using a parsimony-based method to infer probable locations for missing individuals under a stepping-stone model of dispersal (See examples given in Fig. 2, Supplemental Fig. 1).

Specifically, we first used all individuals to generate a pedigree using the R package 'sequoia', which uses a maximum-likelihood framework to identify pairwise relationships between individuals, including those that are inbred (Huisman, 2017).We chose to use sequoia over other pedigree programs as it is robust to moderate inbreeding, standardizes ages across individuals to prevent erroneous assignment, and incorporates the sex of individuals (Huisman, 2017). We also ran the pedigree reconstruction program Colony to assess if the choice of programs could affect our results (Jones and Wang 2010). For our samples, sex was determined at the time of capture via subcaudal scale counts, presence of developing embryos, and/or probing for hemipenal pockets (G. Lipps, pers comm.). Age was determined via counting rattle segments if the rattle was unbroken, and by binning weight classes for those with incomplete rattles (Hileman et al., 2017). Weight classes were based on range-wide estimates for different age groups reported by Hileman et al. (2017), with the following classifications representing 0, 1, 2, 3, 4+ years of age: <20g, 20-31g, 32-50g, 50-500g, >500g. Age estimates were then subtracted from the year of capture to generate probable birth years for all individuals. Once all individuals were matched to a birth year and sex (including unknown), we ran sequoia allowing for one erroneous allele in each pair, and that the most likely relationship to be 95% more likely than any other to be accepted.

2.5 Assigning locations to missing individuals

Once the pedigree was determined, we focused on pairs of related individuals up to half niece/nephew to aunt/uncle or cousins (r=0.125). By only using these types of relationships, we can put a temporal frame on our rate estimates that any dispersal events must have occurred within the last 3 generations. We then generated "dummy" individuals for all pairs other than parent-offspring, where the dummy individual represented a missing recent shared ancestor for a given pair.

To estimate the "dispersal" of a dummy individual, each dummy individual was first assigned to a given genetic cluster based on the following criteria (see Supplemental Fig. 1) First, if both related individuals were in the same genetic cluster, then the dummy was assigned to that location as well. Second, if related individuals were not in the same cluster, then a movement matrix was generated for the dummy individual, where matrix values represented the number of movements required to recreate the observed pattern between known individuals. Specifically, the number of movements were calculated under a simple stepping-stone cost model where it would take one event to reach nearby patches, and an additional event per occupied patch between individual locations under the assumption that *S. catenatus* are unlikely to make extreme long distance movements (Supplemental Fig. 1). We chose a simple model with a single cost per patch moved to represent individuals either making it to another patch or dying in the process. We selected these values, as we do not have information of the relative resistances between sites to have a more detailed cost model. Once movement costs were calculated for every site, each dummy was assigned to the location with the lowest cost. In cases where two or more sites had the same cost, individuals were randomly assigned to one of the sites. Our methodology is appropriate for these populations because in this region, rattlesnakes exist in discrete patches of suitable habitat mostly surrounded by woodlands, active cropland, and impervious surfaces

that makes a simple stepping-stone movement model an appropriate approximation of movement between occupied patches (Fig. 1).

As a comparison using sites in which dispersal likely occurs, we also applied our methods to a single focal area comprised of three distinct occupied fields (Fig. 1, GRLL-4) nested within one genetic cluster where known movement between fields has been documented from mark-recapture data (G. Lipps, unpublished data). For these three sites, we chose to use each field as our *a priori* sampling unit, as compared to the potential genetic units used previously. Despite known movement occurring between each field, the intervening landscape is heavily wooded, and the fields are actively threated by ongoing succession. Here, we also applied a model where the distances between each site was a single step, since it represents a single large field with two smaller satellite patches. Applying our methods to these focal sites allowed us to evaluate how pedigree-based rates perform in areas likely undergoing frequent dispersal events. After dummy individuals were generated and assigned to probable locations, per generation dispersal rates were calculated by taking all pairs of parent-offspring incorporating the pairs with dummy individuals and repeating this procedure 1000 times. We then built Bayesian models using the package 'R2jags' to determine dispersal out of each site. Bayesian models were fit with the number of successful dispersal events to a given site represented as a binomial distribution (p[site[i]], N) with p being the probability of successful dispersal to a given site and N the total number of parent-offspring pairs with at least one individual in the source site. Probability of dispersal was assigned a non-informative prior of beta(1,1). We calculated the 95% credible intervals for dispersal by running 5000 iterations over ten independent chains using the first 2500 iterations as a burnin and the top chain selected based on DIC scores.

2.6 Identifying Management Units

After generating per generation dispersal rates as described above, we set out to quantify dispersal between each pair of genetic clusters identified in sections 2.3 to determine if each genetic cluster should be considered an independent management unit (MU) or not. We applied a cut-off of at least one migrant per generation (Mills & Allendorf, 1996).

As a check on our identification of management units, we tested the prediction that if connectivity is low to zero between sites then individuals within each MU should be more related to each other than any other individual outside the group. To test this hypothesis, we calculated pairwise relatedness between all individuals using Coancestry (version 1.0.1.8; Wang, 2011) to calculate Wang's relatedness for all pairs of individuals. We specifically set inbreeding equal to true in Coancestry, and also calculated the following other pairwise relatedness metrics to assess the sensitivity of Wang's estimator to inbreeding: Lynch-Li, Lynch-Ritland, Ritland, Queller-Goodnight, and Dyad Maximum-Likelihood (Wang 2014).We used relatedness to assess inbreeding, as genomic estimates of relatedness have been shown to be more accurate to quantify inbreeding in wild populations than pedigrees (Wang, 2016). Wang's relatedness is a method of moments relatedness metric that has been shown to be robust to unknown population allele frequencies and having a high proportion of closely related individuals in the dataset (Bink, Anderson, van de Weg, & Thompson, 2008; Wang, 2002). Once pairwise relatedness was calculated, we grouped values for all between and within site comparisons to get mean relatedness for each site pair. We also calculated Nei's pairwise Fst across each group using the 'pairwise.fst' function in the 'adegenet' package with the default settings (adegenet citation).

2.7 Comparison to BayesAss

To assess how well our pedigree-based dispersal rates compared to migration rates calculated using other approaches, we also assessed connectivity between genetic clusters with 'BayesAss' (Wilson &

Rannala, 2003). BayesAss is based on a Bayesian assignment model that uses sampling locations combined with neutral genetic markers to quantify migration rates in the last 5-15 generations (Broquet, Yearsley, Hirzel, Goudet, & Perrin, 2009; Faubet et al., 2007; Wilson & Rannala, 2003). While the migration rates from BayesAss are calculated differently than our dispersal per generation from our pedigree methods, estimates from BayesAss may be closely correlated to actual dispersal rates (Wang & Shaffer 2017). We ran BayesAss on both the data with individuals grouped by genetic units, and on the same subset of focal individuals that may be undergoing frequent dispersal. We followed the recommendations given by Meirmans (2014) and performed 10 independent runs for each of the two datasets. We then used the supplemental code provided by Meirmans (2014) to calculate BIC scores for each run and chose the best migration rates for each of the two models by selecting the run with the lowest BIC score.

Results:

3.1 Bioinformatics and SNP Filtering

We sequenced a total of 132 samples, with a minimum of 1 million raw reads per individual. After alignment and quality control filtering in ipyrad, we recovered 235,057 polymorphic loci across all individuals. Following our initial filtering on minor allele frequencies, we sequentially reduced the proportion of missing data allowed within individuals and across loci until we had a final dataset consisting of 86 individuals with 2996 loci with no missing data.

3.2 Genetic Clustering

Based on the Bayesian information criterion (BIC), adegenet supports six clusters (K), representing each of the five main sampling areas while splitting the largest sample area into two clusters (Fig. 1, GRLL-4). Based on prior capture data, several individuals split between the fifth and sixth cluster were caught

within 5m of each other. Five and seven clusters also had low BIC scores and under K = 5, each of the habitat patch groups was placed with nearby fields. An examination of the assignment probabilities for each individual under K = 5 also showed no evidence for individuals with recent admixture, indicating there may be low connectivity between these sites (Fig. 3). However, one individual, a six-year-old male, was assigned with 100% probability to a different genetic unit than it was captured in using both the prior and prior-less DAPC models (Fig. 3, captured in GRLL-4, assigned to GRLL-3). Given that males are known to make long distance movements to find mates in this species, it is possible that it could have dispersed between units (Hileman et al. 2017).

We then tested support for K = 4, 5, and 6 under the spatially explicit model in conStruct. Out of the ten independent runs for each K value, we first selected the top run based on MCMC chain convergence. After comparing layer contributions, K = 5 was the largest K value tested with all groups contributing at least 0.1 (10%) of the overall genetic variation. The grouping of each habitat patch under K = 5 for conStruct matched those observed using adegenet. Our cross-validation within conStruct supported the spatial model over the non-spatial, and while the cross-validation recommended K = 6, this was eliminated on the basis of the layer contribution thresholds. Based on the agreement between adegenet and conStruct, we used the five genetic clusters (corresponding to the five named boxes in Fig. 1) as the units for determining dispersal.

Estimates of LDNe values ranged from 4.1 to 10.9 with a mean of 7.9 across the five patches. All 95% parametric CI's were well below an Ne of 50, matching findings reported by Sovic et al. (2019). Of note is that our LDNe estimates overlapped with those reported by Sovic et al. (2019) for the two patches (Fig. 1: GRLL-1, GRLL-4) also reported there

3.3 Pedigree Inferences and Dispersal Estimates

Sequoia identified 110 pairs of related individuals that could be assigned to a specific kinship category with a minimum of 95% likelihood. Of the pairs identified, three were parent-offspring, two were between full siblings, 40 were between half-siblings, 58 between 2nd degree relatives that can be identified as either niece/nephew to an aunt or uncle, and five 2nd degree pairs where the type of relationship could not be further identified. The five unknown 2nd degree pairs were excluded from later analyses. Across all 105 related pairs, none contained individuals found between two genetic clusters, and our low-likelihood acceptance model also found no between cluster pairs as did our Colony analyses (results not shown). Since no related individuals were found across genetic clusters, we inferred that there is no contemporary dispersal between genetic units based on the pedigree data. While this runs counter to the evidence above of the male *S. catenatus* in GRLL-4 having a genetic profile of GRLL-3 individuals, that snake had no kin across the pedigree, and thus was not incorporated into the model. GRLL-3 and GRLL-4 represent the two geographically closest clusters, and low dispersal may still be occurring there despite these results. However, the disperser has not successfully bred into the recipient population, and we found no evidence of dispersal events with successful breeding in the recent past.

We then applied our method to the three occupied fields in a single genetic cluster where mark-recapture data has documented movement between fields. Within this cluster, sequoia recovered 48 unique pairs of related individuals. Specifically, two pairs of full siblings, twelve pairs of half siblings, 20 identifiable 2nd degree pairs, and four unknown 2nd degree pairs. After removing the four unknown pairs, we assigned 88 dummy individuals to recreate probable parent-offspring pairs. Across these three occupied fields, related individuals were found between all possible combinations of fields. Thus, we were able to generate dispersal rates and 95% confidence intervals between each set of occupied fields (Fig. 5, Table 2). All dispersal rates were significantly different from zero, and high rates of movement were seen from individuals leaving two of the fields. While individuals are unlikely to leave field one (the

largest of the three), individuals have a 10 - 30% chance of migrating to this patch from either of the other two (Fig. 5, Table 2).

Overall, our technique estimates dispersal rates within the last 9-30 years given a mean generation time of three years for this species (Sovic et al., 2019) and that the oldest known individual captured in this study was approximately ten years old based on capture data. Based on the estimates from our pedigrees, we can conclude that there is little to no contemporary connectivity between each of the previously determined genetic units. We were able to detect frequent movements between smaller fields located within a single genetic unit showing that when dispersal is occurring regularly, our method will be able to derive movement rates.

3.4 Pairwise-relatedness across all individuals shows evidence for inbreeding

Wang's pairwise relatedness within each genetic unit show evidence of high levels of inbreeding, matching the low LDNe values and lack of connectivity. While Wang's estimator can be affected by high levels of inbreeding, it was closely correlated to all other relatedness metrics calculated with a minimum of r = 0.851 to Ritland's estimator and a high of 0.999 to Lynch-Li (Wang 2014). Across four out of the five genetic clusters, more than half of all individuals were as related as outbred cousins (relatedness > 0.125) (Fig. 4). In the genetic unit with the lowest mean pairwise relatedness, 25% of individuals were still more related than cousins. Of those four more inbred clusters, over 25% of individuals were more related than half-siblings, and the single most inbred cluster did not have a single pair of individuals that were not closely related (Fig. 5, GRLL-5). Despite high pairwise relatedness indicating sustained inbreeding in this cluster, we failed to identify any pedigree relationships between pairs of individuals, likely a result of multiple familial relationships, precluding identification of a single best one. While such highly inbred populations do pose a problem for pedigree-based methods, at such high relatedness any

dispersers into or out of the population would be readily detected by a DAPC, further emphasizing the need to check that results from multiple analyses converge.

Between genetic units, all pairwise relatedness values were zero or slightly negative (results not shown). Negative relatedness can arise due to differences in calculations and can be interpreted as individuals being completely unrelated relative to the sample set (Bink et al., 2008). A lack of any relatedness between individuals from different genetic units is further evidence for contemporary isolation for these units.

3.5 BayesAss Migration Estimates

All BayesAss runs converged to similarly low rates of connectivity between sites. For the run with the lowest BIC score, as derived using the code of Meirmans (2014), all between-site rates included zero in their 95% confidence intervals (Table 1). The single highest between group migration rate was 6.5%, but most were less than 3%. The 6.5% rate was from GRLL-3 to GRLL-4, as expected given the male disperser, but the 95% credibility interval included zero indicating any regular dispersal between the pair of sites was negligible. Given that all rates had confidence intervals that overlapped with zero, the BayesAss results are consistent with the inference from the pedigree-based method of little to no connectivity between genetic clusters.

For the single large sample size genetic cluster, BayesAss results deviated significantly from those estimated using our pedigrees. Specifically, BayesAss found low, but significant, migration between nearby sites, but not between the two furthest locations. Rates from BayesAss ranged from 3% to 12% between sites, indicating that most individuals remain in their natal locations (Table 3). However, BayesAss is known to overestimate connectivity in cases where there was moderate historical connectivity and low to zero contemporary connectivity (Samarasin et. al., 2017). Based on the critique

of previous estimates of connectivity in this system (Chiucchi & Gibbs, 2010) as discussed by Samarasin et al. (2017), the values reported here are likely overestimates of contemporary connectivity.

Discussion:

4.1 Estimating contemporary dispersal using pedigrees

Recent advances have been made to infer the probable location of parents based on the location of full-sibling pairs, but such pairs can be rare in many datasets (Kormann et al., 2012). Others have used extended kin pairs as qualitative evidence of connectivity, or to detect the presence of low-permeability barriers (Escoda et al., 2017), but do not explicitly quantify levels of dispersal (Carroll & Gaggiotti, 2019; Vandergast et al., 2019). Here, we have shown that it is possible to use both close and distant kin relations to generate quantitative estimates of dispersal rates. By using distant relationships beyond just parent-offspring (Wang, 2014b), the number of samples is greatly increased (e.g. from three to > 100 in our dataset), allowing for higher confidence in the observed patterns. Furthermore, our approach takes advantage of the fact that in small or inbred populations, many related individuals may be found, even if parent-offspring pairs are rare (Kormann et al., 2012). However, it is worth noting that at very high levels of inbreeding it may become impossible to distinguish between any kinship pairs, such as in our GRLL-5 population. In such situations, it may be impossible to apply the approach we have outlined here, although connectivity between populations is unlikely in such a situation.

A second advantage of our approach is that unlike previous methods, it explicitly takes into account habitat heterogeneity which is typical of threatened species that often exist in highly fragmented habitats. For non-threatened species living in areas with more contiguous habitats, evaluating dispersal based solely on geographic distance between kin pairs may be more reasonable, as individuals are more likely to be located across a gradient of distances (Aguillon et al., 2017). However, many species of

conservation concern persist in fragmented landscapes (Fischer & Lindenmayer, 2007; Mortelliti, Fagiani, Battisti, Capizzi, & Boitani, 2010). Our method explicitly incorporates fragmentation with the underlying assumption that the landscape between occupied patches is inhospitable for the species of interest. Thus, the method described here is likely more broadly applicable to threatened and endangered species.

Finally, another benefit of pedigree-based methods is that unlike genetic assignment methods, pedigrees only incorporate potential movements over an explicit timeframe defined by the depth of the pedigree considered. Therefore, it is possible to put a precise estimate on the period of time over which the observed dispersal events occurred. For example, in this study, the oldest *S. catenatus* recorded at our sites was a 10-year-old female, while the average generation time is approximately three years (Sovic et al., 2019). As a consequence, our movement estimates represent dispersal rates between sites within the last 30 years, well within recent modifications of the landscape for agriculture (McCluskey et al., 2018). This contrasts with the broader and less precise estimates derived from BayesAss, which typically represent the last 5-15 generations (Rannala & Mountain, 1997; Wilson & Rannala, 2003), although this is likely only true within a band of optimal dispersal values described by Meirmans (2014). By knowing dispersal rates are linked to the recent past, we can make inferences on how the observed landscape shaped these rates (Anderson et al. 2010; Boulanger, Dalongeville, Andrello, Mouillot, & Manel, 2020). The ability to link the landscape a species lives in to observed movement patterns allows for better conservation decisions to be made regarding land protection, acquisition, and management (Cayuela et al., 2018; Escoda et al., 2017). Furthermore, unlike genetic assignment methods, our model does not rely on any assumptions of Hardy-Weinberg equilibrium for loci used in the analyses.

One weakness of the methods used here is the need to use a movement cost matrix based on expert opinion. For relatively simple systems with only a few sites this can be done with reasonable ease

based on species biology but can rapidly become more difficult in systems with more diverse habitats. Some potential alternatives would be to use a cost matrix or least cost paths between all sites to represent potential movement costs (Cushman, McRae, & McGarigal, 2015; Spear, Cushman, & McRae, 2015; Zeller, McGarigal, & Whiteley, 2012). Least cost paths and other more quantitative landscape genetic techniques could allow for more explicit linking of movement values to the landscape of the species (Cushman et al., 2018; Cushman, Landguth, & Flather, 2013; Dilts et al., 2016; Shirk, Schroeder, Robb, & Cushman, 2015; Zeller et al., 2012).

Overall, our model was able to quantify a lack of contemporary connectivity between several isolated sites in a Federally listed species, while also showing the capacity to detect high levels of movement in fields separated by only a few hundred meters of unsuitable landscape. Unlike genetic assignment methods that are commonly applied to situations like this, our method is not affected by historical gene flow. Both our pedigree method and assignment methods require a priori groups to be tested, but as show here, the methods can be applied at the level of individual fields up to groups of genetic units. However, it is important to confirm results with multiple analyses. Here, we can verify that each genetic unit is isolated due to the low mean relatedness between units, low Ne values with high inbreeding, and high Fst between each genetic unit. We were able to detect a single potential migrant in the lone male discussed previously, indicating there may be some rare dispersal between the two closest genetic units, but did not find an evidence that it successfully bred into the local population. Both this lone migrant and the broader variability in both sampling and pedigree software demonstrate the need for researchers to confirm results with alternative analyses such as Ne and comparing mean relatedness before making final recommendations.

4.2 *S. catenatus* conservation

These data support recognizing each of the five genetic units as isolated management units (Moritz, 1994). Each genetic unit in this study represents an isolated population with no contemporary connectivity, and thus they are not affected by demographic stochasticity in the other genetic units (Cayuela et al., 2018; Mills & Allendorf, 1996; Moritz, 1994; Waples & Gaggiotti, 2006). However, given the close proximity of these populations and that there was likely historical connectivity (Chiucchi & Gibbs, 2010), restoration of connectivity to form a single management unit for *S. catenatus* should be a conservation goal.

Past research found evidence for low contemporary connectivity between a subset of these populations based on results from BayesAss (Chiucchi & Gibbs, 2010). However, these results were recently called into question on the basis of bias built into the genetic assignment methodology used (Samarasin et al., 2017). Specifically, Samarasin et al. (2017) showed that in populations with high historical connectivity, and low to zero contemporary connectivity, genetic-based programs will often overestimate contemporary connectivity and underestimate historical rates. Our work, which incorporate data from newly discovered occupied patches in the region and a different analytical method (pedigree-based dispersal rates), confirm that connectivity in the very recent past is extremely low – we found no evidence for dispersal between genetic clusters over the past three generations (within the last 30 years). The observed lack of connectivity is further supported by the fact that we observed high mean kinship in every genetic unit (Fig. 4) and a mean kinship of zero between all pairs of genetic units.

These results, and those of Chiucchi and Gibbs (2010) and Samarasin et al. (2017) also suggest that *S. catenatus* populations in this region likely went from occupied patches with regular movement between them to complete isolation in the recent past. This may be due to the increase in forest and agricultural land from anthropogenic events that have occurred over the last 100 years in Northeast Ohio (McCluskey et al., 2018). We note that these genetic clusters show high levels of genetic heterozygosity

and limited genetic differentiation. (Supplemental Table 1). This supports the idea that due to their recent isolation these *S. catenatus* populations may not yet have had the corresponding reduction in genetic variability from drift, but that this cost could be "paid" in within a few generations (Sovic et al., 2019). To prevent genetic erosion due to genetic drift and inbreeding in the future for these populations, translocations of individuals between patches could be a prudent conservation measure (Madsen, Shine, Olsson, & Wittzell, 1999; Madsen, Ujvari, & Olsson, 2004). However, translocations must be taken with care and proper study design used (Dodd & Seigel, 1991; Ochoa et al. 2020), as previous attempts with this species have been mostly unsuccessful (Harvey, Lentini, Cedar, & Weatherhead, 2014; King, Berg, & Hay, 2004).

As a next step to restore connectivity, we first need to determine what landscape features promote or block movement. To do so, landscape resistance models that match genetic distances to differences in landscape features offer a potential route to find possible corridors or important landcover for *S. catenatus* to move through (Cushman & Landguth, 2012). While we found connectivity between close fields within the same clusters, ideally such methods should be applied to additional landscapes where *S. catenatus* are shown to move larger distances.

4.3 Conclusion

We have shown that it is possible to use distant kin and a gap-filled pedigree to reconstruct dispersal rates across fragmented landscapes with disjunct occupied sites. Like other pedigree and assignment-based methods this approach expands our ability to assess patterns of movement over shorter time scales than more traditional genetic approaches which makes them sensitive to the effects of recent anthropogenic impacts. There are two broad improvements that could be made to our methodology in the future: (1) incorporating least cost paths or other alternatives to the expert opinion cost matrix and

(2) adding demographic data into the Bayesian model to estimate sex or age bias in dispersal. Overall, these advances will add to potential of using pedigrees to study of the factors governing the distribution and abundance of organisms over short timescales that might have previously been out of reach for population genetics (Bradburd & Ralph, 2019).

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Data Accessibility: Raw fastq files used here will be uploaded to the Dryad Digital Repository. R scripts used to generate dummy pedigree individuals and calculate dispersal rates will be uploaded to the authors personal Github page and made publicly available.

Author Contributions: SM and HLG designed the study. SM and GL did the field work. GL obtained landowner permission and permits. Funding was under grants provided to HLG and GL. SM performed the lab work and bioinformatics. SM and HLG wrote the first draft, and GL provided feedback and approved the final version.

Tables and Figures

Table 1: Migration rates derived from BayesAss, with 95% credibility intervals in parentheses. All 95% CI for between cluster migration also include zero. Values represent the probability of an individual travelling from the row sites to the column sites.

Cluster	GRLL-4	GRLL-5	GRLL-1	GRLL-3	GRLL-2
GRLL-4	0.947 (0.911-0.983)	0.007 (0-0.021)	0.007 (0-0.021)	0.015 (0-0.035)	0.022 (0-0.047)
GRLL-5	0.019 (0-0.053)	0.926 (0.863-0.987)	0.019 (0-0.053)	0.019 (0-0.053)	0.019 (0-0.053)
GRLL-1	0.026 (0-0.06)	0.013 (0-0.038)	0.920 (0.866-0.974)	0.026 (0-0.06)	0.013 (0-0.038)
GRLL-3	0.065 (0-0.129)	0.019 (0-0.053)	0.019 (0-0.053)	0.876 (0.800-0.952)	0.019 (0-0.053)
GRLL-2	0.024 (0-0.067)	0.024 (0-0.067)	0.024 (0-0.067)	0.048 (0-0.106)	0.88 (0.800-0.960)

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Table 2: Pedigree-based dispersal rates for the largest genetic unit (GRLL-4) of *S. catenatus*, comprised of three habitat patches. Values represent the probability of an individual dispersing from the column patch to the row patch. 95% credibility intervals are given in parentheses.

PATCH 1PATCH 2PATCH 3PATCH 10.893 (0.888-898)0.357 (0.338-0.337)0.115 (0.101-0.129)PATCH 20.089 (0.084-0.095)0.555 (0.535-0.574)0.210 (0.193-0.228)PATCH 30.018 (0.015-0.02)0.088 (0.077-0.100)0.676(0.656-0.696)

Table 3: BayesAss migration rates for the largest genetic unit (GRLL-4) of *S. catenatus*, comprised of three habitat patches. Values represent the probability of an individual dispersing from the column patch to the row patch. Standard deviations are given in parentheses, and all but two rates denoted with a (*) did not include zero in their 95% CI.

PATCH 1PATCH 2PATCH 3PATCH 10.90 (0.842-0.958)0.12 (0.225-0.0218)0.08 (0.002-0.158)PATCH 20.07 (0.031-0.109)0.79 (0.693-0.886)0.08 (0.002-0.158)PATCH 30.03* (0-0.067)0.08 (0.002-0.158)0.84 (0.743-0.938)

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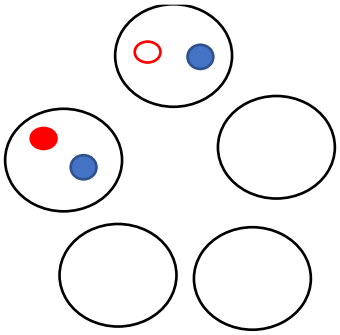
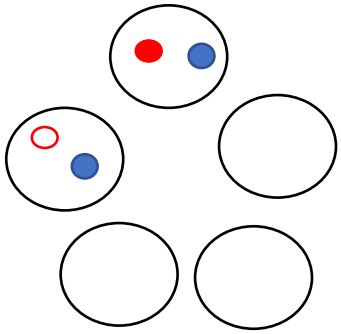
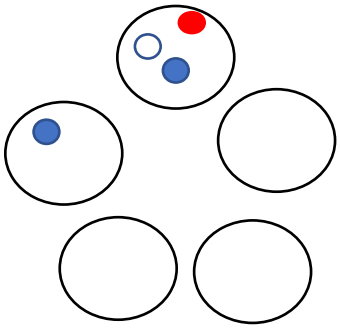
Pathway 1: Shared parent (red dot) reproduces at S1, then moves to S2 and reproduces again. This requires a minimum of 1 dispersal event.



Pathway 2: Shared parent (red dot) reproduces at S2, then moves to S1 and reproduces again. This requires a minimum of 1 dispersal event.



Pathway 3: Shared parent (red dot) reproduces at S1 twice, then one offspring moves to S2. This requires a minimum of 1 dispersal event.



Pathway 4: Shared parent (red dot) reproduces at S2 twice, then one offspring moves to S1. This requires a minimum of 1 dispersal event.

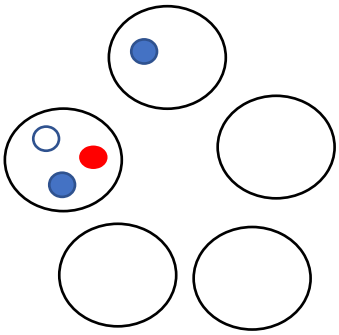


Figure 2: Examples of potential dispersal events to recover a missing shared parent (red dot) from two half-siblings (blue dots) across five potential habitat sites (black circles). For details, see supplemental figure 1. This uses a simple stepping-stone model where individuals may move only to the next nearest site. Across all four pathways, we are able to eliminate the two lower sites as potential locations under the principle of parsimony for movement events. We can then use the uncertainty of the parental location to incorporate error into the stepping stone model to improve dispersal estimates.

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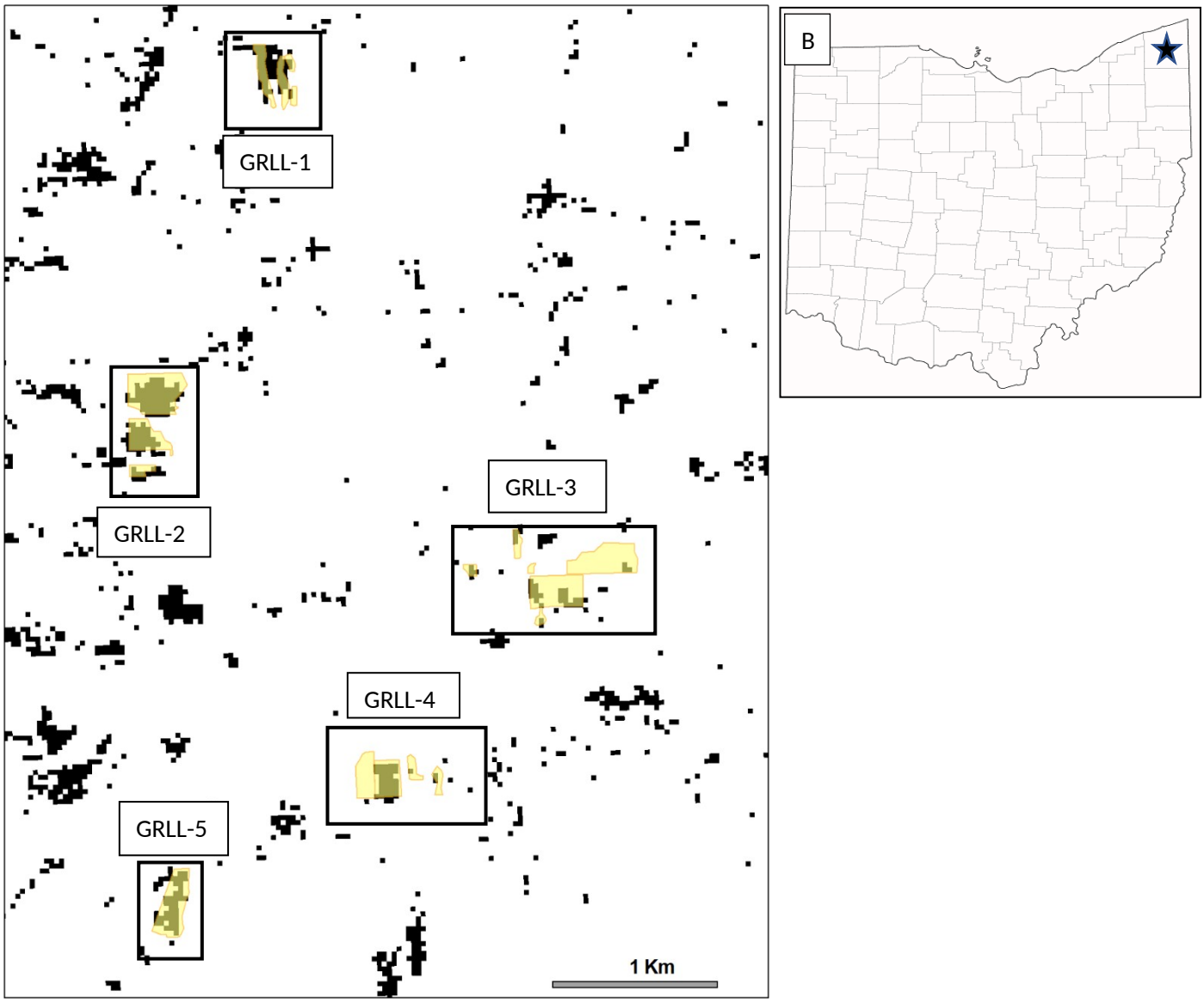

Figure 1: Sites where S. catenatus was sampled in northeast Ohio. Map is based on a habitat suitability model developed by McCluskey (2016) showing potentially suitable habitat (black) and unsuitable habitat (white). Details are not included in order to protect sensitive location information for this rare snake. Occupied sites are identified as yellow shaded polygons, and each site cluster is identified as GRLL-# for later analyses. The trio of occupied fields later focused on form cluster GRLL-4. Figure 1B identifies the Ohio county (starred) where sites are located.

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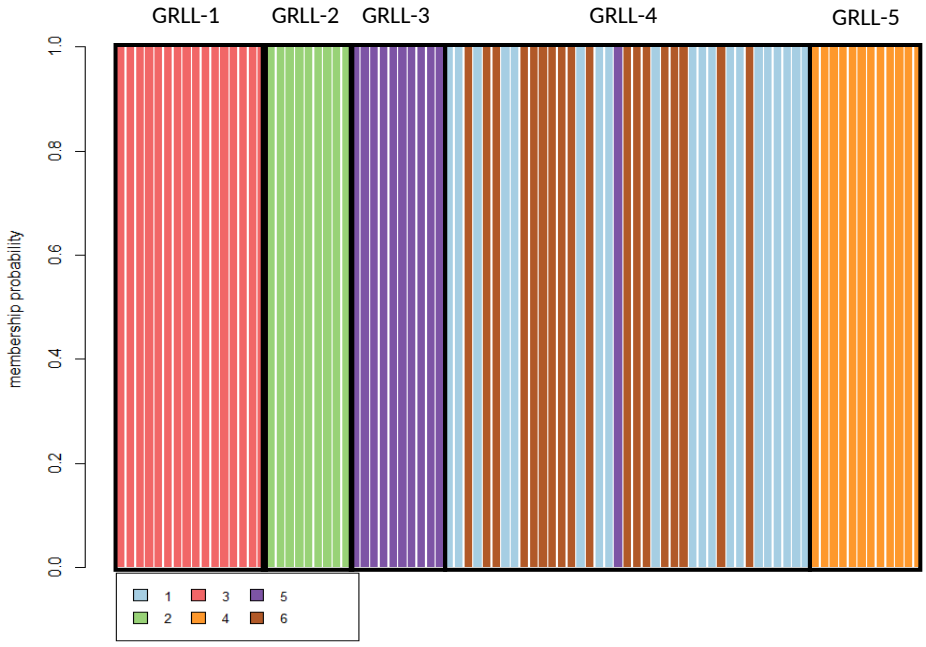

Figure 3: Discriminate Analysis of Principle Components across 86 individual Eastern Massasauga Rattlesnakes in Ashtabula County, Ohio, USA. Assignment plot for K = 6, where each K value is represented by one color in the legend. Each vertical bar represents one individual, and the proportion of the bar assigned to each color represents the probability of assignment to that cluster for the given individual. All individuals from a site are within the black box with the site name above the box, matching sites given in Fig. 1.

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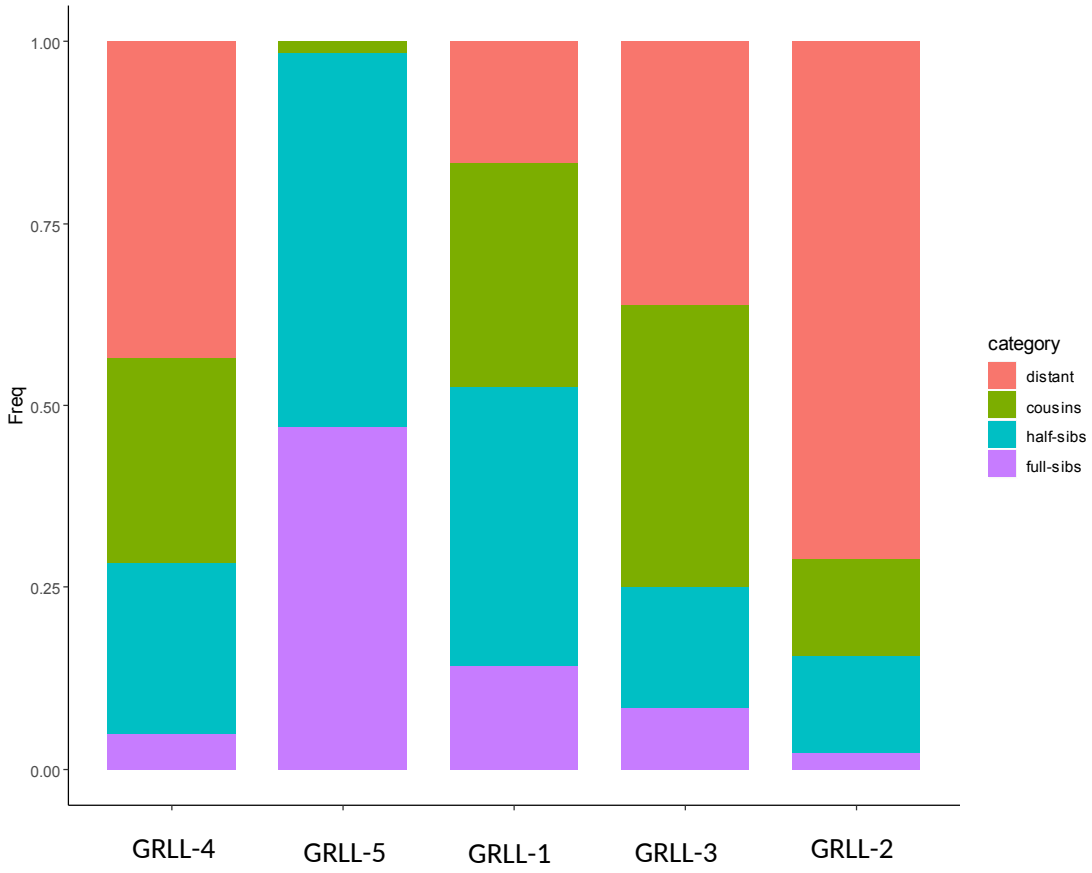

Figure 4: The relative proportion of pairs of individuals within each genetic unit binned by pairwise relatedness, based on expectations for non-inbred populations. Thresholds of 0.125 was used for cousins, 0.25 for half siblings, and 0.5 for full siblings. Between genetic units not shown, as all pairs were below 0 mean relatedness.

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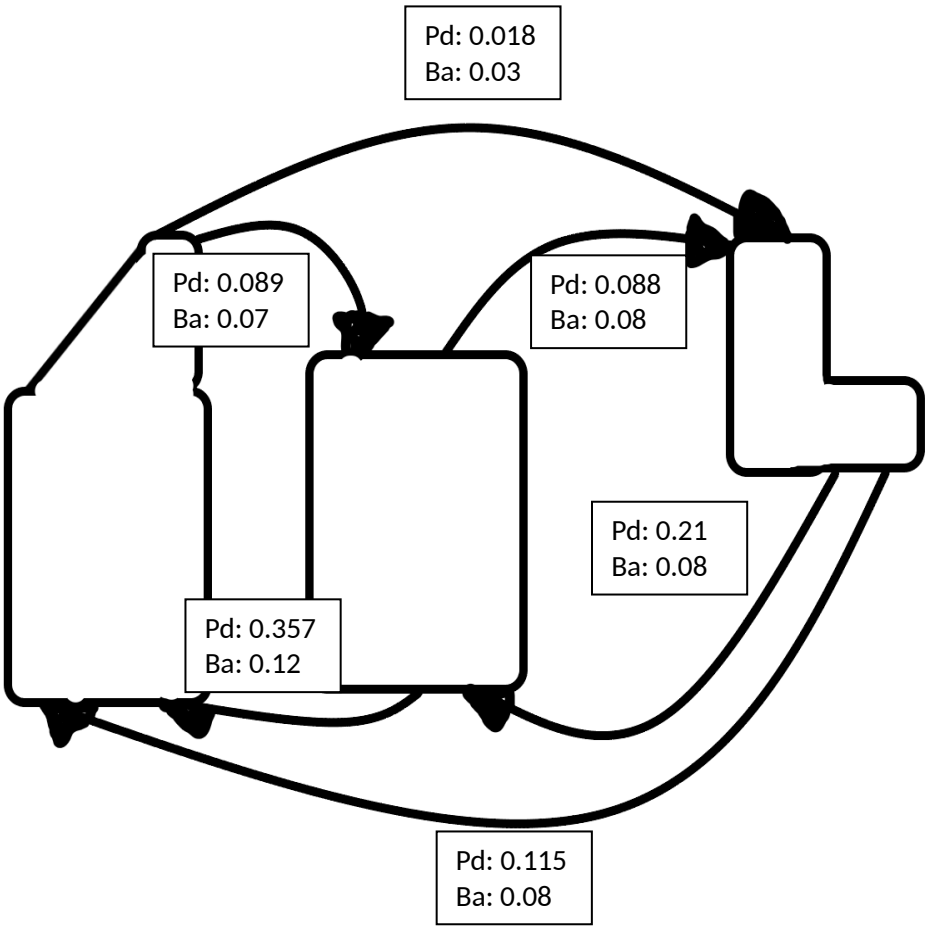

Figure 5: Dispersal estimates between habitat patches for the genetic unit with the largest number of samples. Pd estimates are mean pedigree-derived rates, while Ba values are mean dispersal rates from BayesAss. 95% CIs for the pedigree rates are given in Table 2, while 95% CIs from BayesAss can be found in Table 3.