IDENTIFYING BARRIERS TO MOVEMENT AND THE EFFECTIVENESS OF CORRIDORS FOR CONNECTING CORE AREAS: LANDSCAPE GENETICS OF PRAIRIE GROUSE IN FRAGMENTED LANDSCAPES

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SUMMARY OF FINDINGS

Sharp-tailed grouse (*Tympanuchus phasianellus*) and greater prairie-chickens (*Tympanuchus cupido*) are area-sensitive species that rely on open landscapes of early successional habitats. Although once abundant and widespread, grassland and brushland habitats today are highly fragmented by agriculture and other human land uses. We used a landscape genetics approach to identify landscape features that impede movement and to identify gaps in connectivity for prairie grouse in Minnesota. With the help of numerous cooperators, we collected 509 prairie-chicken and 831 sharp-tailed grouse samples, which included hunter-submitted wings from 82 sharp-tailed grouse and 52 prairie-chickens. After we eliminated juveniles not sampled again as adults, duplicate samples, and samples with genotyping errors, we were left with a unique genetic sample of 294 prairie-chickens and 451 sharp-tailed grouse, including 367 individuals from the northwest (NW) and 84 individuals from the east-central (EC) regions. Results for prairie-chickens indicated good connectivity in the existing range but further improvements along the Prairie Plan corridor in Norman and Clay Counties would be beneficial. For sharp-tailed grouse, both the NW and EC management regions are genetically diverse and distinct, with high connectivity indicated between them. We cannot be sure whether the gene flow indicated between these regions is best explained by contemporary connectivity or a historical connection that has recently been lost. The population in the EC region shows signs of a recent demographic compression, consistent with surveys that indicate recent declines in population size. Inbreeding is not currently a problem in the areas sampled, but if the population size in the EC region continues to decline or stay small, genetic diversity would be expected to be lost gradually and the population may eventually face inbreeding depression. We recommend increasing the quantity and quality of habitat in the EC region to increase population size and maintain genetic diversity. We provide recommendations about where land management can achieve the greatest impact on genetic connectivity.

INTRODUCTION

The grassland habitats that prairie grouse require have become increasingly fragmented as a result of competing pressures on the land (Berg 1997). Core habitat areas are isolated from each other by unsuitable areas that may prevent successful movement and the colonization of newly created habitat. The Minnesota Prairie Conservation Plan recognizes the importance of providing dispersal corridors to connect isolated core areas and identifies the

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greater prairie-chicken as an indicator species for upland prairie and grassland habitat (Minnesota Prairie Plan Working Group 2011). Similarly, for sharp-tailed grouse to move among suitable habitat areas in isolated grassland, brushland, savanna, and peatland habitat patches (Berg 1997), they must traverse areas that may pose difficulty for successful movement. If the resistances of various landscapes to movement are understood, then more effective corridors can be identified, and management efforts can be prioritized using this information (Epps et al. 2007, Braunisch et al. 2010, Spear et al. 2010).

Landscape genetics is an emerging field that provides methods to examine connectivity on the landscape by combining geographic place-based information with information about genetic variation within or among populations (Braunisch et al. 2010, Lowe and Allendorf 2010, Sork and Waits 2010, Haig et al. 2011). This tool can be used to examine effective dispersal (gene flow) on the landscape, without having to rely on telemetry techniques, which can be expensive and may require large numbers of marked animals if successful dispersal events are infrequent (Coulon et al. 2004, Spear et al. 2010). Landscape genetic methods have been used in recent years to identify barriers to dispersal, including human development, non-habitat land cover types, and distance in species like capercaillie (*Tetrao urogallus*, Braunisch et al. 2010), northern bobwhite (*Colinus virginianus*, Berkman et al. 2013a,b), and prairie-chickens (Gregory 2011). Thus, landscape genetics can be used to examine the movements of birds in a spatially explicit manner.

OBJECTIVES

1. To identify barriers to movement for sharp-tailed grouse and greater prairie-chickens in Minnesota (e.g., distance, urban development, treed areas) as measured by genetic connectivity
2. To identify landscape features and types that enable movements of prairie grouse among areas of suitable habitat in Minnesota as measured by genetic connectivity
3. To improve corridor planning and provide guidance to keep connected populations connected

METHODS

Wildlife managers, cooperators, and seasonal technicians surveyed prairie-chickens and sharp-tailed grouse at leks throughout Minnesota in the springs of 2014 and 2015. Feathers lost during male contests, copulations, and as a result of other activities were collected from leks. To maximize the probability of sampling many different individuals, staff and technicians were instructed to spread out the sampling at each lek, sample feathers from discrete locations on the lek, and only collect one sample per location or cluster of feathers encountered. Each sample of feathers, or single feather when necessary to ensure that only one individual was represented, was placed in an envelope and labeled with the lek location (coordinates or Township, Range, Section, and quarter-section information), date, collector name, contents, and species. Information from each envelope was recorded in a database and assigned a unique sample number. Areas underrepresented in 2014 were given greater effort in the spring of 2015. Feather samples from leks were supplemented with samples from hunter-harvested birds in both 2014 and 2015. Wings from harvested birds were aged based on plumage characteristics (Bihrle 1993).

All samples were analyzed at the Wildlife Genetics International Lab in British Columbia. At the lab, DNA was extracted and amplified at 15 microsatellite loci. Microsatellites are highly variable, neutral (non-coding) genetic loci. Recent studies of prairie-chickens and sharp-tailed grouse identified polymorphic microsatellite loci in these species and populations (see citations in Gregory 2011 and Malone 2012). The sex of birds was determined molecularly using techniques such as those in Fridolfsson and Ellegren (1999).

STATISTICAL ANALYSIS

We tested and accounted for the presence of null alleles using Program MicroChecker (Oosterhout et al. 2004). We used Program GenAlEx 6.5 to calculate estimates of genetic diversity (Peakal and Smouse 2012) and to test the Probability of Identity (PI) and the Probability of Identity among siblings (PI-Sibs). PI and PI-Sibs are estimates of the power of the genetic markers to differentiate unique individuals from a population of unrelated individuals (PI) or from a population of siblings (PI-Sibs). GenePop was used for Hardy Weinberg exact tests and to estimate gene flow (F_{ST} and Number of Migrants *Nm*) between the EC and NW sharp-tailed grouse management regions (Raymond and Rousset 1995).

We used Program Structure to implement a Bayesian clustering algorithm to test for genetic isolation first among management zones and then within management zones (Pritchard et al. 2000).[3] More spatially explicit formulations of the clustering algorithm were applied using Package Geneland in Program R (Guillot et al. 2008). Whereas Structure uses a spatially implicit clustering algorithm (you can assign individuals to populations based on locations but not to specific coordinates), Geneland uses map locations of samples to create a Poisson weighting matrix based on Tobler's First Law of Geography (Miller 2004), which essentially assigns individuals to specific locations relative to each other and gives greater weight to samples that are physically closer together (Guillot et al. 2008). The outputs of both Structure and Geneland are a negative log likelihood for a particular number of population clusters and the probability of assignment for each individual to each putative cluster.[4] We used the number of populations and the individuals assigned to each population from Geneland as the putative number of populations for subsequent analyses. Lastly, we used a Standardized Difference Test and a Wilcoxon Test to test for excess heterozygosity, which is a signal of a relatively recent (10-15 generations, or ~15-30 years) population bottleneck (Cornuet and Luikart 1996), and estimated effective population size (N_e) using linkage disequilibrium methods implemented in Program NeEstimator (Waples 2007, Waples et al. 2014).

Spatial Analysis

All analyses were carried out at the spatial extent of the MNDNR greater prairie-chicken or sharp-tailed grouse management regions with the boundaries buffered by 50 km. A 50-km buffer was used because it exceeds the average dispersal distance of most prairie grouse (Johnson et al. 2011; Connelly et al. 1998), completely encompassed the extent of our data, and minimizes possible boundary effects that might occur as a result of an artificially imposed boundary on a dynamic ecological system (Franklin 2009). Restricting the analysis to our region of interest is necessary to reduce the potential for spurious correlation that can sometimes occur with spatial data analysis (Loiselle et al. 2003). For greater prairie-chickens, we conducted a separate, secondary analysis within the extent of the Prairie Plan corridor. For sharp-tailed grouse we

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[3]Program Structure uses a Bayesian clustering algorithm to identify the most likely number of distinct genetic groups, given the observed allele frequencies and levels of linkage disequilibrium within the genetic data. You can choose to include information on the putative population of origin or sampling unit when you run Structure and thereby test the degree to which *a priori* defined populations or management units are distinct genetic populations or subpopulations. Moreover, because Structure uses a Bayesian algorithm it can be implemented in a hierarchical fashion to test for genetic clustering within a local area below the level that would normally be associated with a population or subpopulation level of genetic isolation. This is a useful feature of Structure if you are trying to identify landscape features that are affecting dispersal and gene flow (Pritchard et al. 2000).

[4]You can use the probability of assignment values to identify migrants. A genotype assigned to 1 cluster that was sampled in another cluster is most likely a migrant, genotypes with ~50% assignment to 2 different clusters are most likely offspring of a migrant mating with a local; 25% assignment, with the grandchildren; and so on.

conducted analyses in a hierarchical fashion to assess movement between regions as well as movement and structure within each region.

We used the Multiple Resolution Land Cover Data (MRLCD, usgsmrlcd.org) set clipped to the extent of our study system(s), which included 16 distinct land use classifications (Table 2). We reclassified the land cover and land use data into a resistance surface following the methods outlined in Spears (2010) and Gregory (2011, Table 2). We also acquired data sets for linear features (i.e., highways, railroad lines, and power lines) and anthropogenic disturbance intensity [Wildlife Conservation Society (WCS) 2005]. An index of anthropogenic influence or disturbance intensity was determined by WCS (2005) using information on population density, land use and infrastructure, and human accessibility to create the Global Human Footprint Dataset. These data sets were combined following the methods used by the Washington Connected Landscape Project (waconnected.org; McRae 2006), to produce 8 putative resistance surfaces (Table 2 GRPC, Table 3 STGR, Figure 1).

We used Circuitscape to calculate a metric of functional isolation, called resistance, between each sample location (McRae 2006). Resistance values create a relative index of hypothesized landscape interactions and influences on species movements, and therefore are a hypothesis of how the landscape influences observed genetic structure (Storfer et al. 2007). Resistance values are relatively arbitrary and are typically assigned based on expert opinion or via the use of a species distribution model; pros and cons exist for both approaches (Spears 2010). Here we used an expert opinion optimization based on the literature and knowledge of each species biology. Each of the 8 resistance surfaces is a different hypothesis about how landscape attributes influence prairie-grouse movement and viability on the landscape (McRae et al. 2008; Spear et al. 2010). Hypothesis 1 (H1) predicts that connectivity is a function of land cover and land use with primary habitat (i.e., grasslands, wet meadows, or shrublands) being highly suitable land cover and land use types of low resistance (Tables 2, 3). H2 predicts that connectivity is a function of the amount of grassland within an area where areas of higher grassland availability are of lower resistance and high suitability. H3 is similar to H2 but predicts that grassland suitability is reduced by the presence of cultivation. H4 predicts a similar response to H2 and H3 but also includes an interaction of agriculture and grassland land cover. H5 predicts that genetic structure is a function of avoidance of linear features on the landscape. H6 predicts that land cover and the intensity of anthropogenic disturbance interact to drive genetic structure. H7 predicts the same as H6 but also includes avoidance of linear features. H8 predicts that all attributes previously mentioned combine to influence genetic structure. Isolation by distance is a null hypothesis that tests the assumption that gene flow is not related to land use or its configuration, but simply to physical distance among spatially structured subpopulations. The Reverse model tests the counterintuitive hypothesis that highly modified and disturbed landscapes are highly beneficial to prairie grouse (Tables 2, 3). This model is an important control because if it does not perform poorly relative to the other models, we clearly do not understand the system sufficiently to be using an expert opinion optimization. However, if it does perform poorly relative to the other models, we can be moderately confident that our assigned resistance values provide useful insights about how the landscape is impacting these species, and we can therefore use the results of the analysis to help guide management actions. Collectively, these model formulations allow us to test the influence of 8 hypothesized land cover and land use interactions of greater prairie-chickens or sharp-tailed grouse movement and gene flow.

We used a Partial Canonical Correspondence Analysis (PCCA, also controlling for Euclidean distance, Balkenhol et al. 2009) to ordinate genetic differentiation among subpopulations along explanatory variable axes. In this way, the strength and direction of genetic isolation can be examined, as well as identifying which attributes are driving population subdivision and isolation

(Cushman 2006). The PCCA was implemented using the vegan package in Program R (Oksanen

et al. 2007). We also used PCCA to ordinate the population by cluster based on landscape attribute data. The PCCA included 5 landscape predictor values (i.e., MRLCD resistance surface, Human Footprint, Linear Features, %Grassland, %Agriculture, and %Agriculture×%Grassland; Table 4).

Lastly, we mapped F_{IS} values for both species in ArcInfo 10.4. F_{IS} values are an indication of how much inbreeding is occurring at a sample location or within a population. Values range 0-1, and the higher the value the more inbreeding is occurring (Frankham et al. 2002). We performed a HotSpot analysis using the Getis Ord Gi* procedure in ArcInfo Spatial Analyst Tools to test for significant high×high and low×low clustering of F_{IS} values (Getis and Ord 1992). This analysis identifies areas where the genetic data suggest that either sharp-tailed grouse or greater prairie-chickens are potentially genetically isolated or where movement is strongly structured by landscape characteristics (F_{IS} HotSpots). This can be useful in conservation planning because it can identify areas where targeted investment in habitat improvements can have the largest population-wide benefits for the species by enabling connectivity.

RESULTS

Greater Prairie-Chickens (Statewide)

With the 294 individual greater prairie-chicken genotypes used in this analysis, we had adequate discriminatory power to identify individuals (Prob Identity = 1.0×10^{-20}; Prob Identity Sibs = 2.7×10^{-7}) and identify population structure. We found no indication of null alleles among loci, except for locus SGCA6. SGCA6 was also not at Hardy Weinberg Equilibrium or at Linkage Disequilibrium (Table 1), which means that this marker was not appropriate for use in this analysis. Consequently we censored SGCA6 from the analysis.

Genetic diversity within the greater prairie-chicken population was high (H_O= 0.76 ± 0.04; AR = 35 ± 18.75). The effective population size of greater prairie-chickens was large (Linkage Disequilibrium N_e = 243.1-*infinity*) and a population bottleneck was not indicated (Wilcoxon Test for Excess Heterozygosity P = 0.34; Standardized Difference Test P = 0.08). Collectively, these observations suggest that the greater prairie-chicken population is panmictic and possibly expanding.

Analysis with Program Structure indicated greatest support for K = 2 populations [-LN(K) = 17,542.1 $\pm$ 511.9] with correlated allele frequencies and high genetic exchange (F_{ST} = 0.004). Analysis with Geneland also indicated that greater prairie-chickens were likely 2 highly connected but distinct subpopulations [-LN(K) = 15,344.3 ± 90.567; F_{ST} = 0.0044, F_{IS} Cluster 1= 0.042; F_{IS} Cluster 2 = 0.047; Figure 2]. Collectively, these results indicate a weakly structured population with exchange among genetic clusters.

Based on the PCCA, the most supported resistance landscape is depicted by H6 (Table 2), which indicates that greater prairie-chicken movement across the landscape is partially influenced by land use and land cover and also by anthropogenic disturbance (Table 4). Entering this resistance surface and greater prairie-chicken sample locations into Program Circuitscape yields a connectivity landscape that identifies regions important for maintaining greater prairie-chicken connectivity (Figure 3a). When we overlaid the results of this connectivity analysis with the Prairie Plan corridor, we identified critical gaps in corridor coverage to ensure connectivity of greater prairie-chicken genetic clusters (Figure 3b).

Lastly, HotSpot Analysis indicated the areas where significant high×high (HotSpots) and low×low (ColdSpots) F_{IS} value clustering occurred. With 95% confidence we identified a single

HotSpot near the northern extent of the greater prairie-chicken range in Minnesota (Figure 4) and a ColdSpot near the southern extent of the greater prairie-chicken range (Figure 4). In the context of this analysis a HotSpot indicates an area with low dispersal and genetic exchange, an isolated area; whereas a ColdSpot indicates an area of high genetic exchange, or panmixia (Figure 4). These identified regions roughly correspond to the genetic clusters identified by Geneland (Figure 2).

Sharp-Tailed Grouse (Statewide)

We obtained 84 unique sharp-tailed grouse genotypes from the EC management region, and 367 individual genotypes from the NW management region. We had adequate discriminatory power (Prob Identity = 5.2×10^{-20}; Prob Identity Sibs = 3.8×10^{-7}) to determine localized population genetic structure, gene flow among management regions, and sufficient resolution to link genetic isolation to physical attributes of the landscape. Across both regions, genetic diversity was high ($H_O = 0.77 \pm 0.03$; AR = 29.4 ± 11.9). Bayesian clustering algorithm within Program Structure found greatest support for K = 2 populations, with each management region being a distinct genetic cluster [-LN(K)= 27,781.11 ± 581.8]. However, the populations were admixed and exhibited a high degree of genetic exchange among regions ($F_{ST} = 0.003 \pm 0.001$), suggesting gene flow was recently or is occurring across Itasca county between leks sampled in Aitkin and St. Louis Counties and leks sampled in Koochiching County. To confirm this, we also tested for gene flow across Cass County between grouse in Aitkin and Beltrami counties ($F_{ST} = 0.029 \pm 0.004$), which further supports the notion that gene flow was or is occurring across Itasca county. As previously mentioned, because Structure is a Bayesian clustering algorithm it can be used hierarchically to explore possible latent subpopulation-level clustering. By restricting the analysis to just individual genotypes sampled in Aitkin, St Louis, and Koochiching counties and eliminating the population of origin as a starting point, we allowed Structure to converge on the number of populations in just this subset of sampled leks. Structure again found greatest support for K = 2 populations with correlated allele frequencies and admixture [-LN(K=2) = 12,316 ± 894.2]. However, this was only moderately greater than support found for K = 1 population with admixture and correlated allele frequencies [-LN(K=1) = 16,821 ± 2,113.4]. Models not assuming correlated allele frequencies and panmixia were not supported [-LN(K) = 34,865 - 49,456.9]. Collectively, these analyses suggest that the 2 management regions are 2 distinct genetic clusters of sharp-tailed grouse connected by current or recent migration between regions, most likely across western Itasca and/or eastern Cass counties (e.g., red/orange areas; Figure 5).

Analysis with PCCA to determine the degree to which landscape features were driving genetic structure between sharp-tailed grouse management regions included 5 landscape predictor variables (i.e., MRLCD resistance surface, Human Footprint, Linear Features, %Grassland, %Agriculture, and a %Agriculture×%Grassland interaction) sampled across Aitkin, St. Louis, Carlton, Cass, Itasca, and Koochiching counties. PCCA was able to explain 61% of the variance in genetic clustering with the first 2 components (Dominant Eigenvalue = 0.33, Secondary Eigenvalue = 0.28, Table 5). Based on these results, the most appropriate landscape resistance model would be H1 or H4 (Table 3), which predicted that populations of sharp-tailed grouse are structured by land cover or by the amount of agriculture and grasslands or wet meadows on the landscape. We elected to present the predictions arising from use of H4 in our Circuitscape connectivity analysis (Figure 5), but predictions arising from use of H1 in Circuitscape indicate more connectivity between the NW and EC regions and are thus less conservative. The results of the connectivity analysis showed limited connectivity between the management regions, but connectivity exists to the west and outside of the management regions in Cass County (Figure 5). Because there is limited connectivity between regions we elected to analyze population- and landscape-genetic attributes of sharp-tailed grouse within each management region.

Sharp-Tailed Grouse (EC region)

Overall the EC region has high genetic diversity (AR = 18.27 ± 5.07; H_O = 0.771 ± 0.04; H_E = 0.779 ± 0.04), and the population-wide inbreeding coefficient (Weir and Cockerham 1984) was low (F_{IS} = 0.017 ± 0.0008). This suggested that these 84 samples were collected from a large outbred population with little genetic evidence for inbreeding. Under an infinite alleles model, we detected a significant excess in heterozygosity using the Standardized Difference Test (P = 0.005) and the Wilcoxon Test (P = 0.002). Moreover, there was a significant right mode shift in allele frequency (P = 0.03). Linkage disequilibrium methods for assessing effective population size indicated an effective population size of 224-771 (95% confidence interval).

Analysis of the EC region with Program Structure indicated greatest support for 3 admixed populations [-LN(K) = 4,755 ± 543.5] with relatively high genetic exchange among them (Avg. Pairwise F_{ST} = 0.06). However, when we mapped individuals based on population assignment, we observed that the population clusters themselves are admixed and not localized (Figure 6). Taken in conjunction, the observed excess in heterozygosity, low inbreeding coefficient, and strong signal of genetic population structure with high admixture of the assigned clusters suggest that the EC region has undergone a recent demographic compression, or bottleneck (Cornuet and Luikart 1996, Luikart et al. 1998a).[5]

To better understand drivers, or lack thereof, of observed population structure we used the hypothesized landscape resistance and suitability model of H8 in a PCCA. We used H8 because it approximates a "full model," and given the relative panmixia suggested by the Structure Analysis, we wanted to maximize our ability to detect any anthropogenic influences on population structure. We orientated the analysis using a discriminant function maximizing the Mahalanobis Distance between explanatory variables. This technique essentially reduces the number of explanatory variables needed by creating synthetic components that each explain the most variance possible. We were able to explain approximately 99% of the variance in the data with the first 2 eigenvectors (Dominant Eigenvalue = 0.87; Secondary Eigenvalue = 0.12). We found that the amount of primary habitat and an interaction between primary habitat and agriculture best explained the observed population clusters (Table 6). However, the confusion error matrix suggested that this analysis had relatively low discriminatory power because it was able to classify only 42% of the sampling locations into the correct genetic clusters based on habitat attributes or land cover and land use attributes. Lastly, the Getis Ord GI* HotSpot analysis of inbreeding using sample location F_{IS} values was non-significant, and we detected no Hotspots or ColdSpots.

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[5]Populations lose neutral allelic diversity due to random genetic drift at a rate of approximately $1/2N_e$, where N_e is the effective population size of the population in question (Hartl and Clark 2007). Thus, we expect small populations to have low allelic diversity and that a population bottleneck that reduces N_e will eventually result in a genetically depauperate population (Lynch et al. 1995; Madsen et al. 1996; Cristescu et al. 2010). However, for a few generations immediately following a population bottleneck, the newly bottlenecked population will have an excess of rare to moderate frequency alleles relative to what would be expected given its smaller population size (Luikart and Cornuet 1998; Luikart et al. 1998a). This is because drift is a random process that probabilistically will affect extremely low and high frequency alleles most strongly (Luikart et al. 1998b). The result is a characteristic right mode shift in the frequency of alleles, meaning that a population will have a higher frequency of occurrence of low to intermediate frequency alleles than expected. This has become a classic and powerful test for a population bottleneck (Luikart et al. 1998b; Luikart and Cornuet 1998). A related test for a bottleneck is an excess in heterozygosity, or population-wide estimates of heterozygosity being larger than expected by chance (Cristescu et al. 2010). Again this indication of a bottleneck is only observable for a few generations following a population bottleneck during which time the surviving population has allele frequencies and heterozygosity characteristics of its formerly larger N_e (Luikart et al. 1998b). As the population persists at its new smaller size, drift will remove genetic diversity eventually resulting in the expected genetically depauperate population (Madsen et al. 1996; Hartl and Cark 2007). From a management standpoint, this

suggests a short-lived opportunity during which time recovery of the population could preserve the majority of the genetic diversity found in the pre-bottlenecked population (Bijlsma et al. 2000; Amos 2001; Crisecsu et al. 2010).

Sharp-Tailed Grouse (NW region)

Overall the NW has high genetic diversity (AR = 40.6 ± 23.4, H_O = 0.77 ± 0.04, H_E = 0.80 ± 0.03), and the population-wide inbreeding coefficient was low (F_{IS} = 0.062 ± 0.003). Under an infinite alleles model, we detected a significant excess in heterozygosity using the Standardized Difference Test P = 0.007, but not with the Wilcoxon Test P = 0.07). There was not a significant mode shift in allele frequency (P = 0.99). Linkage disequilibrium methods for assessing effective population size indicated an effective population size of N_e = 185.6-*infinity*. Analysis of the NW subpopulation with Program Structure indicated greatest support for 5 admixed populations, or genetic clusters [-LN(K=5) = 18,346.1 ± 879.9] with relatively limited genetic exchange among them (Avg. Pairwise F_{ST} = 0.756 ± 0.04, Figure 7).

The PCCA analysis was not able to accurately classify the data, as indicated by being able to explain only 39% of the variance with the first 2 eigenvectors (Dominant Eigenvalue = 0.28, Secondary Eigenvalue = 0.11, Table 7). The discriminant analysis ordination also failed to accurately distinguish genetic clusters because we were able to assign only 34% of the sample sites to the correct genetic cluster. Due to the failure of the PCCA to adequately discriminate genetic clusters based on landscape variables, we did not proceed with a landscape connectivity analysis via Circuitscape.

The Getis Ord Gi* HotSpot Analysis indicated both significant high×high clustering of F_{IS} values (HotSpots) and significant low×low clustering of F_{IS} values (ColdSpots, Figure 8). The ColdSpot near Koochiching County along the eastern edge of the NW region suggested more movement than expected at random. We also identified a potential ColdSpot along the western edge of the region near Kittson County. There was also a HotSpot in the center of the western half of the NW along the border of Pennington and Marshall counties, indicating relatively isolated sharp-tailed grouse populations with limited gene flow into the system or among sample locations within that region (Figure 8).

DISCUSSION

Greater Prairie-Chickens (Statewide)

Our analyses suggested that greater prairie-chickens are most likely a single, large, panmictic population with some subpopulation-level structure starting to develop, or developing along the fringe of an expanding range. We found no evidence of inbreeding depression, and our estimates of the effective population size include infinity, both of which are encouraging and suggest that the population was stable, and possibly expanding. A stable population has also been indicated in recent survey data, after declines coincident with losses in CRP enrollments after 2007 (Roy 2016a).

By correlating observed gene flow with land cover and land use data we were able to evaluate the degree to which land cover and land use contributed to greater prairie-chicken genetic structure. The little population structure that does exist is likely due to an interaction between decreasing grassland and increasing cultivated agriculture. Mapping these connectivity arcs with the Prairie Plan corridors revealed 2 potential gaps in prairie corridor coverage that may need to be addressed for the long-term management of greater prairie-chickens (Figure 3).

One area of management concern for the greater prairie-chickens is the observed lack of connectivity through Norman County (Figure 4). Our analysis suggested that greater prairie-chickens in Polk County are separated from the birds in Wilkin and Ottertail Counties. This conclusion is further supported by the Geneland Analysis of genetic clustering. A second area of

potential management concern is the HotSpot band identified in Clay County. The areas south of this band are a ColdSpot, or an area of high gene flow and movement. There are 2 possible
explanations for the existence of this band. First, it could have occurred if our sample contained relatively few inbred or highly related birds from 1 or 2 leks (i.e., an artifact of sampling). Alternatively, the pattern is real and is related to landscape fragmentation and management. Upon further investigation, this band of ~200 km^2 contained samples from 15 unique birds (12 males, 3 females) from 5 different leks, which is sufficient sampling to support the conclusion that the observed pattern is real and *not* a sampling artifact. Based on our landscape connectivity model, cultivation is 6-7 times less permeable to movement than is rangeland land cover, and heavily human-modified landscapes are 22-133 times less permeable to movement than range (Table 2). The 200-km^2 block where we detected restricted movement has a median human footprint value of 41 (which corresponds to a moderately high level of disturbance) and is 56% cultivated agriculture and 34% grassland land cover types. Consequently, the existence of this band may be a concern, as it suggests that high movement south of this band is occurring, likely due to marginal habitat conditions to the south.

Sharp-Tailed Grouse (Statewide)

Our analysis suggested that each of the 2 sharp-tailed grouse management regions is a distinct genetic cluster. However, some gene flow is currently or was recently occurring between the EC region and the eastern end of the NW region, and these regions are not genetically differentiated from each other. Thus, management actions within one region may influence population dynamics of the other region. However, because each management region is a distinct genetic cluster, and contemporary gene flow is uncertain, we make separate management recommendations for each region.

Sharp-Tailed Grouse (EC Region)

The possibility of inbreeding depression is a concern in prairie grouse, because of evidence for inbreeding depression in an isolated population of prairie grouse in Illinois (Westmeier et al. 1998, but see Mussman et al. 2017). Given recent demographic data from the EC region suggesting that the sharp-tailed grouse population within that region is declining (Roy 2016b), we were interested in testing for low genetic diversity and inbreeding depression in this region. Contrary to expectations in a population undergoing inbreeding depression, the EC region exhibited high genetic diversity, a low inbreeding coefficient, significant excess in heterozygosity, a right mode shift in allele frequency (Luikart et al. 1998a), and relatively large N_e size relative to the sample size. Moreover, analysis of genetic structure within this region indicated 3 mixed genetic clusters. These results are inconsistent with genetically clustered groups co-occurring on the landscape. However, given the previously observed excess of heterozygosity and that Structure has a proclivity to weight genetic cluster assignments by the co-occurrence of rare alleles (Pritchard et al. 2000), confusing Structure results might be expected within either a rapidly declining or rapidly expanding population (e.g. after a founding event, following a catastrophic population crash, or in the recipient population after a translocation). In light of the demographic observations, the most probable explanation is that the EC sharp-tailed grouse population is not experiencing inbreeding depression, but is in the process of going through a population genetic bottleneck as a result of a relatively recent and rapid reduction in population size (Luikart et al. 1998a). However, we acknowledge that our conclusions relate only to those areas sampled for the analysis; we did not have samples from Pine and Kanabec counties, where population losses have been most extreme.

Population bottlenecks are a concern for sharp-tailed grouse because they have a lek mating system characterized by repeated female choice of a small subset of available males (but see

Hess et al. 2012). Thus, in lek mating systems, the effective population size is typically much smaller than the actual population size. Because genetic drift removes allelic richness from a population at a rate of $1/(2*N_e)$ per generation (~1 year/generation for sharp-tailed grouse, Hartland Clark 2007), demographic compression can have disproportionately large impacts on inbreeding propensity for lek-breeding species. Continued monitoring of the population genetics of this population is warranted to track the potential loss of genetic diversity that may occur as a result of this population reduction. If the population can be recovered rapidly, inbreeding depression should not be an issue in this population. Management efforts should focus on enhancing population recovery.

Based on the correspondence analysis, the most likely cause for the genetic structure (and associated population decline) is a reduction in the quantity and quality of the primary habitat and an increase in other land uses (Table 6). We did not see a strong effect of fragmentation; however, it is difficult to disentangle the influences of habitat loss from fragmentation. Again, focused habitat restoration, increasing the amount of primary habitat, and improving the quality of existing habitat will provide the greatest benefit to maintain connectivity within the EC region (Figure 5, Table 6).

Sharp-Tailed Grouse (NW Region)

The NW region has high genetic diversity, a low inbreeding coefficient, and a large N_e with a 95% confidence interval that encompasses infinity. However, the population is highly structured with K = 5 distinct clusters with limited genetic exchange among them (F_{ST} = 0.68–0.79). Yet, the populations are not highly structured by landscape (land cover / land use) attributes. A common null hypothesis in landscape genetic analyses is isolation by distance, or migration drift equilibrium (Cushman 2006). Although we did not explicitly test for isolation by distance, we did control for it. Our analysis suggested that much of the NW may actually be at or near migration drift equilibrium conditions, with 2 notable exceptions. First, the eastern edge of the region near Koochiching County was found to be an F_{IS} ColdSpot (Figure 8). As previously noted, this may be indicative of a high gene flow system with higher than anticipated migration into and/or out of the system, perhaps indicating marginal habitat conditions along the edge of the current range. Second, the F_{IS} HotSpot identified near Marshall County (Figure 8) indicates that sharp-tailed grouse in this area may have less than expected migration into and out of the system. However, the genetic assignment data (Figure 7) indicates movement within the NW region, suggesting little immediate concern for the genetics or movement of sharp-tailed grouse in the NW.

In conclusion, genetic data suggest that due to high observed genetic diversity, neither species of prairie grouse is in immediate danger of inbreeding depression. Both species exhibited relatively high observed heterozygosity and high observed allelic richness. In addition, analyses of genetic structure indicated connectivity among local populations of both species of grouse.

We also observed that sharp-tailed grouse in the EC management region are not a distinct genetic population from the sharp-tailed grouse in the eastern portion of the NW region. When we linked the genetic data to patterns of land cover/land use, we observed that limited contemporary or relatively recent historical connectivity has occurred between these 2 regions. Furthermore, genetic data suggest that sharp-tailed grouse in the EC region have experienced a demographic bottleneck recently. The landscape genetic analysis further suggests that this demographic bottleneck is likely due to a reduction in primary habitat throughout this region. These genetic data suggest that current declines within the EC sharp-tailed grouse region that are documented with annual surveys are not due to inbreeding depression but are the result of other factors, such as changes in habitat. This would not have been possible to elucidate without the application of landscape genetic approaches.

Finally, we also observed some genetic structure occurring at the subpopulation level within both greater prairie-chickens and sharp-tailed grouse in Minnesota. This observed structuring resulted in inbreeding HotSpots. Inbreeding HotSpots warrant further investigation and monitoring because they represent areas where further landscape management to improve habitat conditions for landscape connectivity may be needed to [limit further genetic structuring].

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Table 1. Summary of microsatellite marker variability and appropriateness for use in genotypic analysis of greater prairie-chickens (GRPC) and sharp-tailed grouse (STGR) in Minnesota based on data collected during 2014-2015.

Marker name[citation]	NA / H_O / H_E[1] GRPC	NA / H_O / H_E STGR	HWE[2] GRPC	HWE STGR
ADL146[(Cheng et al. 1995)]	7 / 0.63 / 0.68	9 / 0.66 / 0.68	0.053-0.652	0.077-0.555
ADL230[(Cheng et al. 1995)]	14 / 0.84 / 0.87	14 / 0.80 / 0.85	0.048-0.251	0.236-0.919
BG16[(Piertney and Höglund 2001)]	18 / 0.75 / 0.88	18 / 0.81 / 0.89	0.043-0.720	0.029-0.199
BG18[(Piertney and Höglund 2001)]	13 / 0.83 / 0.81	15 / 0.82 / 0.82	0.013-0.751	0.032-0.912
LLSD3[(Piertney and Dallas 1997)]	17 / 0.85 / 0.85	21 / 0.77 / 0.84	0.048-0.345	0.00-0.589
LLSD4[(Piertney and Dallas 1997)]	27 / 0.94 / 0.94	28 / 0.93 / 0.95	0.119-0.324	0.093-1.00
LLSD7[(Piertney and Dallas 1997)]	24 / 0.88 / 0.88	25 / 0.79 / 0.89	0.476-0.589	0.022-0.722
SGCA6[(Taylor et al. 2003)]	296 / 1.0 / 0.75	367 / 1.0 / 0.75	NA	NA
SGCA9[(Taylor et al. 2003)]	27 / 0.94 / 0.94	28 / 0.92 / 0.94	0.249 -0.361	0.320-1.00
TUD3[(Caizergues et al. 2001)]	26 / 0.91 / 0.91	26 / 0.91 / 0.91	0.051-0.975	0.222-0.975
TUT2[(Caizergues et al. 2001)]	3 / 0.47 / 0.47	3 / 0.43 / 0.47	0.199-0.751	0.182-0.751
TUT3[(Caizergues et al. 2001)]	9 / 0.78 / 0.78	10 / 0.79 / 0.79	0.106-0.748	0.033-0.981
TTD1[(Caizergues et al. 2001)]	8 / 0.77 / 0.77	9 / 0.62 / 0.78	0.291-0918	0.104-0.383
TTD3[(Caizergues et al. 2001)]	20 / 0.51 / 0.57	19 / 0.60 / 0.59	0.026-0.535	0.054-0.190
TTD6[(Caizergues et al. 2001)]	16 / 0.70 / 0.81	17 / 0.66 / 0.68	0.062-0.652	0.062-0.739

[1]NA = Number of Alleles, H_O = Observed Heterozygosity; H_E = Expected Heterozygosity
[2]HWE = Results of Hardy Weinberg Exact Tests for Neutrality. If markers are entirely homozygous or heterozygous, or otherwise fail to conform to Hardy Weinberg expectations, they are eliminated from the analysis because they will be uninformative. SGCA6 was removed from the analysis due to failure to conform to Hardy Weinberg expectations.

Table 2. Summary of resistance surface modeling values for greater prairie-chickens[1] in Minnesota 2014-2015.

Land cover and use classifications	H1[2]	H2	H3	H4	H5	H6	H7	H8	REV
Trees	500	-	-	-	-	500	500	500	500
Grassland, Meadows, Shrublands	25	-	-	-	-	25	25	25	10,000
Wet Meadows	75	-	-	-	-	75	75	75	10,000
Fresh Water	800	-	-	-	-	800	800	800	800
Cultivated Agriculture	500	-	-	-	-	500	500	500	1,000
Semi-Natural Vegetation	100	-	-	-	-	100	100	100	100
Recently Disturbed	10,000	-	-	-	-	10,000	10,000	10,000	75
Developed/Urban	10,000	-	-	-	-	10,000	10,000	10,000	25
%Grasslands (10km)	-	1-22	1-22	1-22	-	-	-	1-22	22-1
%Cultivated Agriculture (10km)	-	-	33-0	33-0	-	-	-	33-0	0-33
%Cultivated Agriculture × %Grassland	-	-	-	4-55	-	-	-	55-4	4-55
Human Footprint (1km)	-	-	-	-	-	0-100	0-100	0-100	100-0
Power Lines, Roads & Railroads	-	-	-	-	0-600	-	600	600	0

[1] We calculated resistance values based on a thorough review of the scientific literature (Spears 2010), knowledge of prairie grouse biology, and understanding of how the statistical program works. The absolute values used are essentially meaningless, but the relative differences among values yields insights about how the landscape is influencing greater prairie-chickens or sharp-tailed grouse. Our values (Tables 2 and 3) reflect our hypothesis that primary habitat (grasslands, wet meadows, or shrublands) is beneficial (low resistance), other natural features such as open water or trees are a partial barrier (more so for greater prairie-chickens than sharp-tailed grouse; intermediate resistance), cultivated agriculture is also an intermediate barrier to movements, and urban areas or recently disturbed/modified areas are highly avoided (high resistance). Cultivated agriculture at low-to-intermediate densities on the landscape is beneficial; at high densities (>50%) it is detrimental. We chose an intermediate value of resistance to capture this dynamic interaction threshold between prairie-grouse and cultivated agriculture in our landscape model.

[2] H1 = Hypothesis 1. Same notation for all hypotheses. See Methods section for descriptions of the hypotheses.

Table 3. Summary of resistance surface modeling values for sharp-tailed grouse[1] in both the east-central and northwest management regions in Minnesota 2014-2015.

Land cover and use classifications	H1[2]	H2	H3	H4	H5	H6	H7	H8	REV
Trees	200	-	-	-	-	200	200	200	800
Grassland, Meadows, Shrublands	25	-	-	-	-	25	25	25	10,000
Wet Meadows	75	-	-	-	-	75	75	75	10,000
Fresh Water	800	-	-	-	-	800	800	800	800
Cultivated Agriculture	500	-	-	-	-	500	500	500	1,000
Semi-Natural Vegetation	100	-	-	-	-	100	100	100	100
Recently Disturbed	10,000	-	-	-	-	10,000	10,000	10,000	75
Developed/Urban	10,000	-	-	-	-	10,000	10,000	10,000	25
%Grasslands & Wet Meadows (10km)	-	1-22	1-22	1-22	-	-	-	1-22	22-1
%Cultivated Agriculture (10km)	-	-	33-0	33-0	-	-	-	33-0	0-33
%Cultivated Agriculture × %Grassland & Wet Meadows	-	-	-	55-4	-	-	-	55-4	4-55
Human Footprint (1km)	-	-	-	-	-	0-100	0-100	0-100	100-0
Power Lines, Roads & Railroads	-	-	-	-	0-600	-	600	600	0

[1] We calculated resistance values based on a thorough review of the scientific literature (Spears 2010), knowledge of prairie grouse biology, and understanding of how the statistical program works. The absolute values used are essentially meaningless, but the relative differences among values yields insights about how the landscape is influencing greater prairie-chickens or sharp-tailed grouse. Our values (Tables 2 and 3) reflect our hypothesis that primary habitat (grasslands, wet meadows, or shrublands) is beneficial (low resistance), other natural features such as open water or trees are a partial barrier (more so for greater prairie-chickens than sharp-tailed grouse; intermediate resistance), cultivated agriculture is also an intermediate barrier to movements, and urban areas or recently disturbed/modified areas are highly avoided (high resistance). Cultivated agriculture at low-to-intermediate densities on the landscape is beneficial; at high densities (>50%) it is detrimental. We chose an intermediate value of resistance to capture this dynamic interaction threshold between prairie-grouse and cultivated agriculture in our landscape model.
[2] H1 = Hypothesis 1. Same notation for all hypotheses. See Methods section for descriptions of the hypotheses.

Table 4. Factor loadings from Partial Canonical Correspondence Analysis (PCCA) of greater prairie-chicken genetic structure in Minnesota 2014-2015. We did not standardize variances prior to analysis with PCCA; therefore, effect sizes (loadings) are not directly comparable. However, direction and relative strength of influence are comparable. Larger absolute values indicate greater importance, whereas the sign (+ or -) indicates the direction of the interaction.

Factor	Loading axis 1	Loading axis 2
Powerlines	0.05	-0.02
Human Footprint	1.49	2.83
Multiple Resolution Land Cover Data Resistance	39.59	75.37
%Agriculture	-3.44	-6.55
%Grassland	1.35	2.57
%Grassland × %Agriculture	-4.79	-9.12

Table 5. Factor loadings from Partial Canonical Correspondence Analysis (PCCA) of sharp-tailed grouse genetic structure between regions in Minnesota 2014-2015. We did not standardize variances prior to analysis with PCCA; therefore, effect sizes (loadings) are not directly comparable. However, direction and relative strength of influence are comparable. Larger absolute values indicate greater importance, whereas the sign + or -, indicates the direction of the interaction.

Factor	Loading axis 1	Loading axis 2
Powerlines	0.61	-0.76
Human Footprint	0.02	0.25
Multiple Resolution Land Cover Data Resistance	78.57	59.30
%Agriculture	-4.88	0.85
%Primary Habitat	3.17	-1.07
%Primary Habitat × %Agriculture	-4.55	8.79

Table 6. Factor loadings from Partial Canonical Correspondence Analysis (PCCA) of sharp-tailed grouse genetic structure within the east-central management region in Minnesota 2014-2015. We did not standardize variances prior to analysis with PCCA; therefore, effect sizes (loadings) are not directly comparable. However, direction and relative strength of influence are comparable. Larger absolute values indicate greater importance, whereas the sign + or -, indicates the direction of the interaction.

Factor	Loading axis 1	Loading axis 2
Powerlines	-0.54	-1.73
Human Footprint	0.14	2.43
Multiple Resolution Land Cover Data Resistance	31.66	64.93
%Agriculture	-6.40	-30.10
%Primary Habitat	31.39	84.45
%Primary Habitat × %Agriculture	42.56	-18.42

Table 7. Factor loadings from Partial Canonical Correspondence Analysis (PCCA) of sharp-tailed grouse genetic structure within the northwest management region in Minnesota 2014-2015. We did not standardize variances prior to analysis with PCCA; therefore, effect sizes (loadings) are not directly comparable. However, direction and relative strength of influence are comparable. Larger absolute values indicate greater importance, whereas the sign + or -, indicates the direction of the interaction.

Factor	Loading axis 1	Loading axis 2
Powerlines	-11.88	-12.99
Human Footprint	-4.01	-3.43
Multiple Resolution Land Cover Data Resistance	56.95	-19.14
%Agriculture	-1.89	-12.99
%Primary Habitat	26.87	50.04
%Primary Habitat × %Agriculture	3.90	-3.38

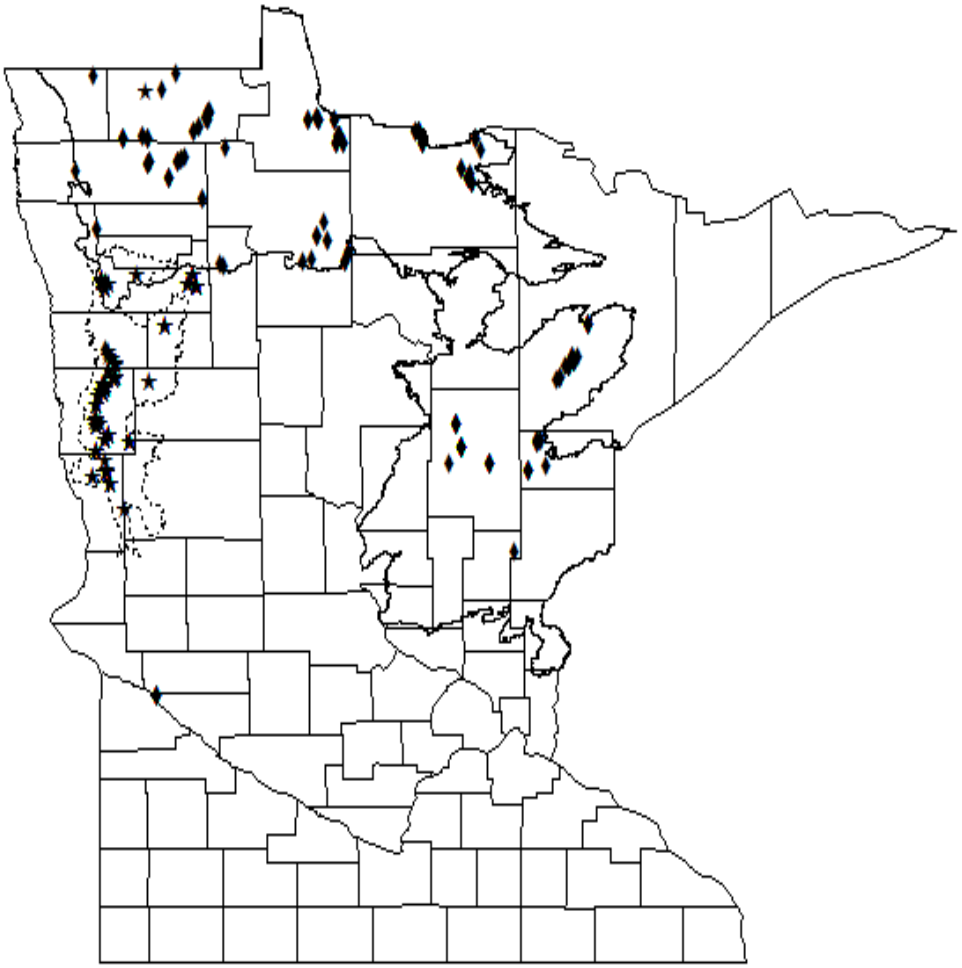

Figure 1. Minnesota sharp-tailed grouse and greater prairie-chicken sample locations (black diamonds and black stars, respectively) during 2014 and 2015 with boundaries of openland focus areas for sharp-tailed grouse (solid line) and approximate current primary range of prairie-chickens (dotted line). The background shows county boundaries.

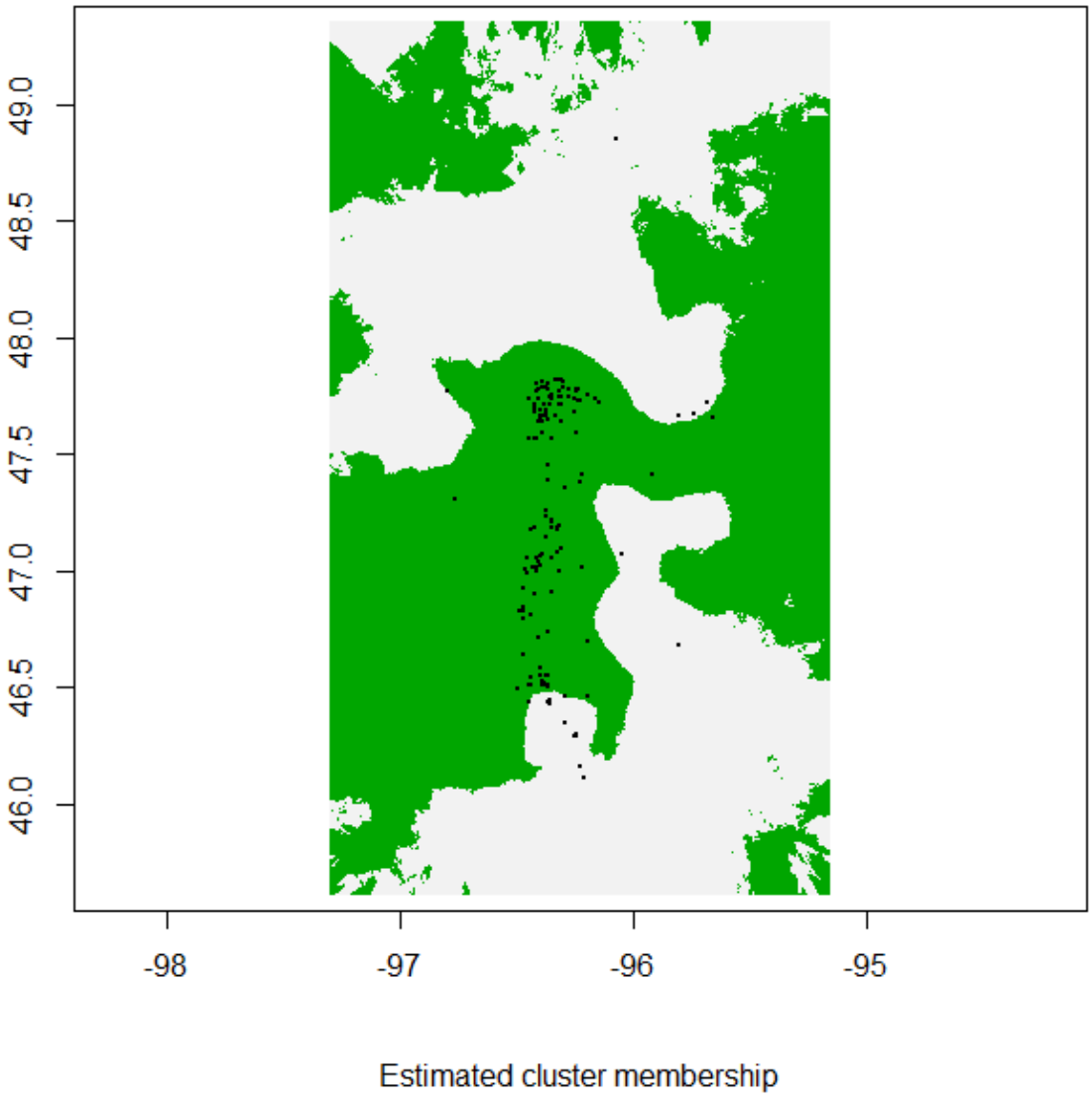

Figure 2. Geneland map of greater prairie-chicken genetic clusters for samples collected in Minnesota during 2014 and 2015. In the figure above the x-axis is degrees of longitude and the y-axis is latitude. Geneland identified 2 distinct genetic clusters—a primary cluster of locations (black dots) in the north and west (green background) and another cluster in the south and east (grey background).

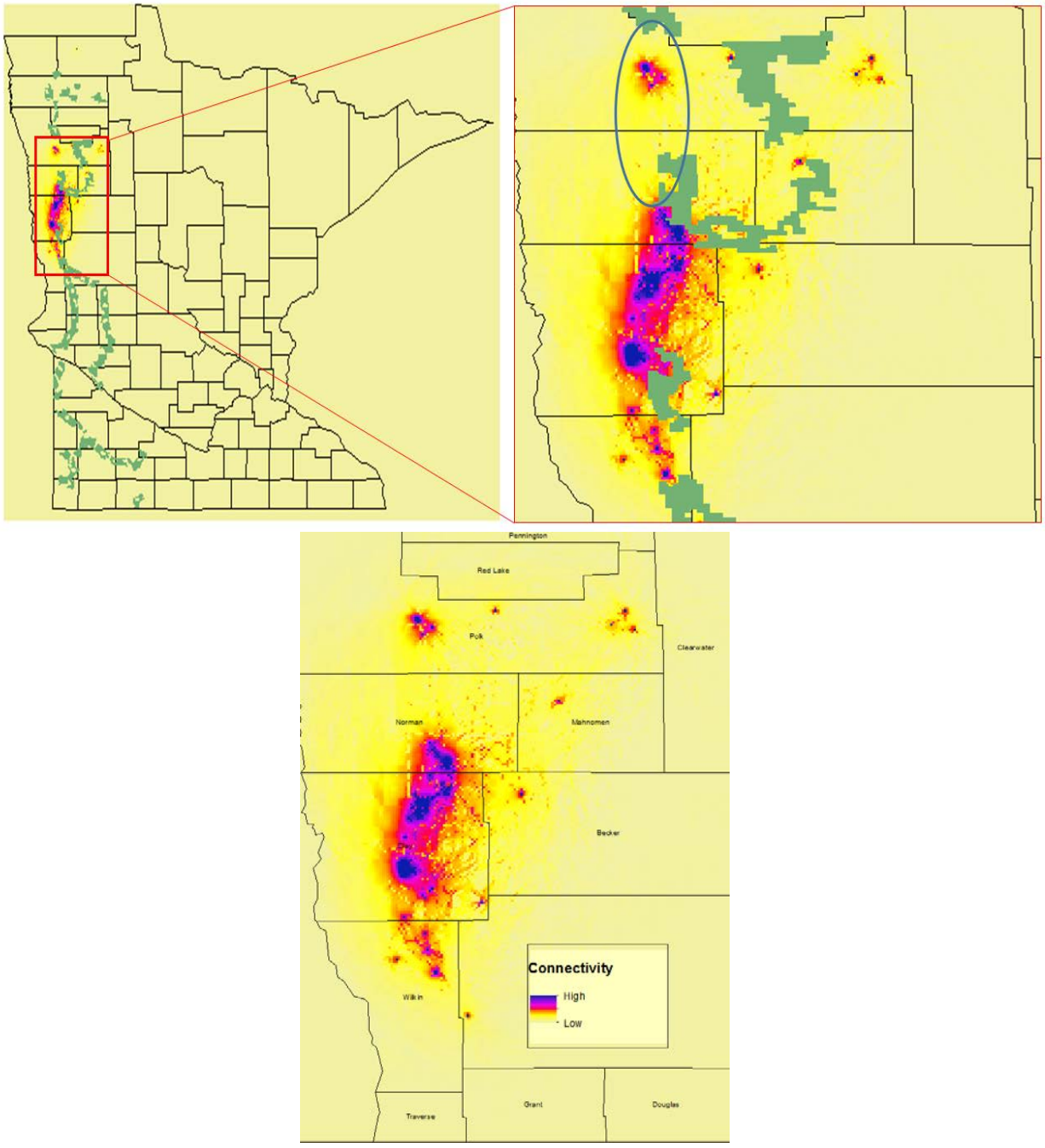

Figure 3a,b. Connectivity model highlighting gaps in corridor coverage for greater prairie-chickens based on the results of the Canonical Correspondence Analysis and Resistance Modeling with Circuitscape for samples collected in Minnesota during 2014 and 2015. When the connectivity map is overlaid with the Prairie Plan corridor area, an area lacking connectivity within the planned corridor is indicated in Norman and Polk counties (blue oval). The top figure depicts the connectivity of the sampled primary prairie-chicken range with the corridors depicted at the statewide scale and the current primary prairie-chicken range scale; the bottom figure excludes the planned corridor areas.

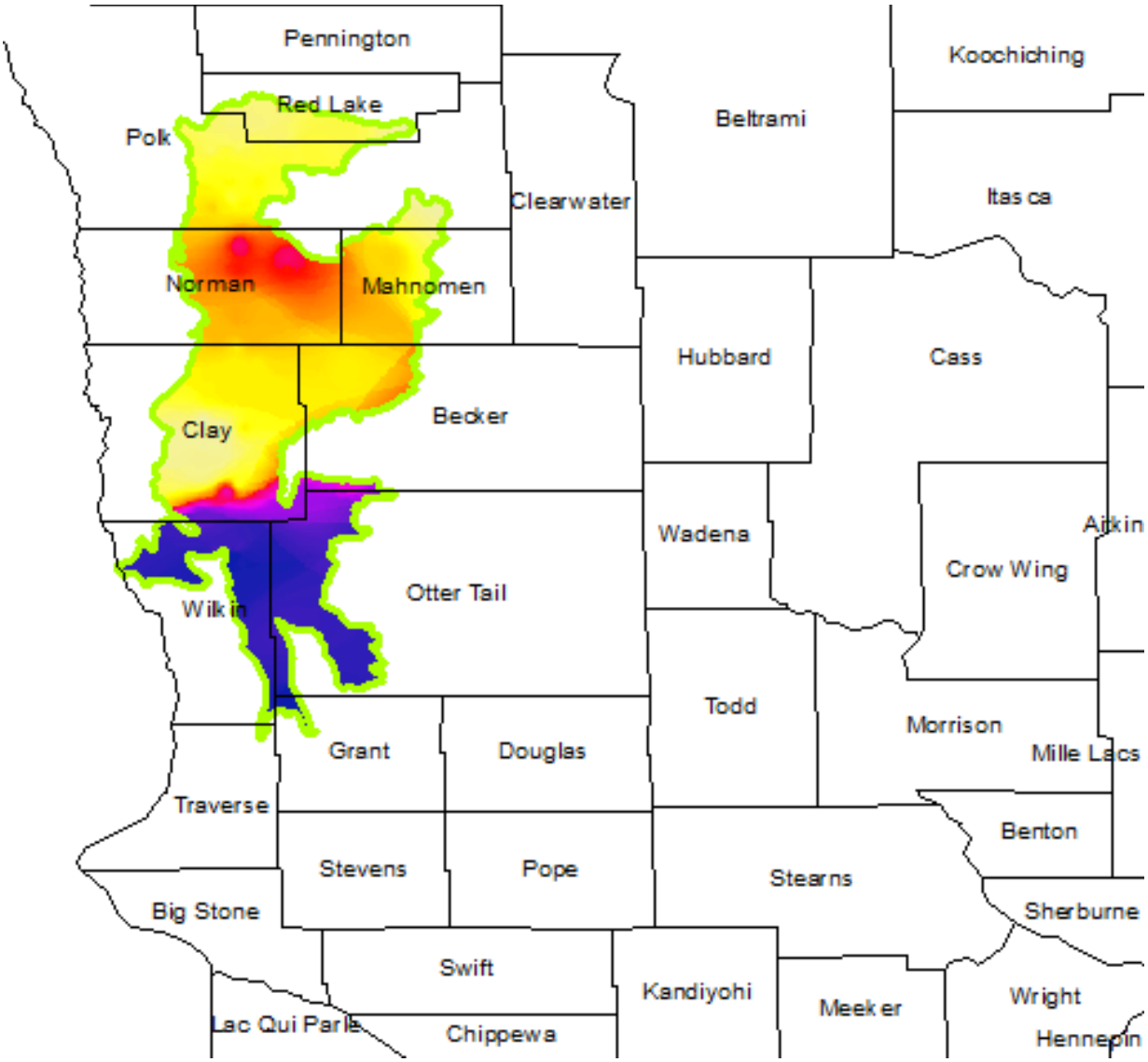

Figure 4. Greater prairie-chicken subpopulation isolation analysis based on HotSpot Analysis of F_{IS} values for samples collected in Minnesota during 2014 and 2015. The map indicates areas with limited migration into or out of the local region. Red areas in the north near Norman and Mahnomen Counties indicate areas where significant high × high clustering of F_{IS} values is occurring (HotSpots). This is indicative of populations that are isolated or becoming isolated due to limited dispersal. Yellow areas in Polk, Clay, and Becker Counties are areas where no clustering was observed; movement is likely unhindered or near migration drift equilibrium. Blue areas in the south near Wilkin and Otter Tail Counties are ColdSpots (low×low F_{IS} value clustering) and are areas where the genetic data suggest that movement is greater than anticipated due to random processes. This might be indicative of high movement due to marginal habitat quality. The red band in Clay County immediately north of the source area suggests a disruption in gene flow that may be indicative of a barrier to dispersal.

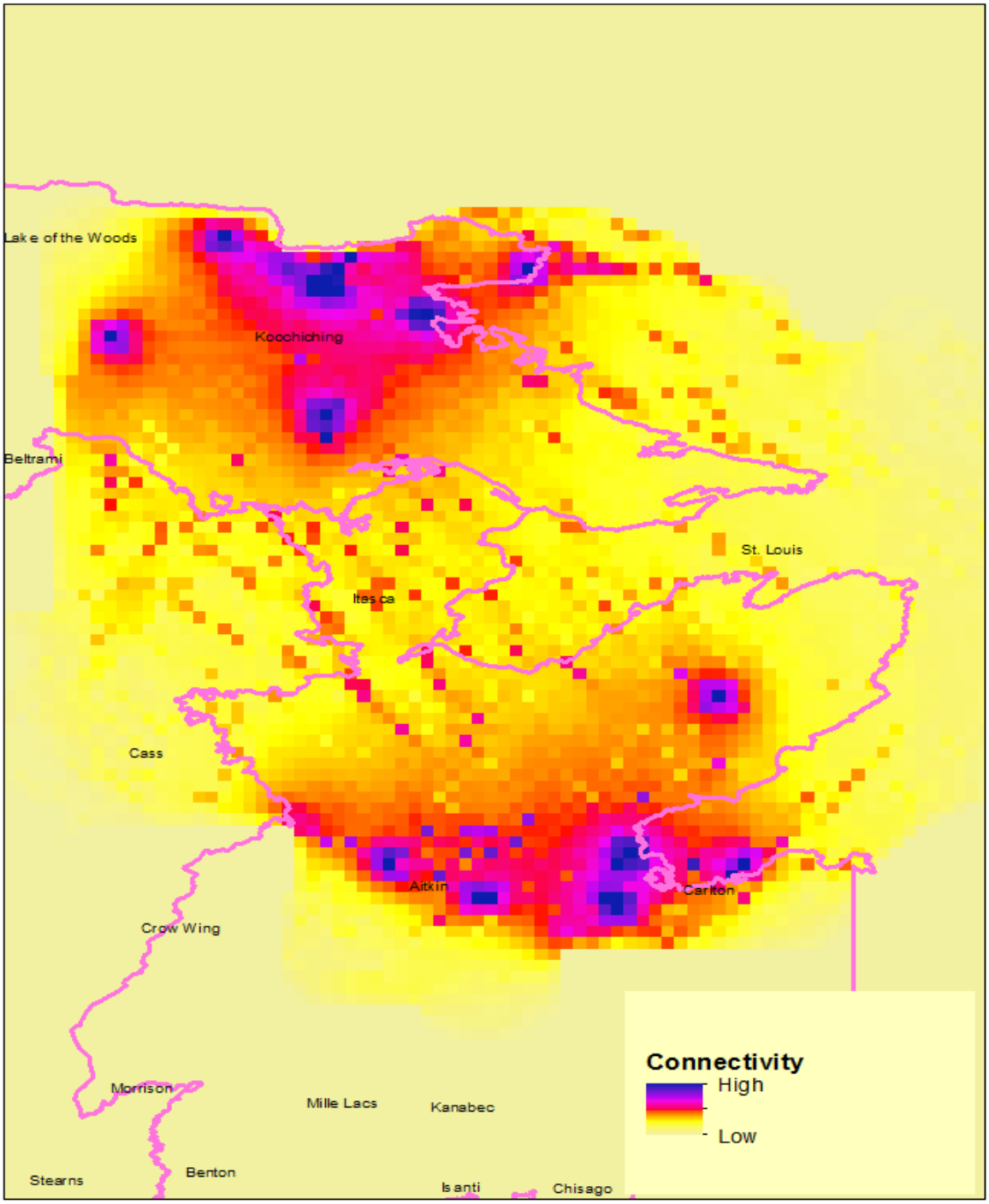

Figure 5. Connectivity model predicting connectivity between sharp-tailed grouse management regions that are delineated along boundaries of Ecological Classification Section subsections (sinuous [color] lines) in Minnesota. The map depicts high connectivity within portions of each management region, but limited connectivity between the northwest and east-central management regions based on samples collected during 2014 and 2015.

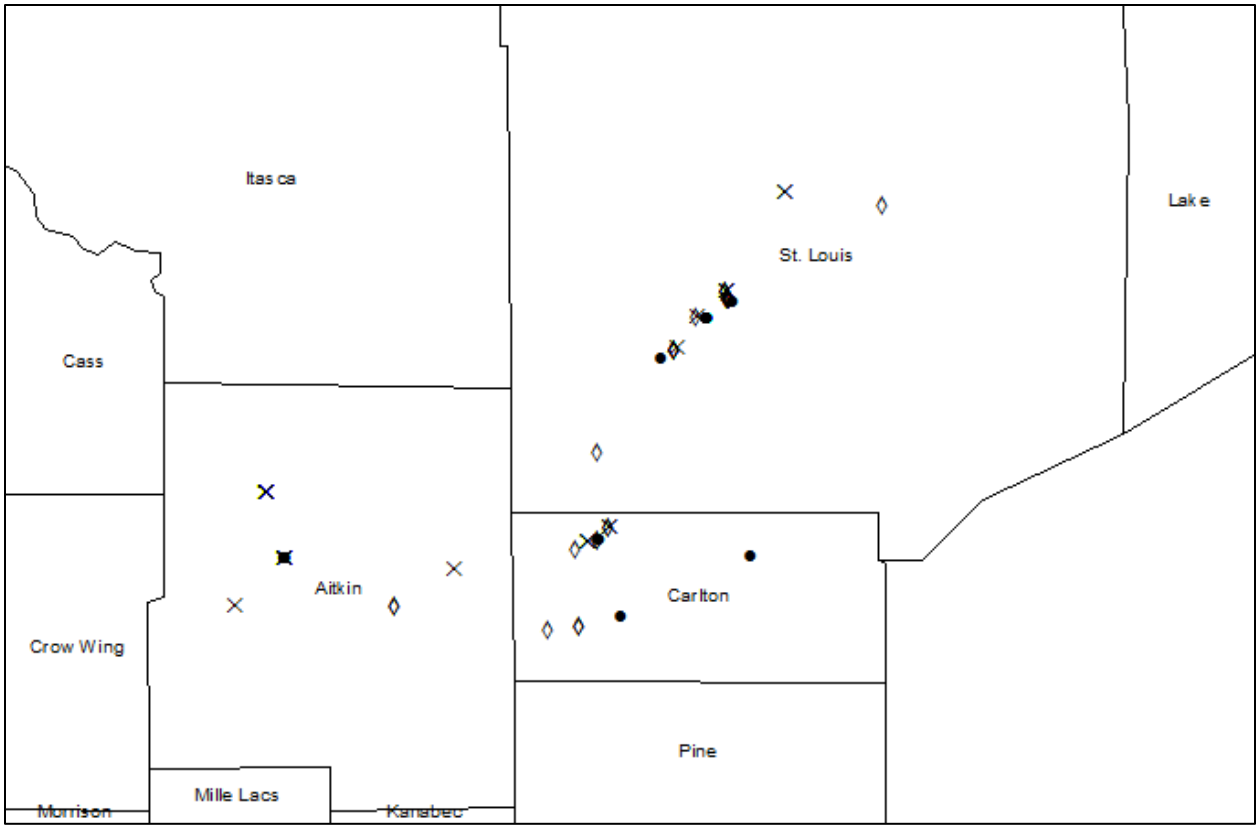

Figure 6. Sampling sites during 2014 and 2015 in the east-central management region of Minnesota mapped based on putative population assignment from Program Structure. Program Structure found greatest support for 3 genetic clusters within the east-central region, indicated symbolically with circles, diamonds, and crosses. The observed clusters do not have a strong spatial pattern, which is indicative of a recent bottleneck or mixing of previously isolated populations, but not structure.

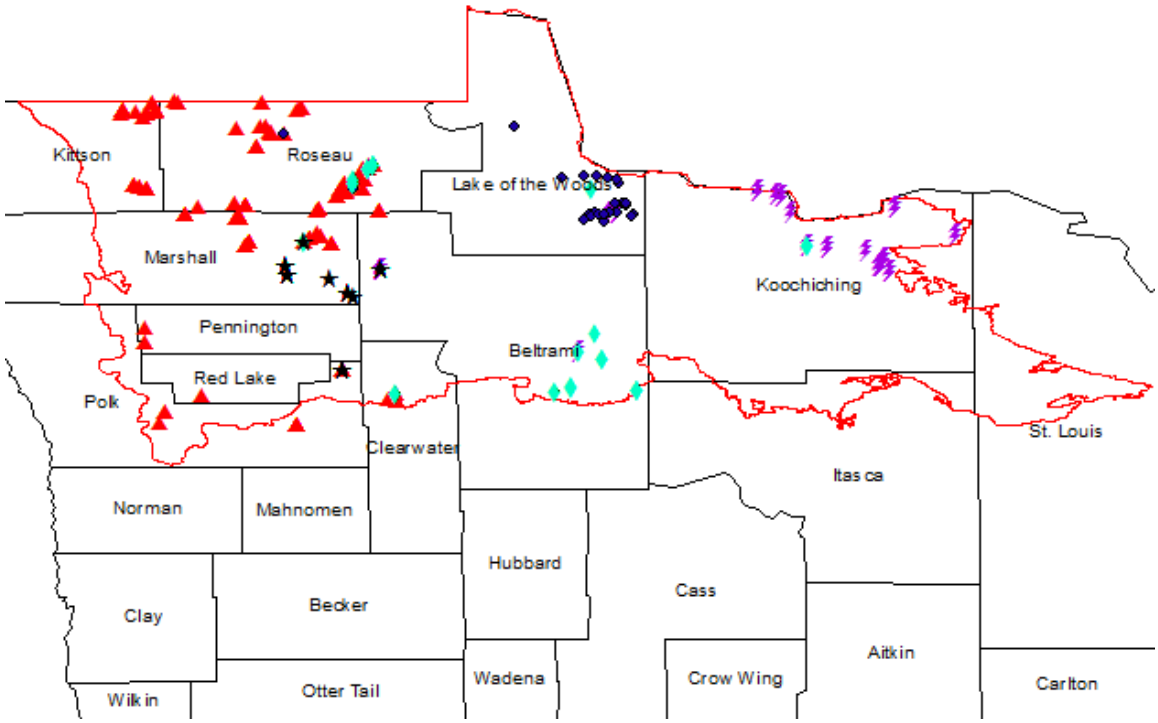

Figure 7. Genetic clusters in the northwest sharp-tailed grouse management region of Minnesota as identified by Program Structure from samples collected during 2014 and 2015. Each set of symbols represents a distinct genetic cluster. Some intermixing of the clusters occurs, but most of the genetic population identities are the same as their sampling location (e.g., all lightning bolts are in Koochiching County). The exception is the cluster centered in Beltrami County, indicating migrants from that area into other areas (i.e., there are diamonds elsewhere in the region).

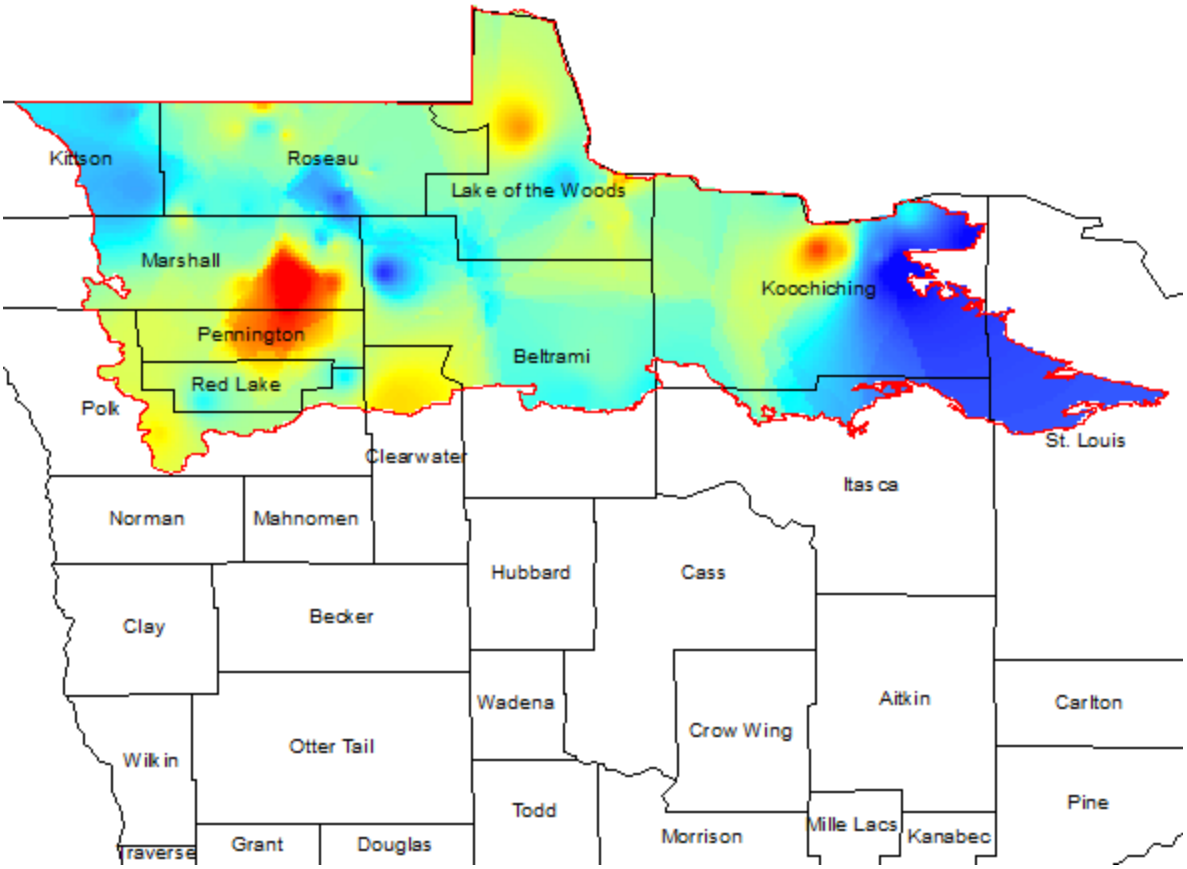

Figure 8. Sharp-tailed grouse subpopulation isolation analysis for the northwest management region in Minnesota, indicating areas with limited migration into or out of the local region. This analysis was based on HotSpot Analysis of F_{IS} values and samples collected during 2014 and 2015. Limited movements are indicated in red (e.g., a foci in Pennington and Marshall counties), but more movement than expected occurred along the eastern and western borders of the range (blue), with intermediate values occurring in yellow. Habitat is most likely marginal in the far eastern part of the range, resulting in more searching and movement, whereas birds in areas with better habitat likely settle closer to their natal areas.