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Hybrid swarm between divergent lineages of mule deer (*Odocoileus hemionus*)

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Abstract

Studies of hybrid zones have revealed an array of evolutionary outcomes, yet the underlying structure is typically characterized as one of three types: a hybrid zone, a hybrid swarm or a hybrid taxon. Our primary objective was to determine which of these three structures best characterizes a zone of hybridization between two divergent lineages of mule deer (*Odocoileus hemionus*), mule deer and black-tailed deer. These lineages are morphologically, ecologically and genetically distinct, yet hybridize readily along a zone of secondary contact between the east and west slopes of the Cascade Mountains (Washington and Oregon, USA). Using microsatellite and mitochondrial DNA, we found clear evidence for extensive hybridization and introgression between lineages, with varying degrees of admixture across the zone of contact. The pattern of hybridization in this region closely resembles a hybrid swarm; based on data from 10 microsatellite loci, we detected hybrids that extend well beyond the F1 generation, did not detect linkage disequilibrium at the centre of the zone and found that genotypes were associated randomly within the zone of contact. Introgression was characterized as bidirectional and symmetric, which is surprising given that the zone of contact occurs along a sharp ecotone and that lineages are characterized by large differences in body size (a key component of mating success). Regardless of the underlying mechanisms promoting hybrid swarm maintenance, it is clear that the persistence of a hybrid swarm presents unique challenges for management in this region.

Keywords: admixture, black-tailed deer, Cascade Mountains, ecotone, hybrid zone, introgression

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Introduction

During the divergence of evolutionary lineages, natural selection drives the evolution of ecological specialization and genetic differentiation of populations in alternate environments. In the classic view of speciation, divergence occurs in allopatry, requiring both geographical and reproductive isolation (Dobzhansky 1937; Mayr 1942). Divergence during speciation, however, is a continuous process, influenced by temporal, genetic and geographic factors (Mallet *et al.* 2007; Nosil *et al.* 2009). As a result, there are numerous examples demonstrating variation in the degree of speciation (e.g. Dopman *et al.* 2005; Nosil 2007). In cases where reproductive isolation is incomplete, secondary contact between allopatrically diverged lineages has the potential to reduce divergence between populations through gene flow and hybridization (Taylor *et al.* 2006; Seehausen *et al.* 2008). The study of hybrid zones thus provides insight into the nature of boundaries between lineages and the process of ecological divergence. In addition to the role of hybrid zones as a fundamental evolutionary process, recent studies of hybrid zones have highlighted their importance for taxonomy and systematics (e.g. Larsen *et al.* 2010; Brelsford *et al.* 2011)

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or to offer insight into mechanisms behind reproductive isolation and speciation (e.g. Nolte *et al.* 2009; Johannesson *et al.* 2010; Munoz *et al.* 2010; Field *et al.* 2011; Lexer *et al.* 2011). Recently, the role of hybridization in species invasiveness has been explored (Ellstrand & Schierenbeck 2000; Wolfe *et al.* 2007), illustrating the potential for hybrid zones to drastically alter the evolutionary trajectory of lineages.

The evolutionary outcomes of hybridization are variable and depend on a variety of factors, including the fitness of hybrid offspring, strength of selection, patterns of gene flow and features of the landscape (Barton 1979, 2001; Barton & Hewitt 1985; Buerkle *et al.* 2000; Jiggins & Mallet 2000; Duenez-Guzman *et al.* 2009; Lepais *et al.* 2009). Despite the complexity of factors involved in hybridization dynamics, the underlying structure of a hybrid zone generally falls into one of three main types: a hybrid zone, a hybrid swarm or a hybrid taxon. Narrow hybrid zones may be produced if reproductive barriers between parental taxa are reinforced (Barton & Hewitt 1985). This can occur if gene flow between parental taxa continues to produce F1 hybrids, but those hybrids exhibit reduced fitness compared to parentals (tension zone model; Bazykin 1969; Barton & Hewitt 1985). Alternatively, reproductive barriers between parental lineages may be reinforced if F1 hybrids exhibit higher fitness than parentals in a limited set of environmental conditions, such as along an ecotone (bounded superiority model; Moore 1977). Hybrid zones produced by a balance between gene flow into a zone of contact and assortative mating, either within parental lineages or in the hybrid population, can remain stable for many generations (Lukhtanov *et al.* 2005; Nosil & Yukilevich 2008; Ortiz-Barrientos *et al.* 2009). If reproductive isolation is weak between parental taxa, F1 hybrid offspring may be able to backcross to parentals or cross with other hybrids to create a hybrid swarm (Harrison 1993; Levin *et al.* 1996; Avise *et al.* 1997; Rubidge & Taylor 2004; Taylor *et al.* 2006; Fitzpatrick & Shaffer 2007). Hybrid swarms may remain stable (Hubbs 1958; Olson 1981; McDevitt *et al.* 2009) or may spread, fusing parental taxa into a single lineage (Avise *et al.* 1997; Fitzpatrick & Shaffer 2007). A new species of hybrid origin may be formed if F1 hybrids are formed and are reproductively viable, but cannot backcross with parentals. Most commonly, this has been shown to occur in cases where hybridization causes a change in chromosome number (Arnold 1997; Rieseberg 1997; Buerkle *et al.* 2000; Otto & Whitton 2000; Gompert *et al.* 2006; Mavarez *et al.* 2006; Mallet *et al.* 2007; Duenez-Guzman *et al.* 2009). Once the evolutionary outcome of hybridization has been characterized, it becomes possible to investigate the relative roles of ecological and genetic factors that influence hybridization and make predictions about the dynamics of the system. These predictions are crucial not only to our understanding of hybrid zone dynamics, but also to our ability to appropriately conserve and manage hybridizing species.

Mule deer (*Odocoileus hemionus*) represent an ideal system for investigations into hybrid zone dynamics. This species is traditionally grouped into two morphologically distinct lineages, mule deer (MD; subspecies *O. h. hemionus*, *fulginatus*, *californicus*, *inyoensis*, *eremicus*, *peninsulae*, *sheldoni* and *cerrosensis*) and black-tailed deer (BTD; subspecies *O. h. columbianus* and *sitkensis*). Morphological differentiation generally centres on facial markings and morphology of the tail and metatarsal glands, although traits are largely overlapping between lineages (Taylor 1956; Wallmo 1981). MD and BTD occur in very distinct habitats; MD are found in coniferous forests, meadows, aspen woodlands and alpine tundra in the summer and montane forest, shrub/grass communities and desert shrub in the winter (Wallmo 1981). Alternatively, BTD do well in early successional habitat (5–20 years following clearcuts) in the southern part of their range, but rely on old growth forests farther north (Brown 1961; Wallmo 1981).

Morphological and ecological distinction between these two groups is supported by genetic data, with the level of divergence between MD and BTD lineages reaching 6–7.7% in mitochondrial DNA (Carr *et al.* 1986; Cronin *et al.* 1988; Cronin 1991; Carr & Hughes 1993; Latch *et al.* 2009). This level of divergence is on par with species-level divergence for most mammals (Avise *et al.* 1998). Nuclear markers exhibit less, although still notable, levels of divergence between lineages (Gavin & May 1988; Cronin 1991; Cathey *et al.* 1998). Lineages of MD and BTD diverged in allopatry in separate refugia during the last glacial maximum (Latch *et al.* 2009). Following glacial re-treat, MD and BTD lineages expanded from refugia to meet in a contact zone that is broadly coincident with the Cascade Mountain range. Despite some evidence for within-lineage mate preferences (Muller-Schwarze & Muller-Schwarze 1975), hybridization between MD and BTD lineages has been documented along the zone of contact (Jackson 1921). However, most incidents of hybridization between these lineages go unreported in the literature because these lineages are currently considered to be a single species. Thus, the extent and pattern of hybridization in the region is unknown. Characterization of the genetic structure of the hybrid zone across the landscape is a necessary first step towards understanding the dynamics of hybrid zones and their role in evolution.

Given the sharp ecotone that separates the eastern and western slopes of the Cascade Mountains, we might expect a smooth clinal transition between

lineages (Bazykin 1969; Moore 1977; Barton & Hewitt 1985). A large difference in body size between lineages (MD often twice as large as BTD; Brown 1961; Wallmo 1981; Kie & Czech 2000) and a correlation between body size and mating success (Miller 1974; Kucera 1978) suggest that patterns of admixture within the zone of contact might also be asymmetric. In the current study, we present a broad-scale survey of genetic variation within *O. hemionus* in the zone of contact between MD and BTD lineages. Our objectives were to characterize the extent and pattern of hybridization using both nuclear and mitochondrial markers, to determine whether hybridization between these lineages has resulted in a hybrid zone, a hybrid swarm or a hybrid taxon. By investigating the patterns of hybridization across the landscape, we also aim to identify processes that may have led to the observed pattern of hybridization. Our findings generate evidence that should be taken into account for future management of deer in the Pacific Northwest and provide the foundation for future inquiries to evaluate potential mechanisms shaping hybrid zone dynamics in this system.

Methods

Sample collection

Deer tissue samples were collected opportunistically during state-specific (Oregon and Washington) hunting seasons. The sex ratio of samples was biased towards males (77% males) given our use of hunter-harvested samples; however, we were able to collect tissue samples from females opportunistically. Tissue samples were placed in vials containing silica desiccating beads and stored at −80 °C until analysis. The location of collection was noted for each sample, most often as precise geographical coordinates. For less specific collection location descriptions [e.g. Game Management Unit (GMU)], we utilized Hawthstools (Beyer 2004) in ArcGIS 9.2 to randomly generate unique points for all deer sampled within that GMU. Originally, 200 deer were collected from 1995 to 2005 and subsequent sampling during 2009 added 210 additional samples. In total, 227 deer were collected from Oregon and 183 were obtained in Washington. Although hybrid zones can move and fluctuate rapidly, we did not observe any qualitative differences in the results obtained between the 2009 sample set and the set of samples collected earlier and thus all samples were pooled for analysis.

Laboratory methods

DNA from tissues collected during 1995–2005 was extracted using a modified ammonium acetate method (Latch *et al.* 2009), and 2009 samples were extracted using Qiagen DNeasy tissue spin column protocols. Extracted products were then amplified at ten microsatellite loci developed in *O. hemionus* (Odh_E, Odh_K, Odh_C, Odh_O, and Odh_G; Jones *et al.* 2000), *Bos taurus* (BM848; Bishop *et al.* 1994), *Cervus elaphus* (C273 and T40; Meredith *et al.* 2005) and *Rangifer tarandus* (RT24; Wilson *et al.* 1997) following PCR protocols previously described in Latch *et al.* (2008). Amplified products were visualized on an ABI3700 DNA Analyzer at the University of Wisconsin–Madison Biotechnology Center (UWBC), and allele sizes were determined using the program GENEMARKER (SoftGenetics, LLC). We assessed overall genotyping repeatability by reamplifying and regenotyping ~10% of the genotypes (n = 384 repeated genotypes, spread across all 10 loci). We observed only one mismatch (1/4100 = 0.024% error) between replicate genotypes that was attributed to allelic dropout and was corrected. The completed data set contained 1.6% missing data (range 0.2–1.9% per locus), with a maximum of three missing genotypes per individual.

We amplified a 730-bp portion of the mitochondrial control region for all putative F1 hybrids (characterized using the STRUCTURE-training method described later), using primers Odh-dloopF (5′-GAGCAACCAATCTCCCTGAG-3′) and Odh-dloopR (5′GTGTGAGCATGGGCTGATTA-3′) using the conditions detailed in the study of Latch *et al.* (2008). Amplified products were sequenced in both directions on an ABI3730xl at the UWBC. Sequences were aligned and edited using GENEIOUS PRO (Drummond *et al.* 2011).

Classification of individuals into parental and hybrid categories

Two Bayesian clustering programmes were utilized to investigate the overall population structure, STRUCTURE (Pritchard *et al.* 2000) and BAPS 5.1 (Corander *et al.* 2006, 2008). In STRUCTURE, we performed five runs at each hypothesized number of subpopulations (K). Runs of 100 000 permutations after 30 000 MCMC burn-in were conducted from K = 1 to 10 under the admixture, correlated alleles model (Pritchard *et al.* 2000). All other parameters were kept as defaults. Our likelihood results exhibited a common phenomenon in which likelihood values plateaued and had increasing variances once the true K is reached (Pritchard & Wen 2003). As such, we used the post hoc ΔK analysis to select the most likely K (Evanno *et al.* 2005). After identifying the most likely K, five longer STRUCTURE runs of 1 000 000 replicates after 1 000 000 MCMC burn-in were performed at the most likely K as well as $K - 1$ and $K + 1$ to verify consistency among runs. Results from longer runs at the

most likely K were also used to calculate q, the proportion of each individual's genome that belongs to each putative subpopulation.

Similar to STRUCTURE, we used BAPS 5.1 (Corander *et al.* 2006, 2008) to estimate the number of subpopulations via the 'Clustering of Individuals' model. The program was run five times at each K for $K = 1$ to 10. When the most appropriate K was indentified, a fixed K analysis was performed using the 'Admixture of Individuals Based on Mixture Clustering' option to estimate ancestry coefficients. Despite robust evidence for the existence for multiple subpopulations in STRUCTURE, the most likely K in BAPS was one. To verify this result, levels of admixture were investigated for $K = 2$ and ancestry coefficients were similar for all individuals. It is likely that the level of genetic differentiation between the lineages and the existence of intermediate individuals limited the ability of the algorithm to detect substructure (Latch *et al.* 2006).

We employed three methods to assign individuals as pure BTD, pure MD and hybrids (F1 and F2/backcross). The first two methods permitted discrimination between parentals and hybrids, but did not permit further classification of hybrids into F1 or F2/backcross categories. In the first method, which we call STRUCTURE-relaxed, individual deer were designated as BTD ($q \leq 0.10$), MD ($q \geq 0.90$) or hybrids ($0.10 < q < 0.90$) based on q-values from STRUCTURE. Although somewhat arbitrary, these relaxed cut-off values are often employed for classifying hybrids in the literature (e.g. Beaumont *et al.* 2001; Vaha & Primmer 2006; Barilani *et al.* 2007; Oliveira *et al.* 2008; Trigo *et al.* 2008; Sanz *et al.* 2009; Bohling & Waits 2011). The second method for classifying individuals of pure and hybrid ancestry we call STRUCTURE-conservative. In this method, parentals and hybrids are classified based on 90% credible intervals (CRs) around observed q-values. Individuals with 90% CRs including 0 or 1 were designated as pure BTD and MD, respectively, whereas those with CRs that did not overlap 0 or 1 were classified as hybrids.

The third method for assigning parental and hybrid individuals (called STRUCTURE-training) not only allowed us to distinguish between parental and hybrid categories, but also permitted further categorization of hybrids into F1 or F2/backcross classes. To facilitate accurate identification of pure BTD and MD within our sample, we used a training data set (Hauser *et al.* 2006; Barilani *et al.* 2007; Pritchard *et al.* 2007; Bohling & Waits 2011). The initial training data set included 191 reference individuals from known pure lineages in British Columbia, Idaho and Montana. These training individuals were run in STRUCTURE ($K = 2$, 100 000 burn-in, 500 000 iterations, admixture model, correlated allele frequency model), and any individuals with a q-value less than 95% were removed to ensure that our training data set was maximally informative. Following this filtering analysis, our final training data set consisted of 56 pure BTD individuals and 106 pure MD individuals. Using the distribution of q-values for training individuals, we then simulated 6000 hybrids (2000 F1, 2000 F1xBTD backcross and 2000 F1xMD backcross) using HYBRIDLAB 1.0 (Nielsen *et al.* 2006). All training individuals and simulated hybrids were analysed in STRUCTURE ($K = 2$, 100 000 burn-in, 500 000 iterations, admixture model, correlated allele frequency model), to determine appropriate threshold values for different parental/hybrid categories (e.g. Lancaster *et al.* 2006; Barilani *et al.* 2007). For this analysis, each parental and hybrid category (BTD, MD, F1, F1xBTD, F1xMD) was considered as a separate population (POPFLAG = 1). The 90% confidence intervals of the distribution of q-values for each parental/hybrid category were used as expected values for the assignment of all individuals in our data set ($n = 410$). Observed q-values for the 410 observed individuals (based on STRUCTURE run with $K = 2$, 100 000 burn-in, 500 000 iterations, admixture model, correlated allele frequency model) were then plotted against expected values to assign deer to discrete hybrid categories. We performed a similar 'trained clustering' analysis in BAPS using the training data set to confirm our STRUCTURE-training results; however, resolution of the two lineages within the data set was still lacking.

Genetic characterization of BTD–MD hybrid zone

We used simulations to determine whether the intermediate allele frequencies observed across the hybrid zone were the result of simple mixing of pure parental types or hybridization with introgression (similar to Nielsen *et al.* 2003, 2006). Three separate potential scenarios, spatial mixing (spatial mixing of distinct lineages without introgression), hybrid zone (hybridization and introgression between individuals from different lineages producing F1 hybrids only) and hybrid swarm (hybridization and introgression producing hybrids beyond the F1 generation), were tested using simulated genotypes generated in HYBRIDLAB 1.0 (Nielsen *et al.* 2006). Spatial mixing was simulated by randomly drawing alleles from the allele frequency distribution of the observed parental BTD and MD genotypes (classified using the STRUCTURE-training method) to simulate a spatially mixed population of pure parental genotypes. The number of simulated genotypes was equal to the number of individuals classified as hybrids in our data set ($n = 180$), and the proportion of each pure parental lineage in the simulated, spatially mixed population was equal to the observed proportion of each lineage

represented in the hybrid sample (the average q-value for classified hybrids in the data set). The observed parental individuals and the simulated spatially mixed population were then run in STRUCTURE (K = 2, 100 000 burn-in, 500 000 iterations, admixture model, correlated allele frequency model) to calculate q-values for the simulated data. A hybrid zone was simulated by randomly mating observed BTD and MD parental genotypes to produce 180 simulated F1 hybrids, the same number of observed admixed individuals. Both observed parentals and simulated F1 hybrids were then run in STRUCTURE as described earlier to calculate q-values for the simulated data. A hybrid swarm was simulated by randomly mating all observed hybrid individuals in their observed proportions. Simulated hybrid individuals and observed parentals were then run in STRUCTURE to calculate q-values as earlier. All sample sizes for simulated hybrids and parentals for each type were equal to the observed number as inferred from STRUCTURE. Distributions of simulated and observed q-values were compared using a Kolmogorov–Smirnov test, with significant results indicating incongruence between observed and expected distributions.

For the overall data set, each pure lineage, and all hybrids, measures of genetic diversity including allelic richness (A_R), observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated in FSTAT (Goudet 1995). Null allele frequencies were estimated for each locus using MICROCHECKER (van Oosterhout *et al.* 2004). For each lineage, the degree of differentiation (F_{ST}) between groups and the deviation from random mating (F_{IS}) within groups were calculated using the program SPAGEDI v. 1.3 (Hardy & Vekemans 2002). Matrices of genetic distances (a; Rousset 2000) and log-transformed geographic distances between all individuals were also generated in SPAGEDI to perform a global test of isolation by distance (IBD) using a simple Mantel test in ZT (MANTELTESTER GUI frontend; Bonnet & Van de Peer 2002).

Patterns of hybridization

MtDNA sequences of all putative F1 hybrids ($0.40 \leq q \leq 0.60$) were compared to published MD and BTD haplotypes in GenBank. Patterns of introgression were examined by comparing the mitochondrial and nuclear (i.e. microsatellite) data sets in two ways. First, we compared the haplotypes (BTD vs. MD) of F1 hybrids to their putative lineage as calculated in STRUCTURE. Second, microsatellite alleles that were identified as unique to either MD or BTD were also examined in putative F1 hybrids.

To distinguish between a clinal and a mosaic hybrid zone structure, we measured the correlation between hybrid index (based on q-values) and geographic distance from the currently recognized management boundary dividing BTD and MD subspecies. Distance to the BTD–MD boundary was calculated using the 'Near' function in ARCGIS 9.2. Pearson's correlation coefficients were calculated using JMP 8 software (SAS Institute). Because most individuals were harvested between 30 and 50 km from the boundary, we also performed the correlation analysis using 17 randomly selected individuals from each 50-km interval (range 0–250 km) to minimize any spurious correlations.

Linkage and Hardy–Weinberg disequilibria were examined in hybrids that were close to the BTD–MD boundary (geographic distance <30 km) and parental BTD and MD far from the boundary (geographic distance >40 km). Significant linkage and Hardy–Weinberg disequilibria at the centre of zone of contact would indicate a hybrid zone, where individuals are not randomly interbreeding (in contrast to hybrid swarms) and where survival and mating are related to genetic identity. Linkage and Hardy–Weinberg disequilibrium were tested within each group using FSTAT. To correct for multiple tests, a false discovery method (FDR; Benjamini & Yekutieli 2001) was used to minimize type II errors (Narum 2006).

Results

Identification of BTD and MD hybrids

Results from STRUCTURE suggested that the most likely number of clusters (K) was two. The maximum calculated mean estimated logarithm of the probability of the data ($\ln \Pr(X \mid K)$) occurred at K = 2, and the ΔK analysis produced a strong peak at K = 2 (Table S1, Supporting information). The two clusters corresponded to the distinct lineages of MD and BTD with hybrids distributed throughout the study area (Fig. 1).

Based on q-values produced by STRUCTURE, the BTD and MD clusters contained 207 and 203 individuals, respectively, when all individuals were assigned to one of the two inferred clusters. The number of parentals and hybrids varied among methods used to delineate categories (Table 1). Using the STRUCTURE-relaxed approach, 108 individuals were pure BTD ($q \leq 0.9$; 26% of the total sample), 116 were pure MD ($q \leq 0.1$; 29% of the total sample) and the remaining 186 were hybrids of varying degrees ($0.1 < q < 0.9$; 45% of the total sample). In contrast, the STRUCTURE-conservative approach yielded 183 pure BTD (90% CR included q = 1.0; 45% of the total sample), 191 pure MD (90% CR included q = 0.0, 47% of the total sample) and 36 hybrids (90% CR did not include 0.0 or 1.0; 8% of the total sample). Numerical results of the STRUCTURE-training method for delineating parental BTD and

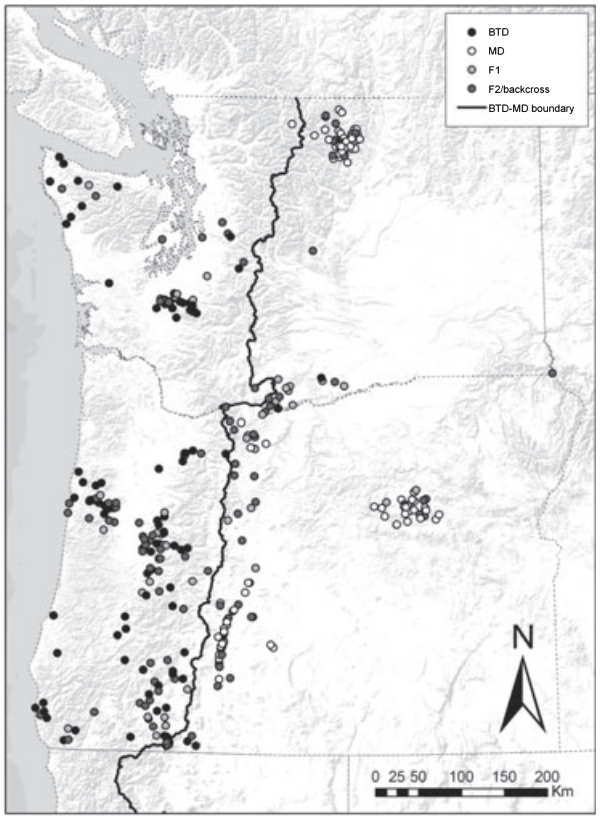

Fig. 1 Geographical distribution of pure and hybrid individuals classified using the STRUCTURE-training method. Individuals are classified as pure black-tailed deer (BTD) ($q > 0.9$), pure mule deer (MD) ($q < 0.12$), F1 hybrid ($0.34 < q < 0.67$) or an F2/backcross ($0.12 \leq q \leq 0.34$; $0.67 \leq q \leq 0.9$). The recognized management boundary between BTD and MD is indicated (bold black line).

Table 1 Number of parental BTD, MD and hybrids identified using three methods in STRUCTURE

	BTD	MD	Hybrid
STRUCTURE-relaxed	108	116	186
STRUCTURE-conservative	183	191	36
STRUCTURE-training	107	123	180

BTD, black-tailed deer; MD, mule deer.
The STRUCTURE-relaxed method defined pure BTD and MD as all individuals with a q-value >0.9 and <0.1, respectively. The STRUCTURE-conservative method designated parentals based on 90% credible regions that included 1 (BTD) or 0 (MD). In the STRUCTURE-training method, pure BTD ($q > 0.9$) and MD ($q < 0.12$) were defined as individuals that fell within 90% confidence intervals of distribution of q-values calculated from training individuals.

MD deer from hybrids were similar to the relaxed approach (Fig. 2). Based on 90% CIs of q-values calculated in training individuals, 107 pure BTD ($q > 0.9$; 26% of the total sample) and 123 MD ($q < 0.12$; 30% of the total sample) were identified while 180 deer were consistent with hybrids ($0.12 \leq q \leq 0.9$; 44% of the total sample). After calculating 90% confidence intervals for simulated hybrids, 55 individuals were further designated as F1 hybrids ($0.34 < q < 0.67$) and the remaining 125 deer were considered F2 or backcross individuals ($0.12 \leq q \leq 0.34$ or $0.67 \leq q \leq 0.9$).

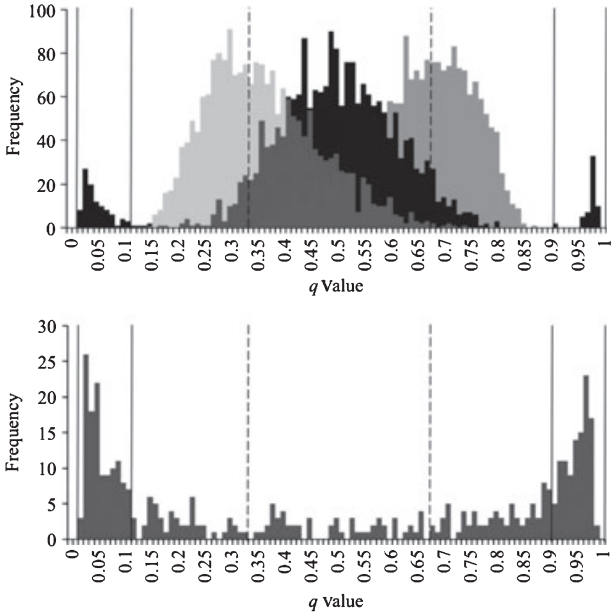

Fig. 2 Distribution of q-values used to define parental and hybrid categories within the STRUCTURE-training analysis. Each group was delineated based on 90% CIs of q-values in training and simulated data sets (above), which were then applied to the observed deer (below). Specific F2 categories were not defined using simulated F1 × black-tailed deer (dark grey) and F1 × mule deer (light grey) due to the overlap in distributions. Solid and dashed lines denote cut-offs for parental and F1 hybrids, respectively.

Genetic characteristics of MD–BTD hybrid zone

Simulations confirmed that population structure across the study area was most likely explained by admixture (hybridization) beyond the F1 generation, rather than by simple spatial mixing of individuals from two divergent lineages or by F1 hybridization alone. Both the simulated and observed q-value distributions yielded sigmoid curves. Kolmogorov–Smirnov tests revealed that the observed distribution of q-values was not significantly different from a simulated hybrid swarm ($D = 0.0732$, $P = 0.2141$) but was inconsistent with a model of spatial mixing ($D = 0.4537$, $P < 0.0001$) and a hybrid zone model ($D = 0.1805$, $P < 0.0001$; Fig. 3).

Measures of genetic diversity for BTD, MD and F1 hybrids were similar (Table 2; locus-specific data for

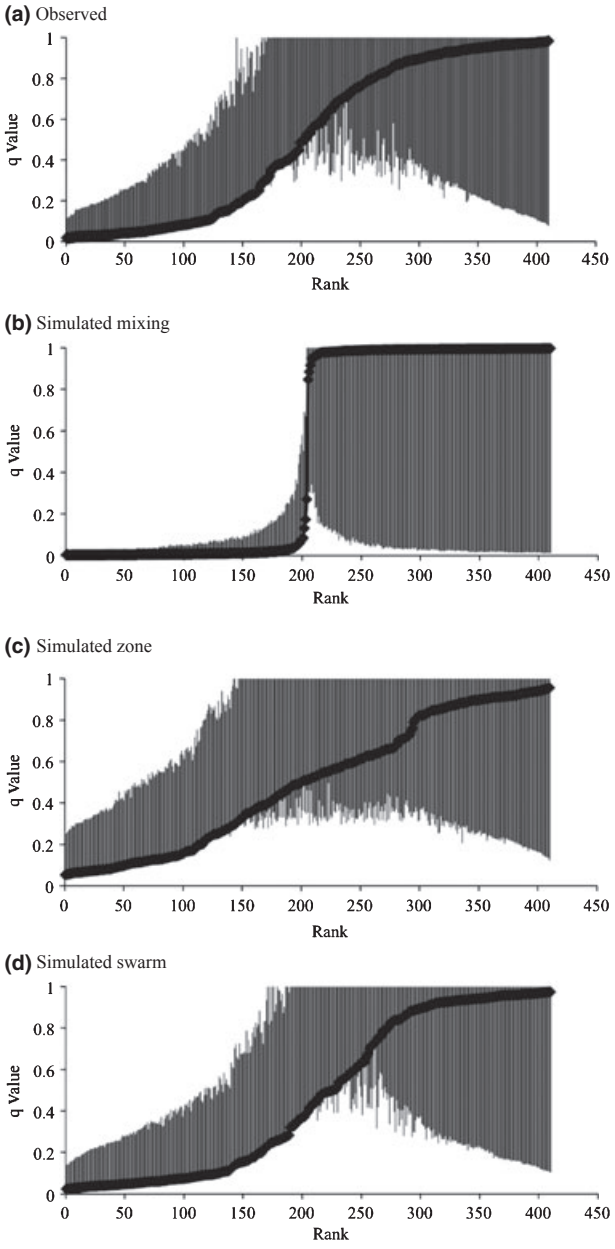

Fig. 3 Distribution of individual admixture proportions (q-value) and 90% posterior probability intervals for empirical (a) and simulated (b–d) populations. The values have been ranked along the x-axis from 0 (pure mule deer, MD) to 1 (pure black-tailed deer, BTD). (a) Represents empirical values from sampled individuals. (b) Represents a simulated hybrid population under a scenario of spatial mixing of pure BTD ($q > 0.9$) and pure MD ($q < 0.12$). (c) Represents a simulated hybrid zone (simulated mating of pure BTD and pure MD that produce only F1 hybrids) (d) Represents a simulated hybrid swarm (simulated random mating of observed hybrids $0.12 \leq q \leq 0.9$).

the total population provided in Table 2, Supporting information), and the total population showed no evidence of null alleles (Table 2, Supporting information). Lineages exhibited significant genetic differentiation from one another, whether all individuals were assigned to a lineage (hybrids included; $F_{ST} = 0.068$, $P < 0.0001$) or only parental individuals were considered ($F_{ST} = 0.124$, $P < 0.0001$). An overall pattern of IBD was detected across the study area ($r = 0.168$, $P < 0.0001$) and within the hybrid population ($r = 0.2688$, $P < 0.0001$). IBD would be expected in any contact zone in which admixture connects two divergent lineages. However, it is unlikely that the observed genetic structure is solely the result of IBD, as relatively large genetic differences were often observed over short geographic distances. Given the regional scale of our study, it is likely that population substructure is also contributing to the IBD pattern we observed. Additional evidence for population substructure is given by positive F_{IS} values observed in each lineage and in the hybrid population (Table 2).

Patterns of hybridization

Hybridization between MD and BTD occurred in both directions. The bimodal distribution of q-values suggests that the hybrid zone represents a relatively even admixture from both lineages (Fig. 4). Of the 55 putative F1 hybrids, we obtained mtDNA sequences for 36 individuals. Eighteen F1 hybrid deer had BTD haplotypes, and 18 had MD haplotypes. According to the STRUCTURE q-values and mtDNA haplotypes, 12 of the 36 sequenced F1 hybrids (33%) exhibited cytonuclear disequilibria; five individuals that had MD haplotypes were assigned to BTD, and seven individuals that had BTD haplotypes were assigned to MD. The remaining F1 hybrids (67%) had mtDNA haplotypes that were concordant with lineage assignment based on nuclear DNA. Lineage-specific alleles for both MD and BTD were also identified in F1 hybrids. F1 hybrids carried 11 of the 27 BTD-specific alleles, and 12 of 17 MD-specific alleles (Table 2, Supporting information).

There was a significant positive correlation between distance to the BTD–MD boundary and hybrid index when all individuals were included ($P = 0.0004$), but not when we controlled for sample sizes within 50-km distance classes ($P = 0.6681$; Fig. 5). This is inconsistent with a clinal pattern of variation across the zone, in which hybrid index would be expected to decrease with distance from the boundary. Overall, we observed very little linkage disequilibrium across the hybrid zone. Two locus pairs (Odh_C x Odh_G and Odh_E x RT24) were in linkage disequilibrium in the pure BTD lineage and three locus pairs (Odh_G x Odh_P, Odh_O x RT24, and Odh_O x T40) were in linkage disequilibrium in the pure MD lineage. We observed no linkage disequilibrium in the hybrids (Table 2). We observed no linkage

Table 2 Genetic diversity estimates for BTD and MD lineages, including allelic richness (A_R; standard errors in parentheses), observed (H_O) and expected (H_E) heterozygosity and F_{IS} estimates (*P*-values and standard errors in parentheses)

	n	A_R	H_E	H_O	F_{IS}	LE	HWE
BTD	107	9.3 (4.96)	0.67	0.59	0.12 ($P < 0.0001$; 0.034)	2	4
MD	123	7.7 (3.11)	0.72	0.66	0.08 ($P < 0.0001$; 0.019)	3	5
Hybrid	180	9.2 (4.26)	0.71	0.68	0.06 ($P = 0.0017$; 0.028)	0	5

BTD, black-tailed deer; MD, mule deer.
The number of loci that deviated from expectations for loci in linkage equilibrium (LE) or Hardy–Weinberg equilibrium (HWE) is listed. Parental and hybrid groups refer to individuals assigned to the BTD ($q > 0.9$), MD ($q < 0.12$) or hybrid ($0.12 \leq q \leq 0.9$) lineage (based on the STRUCTURE-training method of hybrid classification).

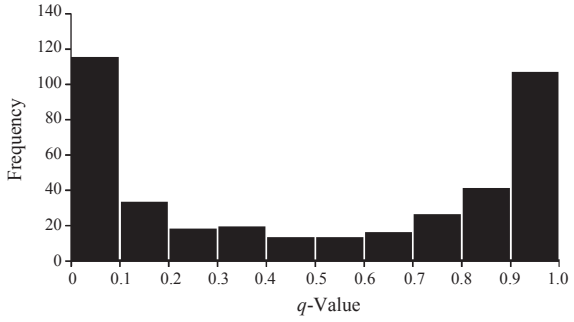

Fig. 4 Bimodal frequency distribution of STRUCTURE *q*-values. Values range from 0 (pure black-tailed deer) to 1 (pure mule deer).

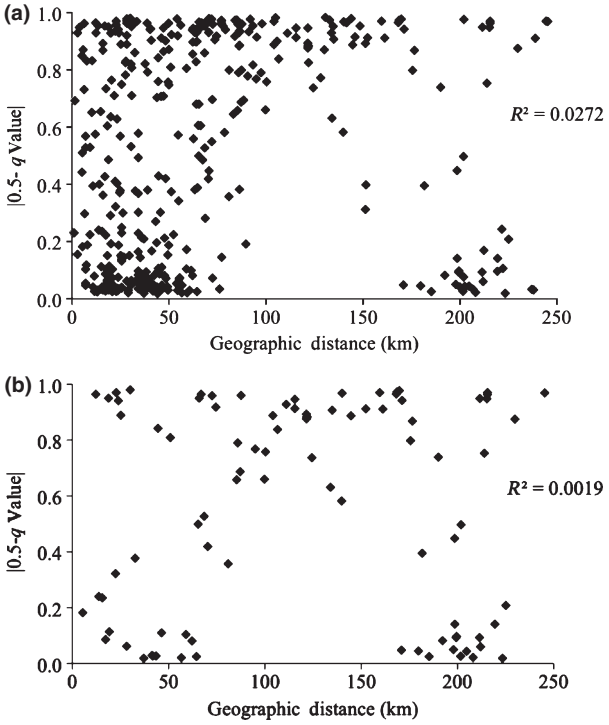

Fig. 5 Relationship between lineage purity and geographic distance to the recognized black-tailed deer–mule deer boundary. (a) A significantly positive correlation between hybrid index (absolute value of $0.5 - q$) and geographic distance was observed ($P = 0.0004$) when variation in sample size per distance class was not considered. (b) The correlation between hybrid index and geographic distance was not significant when sample sizes within 50-km intervals were held constant ($P = 0.6681$).

disequilibrium at the centre of the zone of contact (<30 km) or away from the zone of contact (>40 km; east and west of the boundary analysed separately). Hardy–Weinberg equilibrium was somewhat difficult to interpret, as our sampling design likely introduced a Wahlund effect into the data set by sampling across local populations, obscuring any signature from admixture. The pure BTD lineage (Odh_P, Odh_O, RT24, T40), pure MD lineage (Odh_E, Odh_K, Odh_G, Odh_P, RT24) and hybrids (BM848, Odh_C, Odh_O, RT24, T40) all exhibited significant deviations from Hardy–Weinberg expectations at multiple loci. We observed fewer deviations from Hardy–Weinberg equilibrium near the centre of the zone of contact (two loci; Odh_C and RT24) than away from the zone of contact (seven loci in deer >40 km west of the zone of contact, BM848, Odh_C, Odh_E, Odh_P, Odh_O, RT24, T40; two loci in deer >40 km east of the zone of contact, Odh_E, Odh_O).

Discussion

We found clear genetic evidence for extensive hybridization between BTD and MD. Clustering analyses clearly identified two genetically distinct lineages, with varying degrees of admixture in the zone of contact. Individual admixture analysis indicated that intermediate allele frequencies found in the zone of contact are consistent with the presence of admixed individuals, and cannot be explained by a model of spatial mixing of individuals from the two lineages without introgression.

The zone of hybridization between BTD and MD closely resembles a hybrid swarm, with hybrids extending beyond the F1 generation and a lack of linkage disequilibrium at the centre of the contact zone. By contrast, a

hybrid zone would have been characterized by an absence of F2/backcross individuals, and linkage and Hardy–Weinberg disequilibrium at the centre of the zone of contact (Barton & Hewitt 1985). Hybrids represent a full range of admixture between parental types, rather than comprising a distinct lineage, providing further support for the hybrid swarm hypothesis. A random association of genotypes within the hybrid swarm also suggests that the swarm has existed for many generations.

Although the full range of hybrids was present, the geographical structure of the hybrids was not clinal and was better characterized as a swarm of genotypes across the zone of contact. In a clinal hybrid zone, hybrid index would be expected to decrease with distance from the zone of contact. No correlation between geographic distance from the boundary and hybrid index was observed when sample size was accounted for in our analysis. Similarly, hybrids located near the centre of the zone of contact did not exhibit higher levels of linkage disequilibrium than did individuals away from the centre of the zone, as would be expected if the structure of the hybrid zone resembled a cline. The lack of a clinal transition is unexpected given the sharp ecotone separating the two lineages and suggests that hybridization between BTD and MD is not maintained by a balance between dispersal into the zone of contact and selection against hybrids (Barton & Hewitt 1985). Additionally, it suggests that ecological adaptation within lineages has been insufficient to bring about significant hybrid inferiority. Likewise, although a nonclinal pattern of hybridization could be maintained by a mosaic-bounded superiority model, with hybrid fitness correlated to some environmental factor(s) that are distributed in a mosaic pattern across the landscape, these conditions would be unexpected given the abrupt habitat transition that separates the two lineages. These lines of evidence strongly suggest that the zone of contact between BTD and MD is not a hybrid zone or a hybrid taxon, but is a well-established, freely interbreeding hybrid swarm.

MD and BTD also hybridize with white-tailed deer (*O. virginianus*) in areas where they are sympatric (Carr *et al.* 1986; Stubblefield *et al.* 1986; Cronin *et al.* 1988; Gavin & May 1988; Ballinger *et al.* 1992; Carr & Hughes 1993; Cathey *et al.* 1998; Hornbeck & Mahoney 2000; Bradley *et al.* 2003; Lopez 2006). Although a few of these studies have documented high levels of hybridization within specific populations (e.g. 13.3%, Bradley *et al.* 2003; 13.8%, Stubblefield *et al.* 1986; 18.8%, Hornbeck & Mahoney 2000), most suggest that regionally, rates of hybridization are low (5.6%, Stubblefield *et al.* 1986; 2%, Cronin *et al.* 1988; 3.6%, Hughes & Carr 1993; 5.6%, Hornbeck & Mahoney 2000; 2%, Lopez 2006). Although no formal classification was undertaken for hybrids identified in those studies, most were thought to be F2/backcross individuals. The low regional rates of intraspecific hybridization between *O. virginianus* and *O. hemionus* are in direct contrast to the very high rate of intraspecific hybridization we observed between lineages of *O. hemionus* (44% using the STRUCTURE-training method of hybrid classification), including both F1 and F2/backcross individuals.

The presence of a hybrid swarm between BTD and MD along the zone of contact in the Cascade Mountain range is somewhat surprising, given that the boundary between lineages is marked by an extreme environmental transition. Sharp transitions in habitat features are often marked by hybrid zones that are narrow relative to the dispersal capability of a species (e.g. Moore 1977; Schilthuizen & Lombaerts 1995; Ruegg 2008). In contrast, hybrid swarms are more often found across wide habitat gradients (Keim *et al.* 1989; Nielsen *et al.* 2003), or when a hybridizing species is introduced into habitat occupied by a native congener (Echelle & Connor 1989; Childs *et al.* 1996; Avise *et al.* 1997; Riley *et al.* 2003). In *O. hemionus*, the existence of a hybrid swarm across a sharp environmental transition zone suggests that deer may not perceive these habitats as drastically different. This is interesting, as a primary reason given for hybridization between *O. hemionus* and *O. virginianus* has been habitat-based; namely, the encroachment of mesic *O. virginianus* habitat into xeric *O. hemionus* habitat in west Texas (Ballinger *et al.* 1992; Carr & Hughes 1993; Cathey *et al.* 1998).

Within the hybrid swarm, admixture is bidirectional and symmetric. Both BTD and MD lineage-specific alleles were found in F1 hybrid individuals, and F1 hybrids were found to carry BTD or MD haplotypes in approximately equal frequency. This is in contrast to expectations for ungulates, where dispersal is male-biased and male reproductive success is widely considered to be driven by body size (Miller 1974; Kucera 1978). MD are significantly larger than BTD in this region (MD males 75–115 kg, BTD males 45–96 kg; Brown 1961; Wallmo 1981; Kie & Czech 2000), and thus one might expect male MD to be more successful than male BTD in interactions with females, regardless of lineage. However, an opposite pattern of unidirectional hybridization favouring males of the smaller species has been observed in several other ungulate species. In areas where mule deer (of the MD lineage) and white-tailed deer are in sympatry, hybridization occurs most often between smaller male white-tailed deer and larger mule deer females (Ballinger *et al.* 1992, Carr & Hughes 1993, Cathey *et al.* 1998). Similarly, Gavin &May (1988) observed an influx of genes from the smaller BTD lineage into the larger Columbian white-tailed deer *(O. v. leucurus)*. In areas where red deer *(Cervus elaphus)*

and sika deer *(C. nippon)* hybridize, mating is found primarily between small sika males and large red deer females (Senn & Pemberton 2009, Senn *et al.* 2010). These unexpected hybridization patterns have been attributed to ecological, demographic, and biological factors. In the MD x BTD hybrid swarm, symmetrical hybridization patterns in spite of presumed directional female preferences (direct or indirect) suggests that something other than sexual selection may be influencing mating in this hybrid swarm, or that sexual selection is opposed by some other force. In the MD × BTD hybrid swarm, symmetrical hybridization patterns in spite of presumed directional female preferences (direct or indirect) suggests that something other than sexual selection may be influencing mating in this hybrid swarm, or that sexual selection is opposed by some other force.

It is possible that differential reproductive success of migrant males (higher success for MD males west of the boundary, and lower success for BTD males east of the boundary) could be offset by asymmetrical migration rates. When we consider the number of migrants in our data set, by counting the number of pure individuals found on the opposite side of the inferred boundary, we indeed find asymmetrical migration rates (15 BTD migrants and three MD migrants). Although a crude way in which to infer migration, it demonstrates that migrant MD males could be five times as successful as BTD migrant males and still maintain symmetrical admixture proportions. Although no study has quantified reproductive success for migrant individuals, we would expect that large MD males in BTD-dominated areas to have higher reproductive success than resident BTD males, given observations that reproductive success is driven by body size (Miller 1974; Kucera 1978).

Other demographic factors could potentially introduce additional biases in hybridization rates, though a lack of demographic data for deer in this region, particularly for the BTD lineage, makes it difficult to assess the importance of these factors in biasing observed hybridization rates. For example, population density is likely to be lower in BTD than in MD (estimates in progress; D. Whittaker, personal communication), which could artificially inflate estimates of hybridization between migrant MD males and resident BTD females. This yields an effect opposite to what we observed; however, it illustrates the point that relative population densities could also be a source of variability in our estimates of observed hybridization rates. Gavin & May (1988) found a pattern of hybridization opposite to other studies of *O. virginianus* × *O. hemionus* hybridization, an influx of BTD genes into Columbian white-tailed deer. The authors attributed their findings to population density differences, with high-density BTD populations introgressing into low-density white-tailed deer populations (Gavin & May 1988). Sex ratios in both populations are female-biased, as expected for ungulate populations, and may be slightly higher in MD than in BTD (D. Whittaker, personal communication). This could result in MD females being more likely to mate with BTD males if no MD males were available to mate, artificially skewing hybridization estimates towards symmetry in the face of higher MD reproductive success. Similarly, the timing of the rut is similar in both lineages, but is slightly wider in MD than in BTD (BTD: Nov 22–Dec 3, mean Nov 28; MD: Nov 11–Dec 16, mean Nov 25; Brown 1961, D. Whittaker, personal communication). There is an advantage to conceiving during first oestrous, and thus females may be more likely to mate with males of a different lineage if it meant breeding early. Again, this would seem to favour MD males who breed early, making it unclear how this would lead to symmetrical hybridization rates in the face of presumed MD male advantage.

Alternatively, the pattern of symmetrical admixture given presumed MD male advantage could be driven by females rather than males. A large male advantage within lineages may not translate across lineages, and migrant males may be uniformly unsuccessful. If ecological or behavioural factors consistently limit the success of males, admixture could occur through females only. For example, if lineage-specific behaviours are involved in reproduction (pre- or postcopulatory), or if territory acquisition necessitates some knowledge of the habitat, migrant males may be uniformly unsuccessful regardless of size. Despite the fact that females tend to be more philopatric than males, females can disperse long distances (180 km for a MD female; Severson & Carter 1978). In most ungulates, including *O. hemionus*, females are mated as long as they are receptive (Verts & Caraway 1998), so migrant females may be mated by resident males whenever they occur. Uniform male failure coupled with similar female migration rates between lineages could result in symmetrical observed admixture rates.

A third possible explanation for symmetrical admixture rates in the face of presumed MD male advantage is asymmetric fitness of hybrid offspring. MD male × BTD female hybrid offspring could be produced at a higher rate than BTD male × MD female offspring, which would be expected if body size is correlated with reproductive success. However, genetic or *in utero* incompatibilities could limit the fitness of MD male × BTD female hybrids relative to BTD male × MD female hybrids, resulting in symmetric observed admixture proportions. For example, dystocia, or difficult parturition, is caused by crossing animals that are differently sized and has resulted in mother and calf mortality in ungulates

(Jainudeen & Hafez 1987; Galindo-Leal & Weber 1994). Such a phenomenon could result in realized symmetrical hybridization even if mating were asymmetrical. Dystocia could explain unidirectional hybridization patterns between red deer and sika deer, where the size of red deer males can exceed seven times that of sika deer females (Whitehead 1993; Senn & Pemberton 2009).

Evolutionarily, the hybrid swarm appears to be stable. The persistence of the MD × BTD hybrid swarm, as indicated by the presence of many hybrids beyond the F1 and a random association of genotypes within the swarm, suggests that the swarm may be self-sustaining, and thus more stable than in other species with directional gene flow and restricted hybridization patterns (e.g. Keim *et al.* 1989). It is quite likely that MD and BTD lineages came into secondary contact following glacial recession, *c.* 8000 BP (Latch *et al.* 2009). Thus, it is possible that hybridization between lineages has been ongoing for thousands of years. At the very least, equilibrium patterns within the swarm suggest that sufficient time has passed for any disequilibria caused by interbreeding between divergent lineages to have been eroded. However, it is not known whether this hybrid swarm will continue to be stable over time, particularly if environmental conditions fluctuate. Continued monitoring of hybridization beyond the immediate vicinity of the boundary may reveal temporal trends in hybrid zone dynamics.

From a management perspective, the current designated boundary between lineages seems to adequately divide the two lineages. However, the boundary is porous, with pure individuals found on the 'wrong' side of the boundary, and hybridization is occurring extensively both along the boundary and far beyond it. This represents a challenge to identify animals of pure or hybrid descent, which is critical for the enforcement of hunting regulations and for record-keeping purposes. Morphologically, it would be impossible to distinguish various categories of hybrids based on traits typically used to differentiate MD and BTD lineages. Genetically, it is possible to distinguish pure individuals from hybrids as we have demonstrated in this study. However, it is unlikely that various hybrid categories could be reliably distinguished given the frequency-based differences inherent in these molecular markers. The establishment and maintenance of fixed differences between lineages would be disfavoured, given the level of admixture observed in this system. Even if molecular markers exhibiting fixed differences between lineages were identified, a very large number of loci would be required to attain the statistical power to categorize deer with very similar levels of hybrid status. It would also be unreasonable to expect that hybrids could be removed from the system, if this was desired, given the establishment of a hybrid swarm (Allendorf *et al.* 2001). These characteristics suggest that management recommendations should be modified to include the widespread existence of hybrid individuals where BTD and MD lineages meet.

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Data accessibility

DNA sequences: Genbank accessions FJ189203–FJ189249, FJ189298–FJ189323.

Sample locations and microsatellite data: DRYAD entry doi:10.5061/dryad.hm2047nh.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Results from STRUCTURE analysis to identify the most likely number of clusters in the total dataset. Likelihoods (Ln($P \mid D$)) are averaged across five runs.

Table S2 Locus-specific genetic diversity estimates for the total population, including sample sizes (n), number of alleles per locus (A), observed (H_O) and expected (H_E) heterozygosity, F_{IS} (and associated P value).

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