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Speciation without gene-flow in hybridizing deer

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13 ### *Abstract*

14 Hybridisation in nature is part of the continuum of speciation. Under the ecological speciation

15 model, divergent selection acts on ecological differences between populations, gradually

16 creating barriers to gene flow and ultimately leading to reproductive isolation. Here, we used

17 white-tailed (*Odocoileus virginianus*) and mule deer (*O. hemionus*) to investigate patterns of

18 speciation in hybridising sister species. We quantified genome-wide introgression and

19 performed genome scans to look for signatures of four different selection scenarios. Despite

20 modern evidence of hybridization, we found no sign of introgression, no signature of

21 divergence with gene flow, and localized patterns of allopatric and balancing selection in the

22 genome. Of note, genes under balancing selection were related to immunity and MHC or

23 sensory perception of smell, the latter of which is consistent with deer biology. The deficiency

24 of patterns selection suggests that white-tailed and mule deer were spatially separated during

25 the glaciation cycles of the Pleistocene where genome wide differentiation accrued via drift.

26 Absence of historical introgression signs could suggest Dobzhansky-Muller incompatibilities

27 and selection against hybrids, and that both species are now far along the speciation

28 continuum. Our results suggests that WTD & MD do not conform to a speciation with gene flow

29 scenario, but that they evolved via drift in allopatry during the Quaternary and that both

30 species are currently advanced along the speciation continuum.

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Introduction

32

33 Hybridisation is a widespread phenomenon that occurs at variable rates (Mallet 2005; Ragavan

34 et al. 2017; Iacolina et al. 2019; Taylor and Larson 2019; Adavoudi and Pilot 2021). The

35 prevalence of hybridisation suggests speciation follows a continuum as opposed to the more

36 discrete and allopatric view introduced by Mayr (1942) (Mallet 2005; Stankowski and Ravinet

37 2021). However, both ideas are not incompatible as the speciation continuum ultimately leads

38 to reproductive isolation with hybridisation being a natural outcome (Stankowski and Ravinet

39 2021). Under the ecological speciation model (sensu Darwin, 1859), hybrids are common and

40 can even facilitate speciation (Nosil 2012). Ecological speciation works when ecological

41 differences lead to divergent selection between populations; eventually, the underlying

42 genetic mechanisms (i.e. causative loci) gradually create barriers to gene flow, ultimately

43 leading to reproductive isolation (Rundle and Nosil 2005; Nosil 2012; Shafer and Wolf 2013).

44   The genetic mechanisms involved in ecological speciation can take the form of barrier

45 loci which act as a restraint to gene flow in different ways: for example, such loci can be under

46 divergent selection, involved in mate choice or contribute to assortative mating, or reduce

47 hybrids fitness (Abbott et al. 2013; Ravinet et al. 2017). Divergent selection should produce

48 genomic islands in this context, also referred to as genomic islands of speciation, that are

49 regions of the genome where the differentiation (e.g. F_{ST}) is higher than the neutral genomic

50 background (Ravinet et al. 2017; Campbell et al. 2018). Such loci and islands have been

51 observed in several species, underpinning a wide array of speciation and divergence processes

52 (Poelstra et al. 2014; Momigliano et al. 2017; Lavretsky et al. 2019; Marques et al. 2019).

53 Scanning the genome for just F_{ST} peaks when speciation with gene flow is ongoing, however, is

54 problematic as other mechanisms can create similar F_{ST} profiles such as genetic drift, global

55   adaptation, or simply reduced genetic diversity due to background selection (Cruickshank and

56   Hahn 2014; Burri 2017; Ravinet et al. 2017; Booker et al. 2021). Joint genome scans including

57   F_{ST}, d_{xy} and π facilitate a better depiction of the processes of selection and ecological

58   speciation (Cruickshank and Hahn 2014; Ravinet et al. 2017; Campbell et al. 2018; Shang et al.

59   2021). Of note, Shang et al. (2021) recently conceptualized the expected patterns F_{ST}, d_{xy} and π

60   under the main modes of selection; this approach allows for neutral patterns of evolution to

61   be disentangled from selection based on contrasting patterns of the aforementioned summary

62   statistics.

63   White-tailed deer (*Odocoileus* virginianus; WTD) and mule deer (*O.* hemionus; MD) are

64   abundant in North America with similar morphology, activity patterns and life-history traits,

65   but they differ in several ecological aspects (Douzery and Randi 1997; Gilbert et al. 2006; Pitra

66   et al. 2004; Brunjes et al. 2006; Berry et al. 2019). WTD prioritise security and thermoregulation

67   whereas MD favour food availability (Whittaker and Lindzey 2004); consequently, WTD prefer

68   habitats at lower altitude and with denser visual cover than MD that favour open areas at higher

69   elevation (Anthony and Smith 1977; Brunjes et al. 2006). White-tailed deer and MD also differ in

70   their sociality and associated predator response. Mule deer live in large cohesive groups

71   including both sexes, and, as a group, adopt an aggressive behaviour in presence of predators,

72   whereas WTD live in smaller female-biased groups and flee in response to predators (Lingle

73   2001; Lingle 2003). Both species represent a high economic value in North America as hunting-

74   related activities generate billions of dollars annually (Cambronne 2013), and both species are

75   important cultural component of Indigenous communities (Adams and Hamilton 2011; Peres

76   and Altman 2018).

77   Despite a species divergence date estimated at ~3.13 mya (Wright et al. 2021), WTD and

78   MD hybridise in areas of sympatry with estimated hybridisation rates ranging from 1 to 19%

79   depending on the region (Carr and Hughes 1993; Hornbeck and Mahoney 2000; Combe et al.

80   2021; Russell et al. 2021). Both species likely spent considerable time in allopatry (i.e. separate

81   refugia) during glacial events (e.g Moscarella et al. 2003; Latch et al. 2009). It has also been

82   shown that however restricted, gene flow appears bidirectional as there are signs of

83   introgression at both mitochondrial and nuclear levels, such that some MD acquired WTD

84   mitochondrial DNA around ~1.32 Mya (Carr et al. 1986; Cronin 1991; Derr 1991; Carr and Hughes

85   1993; Cathey et al. 1998; Bradley et al. 2003; Russell et al. 2019; Wright et al. 2021). This pattern

86   also suggests ancestral hybridisation and gene flow has taken place. Given their clear

87   phenotypic and behavioural differences, and their documented hybridisation, white-tailed

88   and mule deer do not conform to the biological species concept (Mayr 1942). Here, we

89   hypothesize that WTD and MD have evolved via ecological speciation, and more specifically a

90   speciation with gene flow scenario. To test this hypothesis, we quantified genome-wide

91   introgression and past admixture events and performed genome scans to look for signatures

92   of four different selection regimes. We expected to find genetic signs of divergent selection,

93   such as speciation islands, consistent with patterns of divergence with gene flow (DwGF). We

94   also predicted higher rates of admixture and historical introgression in areas of sympatry.

95   ### *Results*

96   We sequenced 28 individuals to an average coverage of approximately 4x. We called 10,397,088

97   SNPs, which represented 0.4% of the genome. In the PCA based on allele frequencies, PC1

98   accounted for 59% of the variation and separated both species (Fig.1B). PC2 explained an

99   additional 8% of the variation and showed a spread of MD individuals that is consistent with

100   their longitudinal distribution (Pearson correlation = 0.8; *p*-value = 0.00057). Latitude was not

101   important in shaping PC1 or PC2 variation (*p* > 0.05). Admixture analysis showed a well-defined

102   genetic clustering between species (Evanno's method: best K = 2; Fig.1C). The WTD individual

103   Ov_MX2 was partially admixed in both analyses, with NGSAdmix assigning it 92.5% WTD and

104   7.44% MD ancestry (Fig.1C). This individuals' admixed pattern disappeared in the admixture

105   analysis with higher K values (K = 3 to 7, Fig.S1), though additional within species clusters are

106   observed.

107   We performed an ABBA-BABA test based on genotype likelihoods between all

108   combinations of our 28 individuals in a first analysis and between all four populations in a

109   second, with the caribou as outgroup. We computed 9828 ABBA-BABA individual topologies

110   in ANGSD; inter-species introgression estimates were all very close to 0, with a D-statistic

111   ranging from 0.011 to -0.013 and Z-score from 6.92 to -8.85. D-statistic in intra-species

112   comparisons range from 0.25 to 0.916 (Z-score from 114.54 to 13111.97) in ABBA and from -

113   0.046 to -0.914 (Z-score from -25.42 to -1348.55) in BABA (Fig.2A). In the comparisons between

114   the four populations, three ABBA-BABA topologies presented signatures of introgression on a

115   total of 12. The single inter-species comparison presents a D-statistic of -0.07 (Z-score = -7.66)

116   for an introgression between sympatric MD and WTD. The other two comparisons show a

117   reciprocal introgression between MD in allopatry and sympatry of 0.9 and 0.89 (Z-score =

118   1342.83 & 1376.69; Fig.S2). The treemix analyses showed a topology with no migration that

119   explained over 99% of the variation (Fig.2B).

120   We further analysed introgression through the measure of f_d across 50kb non-

121   overlapping windows in two different introgression scenarios: 1) WTD_allopatry,

122   WTD_sympatry, MD_sympatry, Caribou and 2) MD_allopatry, MD_sympatry, WTD_sympatry,

123   Caribou (Fig.S3). We found 1243 outlier windows with a f_d higher than the 97.5% percentile in

124   the first scenario and 1248 in the second, each representing ~2.45% of the genome with 68

125   windows overlapping between comparisons (Fig.S3).

126   Measures of relative genomic divergence between WTD and MD were elevated (F_{ST} = 0.26) but

127   absolute divergence was relatively low (d_{xy} = 0.011); genetic diversity (π) was higher in WTD than

128   in MD (WTD = 0.008, MD = 0.004, Mann-Whitney test: p < 2.2e-16). We identified a total of 1183

129   windows presenting patterns consistent with one of the four selection scenarios (Fig.3, Fig.S4).

130   Of those, 1016 suggest a pattern of balancing selection, they were distributed across 236

131   scaffolds and represent 1.99% of the genome. Those windows contained 121 genes, some of

132   which showed an enrichment in ontologies associated with the sense of smell and chemical

133   stimuli, including three categories presenting an enrichment above 15-fold (Fig.4A, Fig. S5). We

134   also detected GOs related to the MHC and immunity, these include three categories with an

135   enrichment above 24-fold (Fig. S5). We identified patterns of background selection in 165

136   windows across 58 scaffolds, representing 0.32% of the genome. These windows harboured

137   208 genes identified with a UniProt ID and for which we found enrichment in GOs related to

138   epigenetic factors such as "Unmethylated CpG binding" (25-fold) or "Histone demethylation"

139   (15-fold) (Fig.4B, Fig. S6). These windows were either isolated or clustered together into

140   putative islands of divergence (Fig.S4). We found 2 windows under allopatric selection, each

141   window containing one gene: ACAP2, a GTPase activating protein, and PCDHB4 potentially

142   involved in cell-binding (UniProt 2022a; UniProt 2022b). When we applied a more liberal cut-

143   offs for F_{ST}, d_{xy}, and π we still observed no evidence for divergence with gene flow (Table S3).

144   ***Discussion***

145   We investigated the speciation history of the *Odocoileus* genera through introgression analyses

146   and genome scans. Our results show the absence of historical hybridisation between WTD &

147   MD suggesting speciation between those species took place without gene flow despite

148   contemporary and historical evidence (Stubblefield et al. 1986; Cronin et al. 1988; Cronin 1991;

149   Derr 1991; Hughes and Carr 1993; Cathey et al. 1998; Hornbeck and Mahoney 2000; Combe et

150   al. 2021; Russell et al. 2021). The main pattern of selection from the genome scans is that of

151   balancing selection, with notably patterns of divergence with gene flow absent. Moreover, the

152   scarcity of allopatric selection (i.e., positive selection post divergence) suggests isolation and

153   drift primarily underlies the species differentiation, with WTD and MD likely only recently

154   coming into secondary contact. The genome-wide scans combined with the absence of

155   introgression suggests that WTD & MD are far along the speciation continuum (i.e. Phase 3-4 in

156   Feder 2012), despite contemporary hybridization.

157   **Speciation with an absence of historical introgression**

158   Contemporary hybridisation in wild WTD & MD is documented at rates ranging from 1 to 19%

159   depending on the region (Stubblefield et al. 1986; Cronin et al. 1988; Cronin 1991; Derr 1991;

160   Hughes and Carr 1993; Cathey et al. 1998; Hornbeck and Mahoney 2000; Combe et al. 2021;

161   Russell et al. 2021). Moreover, a proposed historical mtDNA introgression would suggest

162   ancestral hybridisation occurred (Wright et al. 2022). We therefore expected to find signs of

163   hybridisation or introgression in our samples (e.g. Combe et al. 2021). We found evidence for

164   some contemporary hybridisation in one sample (Fig.1C), but no strong signals of historical

165   interbreeding at the genome-wide level were detected. Our sampling surely is limited for the

166   detection of recent hybridisation, but previous studies suggest gene flow is rather restricted

167   (Cronin et al. 1988; Cronin 1991; Derr 1991; Hughes and Carr 1993; Russell et al. 2021). For

168   example, Russell et al. (2019) discovered a 1% hybridisation rate in Alberta by sampling 987

169   individuals in a range overlap of approximately 230,000 km^2. While signatures of fd identified

170   potentially introgressed windows in ~1% of the genome, this metric can be a sign of

171   incomplete lineage sorting (ILS) rather than introgression, particularly with no signs of

172   genome-wide introgression as evidenced in the D statistic. Moreover, the independent treemix

173   analysis does not support historical gene flow, thus collectively, we consider the fd signal to be

174   reflective of ILS which would be expected given the high genome-wide diversity and a relatively

175   recent species-split (Cutter 2013).

176   The absence of signatures of ancestral hybridisation suggest that WTD & MD evolved in

177   allopatry and have recently come in secondary contact. The dynamic history of the North

178   American continent, notably the glacial cycles of the Quaternary, shaped the evolution and

179   distribution of many species (Avise et al. 1998; Hewitt 2000; Shafer et al. 2010). The use of

180   different refugia for prolonged periods of time during the glaciation events has increased the

181   differentiation between populations in several species, including deer (Latch et al. 2014;

182   Dussex et al. 2020; Kinoshita et al. 2020; Colella et al. 2021; Ito et al. 2021). Previous studies

183   show that MD persisted in several refugia during the glacial cycles of the Pleistocene, increasing

184   the intraspecific divergence (Latch et al. 2009; Latch et al. 2014; Wright et al. 2022).

185   Environmental shifts following the LGM also seem to have impacted the divergence between

186   subspecies of WTD & MD in Florida and Mexico for example (Ellsworth et al. 1994; Alminas et al.

187   2021). More recently, a form of allopatry was mediated through overharvest and habitat

188   destruction of WTD where populations greatly decreased to extirpation in some regions

189   (Deyoung et al. 2003; McDonald et al. 2004; Budd et al. 2018; Chafin et al. 2021). Here, the core

190   of the current sympatric zone was greatly affected, notably Colorado, Montana, Idaho,

191   Nebraska and Wyoming where WTD was almost extirpated before restocking efforts took place

192   starting in the mid 40's (McDonald et al. 2004). Historical introgression could further be

193   hindered by selection against hybrids, either through sexual selection, intrinsic or ecological

194   incompatibilities (Rundle and Nosil 2005; Rundle and Nosil 2005; Nosil 2012). Given the

195   genome-wide level of differentiation, we hypothesize that Dobzhansky-Muller incompatibles

196   (DMIs) are in play, noting that they often occur early and rapidly (Schumer et al. 2015)

197   Little is known about hybrid fitness in the *Odocoileus* genera. Assessment of a single F1

198   male suggested it was fertile and it showed no overt genetic defects (Derr et al. 1991), but

199   conclusions can not be drawn from a single observation. Sexual selection can be a powerful

200   driver of selection against hybrids (Servedio 2004). In WTD, while females might favour males

201   with larger antlers (Morina et al. 2018), sexual selection is overall poorly understood in

202   *Odocoileus* and most studies focus on male breeding success rather than sexual selection by

203   females (DeYoung et al. 2006; DeYoung et al. 2009; Jones et al. 2011; Newbolt et al. 2017).

204   Morphologically, however, WTD & MD are very similar and the identification of their hybrids is

205   often unreliable with the metatarsal gland and their escape gaits being the only characters

206   consistently distinguishing hybrids (Lingle 1992; Wishart 1980). Thus, we propose a testable

207   hypothesis that ecological incompatibilities combined with DMI maintain the species

208   boundary. More research is also needed on WTD/MD hybrids to quantify their fitness and

209   genomic regions underlying any selected hybrid phenotypes.

210   **Mixed signatures of selection underly speciation in North American deer**

211   Given ongoing hybridisation between WTD & MD and a history of recurrent glacial cycles, we

212   expected to find deer genomes showing patterns of divergence with gene flow. The absence of

213   introgression and the nonexistence of DwGF in our genome scans, even with more liberal

214   thresholds, is surprising (Table S1). The further absence of allopatric selection suggests a

215   diminished role of selection in deer speciation, lending support to drift-induced model more

216   consistent with Mayr's (1942) view. While the number of windows in allopatric selection

217   increased with more liberal thresholds (Table S1), they are never the dominant pattern. We

218   suggest this absence could be a result of species being far along on the speciation continuum

219   such that patterns of AS (specifically high F_{ST}) are no longer detectable or meaningful, which is

220   consistent with late stages of ecological speciation (Feder 2012). Recombination rates,

221   demography and selection can influence the patterns investigated here (Shang et al. 2021),

222   and thus future studies could integrate those factors to confirm the position of WTD & MD on

223   the speciation continuum.

224   Signatures of balancing selection (BLS) were widespread with windows containing

225   genes consistent with deer biology (olfactory receptors) and previous literature (i.e., MHC

226   genes). The major histocompatibility complex (MHC) is involved in pathogen recognition in

227   vertebrate species (Bernatchez and Landry 2003; Piertney and Oliver 2006). Polymorphism in

228   this complex is directly linked to disease susceptibility, the most diverse are the most resistant,

229   and there is evidence that MHC diversity is maintained by BLS (Bernatchez and Landry 2003;

230   Piertney and Oliver 2006; Aguilar et al. 2004; Pierini and Lenz 2018; Zhang et al. 2018). The same

231   is true for the results on sensory perception and particularly the olfactory receptors genes, both

232   of which are under BLS (Liu et al. 2021). Each allele in the olfactory receptors genes is expressed

233   by a single sensory neuron (Degl'Innocenti and D'Errico 2017), it is therefore plausible that

234   diversity in these genes would be maintained by BLS. Olfactory receptors in WTD and MD, and

235   more broadly deer, is critical to both predator detection and rutting behaviour (Ditchkoff 2011).

236   Anatomically, WTD has an architecture optimised for the delivery of sensory stimuli to the

237   receptors in the nose (Ranslow et al. 2014). Chamaillé-Jammes et al. (2014) showed that MD

238   inspect and respond differently to different predators' olfactory cues, and both human and

239   mammalian predators utilize wind direction and other mechanism to minimize scent

240   detection by deer (Zagata and Haugen 1974; Cherry and Barton 2017). In other ungulates

241   species, olfactory reception is associated with sexual activity (Cann et al. 2019), maternal

242   behaviour (Keller and Lévy 2012; Blank and Yang 2017), territory choice (Deutsch and Nefdt

243   1992), predator response (Kuijper et al. 2014; Wikenros et al. 2015) and foraging (Hirata and

244   Kusatake 2021). Low differentiation (F_{ST}) but high diversity (π) of olfactory receptors in deer is

245   consistent with their biology and behaviour and would be expected to be under strong

246   selection pressures.

247   Conclusion

248   Our results suggests that WTD & MD do not conform to a speciation with gene flow scenario

249   despite evidence of contemporary hybridization. We propose they were spatially separated

250   during the Quaternary glaciation cycles where genome-wide differentiation accrued via drift.

251   This is evidenced by the majority of the genome (> 97%) not matching a selection scenario.

252   Increased sampling and model-based demographic assessment should help clarify the role of

253   glaciers and secondary contact in North American deer. The near absence of patterns of

254   allopatry and hybridisation suggest that white-tailed and mule deer are far along the

255   speciation continuum, the absence of introgression signs could suggest DMIs and selection

256   against hybrids which would contribute to the reinforcement of reproductive isolation. Future

257   studies should focus on assaying hybrid phenotypes and vigour.

258   ***Materials and Methods***

259   Sampling & Sequencing

260   We obtained tissue samples from harvested deer collected across the range of WTD & MD

261   including areas of sympatry and allopatry (Fig.1A, Table 1). These areas were determined from

262   NatureServe records and adjusted with IUCN range data (IUCN 2015a; IUCN 2015b;

263   NatureServe 2021a; NatureServe 2021b). Specifically, the state of Washington and the province

264   of British Columbia, for which the coasts are largely MD allopatric whereas the eastern parts

265   are sympatric, were divided in two by the 120th meridian West. We also removed Manitoba from

266   the sympatric list given the area is quite small and not sampled. We extracted DNA from tissue

267   using the Qiagen DNeasy Blood and Tissue Kit following manufacturer's instructions and

268   checked the samples concentration using Invitrogen Qubit assays. WGS libraries were

269   generated at The Centre for Applied Genomics in Toronto, Canada and sequenced to an

270   average of 4x coverage on an Illumina HiSeqX.

271   **Data processing**

272   Raw reads quality was examined using FastQC (v0.11.9; (Andrews S. 2010). We trimmed the

273   reads using Trimmomatic (v0.36; Bolger et al., 2014), and aligned them to the WTD genome

274   (GCA_014726795.1) with bwa-mem (v0.7.17; Li & Durbin, 2009). We sorted the reads using

275   SAMtools sort (v1.10; Li et al., 2009), identified duplicates reads with Picard MarkDuplicates

276   (v2.23.2; Broad Institute, GitHub Repository., 2019) and removed them using Sambamba view

277   (v0.7.0; Tarasov et al., 2015). We performed a local re-alignment using GATK

278   RealignerTargetCreator and IndelRealigner (v4.1.7.0; McKenna et al., 2010). For further quality

279   checks, we used Sambamba flagstat, mosdepth (v0.3.1; Pedersen and Quinlan 2018) and

280   MultiQC (v1.10; Ewels et al., 2016). Some samples were sequenced to a higher depth on

281   multiple lanes, for those samples, before the duplicate removal step, the read groups were

282   specified and both sequencing files merged using Picard AddOrReplaceReadGroups and

283 MergeSamFiles. The rest of the pipeline was performed as described above with the addition

284 of a final step: we reduced the final coverage to the average coverage of all other samples (4x)

285 using Picard DownsampleSam.

286 We created three datasets for our different analyses. The first contained the variants

287 among all deer individuals (hereafter called DeerSNP dataset). We produced it using ANGSD

288 (v0.918; Korneliussen et al., 2014) and estimated genotype likelihoods following the GATK

289 model (-gl 2) and called genotypes (-doGeno 4) with the following filtering: SNPs with a

290 minimum p-value of $1e^{-6}$, a minimum base quality of 20 and Minimum mapQ quality of 20 (-

291 SNP_pval 1e-6, -minMapQ 20 & -minQ 20). The second dataset additionally included invariant

292 sites (referred to as DeerALL), it was produced following the same method as DeerSNP except

293 for the filtering on the SNP p-value (-SNP_pval 1e-6) which was removed. Our third dataset

294 comprises the 28 deer individuals and one caribou sample, all mapped to the caribou genome

295 (GCA_014898785.1; afterwards called DeerCAR dataset). For this dataset, we first sorted the

296 deer data by read name and converted them to fastq using SAMtools sort and fastq

297 respectively. We mapped the deer data to the caribou reference genome using bwa-mem. We

298 ran ANGSD using the same procedure as for DeerSNP dataset and with the addition of a

299 caribou genome sequence obtained for this analysis (Dedato et al. 2022).

300 Population structure analyses

301 To infer admixture proportions and compute a PCA on allele frequencies based on genotype

302 likelihood data we used PCAngsd (Meisner and Albrechtsen 2018) and NGSadmix (Skotte et al.

303 2013) on the DeerSNP dataset. NGSadmix was run with K = 1 up to K =7 and the best K value

304 was determined with CLUMPAK (Kopelman et al. 2015) following the Evanno method (Evanno

305 et al. 2005) with 7 replicates for each K value. We investigated the correlation between the

306   distribution on each PC and each individual's range in R with a linear model including the

307   species and either latitude or longitude, and a Pearson's correlation.

Genome-wide introgression

309   To evaluate the extent of introgression between sympatric MD and WTD, we used the ABBA-

310   BABA test, which allows for the detection of introgression between three populations P1, P2 &

311   P3 and an outgroup (Green et al. 2010; Durand et al. 2011). We ran the ABBA-BABA analysis

312   implemented in ANGSD on the DeerCAR dataset between individuals (-doAbbababa 1) and

313   between populations (-doAbbababa2 1; Soraggi et al. 2018), using the caribou as outgroup.

314   The D-statistic, standard error and Z-score were computed with ANGSD's accompanying R

315   scripts: jackKnife.R and estAvgError.R. To further our understanding of the admixture events

316   and build a maximum likelihood tree of our system, we used treemix (v1.13; Pickrell &

317   Pritchard, 2012) on both DeerCAR and DeerSNP datasets. We constructed the maximum

318   likelihood trees with migration events ranging from 0 to 5, either WTD_allopatry or Caribou as

319   root, and accounted for linkage using 1000 SNPs per block. Tree and residuals visualisations

320   were performed with associated R script plotting_funcs.R and the variance explained by each

321   migration event was computed with the get_f() function.

In-windows introgression

323   The D statistic is sensitive to genomic variation (π), and should not be used to determine

324   introgression on a small scale (Martin et al. 2015). The f_d statistic, proposed by Martin et al.

325   (2015), is less dependent on diversity than D and therefore allows to detect potentially

326   introgressed regions of the genome. We used the python script ABBABABAwindows.py (Martin

327   2021) to estimate in D and f_d in 50Kbp windows to detect potentially introgressed loci in our

328   DeerCAR dataset on two comparisons: 1) P1 = WTD allopatry, P2 = WTD sympatry, P3 = MD

329   sympatry and 2) P1 = MD allopatry, P2 = MD sympatry, P3 = WTD sympatry, both with the

330   caribou as outgroup. We then identified potentially introgressed windows as those having a f_d

331   value higher than the 97.5% quantile. Their position was used in BEDTools intersect (v2.30.0;

332   Quinlan and Hall 2010) with the caribou annotation file to identify the genes present in

333   putatively introgressed windows.

334   **Genome scans**

335   To detect islands of divergence between WTD & MD, we used the python script

336   popgenWindows.py (Martin 2021) to estimate individual heterozygosity, F_{ST}, D_{xy} and π in 50Kbp

337   windows (note we filtered out scaffolds shorter than 50kb). The accurate computation of D_{xy}

338   and π require a dataset including the invariant sites, we performed this analysis on the DeerALL

339   dataset. To detect the outlier loci, we based our approach on the four selection scenarios

340   developed in Shang et al. (2021): I) Divergence with gene flow that presents as high F_{ST} and D_{xy}

341   but low π; II) allopatric selection that shows high F_{ST}, low π and stable D_{xy}; III) background

342   selection that presents as high F_{ST} but low π and D_{xy}; and IV) balancing selection that displays

343   as low F_{ST} but high π and D_{xy}. Thresholds were set as the upper or lower 5% for a high or low

344   criteria, and between 45 and 55% for a stable condition. Outliers were flagged as belonging to

345   one of the four scenarios when they met the specific criteria shown in Table 2. For each

346   scenario, we identified genes present in outlier regions by comparing the coordinates of the

347   outlier windows and the WTD annotation file in BEDTools intersect. We extracted the gene

348   names from those regions as well as those from their associated scaffolds. The gene list from

349   outlier windows was uploaded to ShinyGO for enrichment analysis with the scaffold's gene list

350   as background information. We downloaded the enrichment analysis results for the molecular

351   function, biological process, and cellular component GOs and analysed the results in R. We

352   also uploaded the gene list to UniProt's Retrieve/ID mapping tool (Pundir et al. 2016) and

353   downloaded the information for visual inspection. All results were analysed in R version 4.1.0

354   "Camp Pontanezen" (R Core Team 2021) .

Data availability

356   Raw reads for the 28 deer individuals were deposited on the NCBI Sequence Read Archive

357   (Accession   number   XXXX).   All   scripts   are   available   on   GitLab

358   (https://gitlab.com/WiDGeT_TrentU/graduate_theses/-/tree/master/Kessler/CH_01)

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691

692

A

Oh_AK1
Oh_BC6
Ov_SK3
Oh_AB1
Ov_SK2
Ov_AB1
Oh_SK5
Oh_BC8
Oh_BC
Ov_ON5
Ov_ON6
Oh_WA1
Ov_MT1
Ov_ON3
Oh_OR2
Ov_SD1
Ov_PA3
Oh_NV2
Oh_UT2
Oh_SD2
Ov_VA3
Oh_CO1
Ov_KS1
Ov_SC1
Oh_CA
Oh_NM2
Ov_AL3
Ov_MX2

B

PC2 (8%)

0.25
0.00
−0.25
−0.50

Ov_MX2

Oh_BC8
Oh_OR2
Oh_WA1
Oh_BC1
Oh_AK1

−0.2   −0.1   0.0   0.1   0.2

PC1 (59%)

C

WTD_allopatry	WTD_sympatry	MD_sympatry	MD_allopatry

1.00
0.75
0.50
0.25
0.00

Ov_ON3 Ov_ON5 Ov_ON6 Ov_PA3 Ov_VA3 Ov_AL3 Ov_SC1 | Ov_AB1 Ov_SK2 Ov_SK3 Ov_SD1 Ov_KS1 Ov_MT1 Ov_MX2 | Oh_AB1 Oh_UT2 Oh_CO1 Oh_SK5 Oh_OR2 Oh_SD2 Oh_NM2 | Oh_NV2 Oh_CA3 Oh_BC6 Oh_BC8 Oh_BC1 Oh_AK1 Oh_WA1

A

MD_sympatry – MD_sympatry – MD_allopatry
MD_sympatry – MD_sympatry – WTD_allopatry

MD_sympatry – WTD_allopatry – MD_allopatry
MD_sympatry – MD_allopatry – MD_sympatry

MD_sympatry – MD_allopatry – MD_sympatry
MD_sympatry – WTD_allopatry – MD_sympatry

MD_sympatry – MD_sympatry – MD_allopatry
MD_sympatry – WTD_allopatry – WTD_allopatry
MD_sympatry – MD_sympatry – WTD_allopatry
MD_sympatry – MD_sympatry – MD_allopatry

MD_sympatry – MD_allopatry – WTD_allopatry
WTD_allopatry – MD_allopatry – MD_sympatry

MD_sympatry – WTD_allopatry – MD_allopatry
MD_allopatry – WTD_allopatry – WTD_allopatry
MD_sympatry – WTD_allopatry – MD_sympatry

WTD_allopatry – WTD_allopatry – MD_allopatry
MD_sympatry – MD_sympatry – MD_allopatry

MD_sympatry – WTD_allopatry – MD_sympatry
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MD_sympatry – WTD_sympatry – WTD_allopatry
MD_sympatry – MD_allopatry – WTD_sympatry

MD_sympatry – MD_allopatry – WTD_allopatry
MD_sympatry – WTD_allopatry – WTD_allopatry
WTD_allopatry – MD_allopatry – MD_sympatry

MD_sympatry – MD_sympatry – MD_allopatry
MD_sympatry = MD_sympatry = MD_allopatry
WTD_sympatry – MD_sympatry – WTD_allopatry
MD_sympatry = MD_sympatry = WTD_allopatry
MD_sympatry = MD_sympatry = WTD_allopatry
WTD_allopatry = WTD_allopatry – MD_allopatry

WTD_allopatry – MD_allopatry – WTD_allopatry
WTD_allopatry – WTD_allopatry – MD_sympatry
MD_sympatry = WTD_allopatry = MD_allopatry

MD_allopatry – WTD_allopatry – MD_sympatry
MD_allopatry – MD_allopatry = MD_sympatry

B

WTD_allopatry
MD_sympatry – WTD_allopatry = WTD_allopatry
MD_sympatry = WTD_allopatry = WTD_allopatry
WTD_allopatry – WTD_allopatry – MD_allopatry

WTD_sympatry
WTD_allopatry – WTD_allopatry – MD_sympatry
WTD_allopatry = MD_allopatry = MD_sympatry

MD_allopatry
WTD_allopatry – MD_allopatry – WTD_allopatry
WTD_allopatry = MD_allopatry = WTD_allopatry
WTD_allopatry – WTD_allopatry – MD_allopatry

MD_sympatry
WTD_allopatry = WTD_allopatry = MD_allopatry
WTD_allopatry – WTD_allopatry – MD_sympatry
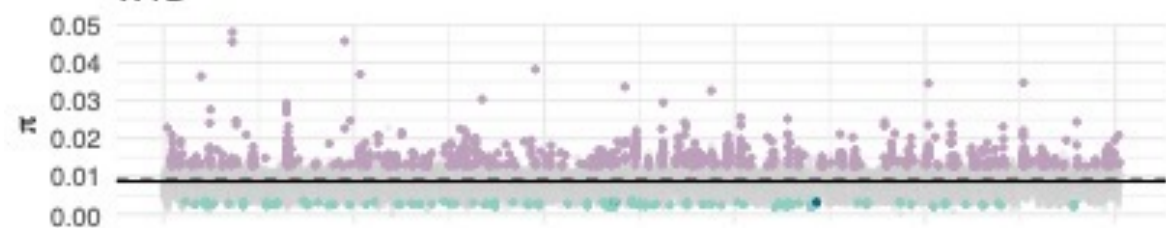
WTD_allopatry = WTD_allopatry = MD_sympatry

Scenarios: ● Allopatric selection   ● Background selection   ● Balancing selection

A

Sensory perception of smell

Detection of chemical stimulus involved in sensory perception of smell

Detection of chemical stimulus involved in sensory perception

Sensory perception of chemical stimulus

Detection of chemical stimulus

Detection of stimulus involved in sensory perception

Fold enrichment

nGenes ● 7.0 ● 7.5 ● 8.0 ● 8.5 ● 9.0

−log10 FDR
2 3 4

B

Unmethylated CpG binding

Lysine−acetylated histone binding

Acetylation−dependent protein binding

Protein demethylase activity

Histone demethylase activity

DNA binding

Nucleic acid binding

Fold enrichment

nGenes ● 20 ● 40 ● 60

−log10 FDR
1.5 1.8 2.1 2.4

Figure legends

Figure 1: Deer individuals in the study. (A) Sampling locations, colours represent the different groups: WTD allopatry (blue), WTD sympatry (violet), MD sympatry (green), MD allopatry (orange). Areas of sympatry are coloured in dark grey and areas of allopatry in light grey, from the IUCN range data for both species (IUCN, 2015a, 2015b). (B) Principal component analysis performed in PCAngsd. (C) Admixture proportions for K = 2 calculated in NGSAdmix.

Figure 2: Introgression analyses. (A) ABBA-BABA analysis between individuals, we only show their assigned populations for clarity. Colour gradient represents Z score, excess of ABBA depicted as points, excess of BABA shown as triangles, arrows illustrate which populations are involved in gene flow. (B) Maximum likelihood tree inferred by Treemix for 0 migration (right) and the variance explained by each migration event (left).

Figure 3: Distribution of windows presenting pattern of selection across the genome for d_{xy} (top), F_{st} (middle) and π (bottom). Windows following a pattern of selection are coloured according to its corresponding evolutionary scenario, grey windows do not exhibit pattern of selection. The continuous and dashed line represent the mean and median respectively.

Figure 4: Gene enrichment analysis from outlier windows. (A) Biological process GOs enriched in the Balancing selection outlier windows. (B) Molecular function GOs enriched in the Background selection windows. Colour represents -log10 FDR, size indicates the number of genes in our dataset that falls under given GO category.

Tables

Table 1: Sample information

Genome_ID	Species	Location	Sex	Group
Ov_ON3	*O_virginianus*	Ontario	Female	WTD_allopatry
Ov_ON5	*O_virginianus*	Ontario	Male	WTD_allopatry
Ov_ON6	*O_virginianus*	Ontario	Male	WTD_allopatry
Ov_PA3	*O_virginianus*	Pennsylvania	Female	WTD_allopatry
Ov_VA3	*O_virginianus*	Virginia	Female	WTD_allopatry
Ov_AL3	*O_virginianus*	Alabama	Unknown	WTD_allopatry
Ov_SC1	*O_virginianus*	South Carolina	Female	WTD_allopatry
Ov_AB1	O_virginianus	Alberta	Female	WTD_sympatry
Ov_SK2	O_virginianus	Saskatchewan	Male	WTD_sympatry
Ov_SK3	O_virginianus	Saskatchewan	Male	WTD_sympatry
Ov_SD1	O_virginianus	South Dakota	Male	WTD_sympatry
Ov_KS1	O_virginianus	Kansas	Female	WTD_sympatry
Ov_MT1	O_virginianus	Montana	Male	WTD_sympatry
Ov_MX2	O_virginianus	Mexico	Male	WTD_sympatry
Oh_NV2	*O_hemionus*	Nevada	Male	MD_allopatry
Oh_CA3	*O_hemionus*	California	Unknown	MD_allopatry
Oh_BC6	*O_hemionus*	British Columbia	Male	MD_allopatry
Oh_BC8	*O_hemionus*	British Columbia	Male	MD_allopatry
Oh_BC1	*O_hemionus*	British Columbia	Female	MD_allopatry
Oh_AK1	*O_hemionus*	Alaska	Unknown	MD_allopatry
Oh_WA1	*O_hemionus*	Washington	Male	MD_allopatry
Oh_AB1	*O_hemionus*	Alberta	Female	MD_sympatry
Oh_UT2	*O_hemionus*	Utah	Male	MD_sympatry
Oh_CO1	*O_hemionus*	Colorado	Female	MD_sympatry
Oh_SK5	*O_hemionus*	Saskatchewan	Unknown	MD_sympatry
Oh_OR2	*O_hemionus*	Oregon	Unknown	MD_sympatry
Oh_SD2	*O_hemionus*	South Dakota	Female	MD_sympatry
Oh_NM2	*O_hemionus*	New Mexico	Male	Oh_sympatry

Table 2: Different scenario and their thresholds for F_{st}, π and D_{xy} percentiles

Scenario	F_{st}	π	D_{xy}	Panel in Fig.1A Shang et al. (2021)
Divergence with gene flow (DwGF)	> 0.95	< 0.05	> 0.95	1
Allopatric selection (AS)	> 0.95	< 0.05	Between 0.45 & 0.55	2
Background selection (BGS)	> 0.95	< 0.05	< 0.05	3
Balancing selection (BLS)	< 0.05	> 0.95	> 0.95	4