Genetics of a reintroduced swift fox population highlights the need for integrated conservation between neighbouring countries

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Abstract

The genetic consequences of reintroductions are rarely considered after releases cease, but long-term viability depends on linked demography and genetic health. Reintroductions of swift foxes Vulpes velox began after 45 years of extirpation from Canada; these have resulted in national down-listing to ‘threatened’ status, and the re-establishment of a small contiguous population in Montana, US. Demographic growth has been associated with stable levels of genetic diversity and growing effective population size, but evidence of two genetic clusters, and a recent decline in abundance could be cause for conservation concern depending on underlying mechanisms. We analysed individuals from two time points at 18 microsatellite loci to investigate whether the genetic structure is a consequence of having used two separate release sites and non-equilibrium population dynamics, but our results suggest that the population is likely at mutation- and migration-drift equilibrium. We examined habitat effects on relative gene flow and found limited evidence for cropland to be a dispersal barrier, but effects of terrain roughness suggest that more rugged landscapes may reduce dispersal capacity. Using parentage analysis we determined maximum dispersal distances of up to 50 km for females and 100 km for males including movements in either direction across the international border, but no mixing of genetic clusters was seen in either country. Greater genetic connectivity among than within respective countries necessitates careful co-management between Canada, where the species has the highest levels of legislative protection, and contiguous areas of the United States where limited trapping for fur is now permitted. We encourage similar analyses of conservation populations across international borders to determine how optimal genetic management can best mesh with different policies and conservation approaches among countries.

Introduction

Collaborative management across international borders is desirable for conservation and can lead to successful outcomes. These broad-scale initiatives typically focus on species that are wide-ranging and/or require an extensive land base for resources, e.g. polar bears (Ursus maritimus, Prestrud & Stirling, 1994), wolves (Canis lupus, Weber & Rabiniwitz, 1996) and migratory birds (Williams, Koneff & Smith, 1999). However, at a smaller scale, there are fewer instances of collaborative management (Kark et al., 2015), where management decisions are driven more by local perceptions and biases (Brichieri-Colombi & Moehrenschlager, 2016). Canada and the United States share the world’s largest international border, and there are a number of instances where species protection differs across the borders. For example, the fisher Pekania pennanti is being reintroduced into areas of Washington State, while it remains a furbearer in the adjacent Canadian province of British Columbia (Lewis et al., 2016).

Differences in management across jurisdictions may not impact the survival of populations significantly, but that will depend on species connectivity. If the spatial scale of population processes can be quantified, we can then identify the jurisdictions directly impacting population viability. Genetic data can be used as a means to identify the scale of connectivity and inform conservation initiatives (Stewart et al., 2017). The swift fox provides an excellent case study for small-scale collaborative management. Swift foxes were extirpated from Canada for over 40 years, and a
reintroduction programme was initiated to re-establish this small native-grassland canid in southern Alberta and Saskatchewan (Carbyn, 1998; Moehrenschlager, List & MacDonald, 2007). Releases of almost 1000 captive-bred or translocated foxes were conducted from 1983 to 1997 at two release sites separated by approximately 200 km (Fig. 1). Efforts were made to ensure that a similar genetic stock was introduced into the two sites. Relative growth and distribution of the population was monitored in 2000/01 and 2005/06 (Moehrenschlager & Moehrenschlager, 2001, 2006; Moehrenschlager & MacDonald, 2003). Based on trends of increasing population size and expanding range, the swift fox was down-listed from ‘Endangered’ to ‘Threatened’ in Canada (COSEWIC 2009). As a result of these efforts, a contiguous population of similar size was established in Montana, and a small quota of swift foxes is allowed to be harvested for fur (https://myfwp.mt.gov/fwpPub/speciesHuntingGuide?wmSpeciesCd=SF, accessed Sept 19 2018).

A recent comprehensive survey, based on mark-recapture and site occupancy analyses across the Canadian/Montana population, suggests that the population’s extent of occurrence is similar to 2006, but there have been substantial declines on both sides of the border in terms of the area of occupancy and abundance (Moehrenschlager & Moehrenschlager, 2018). This finding has prompted a closer investigation of previously collected genetic data, where a small number of microsatellites were used to assess the genetic status of the reintroduced population. These data indicated a stable, mid-range level of genetic diversity, and evidence of increasing effective population size (Cullingham & Moehrenschlager, 2013). However, there was evidence of population substructure, with two subpopulations being identified: “east” and “west”, Fig. 1. This was surprising, given that multiple years of releases using the same stock at both locations should help to reduce genetic differences between sites.

In an effort to understand the impacts of differential management on this species, we genotyped the previous samples at an additional 12 loci to increase genetic resolution. Our aim was to characterize the underlying causes of population substructure, gene flow within population clusters and the range of dispersal distances of individual swift foxes. Population substructure may result from non-equilibrium population dynamics that have resulted from two separate founding locations (Latch & Rhodes, 2005; Brekke et al., 2011; Morales et al., 2017) or from natural barriers to dispersal (Schwalm, Waits & Ballard, 2014). We examine equilibrium dynamics through mutation-drift and migration-drift equilibrium tests (Piry, Luikart & Cornuet, 1999; Beaumont, 2001) to understand whether the population substructure is more likely the result of founding effects and non-equilibrium dynamics. To determine whether there are habitat effects on dispersal, we calculate the amount of spatial genetic structure across the study area (Galpern et al., 2014) and use landscape genetics to identify dispersal barriers (McRae & Beier, 2007). To determine the scale of connectivity, we estimate the dispersal kernel using parentage analysis (Nathan et al., 2003; Cullingham et al., 2009; Waser & Hadfield, 2011; Norman & Spong, 2015). Such indicators of movement could inform how management actions in one jurisdiction might affect population recovery in other jurisdictions, especially as the swift fox has different levels of protection across the international border.

Materials and methods

Sample collection and genotyping

We used hair samples collected in 2000/01 (N = 111) and 2005/06 (N = 193) that were previously analysed at seven microsatellite loci (Cullingham & Moehrenschlager, 2013). To determine parentage among these samples, we genotyped an additional 11 microsatellite loci and a sex-determining locus (ZFX/ZFY; Morin et al., 2005). Individuals were sexed upon hair collection, and we used genetic determination to ensure that the sexing data were error free for parentage analysis. The additional microsatellites were modified from previously published primers (Ostrander, Sprague & Rine, 1993; Holmes et al., 1995; Breen et al., 2001; Cullingham, Smeeton & White, 2007), except where specified (Table 1), or developed using the dog genome online (http://www.ncbi.nlm.nih.gov/ genome/guide/dog/). Amplifications and microsatellite analysis was carried out at Wildlife Genetics International (Nelson, BC). Genotyping was completed on an ABI Prism 310 Genetic Analyzer and the allele scoring was determined using the ABI Prism GeneScan and Genotyper version 2.1 software (Applied Biosystems, Foster City, CA). To ensure a low genotyping error rate, similar to Cullingham & Moehrenschlager (2013), samples that had weak amplification were analysed in duplicate using increasing amounts of DNA for the second amplification.

Genetic diversity measures, including number of alleles, heterozygosity (both observed and expected) and Fst, were estimated using genAlex 6.4 (Peakall & Smouse, 2006). Samples were separated into their genetic clusters (Cullingham & Moehrenschlager, 2013) to look for Hardy–Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) across loci using FSTAT (Goudet, 1995). Significance across loci was
Table 1. Swift fox microsatellite locus information used in this study including the NCBI accession number, primer sequence and population-level statistics: number of alleles \(N_a\), expected heterozygosity \(H_e\), observed heterozygosity \(H_o\), and the inbreeding coefficient \(F_{IS}\).

| Locus | Accession | Forward primer | Reverse primer | \(N_a\) | \(H_e\) | \(H_o\) | \(F_{IS}\) | Reference |
|-------|-----------|----------------|---------------|--------|--------|--------|---------|-----------|
| AHT121 | N/A       | TAT TGC GAA TGT CAC TGC TT\(^a\) | ATA GAT ACA CTC TCT CTC CG\(^c\) | 12     | 0.693  | 0.754  | 0.081   | c         |
| REN144A06 | AJ411278 | TTT TAT GGT TGA GTG CTA TTC C   | GAA ATT GGC CAC AGT TCC AT\(^b\) | 6      | 0.746  | 0.725  | 0.030   | b         |
| REN26212 | AJ411585 | GAC AGG GCT CTC TGA CAA GG\(^b\) | ACT TCA CTG CTC CAG AGG AC | 10     | 0.696  | 0.717  | 0.029   | b         |
| REN145P07 | AJ411284 | TGG AAA GGT TAC CTC GA   | AGC TGC CCT AGG AGT TCG T | 7      | 0.736  | 0.720  | 0.022   | b         |
| REN94H15 | AJ411122 | AGT CAT TGT TTC TAG GCT CTC AC | GCC AAG TTT ATT CTC ACC | 5      | 0.646  | 0.698  | 0.030   | b         |
| REN68B08 | AJ411056 | GCG TTA GCT GCC ATC ATC TTG\(^a\) | TGC ATA AAG TGT CCA GAA GCC | 5      | 0.625  | 0.642  | 0.027   | b         |
| C02.030 | N/A       | GCC TTT TAG GGA GCT TTC TTC\(^a\) | GAG TCT GCT TTC TTC CTC CCG\(^a\) | 9      | 0.650  | 0.632  | -0.028  | b         |
| REN199008 | AJ411439 | A CAA ATG GCT TTC ACT TCC AG | GTG GTG TTC TTC ATC TCG AC | 6      | 0.654  | 0.703  | 0.070   | b         |
| REN66E15 | AJ411051 | CCC TGC TGG GGT AAG TAT GTA | ATA TGG GTA CAA TTC CTC TGG | 6      | 0.429  | 0.476  | 0.100   | b         |
| REN183803 | AJ411403 | A TCA TTC TGA CAG GCA TGA GG\(^b\) | TCC AAA GAA GAC CTG GAG ATG | 9      | 0.621  | 0.675  | 0.079   | b         |
| REN85N14 | AJ411099 | A TTC AGC GAT ACC AGG CAT AC | TAT GGA GAT GGA GGG CAC AC | 7      | 0.568  | 0.578  | 0.017   | b         |
| VVE-M25 | DQ530244DQ530253 | CAA ATG CAA AAA CCC TTC AGA T | G TTA AGA TAA CCA TCA TCG TCG TC | 20     | 0.835  | 0.848  | 0.016   | d         |
| VVE-M19 | DQ530243DQ530252 | GTT GTC CCC AGC TCT TAC | GGC TGC GAT ACT TGT TGG ACA T | 16     | 0.839  | 0.858  | 0.022   | d         |
| VVE5-33 | DQ530255 | TGG AAT TTT TTC GCA CAA GAG ATT | G CCT GAG TCG ATC CAG GAT | 8      | 0.739  | 0.752  | 0.017   | d         |
| VVE5-45 | DQ530249 | AGA CAA TTA TCT GGA GAT CTC TGA | G TGA TGA TGG GGT GGG GCA TT | 7      | 0.714  | 0.776  | 0.079   | d         |
| VVE-M17 | DQ530242DQ530251 | AGA TGG TAA GCA GTC ATC CCT GG | G ATA TAT GGG CAT GTG AAA GAT AC | 6      | 0.704  | 0.683  | -0.030  | d         |
| VVE3-131 | DQ530248 | GAT GAT TCT GTC TAA ACT GAC\(^d\) | G CCT TCT GCA AGA ATC TTA T | 4      | 0.668  | 0.702  | 0.049   | d         |
| VVE2-64 | DQ530245 | ATC TGT TTC CTC ATC TGA CTG CT | G CCA GAA TTT ATC ACT TCT CGT TTC\(^d\) | 6      | 0.579  | 0.588  | 0.016   | d         |

As published in \(^a\)Ostrander et al., 1993; \(^b\)Breen et al., 2001; \(^c\)Holmes et al., 1995, and \(^d\)Cullingham et al., 2007.
indicated using the adjusted nominal level (α = 0.05) estimated in FSTAT. We estimated $F_{ST}$ across the genetic clusters using FSTAT.

**Equilibrium testing**

To test whether the system is in drift-migration equilibrium, we used 2MOD (Beaumont, 2001). This application assesses whether the gene frequencies observed among clusters are explained by the drift-migration equilibrium or a model of drift alone, using a relative likelihood approach (Ciofi et al., 1999). With individuals assigned to their cluster, we ran three iterations of 100 000 MCMC iterations each and discarded the first 10% of values as burn-in, and estimated model probability based on the proportion of runs that supported the drift-migration model. We also examined whether the system was in mutation-drift equilibrium. We first assessed which mutation model best fit our microsatellite data using a likelihood approach. We ran the program MSAT (Nielsen, 1997) for each locus, testing the likelihood of either the stepwise mutation model (SMM; Ohta & Kimura, 1973) or the two-phase mutation model (TPM; Di Rienzo et al., 1994). Each run was completed using 500 000 iterations, and for the two-phase model, we used a minimum proportion of multistep mutations as 0.001 and a maximum of 0.5. We assessed model fit using the Chi-square likelihood test comparing the null model (SMM) to the TPM (Nielsen, 1997). Based on the results from MSAT, we ran BOTTLENECK (Piry et al., 1999) to test for mutation-drift equilibrium for the east and west clusters separately, and the samples were pooled. We looked at whether heterozygosity exceeded what expected at equilibrium using the Wilcoxon signed-rank procedure under the SSM and the TPM with 70% single-step mutations (see Results).

**Landscape connectivity**

Previous analysis of these data used STRUCTURE (Pritchard, Stephens & Donnelly, 2000) and the Evanno, Regnaut & Goudet (2005), (2005) method to identify the number of clusters. Given this method has a propensity to identify two clusters as optimal (Janes et al., 2017), we wanted to ensure that these clusters were associated with genetic structuring. We used non-spatial and spatial methods. First, we used the discriminant analysis of principal components (DAPC; Jombart, Devillard & Balloux, 2010), following k-means clustering using the Bayesian Information Criterion (BIC), to select clusters as executed in the adegenet (Jombart, 2008; Jombart & Ahmed, 2011) R package (R version 3.5.1; R Development Core Team, 2017). Second, we used the R package memgene (Galpern et al., 2014) to describe the spatial structure. This approach has been shown to be more sensitive than other methods in identifying spatial genetic neighbourhoods. It uses Moran’s eigenvector maps (Borcard & Legendre, 2002) combined with a regression framework using the proportion of shared alleles which describes the spatial autocorrelation among sites. The output is a map where each individual is assigned a circle, the size of the circle suggests the strength of the autocorrelation, and the colour indicates whether they are positively or negatively correlated.

To evaluate whether landscape is a driver of spatial genetic patterns, we developed landscape resistance surfaces based on our knowledge of swift fox habitat specialization (Kilgore, 1969; Carbyn 1998, Pruss, 1999; Sovada et al., 1998; COSEWIC 2009) and calculated the correlation of these surfaces with genetic distance data. To develop the landscape resistance surfaces, we completed all data processing in ARCGIS 10.2 (ESRI). We obtained data layers for land type (cropland, native grassland), roughness, homogeneity, slope and roads. To estimate the slope, we used a North American DEM surface at 1 km resolution (NOAA, http://www.ngdc.noaa.gov/mgg/topo/globeget.html, last accessed 11 September 2015) and used the slope estimator in the Spatial Analyst extension. Roughness was derived from a TIN structure (converted from the DEM) using the Surface Tools for Points, Lines and Polygons extension. The resulting layer is a topographic index where a value of 1 indicates a totally flat surface and increasing values indicate higher landscape convolution. The resolution of this layer was 30 × 30 m. For homogeneity, a tasseled cap transformation (Crist & Cicone, 1984) was used on landsat images to estimate the brightness of the grid in the software package ENVI (Exelis, Rochester, NY), again, the resolution of this layer was 30 × 30 m. We resampled all of the data layers to have a cell size of 100 × 100 m. Second, we reclassified the layers using resistance values of 1–100, where 1 indicates no resistance and 100 is very resistant. For details on specific resistance values for each layer, refer to Table 2. Finally, we exported the resistance surfaces in ASCII format for analysis using CIRCUITSCAPE (McRae & Beier, 2007). CIRCUITSCAPE uses the circuit theory to simulate gene flow. Analysis of each resistance layer produces a distance matrix which can be used to test for genetic × distance correlations.

Genetic distances were calculated between individuals using the proportion of shared alleles and a measure of relatedness (Queller & Goodnight, 1989). The proportion of shared alleles was estimated using the ‘propShared’ function in the adegenet package in R, while relatedness was calculated in SPAGEDI (Hardy & Vekemans, 2002). To test the relationship between resistance distance matrices and genetic distance, we used multiple regression on distance matrices (MRM) using the ecodist package (Goslee & Urban, 2007) in R. We analysed each resistance layer separately and then combined all of them in a single model. Each of the resistance layers may explain the same proportion of genetic data; therefore, by incorporating them into a single model, we can identify those factors that are significant when considered jointly. Using the information from the joint analysis we created a final model to explain the genetic structure.

**Parentage**

To estimate dispersal distances, we completed the parentage assignment and calculated the distance from offspring to one or both parents (average from parents when both mother and father identified). Distances among all individuals were...
Table 2. Habitat resistance models developed to assess what factors explain swift fox genetic structure

| Model               | Habitat      | Buffer | Resistance score | R²  | P    | P*  |
|---------------------|--------------|--------|------------------|-----|------|-----|
| Null                | Geographic distance | 1      | 0.016            | 0.001 | 0.001 |
| Slope               | 0.00–4.71    | 1      | 0.019            | 0.001 | 0.002 |
|                     | 4.71–15.49   | 25     |                  |      |      |     |
|                     | 15.49–38.73  | 50     |                  |      |      |     |
| Roughness           | 0.00–13.53   | 1      | 0.003            | 0.003 | 0.004 |
|                     | 13.53–33.49  | 25     |                  |      |      |     |
|                     | 33.49–82.09  | 50     |                  |      |      |     |
|                     | >82.09       | 100    |                  |      |      |     |
| Roads               | 500 m        | 1      | 0.002            | 0.140 | 0.591 |
|                     | 1000 m      | 25     |                  |      |      |     |
|                     | >1000 m      | 50     |                  |      |      |     |
| Homogeneity         | 0.00–0.04    | 1      | 0.005            | 0.013 | 0.064 |
|                     | 0.04–0.13    | 25     |                  |      |      |     |
|                     | 0.13–0.24    | 50     |                  |      |      |     |
|                     | 0.24–0.48    | 100    |                  |      |      |     |
| Crop                | Native grassland | 1       | 0.0017          | 0.255 | 0.445 |
|                     | Cropland     | 100    |                  |      |      |     |
| Null + Slope + Roughness | Null       | 1      | 0.035            | 0.001 |       |
|                     | Slope        | 100    |                  | 0.004 |       |
|                     | Roughness    |        |                  | 0.003 |       |

- **R² and associated P-values are based on multiple regression on distance matrices. Each factor was tested separately, followed by all components being included in one model to identify the top factors, the P-value associated with each factor when all factors were included is P*. The final model in the table is the significant model that explains the largest portion of variation in the genetic data. Bold values indicate significant model variables.**

Calculated using UTM coordinates in R with the ‘dist’ function. Dispersal distances were analysed in R to test for differences in the distribution between years, and sexes using the non-parametric Kruskal-Wallis test. Assigning parentage in wild populations with incomplete sampling can result in incorrect assignments due to statistical uncertainty (Christie, 2010). To ensure high confidence and a low error, we used four methods and assigned family groups based on concordance.

For the consensus pedigree, we used COLONY (Jones & Wang, 2010), CERVUS (Marshall et al., 1998; Kalinowski, Taper & Marshall, 2007), SOLOMON (Christie, 2010) and FRANZ (Riester, Studlar & Klemm, 2009). For the 2000/01 data, we used the age information from the collected field data to separate offspring, putative mothers and putative fathers. For the 2005/06 data, we used all individuals from the 2000/01 data as putative parents. Reliable age was obtained for the adult class, but individuals designated as juvenile were done so with low confidence, therefore all juvenile individuals were included in both the parent and offspring input files. In COLONY, both monogamous and male polygamous behaviours have been observed for swift foxes (Egoscue, 1979; Kamler, 2002; Kitchen et al., 2006); therefore, we compared the results from the two scenarios to generate a consensus set of parent–offspring relationships. Because we did not have detailed information for our population, we used a conservative estimate of 20% sampled parents in CERVUS. For all analyses, we used a genotyping error rate of 0.01, and only considered parent–offspring pairs with high confidence (95%) and allowed for one mismatch. Using SOLOMON (Christie, 2010), we estimated the number of putative false parent–offspring pairs by simulating 1000 datasets, and 50 000 000 genotypes to calculate the probability of a pair being false, given the frequencies of their shared alleles. In FRANZ, we included all years and assigned birthdates based on the age class and capture year. For example, an adult captured in 2000/01 must have been born in 1999 or earlier. To be conservative, we considered all adults captured in 2000/01 to be born in 1999. All adults captured in 2005/06 were considered to be born in 2004, while the juvenile class for 2005/06 was given an unknown age status. We ran the analyses using default parameters, with reproductive years from one to six. Using the high confidence results from each parentage analysis we were able to create a consensus pedigree for both time periods. If two or more methods identified the same parent, and the other methods did not identify an alternative parent, we considered this a family group. Exceptions were noticed for the 2005/06 time period, as we had generational data for better resolution using FRANZ.

**Results**

**Genotyping**

After removing samples with missing data at more than one locus, there were 294 individuals, not including recaptures (N_EAST-2001 = 31, N_EAST-2005 = 58, N_WEST-2001 = 68, N_WEST-2005 = 122 and N_undefined = 15; Fig. 1). Polymorphism across loci ranged from 4 to 20 alleles per locus and observed heterozygosity ranged from 0.476 to 0.858 (Table 1). Genotyping error across duplicate samples was <1%. Genetic
sexing data corresponded to the fox live-capture data for all individuals. All loci were in HWE in each genetic cluster at the adjusted nominal level (α = 0.00139). Of 153 comparisons for LD, four locus pairs (AHT121 × VVE-M19; AHT121 × VVEM5-33; C02.030 × VVE3-131; VVE-M19 × VVE5-33) showed linkage in both clusters (α = 0.00016). Genetic differentiation ($F_{ST}$) between the clusters was 0.025.

**Equilibrium testing**

The probability of a gene-flow drift model ($P = 0.85$) was five times more likely than the drift only model across three iterations in 2MOD. Based on the likelihood model comparison, the TPM was supported ($P > 0.01$) for 11 loci, was marginally supported ($P > 0.1$) for two loci and not supported for five loci. Based on these results, we decided to run both models in BOTTLENECK. Under the TPM, no loci showed deviations from the null expectation under mutation-drift equilibrium for either cluster or the pooled dataset. Under the SMM, only one locus that fit the SMM was significantly deviated from the null model for the pooled dataset. As well, the loci followed the normal L-shaped distribution you would expect based on the mutation-drift equilibrium (Luikart et al., 1998).

**Landscape connectivity**

Using the k-means clustering in adegenet both K = 1 and K = 2 were supported by the BIC analysis, therefore we mapped the two clusters (tagging individuals based on the east and west structuring) using DAPC. These clusters are partly overlapping, suggesting a low level of genetic structure (Figure S1). Similarly, using memgene we found significant spatial genetic structure across the study area that supported the presence of the east and west clusters (Figure S2). There was identification of some additional genetic neighbourhoods in both the east and west clusters. The observed structure explained 9% ($P < 0.01$) of the variation in the data, with the rest of the variation being among individuals. Genetic distance based on the proportion of shared alleles was highly correlated with relatedness ($r = 0.89$, $P = 0.001$); therefore, we present the results using the proportion of shared alleles. Relating genetic structure to potential habitat barriers, we found that four of the resistance layers explained a significant proportion of genetic distance (isolation by distance, homogeneity, roughness and slope; Table 2). However, when considered jointly, only isolation by distance, roughness and slope were still significant. These three variables, in a combined model, explained 3.5% of the variation in the data ($P = 0.001$; Table 2). We were concerned with collinearity among explanatory matrices, therefore we estimated the correlation using Mantel and partial Mantel tests in ‘ecodist’. Roughness was highly correlated with distance ($r = 0.8585$, $P = 0.001$), but roughness still explained significant variance in genetic distance when Euclidean distance was controlled for ($r = 0.0962$, $P = 0.001$), while slope did not. Connectivity based on roughness is visualized in Figure S3.

**Parentage**

Our dataset had high marker resolution to complete parentage analysis: probability of identity = $4.1 \times 10^{-7}$ and probability of identity for siblings = $2.9 \times 10^{-7}$. There was high concordance across the parentage methods for the 2000/01 time period. Twenty-three parents were identified across all four methods, four parents across three methods, four parents across two methods, of which only two were retained, and eight parents across one method, none of which were retained, for details refer to Table S1. This resulted in 25 family groups being identified, which consisted of four triads, 10 father–offspring and 11 mother–offspring relationships.

Concordance of parentage assignment for the 2005/06 time period was not as high, but this is a result of the uncertainty in the age status of some of the foxes, as well as the inclusion of the 2000/01 individuals as putative parents. We identified 39 family groups, which consisted of 20 triads, 9 father–offspring and 10 mother–offspring relationships. Nine parents were identified by all four methods, 20 parents were identified by three methods, 24 parents were identified by two methods and 19 parents were identified by one method (Table S2). The majority of the last group were not retained, except in instances where FRANZ identified more logical candidates based on prior generation information. For both time periods, the distributions were positively skewed (Fig. 2), distances were not different between the time periods ($\chi^2 = 0.56$, $P = 0.45$), therefore we estimated the mean and standard error using a combined dataset. Female swift foxes dispersed significantly less ($\mu_B = 12.59 \pm 1.89$) than male foxes ($\mu_M = 40.64 \pm 7.21$; $\chi^2 = 11.60$, $P = 0.0007$). We detected more female dispersers, however we captured ~3 times more females than males in the first time period. All of the dispersal events that we recorded were within either the east or west clusters; there were no cross-cluster dispersers (Fig. 3). We did identify dispersal events in either direction across the Canada/US international border (2000/01 = 5, 2005/06 = 6; Fig. 3).

**Discussion**

To provide information relevant to the management of reintroduced swift foxes, we have completed a comprehensive genetic analysis of the population that is contiguous across the Canada/US international border. Genetic equilibrium testing of reintroduced populations is not often employed to determine the cause of population substructure. Using multiple approaches we have found evidence supporting equilibrium dynamics in a reintroduced swift fox population, which suggests that the observed population structure is more likely the result of a reduced gene flow between genetic clusters rather than an artefact of multiple reintrouction sites. Landscape genetic analysis identified topography as an important variable in explaining the observed genetic structure. Detailed analysis of parentage has allowed us to develop a dispersal kernel, demonstrating the capacity for swift foxes to disperse large distances. Comprehensive genetic analysis of this population has provided valuable information for
continued cooperative management of the swift fox across the Canada/US international border.

Genetic structure in reintroduced populations is often the consequence of multiple reintroduction sites (Latch & Rhodes, 2005; Taylor & Jamieson, 2008; Biebach & Keller, 2009; Brekke et al., 2011; Moraes et al., 2017). This is because each site will go through a bottleneck, and a period of colonization, where serial founding effects will enhance genetic differences among reintroduction sites (Le Corre & Kremer, 1998). For swift foxes, two release sites were used simultaneously, and the observed genetic structure corresponds to these sites (Fig. 1). We examined genetic equilibrium dynamics to understand whether the structure is attributed to release site locations or is the result of current demographics. Migration-drift and mutation-drift results yielded no evidence to suggest that the population is not in equilibrium. Additional information also supports our assertion that equilibrium has been achieved in this system. In non-equilibrium situations, there can be genetic divergence of clusters, but that divergence does not follow the pattern of isolation by distance (Slatkin, 1993). For instance, analysis of reintroduced river otters in Missouri found significant population structure, but no evidence of isolation by distance suggesting non-equilibrium dynamics (Mowrey et al., 2015). Across our sampling we did find a weak but significant pattern of isolation by distance, further suggesting that equilibrium has been reached.

If equilibrium has been reached, the genetic structure is likely a result of reduced gene flow. From all of the dispersal events, we were able to observe across the two time periods, none were found to occur between the east and west clusters (Fig. 3). This does not mean that these events are not occurring, but does suggest that they occur at a lower frequency than within each region, suggesting some limitations to migration, supported by significant geographic structure in the genetic data. While the amount of variation explained by the geographic structure seems low (9%), it is within expectations, given \( F_{ST} = 0.025 \). The spatial genetic structure corresponds well with the east/west clusters. There are some additional spatial neighbourhoods identified by memgene (Figure S2), and these are potentially the result of family structure (Fig. 3). In testing for landscape effects, we were expecting cropland to pose a significant barrier to dispersal because cropland seems to be avoided by swift foxes (Nicholson et al., 2007), but we found no such effect. This is similar to other studies that have found habitats perceived to be of poor quality or fragmented, actually promote genetic connectivity, as dispersers move quickly through these habitats (Battin, 2004; Haddad & Tewksbury, 2005; Kierepka & Latch, 2016). Instead, we found a marginal relationship of roughness with genetic structure. While the variance explained may relate more to isolation by distance, it could also suggest an avoidance of these areas because they restrict visibility, similar to tall-structured vegetation, which Nicholson et al. (2007) observed in Texas. Also, Schwalm et al. (2014) found that swift fox population structure was affected by topography, and less so by anthropogenic alterations.

Distances between parent and offspring dyads or triads suggested that dispersal kernels were similar over time, and that while most foxes dispersed <20 km, some were capable of moving up to 100 km. The frequency of larger dispersal movements is somewhat greater than in previous swift fox mark-recapture studies (Ausbond & Foresman, 2007; Nicholson et al., 2007; Ausband & Moehrenschlager, 2009). Our finding of males moving greater distances than females is similar to other mammalian studies (Greenwood, 1980; Dobson, 2013) and it indicates that connectivity to other remote populations would most likely be achieved through males.

The estimated swift fox dispersal distances, the fact that reintroductions began almost 20 years prior to our sampling (which is equivalent to 4–10 generations), and release sites being separated by about 200 km, suggest that there has
been sufficient time for colonization and mixing of individuals to allow equilibrium to be reached. Dispersal patterns we identified within, but not between, clusters help to explain slight east–west differences in genetic structure. Clusters with a small amount of migration will ensure the maintenance of genetic variation, while still allowing divergence. Limited gene flow between the clusters may have two advantages; genetic exchange is sufficient to limit inbreeding, but such occurrences are infrequent enough to reduce the likelihood or rate of potential disease spread.

The recent decrease in swift fox abundance (Moehrenschlager & Moehrenschlager, 2018), coupled with our finding that swift fox dispersal is frequent across the international border, implies that demographic recovery in one country is going to be particularly sensitive to management actions in the other country. This highlights the need for coordinated management on a small scale. With swift foxes having the highest level of protection in Canada, and no federal protection in the USA (https://ecos.fws.gov/ecp0/profile/speciesProfile?spcode=A085), accessed 15 September 2018), the risk exists that incentives for protection in Montana could be weaker than in Canada. However, recent developments in Canada have increased the potential use of strychnine in powder or liquid from within the swift fox range (https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pestmanagement/public/consultations/proposed-re-evaluation-dec

Figure 3 Distribution of swift fox family groups identified using genetic parentage analysis for two trapping periods: 2001 (left) and 2005 (right). The top panels are triads, the middle panels are mother-offspring groups, and the bottom panels are father-offspring groups. Individuals that assign to the west population are labelled with a “W”, while the East population are labelled with an “E”, and mothers are indicated with “M”, fathers with “F”, and offspring with “O”. The distribution of native Prairie is included for context. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]
Reintroduction genetics across international borders

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Data accessibility

Genotype data, including sample information (year captured, sex, age, location), will be uploaded to a publicly accessible repository (e.g. Dataverse) upon publication acceptance.

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**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Discriminant analysis of swift fox genetic structure; individuals were labelled with their east and west cluster designations.

**Figure S2.** Visualization of the first memgene (Galpern et al., 2014) axis for swift foxes across the study area demonstrating the spatial pattern of genetic relatedness.

**Figure S3.** Connectivity across the swift fox distribution estimated using Circuitscape (McRae & Beier, 2011) based on roughness.

**Table S1.** Comparison of parentage assignments for swift fox samples collected in 2000/2001 across four different methods: Colony (Jones & Wang 2007), Cervus (Marshall et al. 1998), Solomon (Christie, 2002), and Franz (Riester et al., 2008).

**Table S2.** Comparison of parentage assignments for swift fox samples collected in 2005/2006 across four different methods: Colony (Jones & Wang 2007), Cervus (Marshall et al. 1998), Solomon (Christie, 2002), and Franz (Riester et al., 2008).