

Connectivity of prairie dog colonies in an altered landscape: inferences from analysis of microsatellite DNA variation

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Abstract Connectivity of populations influences the degree to which species maintain genetic diversity and persist despite local extinctions. Natural landscape features are known to influence connectivity, but global anthropogenic landscape change underscores the importance of quantifying how human-modified landscapes disrupt connectivity of natural populations. Grasslands of western North America have experienced extensive habitat alteration, fragmenting populations of species such as black-tailed prairie dogs (*Cynomys ludovicianus*). Population sizes and the geographic range of prairie dogs have been declining for over a century due to habitat loss, disease, and eradication efforts. In many places, prairie dogs have persisted in the face of emerging urban landscapes that carve habitat into smaller and smaller fragments separated by

uninhabitable areas. In extreme cases, prairie dog colonies are completely bounded by urbanization. Connectivity is particularly important for prairie dogs because colonies suffer high probabilities of extirpation by plague, and dispersal permits recolonization. Here we explore connectivity of prairie dog populations using analyses of 11 microsatellite loci for 9 prairie dog colonies spanning the fragmented landscape of Boulder County, Colorado. Isolation-by-resistance modeling suggests that wetlands and high intensity urbanization limit movement of prairie dogs. However, prairie dogs appear to move moderately well through low intensity development (including roads) and freely through cropland and grassland. Additionally, there is a marked decline in gene flow between colonies with increasing geographic distance, indicating isolation by distance even in an altered landscape. Our results suggest that prairie dog colonies retain some connectivity despite fragmentation by urbanization and agricultural development.

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Introduction

Wildlife populations are distributed discontinuously across the landscape, leading to varying degrees of spatial and genetic connectivity among populations. Through the burgeoning field of landscape genetics, we have gained a greater understanding of the natural barriers that structure populations and mediate gene flow across a landscape (e.g. Manel et al. 2003; Perez-Espona et al. 2008; Spear et al. 2005). Human alteration of the landscape further divides populations and may interrupt or redirect existing corridors among them (Collinge 2009). Features with demonstrated effects on

the connectivity of populations include highways (Coulon et al. 2006), deforestation (Pavlacky et al. 2009), urbanization (Telles et al. 2007), agriculture (Levy et al. 2010) and dams (Beneteau et al. 2009). Furthermore, landscape changes can alter migration corridors (Antonio et al. 2007), create asymmetrical gene flow (Barrowclough et al. 2004), or decrease the magnitude of gene flow without altering its direction (Goverde et al. 2002). With an increasing percentage of global land being converted to agriculture (34%; Ramankutty et al. 2008) and urban sprawl (increasing twice as fast as human population growth; DeCoster 2000), many species encounter complex human-modified landscapes. Depending on dispersal ability of the organism, genetic connectivity of different species will be influenced to varying degrees by each form of habitat alteration.

Population connectivity in black-tailed prairie dogs (*Cynomys ludovicianus*, hereafter “prairie dogs”), a social mammal important in prairie ecosystems, was historically maintained by their occurrence in large, continuous swaths of grassland. In natural landscapes, dispersal among colonies probably happens every generation, can occur over relatively large distances, and likely employs corridors such as dry creek beds or ravines (Garrett and Franklin 1988; Roach et al. 2001). In the last 200 years, the occupied range of prairie dogs has declined by over 99% (Miller and Cully 2001) from the combined actions of land conversions, eradication campaigns, and extirpation by sylvatic plague. Consequently, prairie dog populations (equivalent to colonies for the purpose of this paper) in many places now occupy discrete patches of grassland surrounded by a matrix of uninhabitable land including urban sprawl, agricultural fields and hay fields (Johnson and Collinge 2004).

Landscapes in which colonies are mostly bounded by urban or agricultural land may inhibit inter-colony movement of animals, and such conditions can accelerate localized inbreeding and prevent recolonization if the colony is extirpated; however, these landscapes may also limit the spread of diseases that can move through well-connected systems (Hess 1996). At the other end of the spectrum, landscapes that support well-connected colonies most closely resemble the native conditions for prairie dogs and presumably limit localized inbreeding; nonetheless, well-connected colonies may be prone to colonization by pathogens (Hess 1996; Jesse et al. 2008; Lopez et al. 2005; Trudeau et al. 2004) such as the plague pathogen *Yersinia pestis*. Pathogens may experience different influences on connectivity than a host if the pathogen has multiple modes of dispersal (Jones and Britten 2010), but pathogens and parasites that rely on their hosts for dispersal (Brinkerhoff et al. 2011), such as flea transmission of *Y. pestis* (Stapp et al. 2009), will be influenced by similar constraints on connectivity as their hosts.

Recent plague events in Boulder County, Colorado (in 2002 and 2005–2009, confirmed by the Centers for Disease Control and Prevention) were more geographically restricted than in the past, spreading through the county over the course of several years (Boulder Open Space and Mountain Parks, unpublished data), possibly due to the disruption of migration corridors caused by urbanization. In natural habitats and in urbanized landscapes, extirpated colonies are re-colonized within a few years (Roach et al. 2001; Antolin et al. 2006). However, if urbanization and other types of land alteration restrict movement of prairie dogs, re-colonization from a smaller number of source colonies may result in founder effects (Templeton 2006) and inbreeding. If movement is limited, the increase in colony isolation due to plague extirpation may be amplified in an altered landscape such as Boulder County, where humans and prairie dogs share a mosaic of different habitat types. Urbanization that isolates colonies (Magle et al. 2010) may disrupt connectivity; in contrast, habitat conversion to agriculture may facilitate dispersal due to increased vegetation cover or by providing refuge from predators. Here we develop a model for the connectivity of black-tailed prairie dog colonies in a complex landscape by integrating the habitat matrix with estimates of genetic similarity among colonies across Boulder County. We show clear effects of various landscape characteristics and demonstrate that there may be a complex network of corridors that facilitates connectivity among prairie dog colonies, with different forms of landscape alteration contributing to connectivity in distinct ways. Finally, we discuss how interrupted connectivity in complex landscapes may contribute to metapopulation dynamics in the context of pathogen-mediated extinctions.

Methods

Genotyping and estimating differentiation among colonies

Our genetic analysis focused on ten colonies located throughout Boulder County (Table 1) with relatively large sample sizes. Of the 1,200 ha of land occupied by prairie dog colonies in Boulder County, study colonies were chosen to represent populations bounded by varying degrees of urbanization (Johnson and Collinge 2004) and surrounded by a range of habitat matrix types (Fig. 1). Sampled colonies were separated by pairwise distances varying from 1.5 to 36 km. No attempt was made to sample at regular spatial intervals; instead, colonies were selected based upon their surrounding habitat matrix. Eight of the ten colonies were affected by plague in 1994, as determined by local plague records kept by land and wildlife

Table 1 List of colony names and their locations, area and population densities

Colony number	Latitude	Longitude	Area (hectares)	Density (prairie dogs/hectare)
1	40.24454	−105.227273	190.46	64
2	40.21930	−105.310418	22.59	36
3	39.93955	−105.096410	153.43	31
5	40.15440	−105.252508	132.53	11
6	39.91218	−105.180573	19.09	14
9	40.01136	−105.191460	16.18	36
15	40.10811	−105.210192	42.50	42
19	40.10466	−105.276360	201.21	27
20	39.92067	−105.220905	24.10	17
106	40.02252	−105.180065	22.26	19

Population density is based on visual counts averaged from 2003 and 2004. Colony 20 was excluded from IBR analysis due to its relocation history

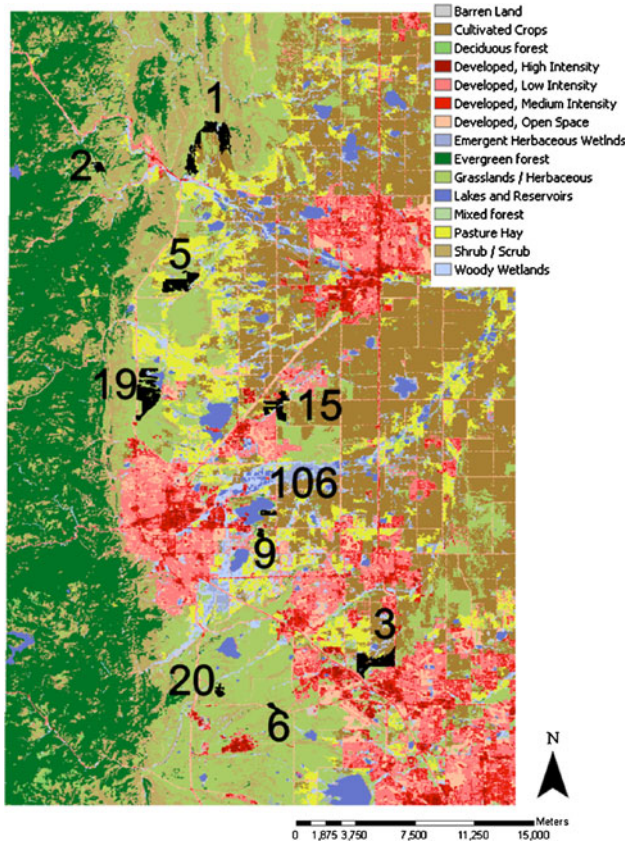


Fig. 1 Map of Boulder County showing land cover types (data from the NLCD 2001) and location of prairie dog colonies used to establish nodes for Circuitscape (in black) and sites for which we determined multilocus microsatellite genotypes (indicated with numbers)

managers (Collinge et al. 2005). Prairie dog trapping and processing were conducted in accordance with protocols approved by the University of Colorado’s Institutional Animal Care and Use Committee and are described in detail therein (available upon request). For each of the ten colonies sampled, 49 Tomahawk traps were set on a 150 × 150 m grid in the approximate center of the colony

and pre-baited with a corn-oat-barley mixture for 5 days with the traps held open. After pre-baiting, traps were baited, set, and left for 3 h at a time for the course of a week. Captured prairie dogs were temporarily immobilized using isoflurane anesthesia while blood, fleas, and ear tissue were collected. One half milliliter of blood was collected from the femoral vein, and tissue was obtained by clipping ½ cm from the outer edge of the ear. Both adult and juvenile prairie dogs were processed, but for animal safety reasons, blood was not obtained from juveniles under 300 g. When each animal had recovered from anesthesia, it was released at the trap location where it was captured.

Tissue samples collected during 2003 and 2004 were stored until extraction at 4°C in a solution of EDTA and DMSO. DNA extraction was performed using a Qiagen Tissue kit according to protocol, and extracted DNA was stored at −80°C until genetic analysis. DNA from 557 Boulder County prairie dogs was amplified at 11 microsatellite loci by polymerase chain reaction (PCR) (Jones et al. 2005). PCR products were analyzed on a LICOR 4200 sequencer and genotypes determined using Gene-ImagIR software. We estimated error rates by repeating amplifications and genotyping for approximately 10% of the data. Because a population of prairie dogs consists of overlapping generations and we did not expect genotype frequencies to change in 1 year, all samples for each colony sampled in 2003 and 2004 were pooled. Animals recaptured in multiple years were typed only once. Tests of Hardy–Weinberg expectations were carried out using Arlequin (Schneider et al. 2000). Colony heterozygosity was calculated using Microsatellite Analyzer (Dieringer and Schlotterer 2003).

We estimated migration between pairs of colonies using assignment methods implemented in GeneClass (Piry et al. 2004) under two scenarios: one in which resident and migrant classification were based on assignment test values (assuming an individual was born in the colony it was

assigned to with highest log likelihood), and one in which we tested for migration in the previous one or two generations using 10,000 MCMC replications and a threshold value of 0.01; that is, if individuals assigned to the sampled colony with probability less than 0.01, they were inferred migrants (Paetkau et al. 1995, 2004).

Models of prairie dog movement potential

Landscape features may influence gene flow among populations by affecting dispersal rates; therefore, models more robust to spatial heterogeneity than simple isolation-by-distance measures are required (McRae 2006). We used an isolation-by-resistance (IBR) approach (McRae 2006; McRae and Beier 2007; McRae et al. 2008) for predicting demographic connectivity among prairie dog colonies in the complex landscape of Boulder County. IBR predicts a positive relationship between genetic differentiation and the resistance distance, a distance metric that exploits relationships between the distances among populations and the ecological resistance estimated during simulated random walks (McRae 2006). IBR is conceptually similar to the least cost path -based distance approach, allowing for the possibility of multiple pathways of connectivity, and pathways of varying width (McRae and Beier 2007). IBR is robust to violations of certain assumptions, including that of migration-drift equilibrium (McRae and Beier 2007).

IBR is based in electric circuit theory, analyzing a landscape as if it were a circuit board and treating organisms (and therefore gene flow) as electrical current. IBR calculates the resistance distance by simultaneously considering all possible pathways connecting population pairs (McRae and Beier 2007), creating a theoretical “circuit board”. Populations (in our case, colonies) are represented as sources or grounds, while the landscape matrix is composed of a raster grid of values that correlate to relative conductance or resistance values associated with landscape features. Conductance or resistance values are fitted to the observed genetic relationships among populations (e.g. pairwise F_{ST} values) through simulation, with fits constrained by known species-habitat associations (McRae 2006). For example, higher conductance values are assigned to landscape cells that are known to contain preferred habitat for dwelling or dispersal (e.g. grassland), and lower conductance values are assigned to cells known to contain habitat that is not preferred or that inhibits dispersal (e.g. water). Using the program Circuitscape, IBR can be analyzed by estimating the resistance encountered along all possible paths (circuits) among colonies (current sources or grounds). The result is that better, more numerous and/or wider pathways between nodes reduce the resistance distance separating them. IBR analysis provides a flexible and efficient tool to understand effects of landscape features on

genetic structure, and to predict genetic and evolutionary consequences of landscape change.

In this study, we modeled gene flow among 10 prairie dog colonies, out of 369 known colonies located in Boulder County. Colonies were represented by their polygon centroids as single raster cells (30×30 m) in ASCII format. Using the National Land Cover Data (NLCD) 2001 layer obtained from USGS, which extended beyond the area of sampled prairie dog colonies, we assigned estimated conductance values to the 15 classes of land cover found within the study area (Fig. 1; Homer et al. 2004). After condensing functionally similar land types into eight single classes (e.g. forest and shrubland), we used a hierarchical approach in which we evaluated 128 initial models representing all possible combinations of eight land cover types in two conductance categories, with each landscape variable (Table 2) initially assigned a conductance value—or ease of prairie dog movement—of either 100 (low resistance, e.g. movement within prairie dog colonies) or zero (“infinite resistance”). For all models, the land use types in which prairie dog colonies were sampled (grasslands and barren land) were included in the high conductance category (failure to include these habitat types in the high conductance, $C = 100$ category resulted in infinite resistance between prairie dog colonies) and lakes were included in the low conductance, $C = 0$ category (since we know prairie dogs do not inhabit or move across lakes).

After determining the ten best models in the initial step, we iteratively refined our models by adding additional intermediate conductance categories (varying degrees of prairie dog movement) to which we assigned land cover types that did not clearly fall into either conductance category in the ten best models (e.g. developed open space). The approach of increasing complexity in subsequent models (as in Lee-Yaw et al. 2009) allowed us to isolate the effects of one land class at a time by assessing the change in model fit after changing its conductance. A total of 203 models were evaluated; among the tested models were those that assigned low conductance values to heavy urbanization, as inferred by Magle et al. (2010), and higher conductance values to low-intensity development, with small roads potentially acting in a similar facilitative fashion to dry creek beds (Roach et al. 2001). Prairie dog colonies were mapped with Geographic Information System tools such that colonies surrounded by heterogeneous land cover were treated as nodes surrounded by discrete areas with varying resistance. Circuitscape was run in pairwise mode (i.e. testing each colony’s connection to every other colony), using a connection scheme where gene flow was allowed between the neighboring 8 cells (through the creation of 8 undirected edges). The edge conductance between any two grid cells (nodes) was based on the average of the conductance (on a scale of 0–100) assigned

Table 2 List and descriptions of NLCD land classes used in Circuitscape resistance model (adapted from Homer et al. 2004)

NLCD land classes		
Code	Title (assigned class)	Description
LR	Lakes and reservoirs (1)	Open water bodies
LID	Developed, low intensity (2)	Impervious surfaces such as roads and suburban environments
MID	Developed, medium intensity (3)	Most commonly include single-family housing units and surrounding areas
HID	Developed, high intensity (4)	Highly developed areas where people reside or work in high numbers (e.g. apartment complexes, row houses and commercial/industrial where impervious surfaces account for 80–100% of the total cover)
HW	Emergent herbaceous wetlands (5)	Areas where perennial herbaceous vegetation accounts for greater than 80% of vegetative cover and the soil or substrate is periodically saturated with or covered with water
WW	Woody wetlands (5)	Areas where forest or shrubland vegetation accounts for greater than 20% of vegetative cover and the soil or substrate is periodically saturated with or covered with water
DOS	Developed, open space (6)	Areas that included large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes
DF	Deciduous forest (7)	Areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover
EF	Evergreen forest (7)	Areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover
MF	Mixed forest (7)	Areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover, deciduous or evergreen species accounting for less than 75% of total tree cover
SS	Shrub/scrub (7)	Areas dominated by shrubs
BL	Barren land (8)	Areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other accumulations of earthen material
CC	Cultivated cropland (8)	Areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards, and land that is actively tilled
GH	Grasslands/ herbaceous (8)	Areas dominated by grasses
PH	Pasture hay (8)	Areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle

to each cell; thus, both nodes and edges were set as conductive. Resistance distance matrices output for each model were compared to pair-wise normalized F_{ST} values for the 9 colonies using partial Mantel tests (controlling for the effects of geographic distance) implemented in the Vegan package for R (The R Foundation for Statistical Computing, <http://www.r-project.org/>).

Finally, using historic records of prairie dog relocation, we assessed whether relocation influenced the genetic structure of prairie dog colonies in our system. Relocation has the potential to interfere with inferences based on genetic differentiation, and because it is a common management strategy in many systems, it is important to assess its effect on these inferences. One relocation event in 1996 totaling 100 individuals involved one of our study sites (20) as a destination, and six additional events from 1995 to 2001 moved 1008 animals to colonies within 2 km of this site (data from the City of Boulder Open Space and

Mountain Parks). Therefore, we re-ran all of our models with this colony excluded to determine whether relocation influenced our estimates of connectivity. Several smaller scale relocation events placed prairie dogs (average = 38 per event) in colonies within 2 km of two other study sites (1 and 3), so we also re-ran our models with these colonies excluded.

Results

Genetic differentiation

Genetic effects of relocation could not be detected in colonies 1 or 3. However, we did detect a signal of relocation in colony 20, the destination of a large relocation effort from 1995 to 2001. Although one-month survival rates in mid-autumn averaged only 21.2%, re-running our models

with this colony excluded improved model fit markedly. Furthermore, excluding this colony from a Mantel test of isolation by distance reversed the pattern (no isolation by distance with colony 20 included, $r = 0.1004$, $P = 0.279$) to one of significant isolation by distance ($r = 0.3654$, $P = 0.033$). Therefore, we removed this colony from analyses of connectivity, and all reported values exclude this colony.

We obtained samples from 557 prairie dogs in 10 colonies (mean 56, range 35–87). After omitting colony 20 from analyses of connectivity because of its history of relocation, 510 individuals in 9 colonies remained. For the eleven loci surveyed, the number of alleles per locus ranged from 6 to 14, with a mean of 9.3. All pair-wise F_{ST} comparisons among the 10 colonies were significant. Values ranged from 0.0588 to 0.194; average pair-wise F_{ST} was 0.109 (Table 3). Average heterozygosity within colonies ranged from 0.595 to 0.767 (Table 3); across all colonies average heterozygosity was 0.663. Removing colony 20 had no effect on average F_{ST} , global allele frequencies or average heterozygosity. Observed heterozygosities were similar to Hardy–Weinberg expectations, with the exception that locus C116 showed a deficiency of heterozygotes in 3 colonies (6, 9, and 20). This locus was the most variable, suggesting that the departure from HW expectations may reflect the presence of null alleles. Because null alleles do not appreciably affect estimates of migration among colonies (Hauser et al. 2006), we included this locus in our analyses. There was no evidence of null alleles at other loci. Based on re-typing of 10% of our data, the estimated genotyping error rate was 2.8%. Errors were approximately randomly distributed across loci and individuals.

Evaluation of connectivity models assumes that animals move across the landscape; therefore, we tested this assumption by estimating the fraction of sampled individuals from a colony inferred to be migrants using Bayesian

assignment methods implemented in GeneClass (Piry et al. 2004). Using a relaxed criterion for assigning individuals to particular colonies (namely that individuals were assumed to be born in the colony to which it was assigned with the highest log likelihood), a total of 70 out of 557 individuals surveyed were inferred migrants. When we used a more stringent criterion for estimating migration between colonies (assuming a threshold value for assignment as resident of 0.01), 22 individuals were inferred migrants. Because we did not sample all colonies across Boulder County, we could not confidently assign whether the inferred migrants moved between sampled colonies; nonetheless, these results support the assumption that prairie dogs move across the landscape of Boulder County.

Explaining connectivity among colonies: evaluating models

Our general approach was to use IBR models for estimating connectivity and to choose among the many possible models using partial Mantel correlations between the estimated connectivity and the degree of genetic differentiation among colonies. For each test of a particular model we controlled for the effect of log-transformed geographic distance because there was a significant effect of geographic distance on the degree of genetic differentiation between colonies (Mantel test, $r = 0.3654$, $P = 0.033$). Of the models evaluated for the two conductance categories (0 and 100), there were some that provided good explanations of the estimated genetic differentiation among colonies; in particular, there were 11 significant ($P < 0.01$) models with high correlation coefficients ($r > 0.6$). Typically, the step-wise addition of habitat types to the resistant ($C = 0$) category initially improved the explanation of the estimated genetic distance between populations followed by a steep decline in model fit (Fig. 2); when all habitat types

Table 3 Linearized F_{ST} values (above diagonal) and heterozygosity (along diagonal)

	1 (61)	2 (67)	3 (59)	5 (37)	6 (48)	9 (57)	15 (35)	19 (87)	20 (47)	106 (46)
1	0.714	0.103	0.064	0.093	0.07	0.136	0.133	0.083	0.061	0.125
2		0.610	0.122	0.109	0.123	0.194	0.119	0.075	0.116	0.183
3			0.767	0.111	0.049	0.095	0.119	0.106	0.064	0.097
5				0.652	0.107	0.131	0.108	0.059	0.091	0.133
6					0.682	0.116	0.107	0.089	0.062	0.112
9						0.595	0.142	0.134	0.063	0.054
15							0.660	0.097	0.1	0.129
19								0.670	0.096	0.141
20									0.659	0.077
106										0.614

Average number of individuals scored for the 11 loci is provided in parentheses below colony number

Fig. 2 Model fit (*r* value) based on the number of habitat classes in the infinite resistance category. Each subsequent model represents the previous model plus one additional land class; the best model for each number of land classes is represented. Because we observe genetic connectivity among colonies in Boulder County, models allowing movement through few land cover types provide poor explanations of the degree of genetic differentiation

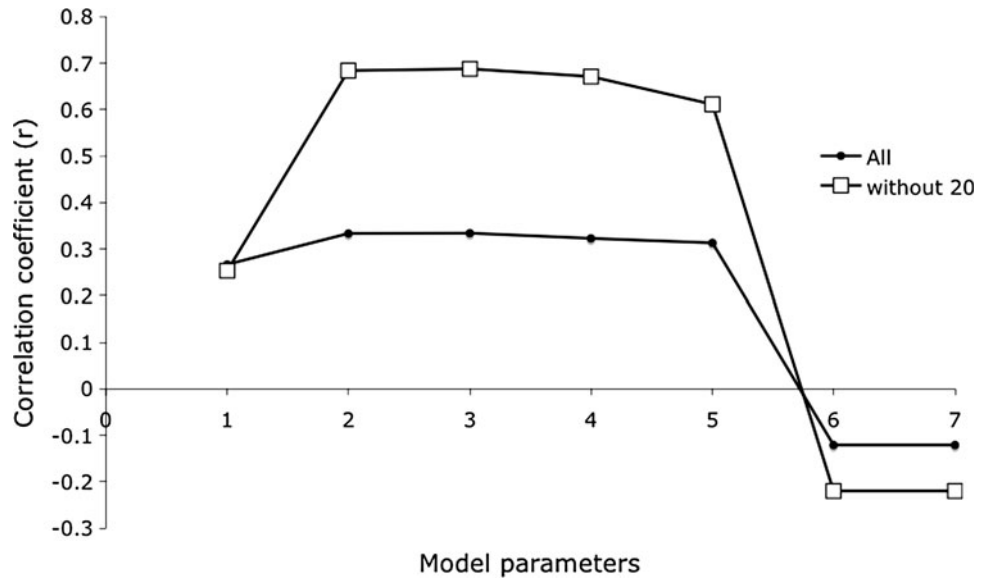


Table 4 Model scores for stepwise addition of variables to a particular conductance class

Model	<i>C</i> = 0	<i>C</i> = 100	<i>r</i>	<i>P</i>	
<i>Conductance categories = 2</i>					
N0		1, 2, 3, 4, 5, 6, 7, 8	-0.277	0.972	
N1	1	2, 3, 4, 5, 6, 7, 8	0.2523	0.089	
N5	1, 5	2, 3, 4, 6, 7, 8	0.6835	0.001	
N20	1, 5, 4	2, 3, 6, 7, 8	0.6870	0.001	
N55	1, 5, 4, 6	2, 3, 7, 8	0.6701	0.001	
N85	1, 5, 4, 6, 3	2, 7, 8	0.6103	0.002	
N100	1, 5, 4, 6, 3, 2	7, 8	-0.2201	0.926	
N121	1, 5, 4, 6, 3, 2, 7	8	-0.2201	0.930	
Model	<i>C</i> = 0	<i>C</i> = 25	<i>C</i> = 100	<i>r</i>	<i>P</i>
<i>Conductance categories = 3</i>					
N5	1, 5		2, 3, 4, 6, 7, 8	0.6835	0.001
N142	1, 5	2	3, 4, 6, 7, 8	0.6916	0.001
N147	1, 5	2, 4	3, 6, 7, 8	0.6910	0.003
N154	1, 5	2, 4, 6	3, 7, 8	0.6885	0.001
N171	1, 5	2, 4, 6, 3	7, 8	0.6810	0.001
N5	1, 5		2, 3, 4, 6, 7, 8	0.6835	0.001
N145	1, 5	6	2, 3, 4, 7, 8	0.6905	0.002
N150	1, 5	6, 3	2, 4, 7, 8	0.6915	0.003
N155	1, 5	6, 3, 4	2, 7, 8	0.6865	0.002
N171	1, 5	6, 3, 4, 2	7, 8	0.6810	0.001

Representative models are shown from two sets of models evaluated with respect to the number of conductance categories (4 conductance categories not shown). Landscape features: 1 = lakes and reservoirs; 2 = low intensity development; 3 = medium density development; 4 = high intensity development, 5 = wetlands; 6 = developed open space; 7 = forest (all types included), shrubs; 8 = grassland, pasture/hay, cropland, barren land. Model 5, the simplest model with good fit, is indicated in bold for comparison. A list of additional models is available upon request

except type 8 (grassland, barren land, pasture/hay and cropland) were resistant to movement, the model explained the estimated genetic differentiation poorly ($r = -0.2201$, $P = 0.93$). The simplest model that was among the eleven best models included only two habitat types that acted as barriers to conductance: lakes (type = 1) and wetlands (type = 5) (Table 4).

In all but one of the models with more than two conductance categories ($N = 24$ models), the correlation between genetic differentiation and resistance increased, although the magnitude of increase relative to the two conductance class models was slight (Table 4). Nonetheless, when the habitat categories with roads (namely 2 and 6) were moved from high to low conductance, there was an improvement in model performance, suggesting roads may reduce (but not eliminate) connectivity. Similarly, when medium and high intensity development (namely suburbanization and urbanization) were included in the lower conductance category ($C = 25$), there was a modest increase in the correlation coefficient, suggesting that buildings (and associated landscape features) inhibit prairie dog movement, although the magnitude of effect is much smaller than the estimated effects of lakes and wetlands. Placing both road types in the infinite resistance category resulted in the worst models ($r < 0$, $P > 0.9$). Including an additional conductance category ($C = 0, 25, 60$ and 100) did not notably improve model performance.

Of the many models that yielded roughly equivalent explanations of genetic differentiation, we selected two for visualization. The model with only lakes and wetlands in the $C = 0$ category (model N5) is perhaps the most defensible model because it involves postulating the fewest habitat features as barriers to prairie dog movement (Fig. 3a). We also chose to visualize the model with roads (LID = 2 and OS = 6, model N148) in the low conductance ($C = 25$) category (Fig. 3b). These models demonstrate that much of the land near where colonies occur is characterized by high connectivity, but colonies are separated from other populations by land cover allowing less movement. For instance, in model N148 (Fig. 3b), the City of Boulder (directly west of the “blue zone”), which consists of numerous small roads, appears as a moderate barrier to movement. Roads in the northeastern portion of the county appear as light blue lines inhibiting dispersal across them. A model with suburbanization and urbanization (MID = 3 and HID = 4, model N158) in the $C = 25$ category was not noticeably different than the simple model (model 5). The degree of similarity between models N5 and N158 showed that modification of the landscape by urbanization had little influence on inferred connectivity.

Overall, the set of best models of prairie dog connectivity (or conductivity) for Boulder County exhibited several features. First, there were several “islands” where

colonies were relatively isolated from all other colonies; especially the northernmost colonies (e.g. model N148, Fig. 3b). Second, the county was bisected into a northern and southern section by a large swath of area in the center of the county with very low probabilities of movement (i.e. the “blue zone”; Fig. 3). The blue zone included lakes and a perennial stream (Boulder Creek), and surrounding wetlands that trend west to east. Third, roads in the eastern part of the county appeared as barriers or areas of low connectivity, but in other places, roads (and the strip of grassland along the edges of roads) appeared to be corridors of connectivity, especially across wetlands.

Discussion

Our estimated maps of connectivity among prairie dog colonies within Boulder County provide evidence for a network of corridors that connect colonies separated by wetlands and urbanization. To test the effects of roads on connectivity, we moved the land types with roads (developed open space and low intensity development) from a high to low conductance category; the resulting models provided better estimates of the degree of genetic differentiation, suggesting that roads may inhibit movement. However, moving roads to a zero conductance category resulted in very poor models. One explanation is that roads may have contradictory effects depending on context. In some cases, roads may facilitate movement because open space along the margins of roads may provide an easy means of movement. Remarkably, prairie dog colonies often exist in the median of highways, completely bounded by busy roads, an observation underscoring that prairie dogs do manage successful crossings of major roads. In other contexts, roads may inhibit movement, especially relatively large roads that dissect the County. Furthermore, perpendicular road crossings and parallel road corridors running in the direction of prairie dog movement may exhibit potentially contrasting influences on connectivity.

The overall inhibitory effect of roads on prairie dog movement has implications for the spread of plague among populations, as well as subsequent recolonization of extirpated colonies. The existence of roads likely suppresses prairie dog mediated movement of *Y. pestis* among colonies (Collinge et al. 2005). This hypothesis is consistent with the restricted geographic distribution and slower spatial spread that characterized the most recent plague outbreak in Boulder County (Boulder Open Space and Mountain Parks, unpublished data). However, once populations are extirpated, the existence of roads could slow the recolonization process, limit the number of source populations, or prevent colonies from being recolonized altogether. Colonization from few sources is predicted to lead

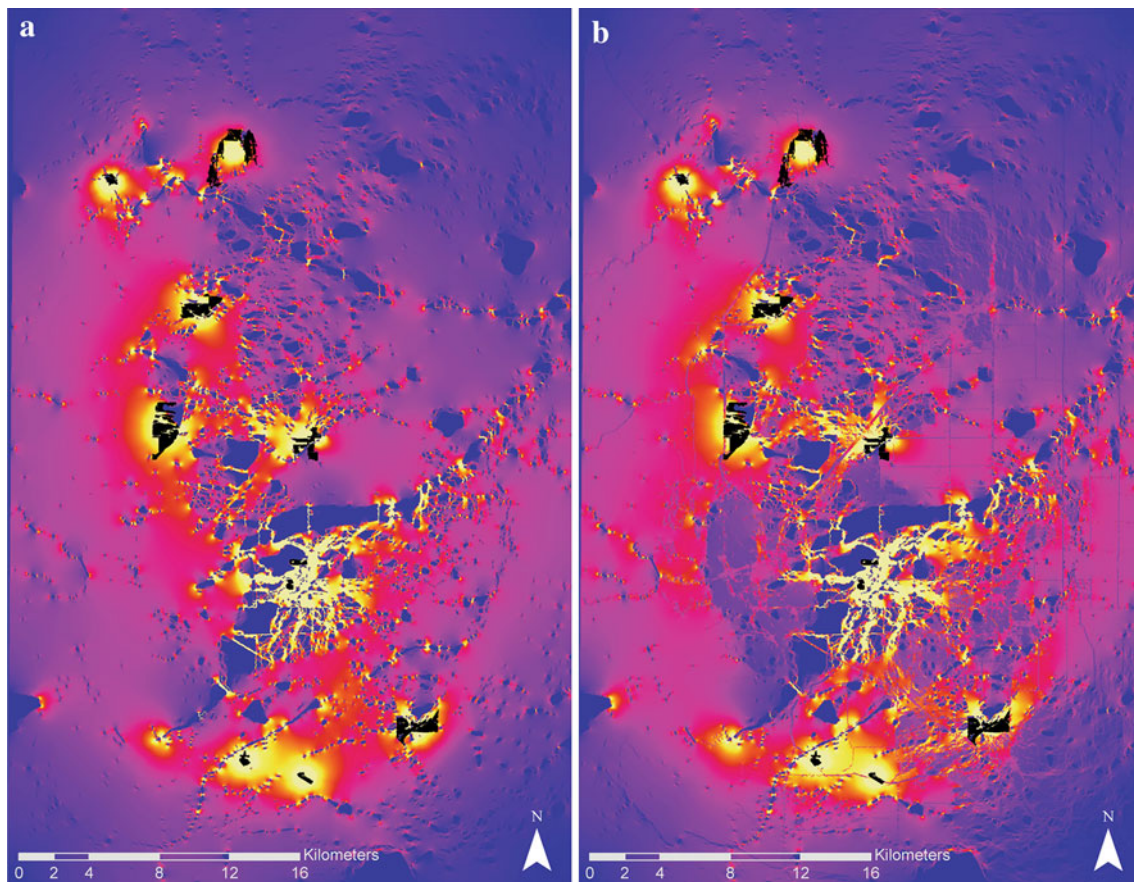


Fig. 3 Heat maps of the inferred connectivity of prairie dogs across Boulder County based on landscape models from Circuitscape: **a** the simplest model with good fit (model 5; $r = 0.6835$, $P = 0.001$) and **b** a model with roads at low conductance (model 148; $r = 0.6894$,

$P = 0.001$); *black* represents prairie dog colonies, *brighter colors* indicate greater connectivity, *bluedarker shades* indicate no connectivity

to founder effects and inbreeding (Templeton 1980). If extirpated populations are unable to be recolonized, the species may go locally extinct. The concomitant suppression of extinction and recolonization suggests that intermediate degrees of connectivity may lead to persistence of metapopulations where a virulent pathogen extirpates populations (via moderate barriers slowing the spread of the pathogen while still allowing sufficient recolonization).

Our results provide a foundation for further refinement of various models of connectivity, which can be assessed with data from a large number of populations. For instance, the effects of roads could be estimated directly by sampling on either side of multiple types of roads (e.g. divided vs. undivided highways, dirt roads, and roads running parallel vs. perpendicular to potential dispersal corridors). Landscape modifications may change the direction or overall length of corridors, leading to gene flow patterns that are altered in magnitude (Riley et al. 2006; Templeton et al. 2007) or direction (Moore et al. 2008; Spear and Storfer 2010). In fact, estimates of the number of effective migrants in our system are an order of magnitude lower

than those inferred in the natural landscape of the nearby Pawnee National Grasslands (Roach et al. 2001). Therefore, it is important to note that in altered landscapes, the magnitude of dispersal can be dramatically lower than in natural landscapes. Understanding the magnitude and direction of prairie dog movement will allow us to better predict when populations may be prone to invasion by *Y. pestis*, and to control plague outbreaks in areas of concern. For instance, knowledge of the most widely used habitat types for dispersal could allow for quarantine of infected populations, culling to prevent cross-species transmission or human exposure, or flea dusting of colonies located on dispersal corridors from infected populations. Furthermore, knowledge of dispersal corridors in a complex landscape could lead to predictions of the speed, spatial extent and pattern of local extinctions within a metapopulation that experiences extirpations in a non-random, spatial context (e.g. when extinctions are caused by a transmissible disease).

One challenge to evaluating models in the immense parameter space inherent in complex landscapes is that

there are a large number of potentially suitable models. The more complex the landscape, the more data are required to distinguish among potential hypotheses. In systems with a high degree of gene flow, data from many populations are likely needed to discern the effects of different combinations of land cover conductance (for example, low conductance for OS and high conductance for LID versus the opposite). We have restricted our search through the parameter space to a limited number of land cover conductance combinations with potentially large effects, in keeping with our modest dataset on population genetics. In some cases, estimates of genetic differentiation among nine colonies were not sufficient to choose among very different models of connectivity. For instance, correlation coefficients of models with high-intensity urbanization allowing high conductance (e.g. model 142, $r = 0.6916$) or providing high resistance (model 169, $r = 0.6906$) were very similar, suggesting the effect of HID development on connectivity is very small. While this may be true, it is more likely that the lack of effect of HID reflects the location of sampled colonies, and sampling a set of colonies around HID may provide a stronger signal. Nonetheless, urbanization, by itself, does not appear to strongly inhibit prairie dog movement.

An additional consideration in estimates of population connectivity is whether humans have managed those populations. Breeding programs and relocation are common management practices for many wildlife populations, and both have the potential to change genetic structure in a landscape. Our results, which inferred that one colony was affected by relocation practices, demonstrate the importance of explicitly addressing the effects of relocation on inferences drawn from genetic structure of populations. Many studies ignore the potential effects of management actions on population structure, but it is important to address human sources of genetic structure as they become increasingly common.

Prairie dogs in Boulder County inhabit an increasingly modified grassland environment that is carved into patches of grassland separated by urbanization, agriculture, and other land uses. As in most landscapes, some populations are more isolated than others by landscape features (e.g. roads and rivers) and changes in land cover (e.g. from grassland to agriculture or forest). Isolated colonies may also contribute to preserving regional genetic diversity (Templeton 2006) and be more insulated from the spread of pathogens such as *Y. pestis* (Collinge et al. 2005; Hess 1996). However, colonies that evolve in greater isolation may lose genetic variation over time, be more subject to genetic drift that overwhelms selection, accumulate deleterious mutations, or diverge from other populations (Templeton 2006). Thus, if roads isolate prairie dog colonies, they may be more protected from plague; however,

they may see a concomitant loss of genetic diversity because of reduced recolonization. It is important to consider the mechanisms that contribute to isolation in certain populations, and how isolated populations may contribute to evolution of the species (e.g. Templeton et al. 1990; Wright 1931) and disease transmission among populations.

Conclusions

The conceptual approach of landscape resistance modeling is a useful means of inferring how various land cover types affect an organism's ability to move from one population to another, with implications for trafficking of diseases such as sylvatic plague. Prairie dog connectivity in a complex landscape matrix is sustained by pasture, cropland and small roads, but impeded by large highways and heavy urbanization. Dispersal corridors among populations may promote the spread of pathogens, but they are critical to maintain genetic diversity within populations and to allow for re-colonization of extirpated demes. An intermediate degree of connectivity (both in terms of distance to nearest population and number of connected colonies) may be ideal in systems where a virulent pathogen periodically extirpates populations, such that disease transmission among populations is slowed, but recolonization is not hampered. The impacts of human alteration of natural landscapes change connectivity in complex ways (Collinge 2009; Storfer et al. 2010), thereby influencing which populations are connected to each other. Thus, understanding how various landscape matrices influence connectivity of different species will inform strategies for preserving and managing these corridors. Maintaining some degree of connectivity in complex, human-altered landscapes is crucial to the persistence of species across the globe, as an increasing percentage of land is converted to human uses such as urban centers and agriculture. Connectivity among populations allows recolonization to rescue extirpated populations, maintains genetic diversity within populations, and ultimately facilitates the long-term persistence of species.

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References

- Antolin MF, Savage LT, Eisen RJ (2006) Landscape features influence genetic structure of black-tailed prairie dogs (*Cynomys ludovicianus*). *Landsc Ecol* 21:867–875
- Antonio RR, Agostinho AA, Pelicice FM, Bailly D, Okado EK, Dias JHP (2007) Blockage of migration routes by dam construction: can migratory fish find alternative routes? *Neotrop Ichthyol* 5:177–184
- Barrowclough GF, Groth JG, Mertz LA, Gutiérrez RJ (2004) Phylogeographic structure, gene flow and species status in blue grouse (*Dendragapus obscurus*). *Mol Ecol* 13:1911–1922
- Beneteau CL, Mandrak NE, Heath DD (2009) The effects of river barriers and range expansion of the population genetic structure and stability in Greenside Darter (*Etheostoma blennioides*) populations. *Conserv Genet* 10:477–487
- Brinkerhoff RJ, Martin AP, Jones RT, Collinge SK (2011) Population genetic structure of the prairie dog flea and plague vector, *Oropsylla hirsuta*. *Parasitology* 138:71–79
- Collinge SK (2009) Ecology of fragmented landscapes. Johns Hopkins University Press, Baltimore
- Collinge SK, Johnson WC, Ray C, Matchett R, Grensten J, Cully JF Jr, Gage KL, Kosoy MY, Loye JE, Martin AP (2005) Landscape structure and plague occurrence in black-tailed prairie dogs. *Landsc Ecol* 20:941–955
- Coulon A, Guillot G, Cosson JF, Angibault JMA, Aulagnier S, Cargnelutti B, Galan M, Hewison AJM (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Mol Ecol* 15:1669–1679
- Decoster LA (2000) How forests are being nibbled to death by DUCs, and what to do about it: summary of the Forest Fragmentation 2000 Conference. Annapolis, MD
- Dieringer D, Schlotterer C (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Mol Ecol Notes* 3:167–169
- Garrett MG, Franklin WL (1988) Behavioral ecology of dispersal in the black-tailed prairie dog. *J Mamm* 69:236–250
- Goverde M, Schweizer K, Baur B, Erhardt A (2002) Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biol Conserv* 104:293–299
- Hauser L, Seamons TR, Dauer M, Naish KA, Quinn TP (2006) An empirical verification of population assignment methods by marking and parentage data: hatchery and wild steelhead (*Oncorhynchus mykiss*) in Forks Creek, Washington, USA. *Mol Ecol* 15:3157–3173
- Hess G (1996) Disease in metapopulation models: implications for conservation. *Ecology* 77:1617–1632
- Homer C, Huang CQ, Yang LM, Wylie B, Coan M (2004) Development of a 2001 national land-cover database for the United States. *Photogramm Eng Rem S* 70:829–840
- Jesse M, Ezanno P, Davis S, Heesterbeek JAP (2008) A fully coupled, mechanistic model for infectious disease dynamics in a metapopulation: Movement and epidemic duration. *J Theor Biol* 254:331–338
- Johnson WC, Collinge SK (2004) Landscape effects on black-tailed prairie dog colonies. *Biol Conserv* 115:487–497
- Jones PH, Britten HB (2010) The absence of concordant population genetic structure in the black-tailed prairie dog and the flea, *Oropsylla hirsuta*, with implications for the spread of *Yersinia pestis*. *Mol Ecol* 19:2038–2049
- Jones RT, Martin AP, Mitchell AJ, Collinge SK, Ray C (2005) Characterization of 14 polymorphic microsatellite markers for the black-tailed prairie dog (*Cynomys ludovicianus*). *Mol Ecol Notes* 5:71–73
- Lee-Yaw JA, Davidson A, McRae BH, Greens DM (2009) Do landscape processes predict phylogeographic patterns in the wood frog? *Mol Ecol* 18:1863–1874
- Levy E, Kennington WJ, Tomkins JL, Lebas NR (2010) Land clearing reduces gene flow in the granite outcrop-dwelling lizard, *Ctenophorus ornatus*. *Mol Ecol* 19:4192–4203
- Lopez JE, Gallinot LP, Wade MJ (2005) Spread of parasites in metapopulations: an experimental study of the effects of host migration rate and local host population size. *Parasitology* 130:323–332
- Magle SB, Ruell EW, Antolin MF, Crooks KR (2010) Population genetic structure of black-tailed prairie dogs, a highly interactive species, in fragmented urban habitat. *J Mamm* 91:326–335
- Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends Ecol Evol* 18:189–197
- McRae BH (2006) Isolation by resistance. *Evolution* 60:1551–1561
- McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proc Natl Acad Sci USA* 104:19885–19890
- McRae BH, Dickson BG, Keitt TH, Shah VB (2008) Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology* 89:2712–2724
- Miller SD, Cully JF Jr (2001) Conservation of black-tailed prairie dogs (*Cynomys ludovicianus*). *J Mamm* 82:889–893
- Moore JA, Miller HC, Daugherty CH, Nelson NJ (2008) Fine-scale genetic structure of a long-lived reptile reflects recent habitat modification. *Mol Ecol* 17:4630–4641
- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mol Ecol* 4:347–354
- Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Mol Ecol* 13:55–65
- Pavlacky DC, Goldizen AW, Prentis PJ, Nicholls JA, Lowe AJ (2009) A landscape genetics approach for quantifying the relative influence of historic and contemporary habitat heterogeneity on the genetic connectivity of a rainforest bird. *Mol Ecol* 18:2945–2960
- Perez-Espona S, Perez-Barberia FJ, McLeod JE, Jiggins CD, Gordon IJ, Pemberton JM (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Mol Ecol* 17:981–996
- Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. *J Hered* 95:536–539
- Ramankutty N, Evan AT, Monfreda C, Foley JA (2008) Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. *Global Biogeochem Cycles*. doi:10.1029/2007GB002952
- Riley SPD, Pollinger JP, Sauvajot RM, York EC, Bromley C, Fuller TK, Wayne RK (2006) A southern California freeway is a physical and social barrier to gene flow in carnivores. *Mol Ecol* 15:1733–1741
- Roach JL, Stapp P, VH B, Antolin MF (2001) Genetic structure of a metapopulation of black-tailed prairie dogs. *J Mamm* 82:946–959
- Schneider S, Roessli D, Excoffier L (2000) Arlequin version 2.00: a software for population genetics data analysis. U Geneva, Geneva

- Spear SF, Storfer A (2010) Anthropogenic and natural disturbance lead to differing patterns of gene flow in the Rocky Mountain tailed frog, *Ascaphus montanus*. *Biol Conserv* 143:778–786
- Spear SF, Peterson CR, Matocq MD, Storfer A (2005) Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Mol Ecol* 14:2553–2564
- Stapp P, Salkeld DJ, Franklin HA, Kraft JP, Tripp DW, Antolin MF, Gage KL (2009) Evidence for the involvement of an alternate rodent host in the dynamics of introduced plague in prairie dogs. *J Anim Ecol* 78:807–817
- Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: where are we now? *Mol Ecol* 19:3496–3514
- Telles MPC, Diniz JAF, Bastos RP, Soares TN, Guimaraes LD, Lima LP (2007) Landscape genetics of *Physalaemus cuvieri* in Brazilian cerrado: correspondence between population structure and patterns of human occupation and habitat loss. *Biol Conserv* 139:37–46
- Templeton AR (1980) Modes of speciation and inferences based on genetic distances. *Evolution* 34:719–729
- Templeton AR (2006) Population genetics and microevolutionary theory. John Wiley, Hoboken
- Templeton AR, Shaw K, Routman E, Davis SK (1990) The genetic consequences of habitat fragmentation. *Ann MO Bot Gard* 77:13–27
- Templeton AR, Neuwald JL, Brazeal H, Robertson RJ (2007) Restoring demographic processes in translocated populations: the case of collared lizards in the Missouri Ozarks using prescribed forest fires. *Israel J Ecol Evol* 53:179–196
- Trudeau KM, Britten HM, Restani M (2004) Sylvatic plague reduces genetic variability in black-tailed prairie dogs. *J Wild Dis* 40:205–211
- Wright S (1931) Evolution in Mendelian populations. *Genetics* 16:97–159