Multiscale population genetic analysis of mule deer (*Odocoileus hemionus hemionus*) in western Canada sheds new light on the spread of chronic wasting disease

C.I. Cullingham, S.M. Nakada, E.H. Merrill, T.K. Bollinger, M.J. Pybus, and D.W. Coltman

Abstract: To successfully manage wildlife diseases, it is necessary to understand factors that influence spread. One approach is to analyze host movement and social structure, as these behaviors can be associated with the probability of transmission. Some populations of mule deer (*Odocoileus hemionus hemionus* (Rafinesque, 1817)) in western Canada are infected with chronic wasting disease (CWD), a transmissible and fatal neurodegenerative disease. We used population analysis of spatial genetic structure of mule deer at broad and local scales to understand factors that influence spread. We genotyped 2535 mule deer sampled from Alberta, Saskatchewan, and portions of British Columbia using 16 microsatellite loci. We found weak genetic structure at broad spatial scales (overall F_{ST} = 0.008) that was well defined by geographic distance, indicating the risk of CWD spread from the focus of infection will decline gradually with increasing distance, but there are no barriers to the spread over time. At the local scale of approximately 2 km, elevated relatedness among CWD-infected individuals suggests transmission rates within social groups. Sex-biased spatial autocorrelation in genetic relatedness also indicates that female philopatry underlies the social structure, and therefore transmission among relatives is potentially driving local disease persistence.

Résumé : Afin de gérer avec succès les maladies de la faune sauvage, il est nécessaire de comprendre les facteurs qui influencent leur dissémination. Une méthode est d'analyser les déplacements et la structure sociale des hôtes puisque ces comportements peuvent être associés à la probabilité de transmission. Certaines populations de cerfs-mulets (*Odocoileus hemionus hemionus* (Rafinesque, 1817)) de l'Ouest canadien sont infectées par la maladie du dépérissement chronique (CWD), une maladie neurodégénérative transmissible et fatale. Une analyse de la structure spatiale génétique de la population de cerfs-mulets à des échelles étendues et locales nous a servi à comprendre les facteurs qui affectent la dissémination. Nous avons déterminé le génotype de 2535 cerfs-mulets échantillonnés en Alberta, en Saskatchewan et dans des régions de Colombie-Britannique à l'analyse de 16 locus microsatellites. Il existe une faible structure génétique aux échelles spatiales étendues (F_{ST} global = 0,008) qui est bien définie par la distance géographique, ce qui indique que le risque de transmission de la CWD à partir du foyer d'infection diminue graduellement en fonction de l'accroissement de la distance, mais qu'il n'y a pas de barrière à la transmission dans le temps. À l'échelle locale d'environ 2 km, la parenté accrue entre les individus infectés de la CWD laisse croire à une transmission à l'intérieur des groupes sociaux. L'autocorrélation spatiale de la parenté génétique avec biais sexuel indique aussi que la philopatrie des femelles sous-tend la structure spatiale et qu'ainsi la transmission entre individus apparentés explique potentiellement la persistance locale de la maladie.

[Traduit par la Rédaction]

Introduction

Populations of mule deer (*Odocoileus hemionus hemionus* (Rafinesque, 1817)) and white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)) in portions of the Canadian prairies are affected by chronic wasting disease (CWD). Chronic wasting disease is a transmissible spongiform encephalopathy that disrupts neurological function and ultimately leads to death (Williams and Young 1980; Williams 2005). This disease does not have a cure or preventative vaccine and can be transmitted from one animal to another potentially through saliva, urine, or blood from diseased individuals (Miller and Williams 2003; Mathiason et al. 2006; Haley et al. 2009; Tamgüney et al. 2009). Additional sources of infection may include environmental sources (Mathiason et al. 2009) caused by contamination from faeces (Tamgüney et

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al. 2009) and carcasses (Miller et al. 2004). Controlling and limiting spread of CWD is important, as it affects the economy (Bishop 2004; Kahn et al. 2004) and potentially the viability of wildlife populations (Gross and Miller 2001; Miller et al. 2008). There is no evidence for the transmission of CWD to humans; however, the potential implications for human health are not well studied (Belay et al. 2004; Decker et al. 2006).

The spread of a contagious and incurable disease in wide-ranging species like deer clearly presents a challenge to disease management. The first case of CWD in the prairie provinces was detected in 1996 in an elk (*Cervus elaphus* L., 1758) from a Saskatchewan cervid farming facility (Kahn et al. 2004). Surveillance programs in wild cervids were implemented in high-risk areas resulting in detection of CWD in a free-ranging mule deer from western Saskatchewan in 2000 (Bollinger et al. 2004). Hunting quotas were increased in areas with disease to survey the population and decrease deer densities to reduce infection rates (Bollinger et al. 2004). However, the spread continued and the first case in a wild mule deer in Alberta was detected in 2005. In Alberta, an aggressive management program was implemented where harvest was liberalized and targeted culls were performed from 2005 to 2008 in areas where CWD-positive individuals were identified through hunter submissions (Pybus 2007). Despite these control efforts, the disease persists and continues to spread.

Spatial patterns of population genetic structure at broad and local scales can provide useful information for understanding disease spread. For example, at broad scales, correlation between patterns of genetic differentiation and the presence of environmental or anthropogenic features can be used to assess barriers and corridors that regulate gene flow and dispersal (Manel et al. 2003; Balkenhol et al. 2009) and hence the direction of potential disease spread. At a local scale, spatial genetic autocorrelation indicates whether populations are socially structured by the clustering of relatives, and the scale of this process (Hardy and Vekemans 1999). Information on genetic structure has lead to the identification of the source of introduction of the West Nile virus to the Galápagos Islands (Bataille et al. 2009), and how landscape features affect vector (raccoons, *Procyon lotor* (L., 1758) movement of rabies (Real and Biek 2007; Cullingham et al. 2009).

Recently, population genetics has been used to understand how both landscape features and social structure affect CWD spread in white-tailed deer (Blanchong et al. 2008; Cullingham et al. 2011; Grear et al. 2010). Although these genetic studies on white-tailed deer have resulted in some relevant findings regarding CWD spread, understanding genetic structure of populations of mule deer in Canada may be particularly useful because in sympatric populations, CWD prevalence in mule deer is generally much higher than in white-tailed deer. Mule and white-tailed deer are closely related species (Polziehn and Strobeck 1998) and have similar life-history characteristics. They are both wide ranging, capable of dispersing >50 km (Robinette 1966; Rosenberry et al. 1999; Conner and Miller 2004), migratory in portions of their range (Nicholson et al. 1997; Nelson 1998), and have strong matriarchal social structure (Hawkins and Klimstra 1970; Kie et al. 2002). Despite these similarities, there are important differences that could result in different disease dynamics. Lingle (2003) and Lingle et al. (2007) studied sympatric populations of mule and white-tailed deer and found mule deer formed larger social groups, and physical associations within the social groups were more frequent. As well, Habib (2010) found group size to be greater for mule deer than white-tail deer with comparable population densities. The potential for increased social interactions provide opportunity for disease transmission (Altizer et al. 2003). Mule deer also have different responses to habitat than white-tailed deer (Whittaker and Lindzey 2004). For instance, mule deer tend to have larger home ranges than white-tailed deer (Whittaker and Lindzey 2004), which is further affected by landscape spatial heterogeneity (Kie et al. 2002; Brunjes et al. 2006). Larger home ranges could lead to increasing opportunities for contact, resulting in an increased chance of disease spread.

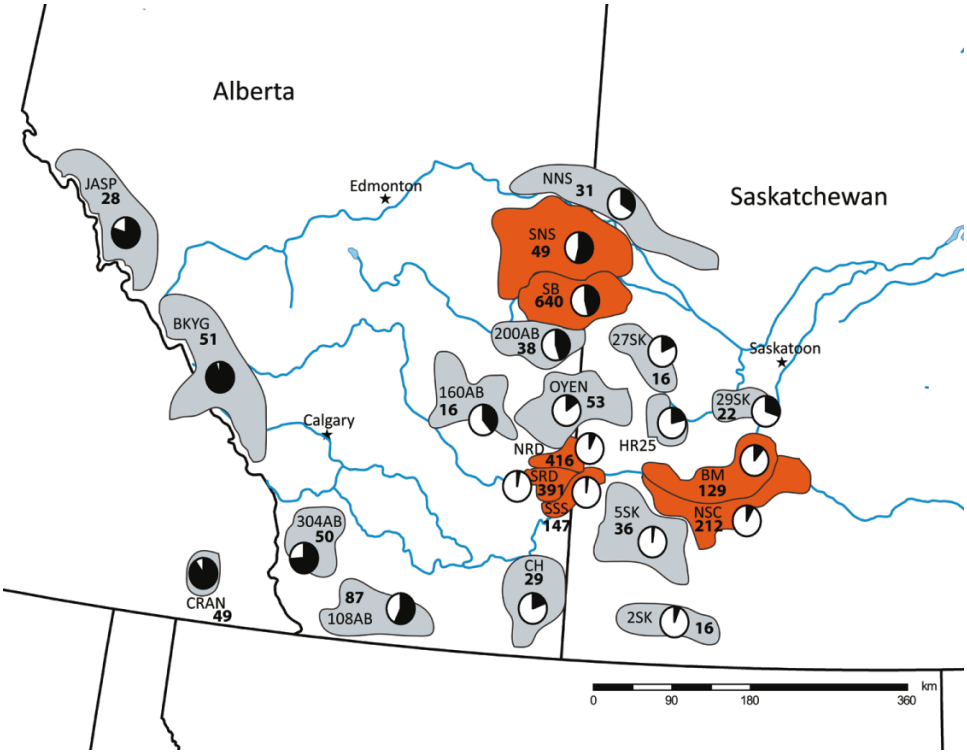
To investigate broad- and fine-scale population dynamics, we examined genetic structure of mule deer over an area extending from eastern British Columbia across central and southern Alberta to central Saskatchewan. We hypothesized that genetic differentiation would increase with spatial distance and across landscape features that are considered to restrict dispersal. Rivers have been identified as an impediment to dispersal for mule deer (Robinette 1966) and white-tailed deer (Blanchong et al. 2008); therefore, we tested for the effects of major rivers on differentiation in Alberta and Saskatchewan. As well, we also considered the Canadian Rocky Mountains, as they restrict dispersal in white-tailed deer (Cullingham et al. 2011). At the local scale, we expected female social structure to generate local-scale genetic structure, and therefore predicted relatedness among females to be significantly positive but not among males. Finally, social interactions have been shown to influence CWD spread in white-tailed deer (Grear et al. 2010), therefore we expected CWD-positive individuals to be more related than CWD-negative individuals.

Materials and methods

Study area and sample collection

We used 2535 samples collected from mule deer (1047 males, 1330 females, 158 unknown) across western Canada (Fig. 1). The sampled area consists of three terrestrial ecozones: (1) the montane–cordillera, which has a rugged, mountain terrain; (2) the boreal plains, which consists of low-lying valleys and plains dominated by boreal forest; and (3) the prairies, which have similar terrain to the boreal plains but are dominated by grassland–farmland (Natural Resources Canada 2010). The majority of the Alberta samples (N = 1930) were collected through the Alberta Fish and Wildlife CWD surveillance and control programs, consisting of hunter submissions and mule deer culled around the location of infected individuals. Additional samples of mule deer from their current distribution and high-risk areas for CWD transmission were obtained from a forensic database of Alberta mule deer (Jobin et al. 2008). Samples consisted of muscle biopsies and ear punches collected from 2005 to 2008 (November–April) and stored dry or in 98% ethanol at –20 °C. Samples from Saskatchewan (N = 556) were provided through Saskatchewan Environment surveillance and

Fig. 1. Location of 22 geographic areas sampled for mule deer (*Odocoileus hemionus hemionus*) in western Canada. Numbers in boldface type indicate sample sizes. Dark grey (in print but orange–brown on the Web) sample areas are the primary areas with chronic wasting disease in mule deer. Pie charts indicate cluster membership for $K = 2$ calculated in STRUCTURE version 2.3.1 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). Included are the major tributaries that run east–west across Alberta and Saskatchewan.



disease programs consisting of hunter submissions. Additional samples from Saskatchewan consisted of ear punches collected from anesthetized deer by researchers from the University of Saskatchewan and the Canadian Cooperative Wildlife Health Centre from 2003 to 2007 (November–April). Samples from British Columbia ($N = 49$) were acquired through road kills or hunter submissions as part of their CWD surveillance program and provided as DNA from 2002 or ear punches collected in 2006 and 2007. All samples were marked with a unique identifier given by the collecting agency with geo-referenced data including UTM coordinates, township coordinates, or wildlife management units, zones, or areas (WMU Alberta; WMZ Saskatchewan; WMA British Columbia). The majority of the samples had UTM coordinates with an accuracy of 500 m – 1 km ($N = 2185$), while the remainder ($N = 287$) had a maximum accuracy of 10 km. Additional information included sex, age, CWD status, and collection date.

Samples from Alberta were tested for CWD using the Bio-Rad TeSeE® test kit (Bio-Rad, Hercules, California, USA). This method is nationally approved in Canada and uses monoclonal antibodies to detect CWD-specific prion proteins. In Saskatchewan, CWD status was determined using standard immunohistochemistry with anti-TSE monoclonal F99/97.6.1 (VMRD Inc., Pullman, Washington, USA) on an automated immunostainer (Ventana Medical System, Tucson, Arizona, USA).

DNA extraction and microsatellite genotyping

DNA extractions were performed using the Qiagen 96 DNeasy® blood and tissue kit following the manufacturer's instructions (Qiagen, Mississauga, Ontario, Canada). Sixteen microsatellite loci were amplified in three multiplex reactions using a QIAGEN® multiplex PCR kit. The forward primer of each pair was fluorescently labeled. Details on primer sequences, multiplex panels, fluorescent labels, and PCR chemistry are provided in Table 1. The 5′ end of the reverse primers for markers P and Cervid1 were modified according to Brownstein et al. (1996). This allowed for complete adenylation of the 3′ forward strand to enhance accurate genotyping and to avoid overlapping allele ranges. All amplifications were performed on Mastercycler ep gradient thermocyclers (Eppendorf, Mississauga, Ontario, Canada) with the following cycles: 15 min at 95 °C; 33 cycles of 94 °C for 30 s, 60 °C for 90 s, and 72 °C for 90 s; and final extension at 72 °C for 30 min. PCR product was diluted to 1:40 and 3 μL of this was run on an ABI 3730 (Applied Biosystems, Foster City, California, USA) using GeneScan™ 500 LIZ™ as the size standard (ABI). Fragment analysis was performed manually using GeneMapper® version 4.0 (Applied Biosystems, Foster City, California, USA).

Genetic diversity measures

Four measures of global genetic diversity were calculated for each locus. Observed (H_O) and expected (H_E) heterozygosity (Nei 1978) were calculated using GenAlEx version 6 (Peakall and Smouse 2006), and mean number of alleles per locus (k) and Weir and Cockerham's F_{IS} (Weir and Cockerham 1984) per locus and overall were calculated using FSTAT version 2.9.3 (Goudet 2001). All individuals

were pooled to determine Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between all pairs of loci using GENEPOP version 3.4 (Raymond and Rousset 1995*a*). The Bonferroni correction (Bonferroni 1936) was applied to tests of statistical significance for HWE and LD.

Broad-scale population structure

Broad-scale population structure was analyzed using both a priori groups and an individual based analysis. For the a priori analysis, individuals were assigned to 22 sample areas based on spatial clustering and major rivers (Fig. 1). Exact tests of allelic differentiation were calculated among sample areas using GENEPOP version 3.4 (Raymond and Rousset 1995*a*). The effect of distance on genetic differentiation was estimated by the correlation between matrices of pairwise genetic distance, calculated as $F_{ST}/(1 - F_{ST})$, and the pairwise Euclidean distance (Rousset 1997) using a Mantel test (Mantel 1967). A partial Mantel test was used to test for the effect of rivers on genetic distance while correcting for geographic distance. The river matrix was generated manually and consisted of zeros between populations not separated by a river and ones if they were separated by a river. Genetic and geographic matrices were calculated using SPAGEDi 1.2 (Hardy and Vekemans 2002) and Mantel tests were conducted in zt (Bonnet and Van de Peer 2002).

Individual analysis was conducted on 2535 individuals from the 22 sample areas using a Bayesian clustering analysis implemented in the program STRUCTURE version 2.3.1 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). This analysis determines the number of populations (K) by assigning individual genotypes into clusters that minimize HWE and LD. Given the dispersal capability of deer, we used the sample area as a prior, as it has been shown to improve assignment when genetic differentiation is low (Excoffier and Heckel 2006; Hubisz et al. 2009). Five independent runs from K = 1–10 were used in the admixture model with correlated allele frequencies with a burn-in of 500 000 replicates and 1 000 000 Markov chain Monte Carlo steps. To infer the most likely K, we used the method outlined by Evanno et al. (2005) that looks at the relative rate of change in the mean posterior probabilities across the inferred K values. For the most likely K, we summarized the assignment of individuals across multiple runs using CLUMPP version 1.1.1 (Jakobsson and Rosenberg 2007). Individuals were assigned to a cluster if their probability was greater than 0.7.

Local population structure

We examined local population structure in three extensively and contiguously sampled regions: north border (n = 553), south border (n = 944), and the south Saskatchewan River valley (SSRV; n = 339) (Fig. 2). Fawns were included in the analysis, which was also restricted to individuals with UTM coordinates with an accuracy within 500 m. We estimated spatial autocorrelation of genetic relatedness for each region and sex using Moran's I statistic (Moran 1950). We used 2 km distance classes from 0 km (individuals sampled at the same geographic coordinates) to 22 km. Our choice of 2 km was based on sample density and distribution. Mean Moran's I and standard error (SE) in each distance class were estimated by jackknife resampling over loci. Significance was evaluated using the progressive Bonferroni approach (Hewitt et al. 1997). We used the lower limit of the distance class at which Moran's I first reached zero (Clark and Richardson 2002) to indicate the spatial scale at which animals were no longer related. To compare spatial genetic structure among regions, we quantified genetic relatedness based on a Sp statistic calculated as $-b/(1 - F_{(1)})$ (Vekemans and Hardy 2004), which is the slope of the correlogram (b) standardized by the relatedness at the first distance class ($F_{(1)}$). We calculated the error for this statistic using the standard deviation of the slope. This measure is recommended because it is not affected by sample distribution (Vekemans and Hardy 2004). Mean pairwise relatedness, R (Queller and Goodnight 1989), was also calculated for same-sex pairs of deer sampled from the same location (500 m resolution) within regions. All analyses were conducted using SPAGEDi version 1.2 (Hardy and Vekemans 2002).

Samples of 85 CWD-positive deer from the SSRV (39 females and 46 males) were matched to their closest noninfected, same-sex neighbor. This was the only region in which the sample size was sufficient to perform this analysis, and sample size necessitated analysis of the sexes combined. We calculated pairwise relatedness matrices separately for infected and noninfected neighbors using the estimator R (Queller and Goodnight 1989) in SPAGEDi version 1.2. This estimator requires allele frequency information, therefore we used all mule deer sampled within the SSRV (N = 339) as the reference population. To compare the distributions of the pairwise R estimates between infected and noninfected deer, we used a contingency test where values were grouped by 0.1 intervals from less than –0.4 to greater than 0.4. Because the distribution of pairwise relatedness are not independent points, we also used the nonparametric method employed in Cullingham et al. (2011), where two random samples are generated without replacement from all pairwise relatedness values and the difference in the means is calculated. Permutation of 10 000 samples generates a distribution to assess the significance (95%) of the actual difference in means.

Results

Genetic diversity measures

The mean number of alleles per locus (Table 2) was 10.3, ranging from 5 (INRA011) to 17 (BM4107 and Rt30). Mean measures of H_O and H_E per locus were 0.672 (SE = 0.179) and 0.683 (SE = 0.181), respectively. F_{IS} had a narrow range from 0.001 to 0.047 (mean = 0.016, SE = 0.012). Deviation from HWE ($F_{IS} > 0$) was nominally significant (α = 0.05) at six loci and overall, but only two loci and global F_{IS} remained significant after Bonferroni correction (α = 0.0031). No pairs of loci were in significant linkage disequilibrium after correction for multiple comparisons (α = 0.0022).

Broad-scale population structure

Global F_{ST} was significant but weak (0.008, $P < 0.0001$). Exact tests of allelic differentiation among the 22 sample areas (supplementary Table S1)[3] also supported genetic

[3] Supplementary Table S1 is available with the article through the journal Web site (http://cjz.nrc.ca).

Table 1. Characteristics of the 16 microsatellite multiplex kit including PCR chemistry and thermocycling parameters from white-tailed deer (*Odocoileus virginianus*), bison (*Bison bison* (L., 1758)), caribou (*Rangifer tarandus* (L., 1758)), bovine, and mule deer (*Odocoileus hemionus hemionus*).

Panel	Locus	Origin	Primer sequences	Primer conc. (μmol/L)	Size range (bp)	Label	References
1	Cervid1	White-tailed deer	5′-AAATGACAACCCGCTCCAGTATC-3′; **5′-GTTTCCGTGCATCTCAACATGAGTTAG-3′**	0.20	158–200	PET	DeWoody et al. 1995
	BBJ2	Bison	5′-GCACTTTAGCTCACTTCCTG-3′; 5′-ACACTGCCCCGGTATCTTTG-3′	0.04	168–190	VIC	DeWoody et al. 1995; Wilson and Strobeck 1999
	Rt30	Caribou	5′-CTGGTGTATGTATGCACACT-3′; 5′-CACTTGGCTTTTGGACTTA-3′	0.24	172–210	FAM	Wilson et al. 1997
	INRA011	Bovine	5′-CGAGTTTCTTTCCTCGTGGTAGGC-3′; 5′-GCTCGGCACATCTTCCTTAGCAAC-3′	0.02	191–215	NED	Vaiman et al. 1992; DeWoody et al. 1995
2	K	Mule deer	5′-GCAGGAAGGAGGAGACAGTA-3′; 5′-GCTGGTTCGTTATCATTTAGC-3′	0.10	175–215	PET	DeWoody et al. 1995; Jones et al. 2000
	BL25	Bovine	5′-AACAGTGGCAATGGAAGTGG-3′; 5′-AGTCAGGATCTAGTGGGTGAGTG-3′	0.06	178–194	VIC	Bishop et al. 1994; DeWoody et al. 1995
	Rt7	Caribou	5′-CCTGTTCTACTCTTCTTCTC-3′; 5′-ACTTTTCACGGGCACTGGTT-3′	0.24	208–242	VIC	DeWoody et al. 1995; Wilson et al. 1997
	BM6438	Bovine	5′-TTGAGCACAGACACAGACTGG-3′; 5′-ACTGAATGCCTCCTTTGTGC-3′	0.40	251–281	PET	Bishop et al. 1994; DeWoody et al. 1995
	BM848	Bovine	5′-TGGTTGGAAGGAAAACTTGG-3′; 5′-CCTCTGCTCCTCAAGACAC-3′	0.56	365–383	VIC	Bishop et al. 1994; DeWoody et al. 1995
3	Rt5	Caribou	5′-AATTCCATGAACAGAGGAG-3′; 5′-CAGCATAATTCTGACAAGTG-3′	0.32	140–162	VIC	DeWoody et al. 1995; Wilson et al. 1997
	BM4107	Bovine	5′-AGCCCCTGCTATTGTGTGAG-3′; 5′-ATAGGCTTTGCATTGTTCAGG-3′	0.40	140–172	PET	Bishop et al. 1994; DeWoody et al. 1995
	D	Mule deer	5′-AGAGCCTCGTCTTTTCATTC-3′; 5′-TTGCTGCTTGCTTGTCTAAT-3′	0.08	156–192	NED	DeWoody et al. 1995; Jones et al. 2000
	ETH152	Bovine	5′-AGGGAGGGTCACCTCTGC-3′; 5′-CTTGTACTCGTAGGGCAGGC-3′	0.16	174–208	FAM	Steffen et al. 1993
	BM6506	Bovine	5′-GCACGTGGTAAAGAGATGGC-3′; 5′-AGCAACTTGAGCATGGCAC-3′	0.28	186–206	VIC	Bishop et al. 1994; DeWoody et al. 1995

Table 1 (*concluded*).

Panel	Locus	Origin	Primer sequences	Primer conc. (μmol/L)	Size range (bp)	Label	References
	P	Mule deer	5′-TTTCACTGTTTTTCTCCTTCAGA-3′; **5′-GTTTCTTTGCCCAATCAGATGTTGTAG-3′**	0.16	213–248	FAM	Jones et al. 2000
	N	Mule deer	5′-TCCAGAGAAGCAACCAATAG-3′; 5′-GTGTGCCTTAAACAACCTGT-3′	0.32	282–342	NED	DeWoody et al. 1995; Jones et al. 2000

Note: Changes to original primer sequences are in boldface type. PCR chemistry: 10 μL total PCR reaction using ~25 ng of DNA, 5 μL of 2× Qiagen multiplex PCR master mix, 2 μL of 10× primer mix with varying final concentrations of each primer (see above) and 0.5 μL mQH_2O.

differentiation because there were 125 significant vs. 106 nonsignificant comparisons (α = 0.0002). Subpopulations along the Rocky Mountains ("mountains": CRAN, BKYG, JASP, 108AB, and 304AB; Fig. 1) were most different from other populations. Genetic distance was positively correlated with geographic distance between sample areas (Fig. 3; Mantel test, r = 0.78, P = 0.0001). There was no association of rivers with genetic distance when controlling for geographic distance (partial Mantel test, r = –0.05, P = 0.29).

From the individual-based analysis, we found weak support for two clusters (Table 3). The assignment of individuals into the two clusters was logical based on an isolation by distance pattern: mountain (304AB, BKYG, CRAN, and JASP) sample areas assigned to one cluster and prairie sample areas assigned to the other cluster (Fig. 1). The exceptions were sample areas that occur between the two clusters (108AB, 160AB, 200AB, SB, and SNS); the individuals here had equally likely probabilities of assigning to either cluster.

Local population structure

Similar patterns of spatial genetic autocorrelation were observed for the sex classes in each of the regions except for male deer in the SSRV (Fig. 4C). Female deer were genetically more related than males at distances up to 2 km (Figs. 4A–4C) with the highest autocorrelation at small lags in the south border (Sp = 0.005, 0.004–0.005), followed by the SSRV (Sp = 0.003, 0.002–0.004), and the north border (Sp = 0.002, 0.002–0.003). Females sampled at the same geographic coordinate in the SSRV (mean R = 0.105, SE = 0.024) and south border (mean R = 0.107, SE = 0.011) were approximately three times more related than those in the north border (mean R = 0.036, SE = 0.012). Small sample size prevented pairwise comparisons of male relatedness in the SSRV, but relatedness within the north border (mean R = 0.018, SE = 0.014) and south border (mean R = 0.009, SE = 0.012) were similarly low.

Within the SSRV, the distributions of pairwise related values (R) among CWD-infected and noninfected matched pairs each exhibited bell-shaped distributions (Fig. 5), and they were significantly different (χ^2 = 41.70, P < 0.0001). The difference in mean R between pairs of CWD-infected deer (0.009, SE = 0.011) and noninfected deer (–0.012, SE = 0.003) was significantly greater than expected based on permutation testing.

Discussion

The low levels of broad-scale genetic differentiation observed in mule deer is characteristic of species with high dispersal and associated gene flow across a large, continuous area (Slatkin 1981). The low global F_{ST} (0.008) of mule deer in western Canada was comparable with values reported in other populations of mule deer (0.009–0.032) across North America (Smith et al. 1990; Cronin 1991; Scribner et al. 1991; Travis and Keim 1995), and similar to what was found for sympatric populations of white-tailed deer (F_{ST} = 0.006; Cullingham et al. 2011). Although genetic differentiation was weak, the pattern is well described by geographic distance. This result contrasted sharply with

Fig. 2. Locations of sampled mule deer (*Odocoileus hemionus hemionus*) from three areas: north border, south border, and the south Saskatchewan River valley (SSRV).

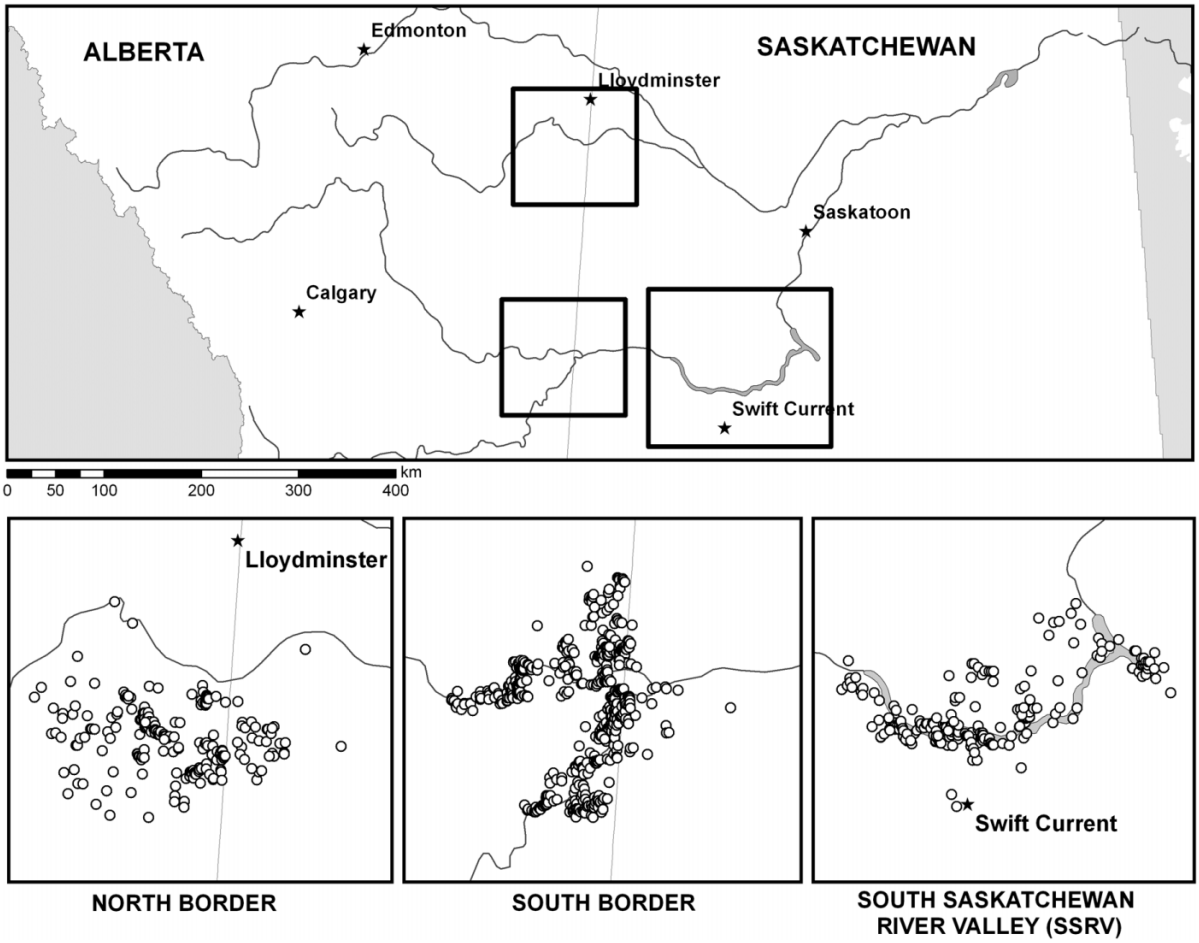


Table 2. Diversity statistics for 16 microsatellite loci and overall calculated from the global population of mule deer (*Odocoileus hemionus hemionus*) (2535 individuals).

Locus	*k*	H_O	H_E	F_{IS} (W&C)	*P*
BBJ2	9	0.768	0.775	0.009	0.1938
BL25	8	0.672	0.689	0.025	0.0219
BM4107	17	0.635	0.667	0.047	0.0031
BM6438	9	0.605	0.606	0.003	0.4688
BM6506	8	0.749	0.749	0.001	0.475
BM848	8	0.758	0.774	0.021	0.025
Cervid1	15	0.757	0.786	0.037	0.0031
D	7	0.412	0.415	0.008	0.3219
ETH152	12	0.861	0.869	0.009	0.1281
INRA011	5	0.155	0.159	0.024	0.0563
K	7	0.653	0.661	0.011	0.1531
N	14	0.840	0.853	0.015	0.0375
P	7	0.564	0.572	0.014	0.1656
Rt30	17	0.794	0.801	0.010	0.1125
Rt5	9	0.765	0.772	0.010	0.1688
Rt7	13	0.771	0.783	0.015	0.0469
Overall	10.3	0.672	0.683	0.016	0.0031

Note: *k* is the number of alleles per locus; H_O is the observed heterozygosity; H_E is the heterozygosity expected under Hardy–Weinberg equilibrium; and F_{IS} (W&C) is the measure of inbreeding according to Weir and Cockerham (1984).

sympatric white-tailed deer, where geographic distance explained very little of the variation in genetic differentiation (Cullingham et al. 2011). This could be the result of differences in genetic equilibrium between the species. White-tailed deer have experienced population increases in the western provinces (Wishart 1984; Natural Resources Service 1995); consequently, their population is not likely at mutation–drift equilibrium and there will therefore be little association of geographic distance with genetic distance (Hutchison and Templeton 1999) Increased relatedness among female mule deer at the local level supports previous observations that the social structure of mule deer is driven by female philopatry (Bowyer 1984; Lingle 2003). Pairwise relatedness among infected mule deer was significantly higher than among sympatric noninfected mule deer, providing evidence that interactions within related social groups may increase the risk of local CWD transmission.

Although we did not observe strong genetic discontinuity in mule deer across western Canada, significant allelic differentiation, strong isolation by distance, and a weak but statistically significant Wahlund effect (i.e., a deficit of heterozygosity which arises as a result of the pooling of subpopulations that have slightly different allele frequencies; Hedrick 2000) indicate that they are not panmictic either. The isolation by distance relationship suggests a high potential

Fig. 3. Genetic distance ($F_{ST}/(1 - F_{ST})$) and geographic distance (km) for pairwise comparisons of 22 sample areas with mule deer (*Odocoileus hemionus hemionus*). Global F_{ST} was 0.008. Genetic and geographic distances were positively correlated ($r = 0.78$, $P < 0.001$).

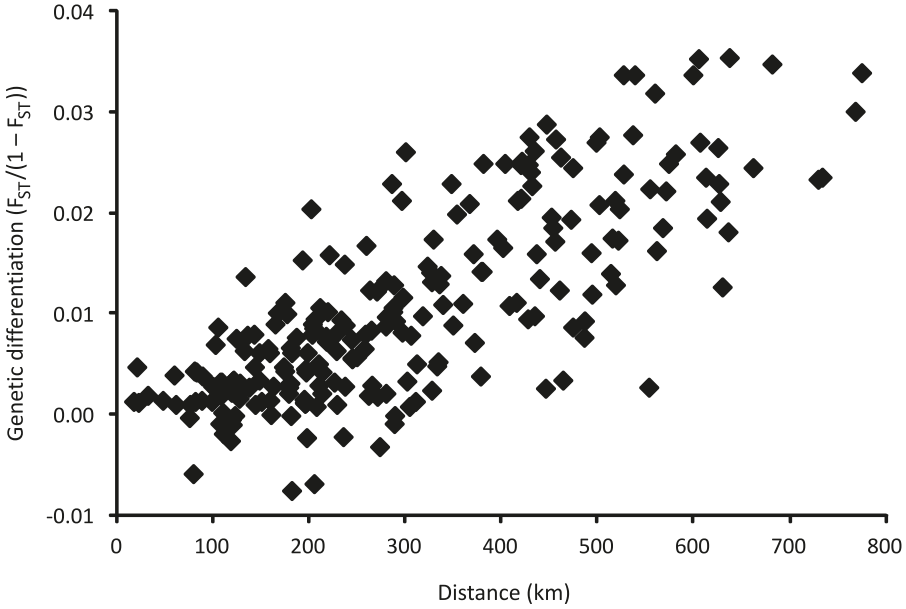


Table 3. Results from Bayesian clustering analysis of 2535 mule deer (*Odocoileus hemionus hemionus*) across British Columbia, Alberta, and Saskatchewan using the admixture model with correlated allele frequencies in STRUCTURE version 2.3.1 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009).

	L(*K*)		
K	Mean	SD	Δ*K*
1	–119028	0	0
2	**–118365**	**27**	**17.6**
3	–118177	84.6	1.1
4	–118085	126	1.8
5	–118226	197.1	0.7
6	–118222	201.7	1.2
7	–118675	410.1	0.1
8	–118736	140.9	2.5
9	–119366	1074.7	0.1
10	–119853	1710.8	0.3

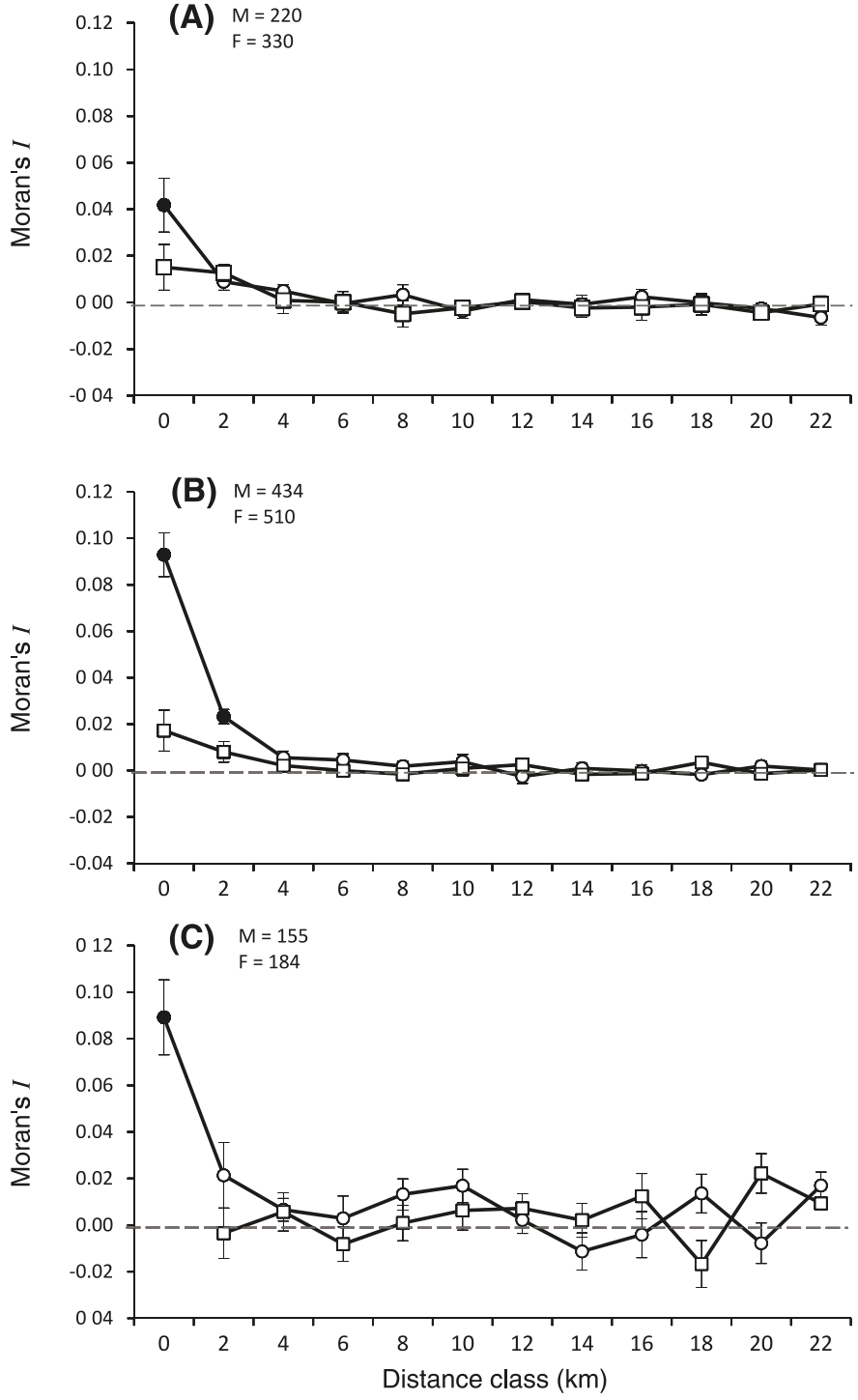
Note: *L*(*K*) is averaged across five separate iterations for each value of *K*. Selection of the most likely value of *K* is two (indicated in boldface type) based on the degree of change in the mean value of *L*(*K*) (Evanno et al. 2005), indicated as Δ*K*.

for continued spread of CWD through dispersal of infected individuals, with a risk function that decreases with geographic distance from foci of infection. This should result in a pattern of CWD spread that resembles a point source with outward diffusion, as documented for bovine tuberculosis in white-tailed deer (Schmitt et al. 1997). However, there has been considerable heterogeneity observed in the pattern of CWD prevalence that has been related to different spread parameters, including history of disease in the area and habitat diversity (Conner and Miller 2004; Miller et al. 2004; Wolfe et al. 2004; Heisey et al. 2010). Migratory behavior may also contribute to CWD spread (Conner and Miller 2004) and the populations we studied are partially migratory, with rates and distances migrated varying across the study area. For example, within the SSRV, 42% of adult mule deer are migratory with median migration distances of 16 km for males and 19 km for females and maximum migration distances of 65 and 113 km, respectively (Skelton 2010); within the north border, approximately 25% of deer migrate for winter, generally <50 km (E.H. Merrill, personal communication). Interactions among migratory deer could result in increasing the geographic extent of the disease more rapidly than dispersal.

At the local scale, host density can also play an important role in transmission patterns (Arneberg et al. 1998), especially in social mammals where interactions within social groups can increase the probability of disease spread (Altizer et al. 2003). Because we found infected individuals to be more related than noninfected matched pairs (Fig. 5), social structure likely plays a role in local transmission of CWD among mule deer. The elevated transmission of disease among relatives has also been observed in bovine tuberculosis and CWD among white-tailed deer (Blanchong et al. 2007; Grear et al. 2010).

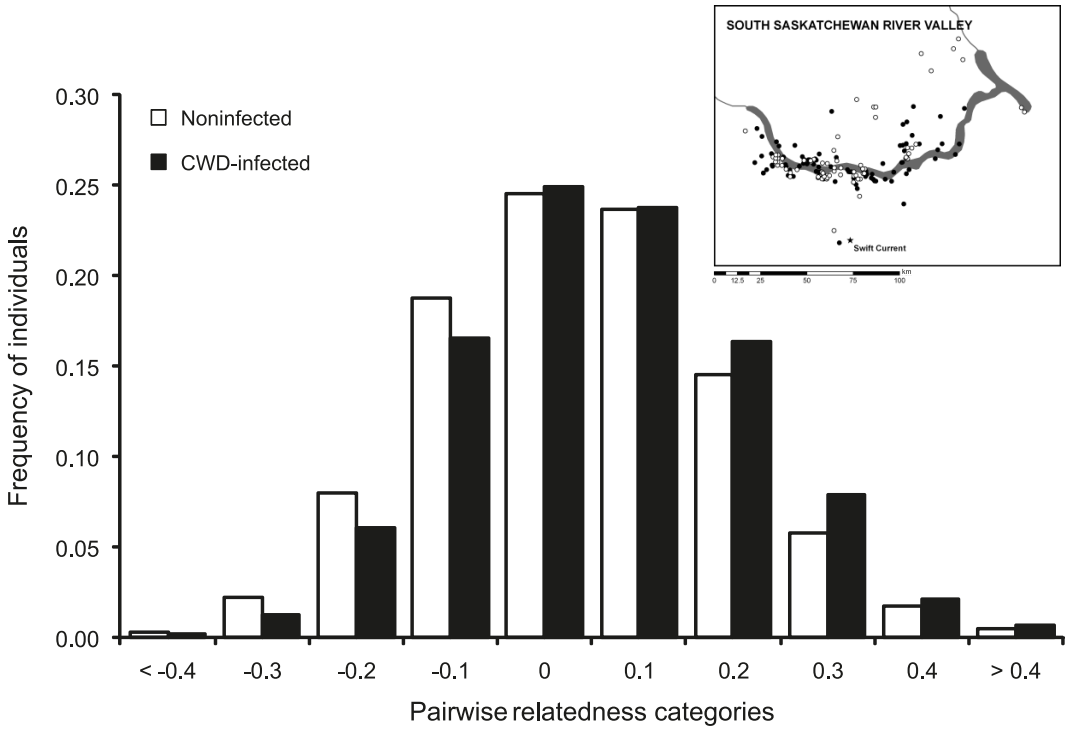
Estimating the extent of social structure might help to resolve the relevant scale of local CWD transmission risk for management. Patterns of genetic spatial autocorrelation reveal areas of homogeneity at local scales that reflect family size (Sokal and Oden 1978), or the radius of genetic neighborhoods (Clark and Richardson 2002). Because female mule deer tend to form small matrilineal groups consisting of does, their fawns, and sometimes female yearlings (Mackie et al. 1982; Bowyer 1984; Mathews and Porter 1993), local-scale structure is likely driven by female philopatry. Accordingly, we found short distance positive autocorrelation in all three regions with significant relatedness only among females (Figs. 4A–4C). Matrilineal social structure is considered typical of mammals (Greenwood 1980)

Fig. 4. Spatial autocorrelation measured by Moran's *I* coefficient for pairs of male (□) and female (○) mule deer (*Odocoileus hemionus hemionus*) in the north border (A), south border (B), and the south Saskatechwan River valley (SSRV) (C). The mean of all pairwise comparisons is shown for each distance class. At 0 km, individuals (500 m resolution) were sampled at the same geographic coordinates. Error bars are ±1 standard error. Open and solid markers are nonsignificant and significant distance classes, respectively, after progressive Bonferroni correction. There were insufficient data to estimate Moran's *I* for males at 0 km in C.



and has been documented for white-tailed deer (Purdue et al. 2000; Comer et al. 2005; Cullingham et al. 2011). Given the relationship between CWD status and social structure, it is reasonable to hypothesize that regions with more pronounced social structure will have higher disease prevalence because of elevated rates of transmission. Given this, the scale for local management to control disease spread among relatives would be within approximately 2 km of infected individuals. However, this is based on female social structure, and given the differences between the sexes, gender-based

Fig. 5. Distribution of relatedness in CWD-infected and noninfected mule deer (*Odocoileus hemionus hemionus*) in the south Saskatchewan River valley (SSRV). The map indicates the location of all 85 CWD-positive deer (●) and their respective same sex, noninfected, closest spatial neighbor (○). The distributions of relatedness between CWD-infected and noninfected deer were significantly different ($\chi^2 = 41.69$, $P < 0.001$).



management should be considered (Fenichel and Horan 2007). This also does not take into account disease transmission as a result of the environment, which over time may play a larger role in local transmission (Miller et al. 2006).

The relationship between social structure and increased transmission is also supported by the patterns of disease prevalence and female-biased fine-scale genetic structure of the north border, south border, and SSRV. Prevalence is greatest in the SSRV (~5%), where females are more highly related. Prevalence is lowest in the north border (~0.13%), where females are less related (Figs. 4A, 4B). In the south border, relatedness among females is as high as in the SSRV yet prevalence is not at the same level. However, based on the history of CWD in western Canada, prevalence should be greatest in the SSRV because the first case was documented here 3 years prior to the first case in the south border (Bollinger et al. 2004). Differences in relatedness among female mule deer in these regions could be attributed to habitat differences. The north border encompasses aspen parklands, whereas the river valley to the south (south border and SSRV) is surrounded by more open prairie. Where woody cover is reduced, there are higher pairwise contact rates (Habib 2010) and the formation of tightly linked social groups for effective defense strategies (Lingle 2003; Lingle et al. 2007). As well, differences in density and sampling could also affect the estimation of relatedness parameters.

Eradication of CWD is no longer deemed feasible in Alberta and Saskatchewan (Langenberg et al. 2008; Saskatchewan Ministry of the Environment 2008); however, limiting spread of the disease is considered possible. Our results lead to two recommendations for CWD management and surveillance. At the broad scale, there are no barriers to gene flow. Therefore, dispersing infected deer could carry the disease across long distances and spark new foci of infection. Hence, surveillance should extend to noninfected areas at least to the distance of maximum dispersal of mule deer, which can exceed 100 km (Skelton 2010). At the local scale, we have found that risk of CWD infection is higher among kin. We have also found that the scale of kin structure extends to approximately 2 km. Therefore, management should focus control measures such as targeted culls with an approximate 2 km radius to reduce persistence. This distance should be expanded based on knowledge of home-range size and seasonal migratory patterns in the area. Not only do our results help inform current management, this information will be beneficial to future management options. For instance, if an effective vaccine is developed (Pilon et al. 2007), we now have an understanding of the spatial scale where we expect to see disease transmission within social groups among relatives. This information can be used to develop strategies to reduce disease prevalence.

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