Associations entre la diversité génétique et la performance individuelle chez le caribou migrateur (Rangifer tarandus) du nord du Québec et du Labrador

Mémoire

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Associations entre la diversité génétique et la valeur adaptative chez le caribou migrateur (*Rangifer tarandus*) du nord du Québec et du Labrador

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Résumé

Les liens entre la diversité génétique et la performance individuelle peuvent révéler l'existence de dépression de consanguinité ou de sélection naturelle à certains loci fonctionnels dans une population. Dans cette étude, nous avons étudié l'association de la diversité génétique avec des traits de performance (survie annuelle et masse corporelle) chez le caribou migrateur appartenant à deux populations en déclin au Québec et au Labrador. Nous avons évalué la diversité génétique à deux échelles génétiques : i) à l'échelle du génome (22 073 polymorphismes nucléotidiques simples) et ii) chez un locus (DRB) du complexe majeur d'histocompatibilité (CMH), un gène d'immunité. Pendant les 20 années de notre période d'échantillonnage, la diversité génétique globale et celle du locus CMH-DRB sont restées stables. La diversité génétique globale n'était pas associée aux traits de performance, mais la diversité fonctionnelle de CMH-DRB était associée négativement à la survie annuelle des adultes. Par ailleurs, nous avons détecté une faible différentiation génétique entre les deux troupeaux qui étaient considérés jusqu'ici comme une seule population d'un point de vue génétique. Nos résultats suggèrent que le déclin rapide des deux troupeaux de caribous n'a pas entraîné de perte de diversité génétique, même si une augmentation de la dérive génétique pourrait être à l'origine de la structure génétique observée. Ils supportent aussi l'hypothèse qu'il n'y aurait pas de dépression de consanguinité dans les troupeaux, malgré leur déclin démographique marqué. L'association négative de la diversité au CMH avec la survie que nous avons détectée va à l'encontre de la majorité des études publiées sur ce sujet, qui montrent en général une association positive. Nos résultats suggèrent que la diversité au CMH n'est peut-être pas avantageuse dans des écosystèmes où la diversité de pathogènes est faible ou dans lesquels l'abondance de pathogènes change rapidement en réponse aux changements climatiques.

Abstract

Associations between genetic diversity and individual performance may indicate inbreeding depression or selective pressures applied on some functional loci in a population. In this study, we looked at the association of genetic diversity with performance traits (annual survival and body mass) in migratory caribou of two declining herds in Québec and Labrador. We assessed genetic diversity at two genetic scales: i) genome-wide diversity estimated with 22,073 single nucleotide polymorphisms and ii) diversity of one locus (DRB) of the major histocompatibility complex (MHC). During the 20-year sampling period, genome-wide and MHC-DRB diversity remained stable. Genome-wide diversity was not associated with performance, but MHC-DRB functional diversity showed a negative association with annual survival of adults. Furthermore, we found a slight differentiation of the two herds that were considered until now as a single population from a genetic point of view. Our results suggest that the rapid decline of both herds did not lead to a loss of genetic diversity, even though an increase in genetic drift could be responsible for the genetic structure we observed. They also suggest that the herds do not suffer from inbreeding depression despite their marked decline. The negative association of MHC-DRB diversity with survival is opposite to the majority of studies published on this matter that usually show a positive association. Our results suggest that MHC diversity might not be beneficial in ecosystems with low pathogen diversity or in which pathogen abundance changes quickly in response to climate change.

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a.a. : *Amino acid*
ABS : *Antigen binding site*
AICc : *Akaike's information criterion for small sample sizes*
bp : Base pair
CI : *Confidence interval*
CMH : Complexe majeur d'histocompatibilité
CMH-DRB : exon 2 du gène DRB du complexe majeur d'histocompatibilité
DAPC : *Discriminant analysis of principal components*
ddRADSeq : *double-digest restriction-site associated DNA sequencing*
DTT : *Dithiothreitol*
EDTA : *Ethylenediaminetetraacetic acid*
GRM : *Genetic relatedness matrix*
H_e : *Expected heterozygosity*
HFC : *Heterozygosity-fitness correlation*
HHC : *Heterozygosity-heterozygosity correlation*
H_o : *Observed heterozygosity*
HWE : *Hardy-Weinberg equilibrium*
MFFP : Ministère des Forêts, de la Faune et des Parcs
MHC : *Major histocompatibility complex*
MHC-DRB : *Second exon of MHC gene DRB*
mx.lcs : *Max_locus_stacks*
OR : Odds ratio
PCA : *Principal component analysis*
PCR : *Polymerase chain reaction*
PSS : *Positively selected sites*
RAF : Rivière-aux-Feuilles
RG : Rivière-George
sd : *Standard deviation*
se : *Standard error*
sMLH : *Standardized multi-locus heterozygosity*
SNP : Single nucleotide polymorphism

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Avant-propos

Ce mémoire comporte quatre chapitres dont le premier est une introduction générale présentant les concepts clés de mon projet ainsi qu'une mise en contexte et le dernier est une conclusion rappelant les résultats principaux de ma recherche et leurs implications. Les deux chapitres (2 et 3) formant le corps du texte sont des articles scientifiques rédigés en anglais qui seront soumis à des journaux prochainement. Voici les détails concernant ces publications :

Chapitre 2 : No evidence of inbreeding depression in fast declining herds of migratory caribou
Soumission prévue à *Journal of Evolutionnary Biology*
Auteure principale : Marianne Gagnon (MG)
Coauteurs : Glenn Yannic (GY), Charles Perrier (CP), Steeve D. Côté (SC)

MG, GY et SC ont défini le projet. Les échantillons ont été récoltés par SC et son équipe. MG et GY ont réalisé les travaux de laboratoire. MG et CP ont réalisé la bioinformatique sur les données de séquences et le reste des analyses a été fait par MG (HFCs) et GY (Structure). MG a écrit l'article, en collaboration avec GY et SC.

Chapitre 3 : Adult survival in migratory caribou is negatively associated with MHC functional diversity
Soumission prévue à *Proceedings of the Royal Society B*
Auteure principale : Marianne Gagnon (MG)
Coauteurs : Glenn Yannic (GY), Frédéric Boyer (FB), Steeve D. Côté (SC)

MG, GY et SC ont défini le projet. Les échantillons ont été récoltés par SC et son équipe. MG et GY ont réalisé les travaux de laboratoire. MG et FB ont réalisé la bioinformatique sur les données de séquences et le reste des analyses a été fait par MG. MG a écrit l'article, en collaboration avec GY et SC.

1. Introduction

1.1 Valeur adaptative, dynamique des populations et diversité génétique

La valeur adaptative d'un individu peut être définie comme sa capacité à survivre et à se reproduire (Orr 2009). Elle peut être quantifiée par le nombre ou la proportion de ses descendants qui contribuent à la génération suivante (Orr 2009, Allendorf et al. 2013, Saether and Engen 2015). En pratique, mesurer la valeur adaptative est très difficile, voire impossible, et il s'avère souvent plus aisé de mesurer des traits associés à la valeur adaptative ; par exemple la survie ou la reproduction, ou d'autres traits qui les affectent. On suppose alors qu'une partie des composantes de la valeur adaptative suffisent à en dresser un portrait réaliste. Par rigueur, dans ce texte, nous réfèrerons à ces traits comme des indices de la performance individuelle, plutôt que de parler directement de valeur adaptative. Comme la survie et la reproduction sont affectées en partie par le génotype des individus et en partie par l'environnement, la valeur adaptative dépend de facteurs génétiques, de facteurs environnementaux et de leurs interactions (Orr 2009, Allendorf et al. 2013). Par conséquent, des variations de l'environnement peuvent entraîner des changements dans la valeur adaptative des génotypes dans une population. Si les nouvelles contraintes sont suffisamment fortes, il en résultera un épisode de sélection où les individus possédant les génotypes les plus performants auront des probabilités de survie et de reproduction plus élevées que celles des autres individus (Allendorf et al. 2013). À l'échelle populationnelle, des variations dans la valeur adaptative des individus peuvent donc influencer la dynamique de la population (Gaillard et al. 2000b). Identifier les paramètres qui modulent la valeur adaptative peut contribuer à une meilleure compréhension des processus évolutifs et démographiques qui affectent les populations.

Les effets des variables environnementales ou extrinsèques sur les traits associés à la performance individuelle ont été largement étudiés (Pioz et al. 2008, Post and Forchhammer 2008, Ballesteros et al. 2012, Simard et al. 2014, Lo and Shaner 2015, Pansch et al. 2018). On sait, par exemple, que les variations météorologiques et la prévalence d'infections bactériennes dans l'environnement peuvent expliquer chacun jusqu'à un tiers de la variance observée dans le succès reproducteur des individus d'une population de chamois (*Rupicapra rupicapra*; Pioz et al. 2008). Chez la même espèce, la phénologie du printemps et la qualité de la nourriture ainsi que la densité de population affectent la

masse corporelle des juvéniles (Garel et al. 2011), une variable généralement positivement corrélée à la probabilité de survie et au succès reproducteur chez les grands mammifères (Festa-bianchet et al. 1997, Gaillard et al. 2000a, Beauplet and Guinet 2007, Pachkowski et al. 2013). Des études génétiques ont aussi permis d'identifier des loci étroitement associés à la performance chez des populations naturelles de mouton de Soay (*Ovis aries*; Beraldi et al. 2007). Des paramètres génétiques plus généraux tels que la diversité génétique pourraient eux aussi avoir des effets positifs (Olano-Marin et al. 2011, Hoffman et al. 2014, Brambilla et al. 2015, 2018) ou négatifs (Charbonnel et al. 2010, Olano-Marin et al. 2011, Acevedo-Whitehouse et al. 2018) sur la valeur adaptative, en fonction de l'environnement.

À l'échelle populationnelle, la diversité génétique augmente le potentiel adaptatif et la résilience des populations aux changements environnementaux (Booy et al. 2000, Ehlers et al. 2008, Hughes et al. 2008, Agashe 2009, Forsman and Wennersten 2016) et représente donc un avantage. En conséquence, la conservation des espèces et des populations devrait inclure la protection de la diversité génétique à cette échelle, surtout dans le contexte des changements globaux (Frankham 2005, Allendorf et al. 2013, McMahon et al. 2014). Par ailleurs, puisque la dynamique d'une population est susceptible d'affecter sa diversité génétique, l'étude de la diversité génétique peut s'avérer être un outil pertinent pour le suivi des populations (Russel 1988). Par exemple, une réduction de la diversité génétique d'une population dans le temps pourrait indiquer que son effectif est en baisse ou que la connectivité avec les populations voisines a été interrompue ou fortement diminuée (Allendorf et al. 2013). À l'inverse, une augmentation de la diversité génétique pourrait par exemple indiquer que de nouveaux individus ont immigré dans cette population (Steinbach et al. 2018).

L'utilité de la diversité génétique intra- et interpopulationnelle pour le suivi et la gestion des populations est largement reconnue (Russel 1988, Frankham 2005). À l'échelle individuelle, l'utilisation de la diversité génétique comme outil de suivi demande encore qu'on acquière des connaissances fondamentales sur ses liens avec la valeur adaptative. En effet, différents mécanismes sont à l'origine d'associations entre la diversité génétique et la valeur adaptative et ceux-ci peuvent générer des associations positives ou négatives (Charbonnel et al. 2010, Olano-Marin et al. 2011, Hoffman et al. 2014, Brambilla et al. 2015, Acevedo-Whitehouse et al. 2018). Néanmoins, si la diversité génétique était aussi avantageuse pour l'individu qu'elle peut l'être pour les populations, elle devrait elle aussi

faire l'objet d'efforts de conservation. Dans l'éventualité où elle serait associée à la valeur adaptative, la diversité génétique individuelle pourrait aussi fournir d'autres renseignements sur l'état d'une population et des individus qui la composent tels que la présence de dépression de consanguinité.

1.2 Corrélations diversité génétique – performance

L'association de la performance individuelle avec la diversité génétique a été mise en évidence chez plusieurs populations animales (de Assunção-Franco et al. 2012, Herdegen et al. 2013, Hoffman et al. 2014, Brambilla et al. 2015, 2018). Ces corrélations sont habituellement appelées des corrélations entre hétérozygotie et valeur adaptative (en anglais : *heterozygosity-fitness correlations ;* HFCs), bien que les mesures de diversité génétique intra-individuelle ne se limitent pas à l'hétérozygotie. En effet, la diversité génétique individuelle peut aussi être estimée par le nombre d'allèles que possède un individu pour des gènes dupliqués (Wegner et al. 2003), par la distance entre des allèles de marqueurs microsatellites (Hansson et al. 2004), par la diversité nucléotidique ou d'haplotypes (McDevitt et al. 2009) ou encore par la diversité en acides aminés (Oosterhout et al. 2006). Lorsqu'ils sont basés directement sur la séquence d'ADN, ces indices témoignent d'une diversité *purement* génétique. Quand ils se basent sur la séquence d'acides aminés traduite à partir d'une séquence d'ADN, ils reflètent davantage la diversité *fonctionnelle* de cette séquence. Par ailleurs, comme il s'avère difficile de mesurer la valeur adaptative (Orr 2009), les variables réponses utilisées dans les HFCs sont généralement plutôt des indices de la performance individuelle. Des traits comme la masse corporelle (Herdegen et al. 2013, García-Navas et al. 2014, Brambilla et al. 2015), la croissance (Han et al. 2013, Brambilla et al. 2015) ou le taux d'infestation par des parasites (Paterson et al. 1998, Oliver et al. 2009, Brambilla et al. 2015) sont fréquemment utilisés parce qu'ils affectent la survie et le succès reproducteur. Il importe par contre d'être prudent dans l'interprétation d'associations entre la diversité génétique et des traits de performance individuelle, puisque ceux-ci peuvent dresser un portrait incomplet de la valeur adaptative d'un individu.

D'une étude à une autre, le sens des HFCs varie selon l'échelle génétique étudiée et le contexte évolutif des populations (Chapman et al. 2009). De façon générale, les mécanismes à l'origine des HFCs observées peuvent être regroupés en deux catégories : ceux découlant des *effets généraux* (David et al. 1995, Hansson and Westerberg 2002), intimement liés à la consanguinité ou au degré d'exogamie des individus, et ceux résultants d'*effets locaux* ou *directs* (David et al. 1995, David 1998),

associés au régime de sélection sur certains loci fonctionnels. Parce que le génome n'est pas indépendant des loci qui le composent et inversement, les effets généraux, locaux ou directs ne sont pas mutuellement exclusifs. Il est même possible que dans une même population, une HFC ait un sens différent selon le type de marqueur génétique utilisé – les marqueurs neutres témoignant des effets généraux et les marqueurs fonctionnels témoignant d'effets directs (Olano-Marin et al. 2011). De plus, si la diversité génétique d'un locus était corrélée à celle du génome, une HFC à ce locus pourrait aussi refléter des effets généraux plutôt que directs ou locaux (García-Navas et al. 2014).

Parce qu'elles impliquent à la fois une composante phénotypique et une composante génétique, les HFCs raffinent notre compréhension de l'écologie et de l'évolution. Chaque type de HFC nous renseigne sur différents mécanismes qui peuvent agir de façon concomitante dans une même population, tels que la dépression de consanguinité ou d'exogamie, la sur- ou la sous-dominance et la sélection balancée, fluctuante ou dépendante de la fréquence (Stearns and Hoekstra 2005). Ces concepts seront définis dans les sections suivantes.

1.2.1 Effets généraux

Il est souvent admis que la diversité génétique d'un individu, généralement exprimée par son degré d'hétérozygotie, reflète son degré d'exogamie ou inversement, son degré de consanguinité. La consanguinité et l'exogamie représentent deux extrêmes d'un gradient d'apparentement mesuré entre les parents d'un individu, allant de très apparentés (consanguins) à très éloignés (exogames). Ainsi, les individus les plus consanguins ont une diversité génétique faible alors que les individus exogames présentent un degré de diversité génétique élevé (Wright 1977).

Une corrélation entre la diversité génétique individuelle et la performance peut émerger dans une population en présence de dépression de consanguinité ou d'exogamie (*hypothèse des effets généraux* ; David et al. 1995, Hansson and Westerberg 2002). La dépression de consanguinité est une réduction de la valeur adaptative chez les individus dont les parents sont très apparentés. Les effets négatifs de la consanguinité résultent de l'expression de mutations délétères récessives ou du génotype homozygote moins performant à des gènes surdominants (dont le génotype hétérozygote est celui qui confère la plus grande valeur adaptative; Charlesworth and Charlesworth 1987). Ainsi, en présence de dépression de consanguinité dans une population, les individus consanguins, qui

présentent une diversité génétique réduite, ont également une valeur adaptative moindre. Il en résulte une corrélation positive entre la diversité génétique et la valeur adaptative. À l'inverse, la descendance d'individus dont le degré d'apparentement est très faible peut aussi montrer une performance réduite. C'est ce qu'on appelle la dépression hybride ou la dépression d'exogamie. Les effets négatifs de l'exogamie résultent d'une incompatibilité entre le génotype hybride et l'environnement, surtout observée dans la première génération de la descendance, ou de bris de combinaisons de gènes coadaptés, observé plutôt dans les générations suivantes (Freeland et al. 2011). On observe ces effets négatifs dans des cas extrêmes d'hybridation de deux espèces (Costa e Silva et al. 2012), mais aussi dans les cas d'hybridation de deux populations génétiquement différenciées (Gilk et al. 2004). En présence de dépression d'exogamie, une corrélation négative entre la diversité génétique individuelle et la valeur adaptative est susceptible d'émerger, puisque les individus exogames ont une diversité génétique élevée, mais une faible valeur adaptative.

Comme la diversité génétique dépend en partie du mode d'appariement des individus lors de la reproduction, elle peut répondre à des tendances dans la dynamique d'une population. En particulier, le déclin d'une population peut entraîner une augmentation de la consanguinité et résulter en une diminution du degré de diversité génétique individuelle (Allendorf et al. 2013). Puisque la consanguinité s'accompagne souvent d'une réduction de la valeur adaptative, qui peut se traduire par une baisse de la reproduction ou de la survie, elle peut contribuer au déclin d'une population. Cette rétroaction positive entre le déclin d'une population et la réduction de la diversité génétique individuelle (ou l'augmentation de la consanguinité) peut générer un vortex d'extinction (Tanaka 1997, 1998, 2000, Blomqvist et al. 2010), aussi connu sous le nom d'effets Allee génétiques (Luque et al. 2016). Pour cette raison, connaître les liens de parenté et le degré de consanguinité des individus d'une population peut permettre d'anticiper la trajectoire démographique future de cette population (Gelatt et al. 2010, Allendorf et al. 2013, Wittmann et al. 2018).

En nature, il s'avère difficile, voire impossible, de déterminer le degré de consanguinité ou d'exogamie d'un individu puisque cela nécessite de connaître le pedigree exhaustif de l'ensemble de la population (Cecchi et al. 2016, Gholizadeh and Ghafouri-Kesbi 2016). Par conséquent, il est ardu d'évaluer les effets négatifs de la consanguinité ou de l'exogamie dans une population naturelle. Une façon indirecte d'y arriver consiste à évaluer le lien entre la diversité génétique et la performance individuelle. La

logique derrière cette approche est qu'une corrélation significative devrait apparaître dans des populations où des individus « normaux » sont présents en même temps que des individus consanguins ou exogames. Une corrélation positive révélerait la présence de dépression de consanguinité dans une population alors qu'une corrélation négative reflèterait les effets de la dépression d'exogamie (Chapman et al. 2009). Les HFCs attribuables à des effets généraux supposent qu'il existe une corrélation négative entre (1) la valeur adaptative et le degré de consanguinité (ou d'exogamie, à l'autre extrême) et entre (2) la diversité génétique et le degré de consanguinité (ou une corrélation positive entre la diversité génétique et le degré d'exogamie; Slate et al. 2004, Szulkin et al. 2010; figure 1.1).

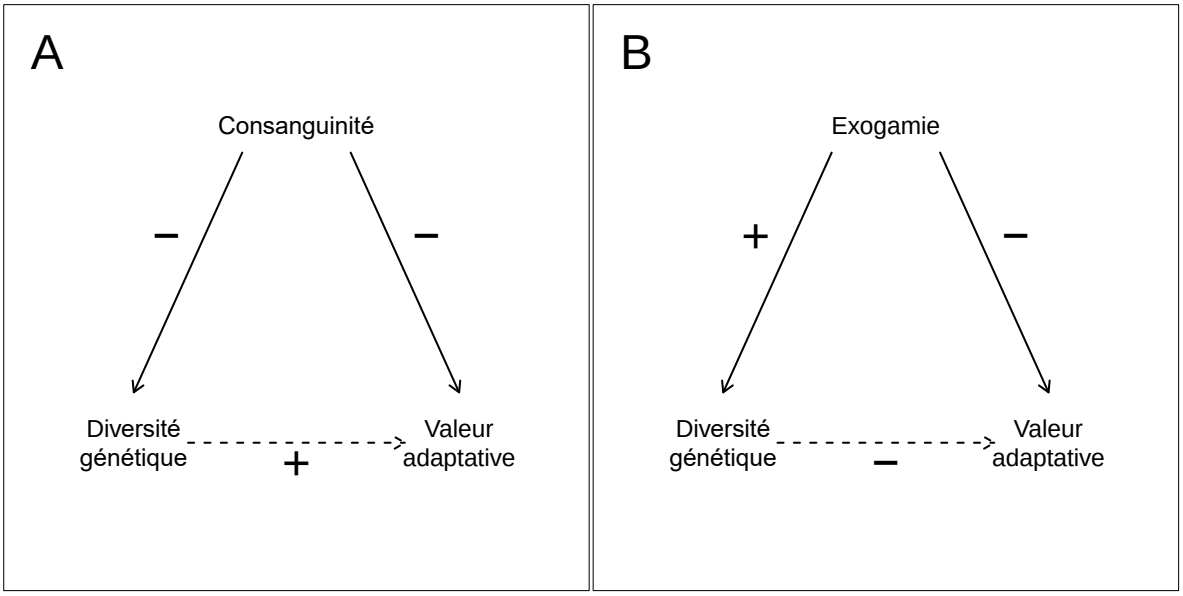

Figure 1.1 (A) Les corrélations positives entre la diversité génétique et la valeur adaptative attribuables à la dépression de consanguinité résultent de deux corrélations négatives entre la consanguinité et (1) la diversité génétique et (2) la valeur adaptative. (B) La dépression d'exogamie peut quant à elle générer une corrélation négative entre la diversité génétique et la valeur adaptative découlant d'une corrélation positive entre l'exogamie et la diversité génétique et d'une corrélation négative entre l'exogamie et la valeur adaptative.

Si la première est largement reconnue (Charlesworth and Charlesworth 1987, Crnokrak and Roff 1999, Allendorf et al. 2013), la deuxième reste un peu plus débattue (Balloux et al. 2004). En effet, même si la corrélation entre la diversité génétique et le degré de consanguinité est généralement acceptée en théorie, peu d'études arrivent à obtenir une mesure de diversité génétique représentative de la diversité du génome, en raison du faible nombre de marqueurs génétiques utilisés (Balloux et al. 2004).

Néanmoins, l'avènement de nouvelles technologies de séquençage permettant de génotyper des milliers de polymorphismes nucléotidiques simples (*en anglais : Single Nucleotide Polymorphisms ;* SNPs) à faible coût pourrait faciliter l'étude des HFCs. En effet, l'utilisation d'un grand nombre de marqueurs couvrant une plus grande part du génome permet d'obtenir une valeur d'hétérozygotie qui reflète plus exactement le degré de diversité génétique d'un individu (Kardos et al. 2016). Avec un estimé plus réaliste, les chercheurs gagnent en puissance pour détecter les HFCs (Balloux et al. 2004, Miller and Coltman 2014, Miller et al. 2014). Par exemple, chez le phoque commun (*Phoca vitulina*), la variance dans les traits de performance expliquée par l'hétérozygotie estimée à l'aide de 14 585 SNPs est jusqu'à cinq fois plus élevée que celle expliquée par l'hétérozygotie estimée à partir de 27 microsatellites (Hoffman et al. 2014). En outre, les études ayant recours à des marqueurs microsatellites (souvent entre 5 et 15, rarement plus que 30 ; Coltman and Slate 2003, Herdegen et al. 2013, Velando et al. 2015) pour estimer l'hétérozygotie individuelle ont été critiquées par le passé. Il leur est reproché de ne pas pouvoir conclure à la présence d'effets généraux étant donné le nombre trop petit de marqueurs utilisés et la faible représentativité du génome qui en résulte (Balloux et al. 2004). D'ailleurs, plusieurs études ayant utilisé des microsatellites ont montré que la diversité d'un ou de quelques marqueurs seulement avait un effet disproportionné sur la performance individuelle (Lieutenant-Gosselin and Bernatchez 2006, Guinand et al. 2013, García-Navas et al. 2014), suggérant que des effets directs ou locaux pouvaient être à l'origine de ces associations.

1.2.2 Effets directs ou locaux

L'étude des HFCs à une échelle génétique restreinte, c'est-à-dire à l'échelle d'un locus ciblé, est utile pour identifier les contraintes évolutives qui affectent ce locus. Les HFCs détectées à cette échelle reflètent non seulement des processus comme la dépression de consanguinité ou d'exogamie, mais en plus, pour les loci affectant directement ou indirectement un phénotype, des différences de valeur sélective entre différents génotypes dans un environnement donné (*hypothèses des effets directs* ou *locaux* ; David et al. 1995, David 1998). Une corrélation entre la diversité génétique et la performance émerge si le locus étudié est sur- ou sous-dominant, c'est-à-dire que les génotypes hétérozygotes ou homozygotes, respectivement, sont avantagés. Le type de marqueur génétique employé permet généralement de déterminer si une HFC est attribuable à des effets directs ou locaux. Dans le premier cas, les marqueurs utilisés font partie de régions codantes du génome, impliquant qu'ils ont un rôle fonctionnel. Les HFCs détectées à l'aide de ces marqueurs reflètent donc *directement* les fonctions

des loci étudiés et la sélection appliquée sur ces loci (David 1998). À l'inverse, les HFCs attribuables aux effets locaux sont détectées à l'aide de marqueurs non codants mais liés génétiquement à des loci fonctionnels (David et al. 1995). À moins de connaître les loci liés génétiquement aux marqueurs utilisés, les HFCs attribuables à des effets locaux sont peu informatives des mécanismes évolutifs à l'origine des HFCs détectées. Il arrive que des effets locaux soient détectés involontairement dans des études qui visaient à l'origine à étudier des effets généraux, quand un seul des marqueurs utilisés pour estimer la diversité globale des individus montre une association avec les indices de performance (Lieutenant-Gosselin and Bernatchez 2006, Guinand et al. 2013, García-Navas et al. 2014). Néanmoins, il est aussi possible d'étudier un locus fonctionnel pour mieux comprendre les processus évolutifs qui agissent sur ce locus (Da Silva et al. 2009, Oliver et al. 2009). Les gènes dont la diversité est très élevée sont souvent choisis dans ce contexte. On cherche à comprendre comment de tels degrés de diversité peuvent être maintenus en nature. De plus, il est pertinent d'étudier ces gènes si on connaît leur fonction et qu'ils revêtent un intérêt particulier dans la détermination de la performance individuelle. C'est le cas des gènes du complexe majeur d'histocompatibilité (CMH), qui présentent ces deux caractéristiques (Klein 1986, Yeager and Hughes 1999, Bernatchez and Landry 2003).

1.2.2.1 Cas spécifique du complexe majeur d'histocompatibilité

Le complexe majeur d'histocompatibilité est un ensemble de gènes qui jouent un rôle clé dans l'immunité acquise, spécifique. Les gènes du CMH codent pour des glycoprotéines dont la fonction est de reconnaître des antigènes et qui sont exprimées à la surface des cellules présentatrices d'antigènes (lymphocytes, macrophages, cellules somatiques, etc. ; Klein 1986). Chaque protéine codée par les gènes du CMH possède un site de liaison aux peptides avec des caractéristiques physicochimiques particulières qui déterminent quels antigènes peuvent être reconnus (liés). Une fois qu'un antigène est lié par une protéine du CMH, il est présenté aux cellules T de l'organisme. Si les cellules T reconnaissent l'antigène, une réponse immunitaire spécifique contre le pathogène reconnu est enclenchée (Klein 1986). Par conséquent, les gènes du CMH interviennent au tout début d'une réponse immunitaire spécifique et ont le potentiel d'influencer radicalement l'immunocompétence d'un organisme, c'est-à-dire sa capacité à combattre une infection. Puisque les fonctions immunitaires sont coûteuses (Hanssen et al. 2004, Kurtz et al. 2006, Mills et al. 2010), on peut généralement observer un compromis entre l'énergie allouée à l'immunité et celle allouée aux autres fonctions. Ces compromis peuvent se manifester par une réduction de la condition corporelle (Bize et al. 2010, Moreno-Rueda

2011), du succès reproducteur (Griesser et al. 2017) ou de la survie (Hanssen et al. 2003, Souchay et al. 2013) chez les individus infectés. Par conséquent, si la fréquence ou la virulence des infections chez un individu sont influencées par son génotype aux gènes du CMH, alors des corrélations entre le génotype des individus au CMH et leur performance sont susceptibles d'émerger.

Des corrélations positives entre la diversité génétique du CMH et la performance individuelle ont été observées dans plusieurs populations (Oliver et al. 2009, Brouwer et al. 2010, Worley et al. 2010, de Assunção-Franco et al. 2012, Lenz et al. 2013, Brambilla et al. 2018). Ce type d'association existe généralement dans des contextes où la diversité de pathogènes présents dans l'environnement est élevée dans le temps ou l'espace. Les contraintes alors imposées par le régime de pathogènes sur l'immunité de l'hôte favorisent les individus dont la diversité au CMH est élevée puisque ces individus peuvent reconnaître une large gamme de pathogènes (*avantage aux hétérozygotes,* Doherty and Zinkernagel 1975; *sélection fluctuante dans le temps,* Hill 1991*; sélection dépendante de la fréquence,* Clarke and Kirby 1966, Takahata and Nei 1990). Parfois, certains allèles ou génotypes au CMH peuvent avoir un effet plus important que la diversité sur la performance individuelle (Langefors et al. 2001, Charbonnel et al. 2010, de Assunção-Franco et al. 2012, Buczek et al. 2016, Lukasch et al. 2017). Dans de rares cas, on peut également observer des HFCs négatives ou non linéaires au CMH (McClelland et al. 2003, Wegner et al. 2003, Charbonnel et al. 2010). Il arrive qu'un génotype à lui seul ait assez d'influence pour augmenter la performance moyenne des génotypes homozygotes ou diminuer celle des génotypes hétérozygotes, ce qui génère une HFC négative (McClelland et al. 2003, Charbonnel et al. 2010). Par ailleurs, au-delà d'un certain seuil, la diversité au CMH peut avoir un effet négatif sur la performance chez des espèces dont les gènes du CMH sont hautement dupliqués (p. ex. chez l'épinoche à trois épines; *Gasterosteus aculeatus*; Wegner et al. 2003). Chez celles-ci, le fait de posséder un grand nombre d'allèles pour un même gène du CMH augmente le risque de posséder un allèle permettant de lier des molécules produites par l'hôte plutôt que par un pathogène. Lorsque de tels allèles sont exprimés, les cellules T permettant de reconnaître les molécules non pathogéniques sont éliminées de l'organisme afin d'éviter les problèmes d'auto-immunité (Mueller et al. 1989). Par conséquent, il est suggéré que les individus dont la diversité au CMH est grande et qui possèdent de tels allèles délétères ont un répertoire de cellules T inférieur et une immunocompétence réduite par rapport aux individus dont la diversité au CMH est intermédiaire (Nowak et al. 1992), ce qui pourrait

générer une relation non linéaire entre la diversité du CMH et la performance individuelle (Wegner et al. 2003).

Les associations entre la diversité au CMH et la performance individuelle fournissent des renseignements sur les relations entre hôtes et pathogènes au moment où l'étude est conduite (Charbonnel and Pemberton 2005, Osborne et al. 2015, Minias et al. 2017). Le degré de diversité au CMH observé peut quant à lui témoigner du régime de sélection passé sur les gènes du CMH ou sur la dynamique de population passée (Mikko et al. 1999, Aguilar et al. 2004). La récolte de ces données dans le cadre d'études longitudinales peut contribuer à parfaire notre compréhension des relations entre hôtes et pathogènes et des changements des communautés de pathogènes au fil du temps. Ces connaissances peuvent s'avérer utiles dans le contexte des changements climatiques actuels, dont l'une des conséquences anticipées est la modification des communautés de pathogènes (Kutz et al. 2005, Chen et al. 2011, Altizer et al. 2013, Dobson et al. 2015).

1.3 Effets directs ou locaux

Deux troupeaux de caribous migrateurs (*Rangifer tarandus*) occupent le nord du Québec et du Labrador : les troupeaux Rivière-George (RG) et Rivière-aux-Feuilles (RAF). Ensemble, leurs aires de répartition, qui se chevauchent partiellement, s'étendent sur plus de 1 000 000 km^2 (Le Corre et al. 2014). Les caribous des troupeaux RG et RAF effectuent des migrations saisonnières entre leurs sites d'estivage, situés dans la toundra, et leurs sites d'hivernage, situés plus au sud, en partie dans la forêt boréale (Le Corre et al. 2014). Certaines années, les aires des deux populations se chevauchent, notamment en période de rut, ce qui peut donner lieu à un flux génique important (Boulet et al. 2007). Les troupeaux RG et RAF sont d'ailleurs considérés comme une seule métapopulation d'un point de vue génétique (Boulet et al. 2007). Les deux troupeaux ont connu une phase de croissance suivi d'une phase de décroissance depuis le début d'un suivi instauré en 1973 (RG) et 1994 (RAF), respectivement par le ministère des Forêts, de la Faune et des Parcs (MFFP) du Québec et le gouvernement de Terre-Neuve. En effet, le troupeau RG est passé d'environ 61 000 individus en 1963 (Des Meules and Brassard 1964) à 823 000 ± 104 000 individus en 1993 (Couturier et al. 1996), puis a décliné pour atteindre un effectif de 8900 ± 668 individus en 2016 (MFFP, non publié). Quant au troupeau RAF, sa taille était estimée à environ 56 000 individus en 1975 (Le Hénaff 1976), puis à plus de 628 000 individus (1 193 000 ± 565 482) en 2001 (Couturier et al. 2004). La taille du troupeau a ensuite décliné

pour atteindre 199 000 ± 15 920 individus en 2016 (MFFP, non publié). Les détails des estimations de tailles de populations sont disponibles à la figure 2.S1. Même si les variations observées au cours des dernières décennies pourraient être attribuées à des fluctuations naturelles (Gunn 2003), nous ne possédons pas d'information sur l'ampleur de ces fluctuations avant le début du suivi. Par ailleurs, la vitesse à laquelle se produit le déclin et le fait que, pour le troupeau RG, les effectifs soient à leur valeur la plus basse enregistrée soulèvent des inquiétudes quant à la capacité des troupeaux à se stabiliser puis à croitre à nouveau. Le changement des conditions environnementales dans la Péninsule d'Ungava depuis les dernières décennies, notamment en lien avec le climat et le développement anthropique (Sharma et al. 2009, Plante et al. 2017), génère aussi des incertitudes quant à la possibilité de rétablissement des troupeaux.

La situation des troupeaux RG et RAF s'inscrit dans une tendance de déclin observée chez la majorité des troupeaux de caribous et de rennes à travers le monde (Vors and Boyce 2009). La prévalence du phénomène suggère qu'un mécanisme à large échelle comme les changements climatiques ou l'intensification de l'activité humaine dans le Nord pourrait être à l'origine de la décroissance observée (Vors and Boyce 2009, Fauchald et al. 2017). Le mécanisme proximal à l'origine du déclin de dizaines de troupeaux à l'échelle circumpolaire demeure toutefois inconnu.

Une des conséquences anticipées des changements climatiques et de l'activité humaine dans les régions nordiques est la modification des communautés de pathogènes (Kutz et al. 2004, 2005). En effet, le développement ou la transmission des pathogènes est souvent limité par la température et les conditions hydriques de leur environnement (Altizer et al. 2006). La modification rapide de ces paramètres pourrait entraîner des changements drastiques de la diversité et de l'abondance de pathogènes dans les écosystèmes. En outre, l'activité humaine peut également mener à l'introduction de nouveaux pathogènes, notamment par le contact entre les animaux sauvages et les animaux d'élevage (Rudolph et al. 2003) ou le transport involontaire de nouvelles espèces (Hatcher et al. 2012). Par ailleurs, il a été montré que l'impact des pathogènes sur la performance individuelle peut être suffisamment grand pour influencer la dynamique d'une population de caribou (Albon et al. 2002). Par conséquent, la modification des communautés de pathogènes dans les écosystèmes arctiques et subarctiques pourrait représenter un mécanisme (Gilg et al. 2012, Altizer et al. 2013) plausible par

lequel les changements climatiques ou anthropiques influencent la dynamique des populations de caribous à l'échelle planétaire.

Peu importe le mécanisme qui est à l'origine des déclins des populations de caribous, une augmentation du risque de consanguinité en réponse aux déclins observés, et particulièrement s'ils se poursuivent, est une menace supplémentaire qui pèse sur ces populations (Tanaka 1997, 1998, 2000, Luque et al. 2016, Wittmann et al. 2018). Par conséquent, la situation du caribou migrateur au Québec et au Labrador est préoccupante pour plusieurs raisons. Le déclin rapide d'une population peut engendrer une perte de diversité génétique à l'échelle populationnelle, puis éventuellement à l'échelle individuelle (Allendorf et al. 2013). À long terme, et surtout si le déclin observé actuellement se poursuivait, les populations pourraient être à risque de souffrir de dépression de consanguinité. La dépression de consanguinité pourrait à son tour contribuer au déclin des populations et provoquer des effets Allee génétiques (Luque et al. 2016, Wittmann et al. 2018), menaçant le maintien des troupeaux. Bien que les troupeaux RG et RAF soient actuellement en déclin, leur taille actuelle ne laisse pas présager la présence de consanguinité. Certains mécanismes peuvent néanmoins augmenter la probabilité de consanguinité dans des populations relativement grandes. C'est le cas de certains systèmes d'appariement comme la polygynie et de l'inégalité du succès reproducteur des individus (Balloux et al. 2004), des caractéristiques retrouvées chez le caribou (Holand et al. 2004, Taillon et al. 2012). La présence de sous-structure dans les populations pourrait aussi augmenter la probabilité d'observer de la consanguinité (Balloux et al. 2004), même si ce phénomène n'a pas été documenté chez les troupeaux RG et RAF.

Dans un autre ordre d'idées, l'environnement dans lequel évoluent les troupeaux RG et RAF connaît probablement déjà des perturbations importantes et continuera de subir les conséquences de l'intensification des activités humaines et d'un changement rapide des conditions climatiques (Kutz et al. 2012, Dobson et al. 2015, Plante et al. 2017). Ces changements pourraient entraîner de nouvelles pressions de sélection, notamment associées au régime de pathogènes. En réponse à ces nouvelles contraintes, les caribous devront s'adapter ou s'éteindre. Dans les deux cas, les troupeaux devraient transiter par une période de déclin telle que l'on observe aujourd'hui.

1.4 Objectifs

La mise en place du programme Caribou Ungava en 2009 a permis d'identifier plusieurs facteurs extrinsèques pouvant avoir un effet sur la performance du caribou. Par exemple, il a été montré qu'une grande productivité de l'habitat pendant l'été affecte positivement la masse corporelle des faons à l'automne et qu'à l'inverse, la sévérité des conditions hivernales a un effet négatif sur leur masse (Couturier et al. 2009b). Il a aussi été démontré que les femelles présentant un haut taux d'infestation par les hypodermes (*Hypoderma tarandi*) avaient une probabilité de gestation plus faible (Pachkowski et al. 2013). Puis, une étude a révélé que des caractéristiques de l'habitat pouvaient avoir un effet négatif sur la survie du caribou quand elles le rendait plus accessible ou visible aux chasseurs (Plante et al. 2017). Néanmoins, peu d'attention a été portée aux caractéristiques génétiques individuelles des caribous qui peuvent affecter sa performance. Notre étude visait donc à déterminer si la diversité génétique est associée à la performance individuelle, telle que définie par la survie annuelle et la masse corporelle, dans les troupeaux RG et RAF. Plus particulièrement, nous avons étudié la diversité génétique globale (22 073 SNPs répartis sur 14 847 loci) des individus et celle d'un locus de leur CMH (exon 2 du gène DRB, ci-après CMH-DRB) afin de déterminer si des effets généraux et directs étaient potentiellement à l'origine de HFCs dans ces populations. De plus, nous avons contrasté les effets d'indices de diversité purement génétique avec ceux d'indices de diversité potentiellement fonctionnelle afin de discriminer les effets directs des effets généraux qui pouvaient agir à l'échelle du CMH.

2. No evidence of inbreeding depression in fast declining herds of migratory caribou

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2.1 Résumé

Il est essentiel d'identifier rapidement la dépression de consanguinité dans les petites populations en déclin pour adapter les mesures de gestion et de conservation en conséquence. Les corrélations entre hétérozygotie et valeur adaptative (HFCs) permettent de détecter la dépression de consanguinité sans nécessiter de connaissances préalables sur l'apparentement des individus d'une population. Au nord du Québec et au Labrador, la taille de deux troupeaux de caribou migrateur (Rivière-George ; RG, et Rivière-aux-Feuilles ; RAF) a décliné d'un à deux ordres de grandeur dans les trois dernières décennies. Cette situation soulève des questions quant à la possible augmentation de la consanguinité dans ces troupeaux résultant du déclin démographique. Dans cette étude, nous avons testé l'association de l'hétérozygotie globale (estimée à l'aide de 22 073 SNPs) avec la masse corporelle et la survie annuelle de 400 caribous des troupeaux RG et RAF échantillonnés entre 1996 et 2016. Nous n'avons pas trouvé d'association entre l'hétérozygotie et les deux variables de performance étudiées. De plus, les degrés d'hétérozygotie individuelle semblent être restés stable pendant la période étudiée. Ces résultats suggèrent que notre jeu de données ne permettait peut-être pas de détecter des HFCs étant donné la faible variance dans le degré de consanguinité ou que le déclin démographique des deux troupeaux n'ait pas provoqué d'augmentation de la dépression de consanguinité, même chez le troupeau RG qui a perdu 99 % de ses effectifs. Par ailleurs, nous avons détecté une faible différentiation génétique entre les deux troupeaux qui étaient considérés auparavant comme une seule métapopulation. Même si nous n'avons pas trouvé d'évidences de dépression de consanguinité dans ces populations, leur déclin rapide pourrait avoir entraîné une augmentation de la dérive génétique, une perte de variants génétiques et une augmentation de la différentiation génétique entre les deux troupeaux. De futures études devraient se concentrer sur les HFCs directes dans ces populations pour comprendre comment ces processus pourraient affecter la performance individuelle et limiter le potentiel adaptatif.

2.2 Abstract

Identifying inbreeding depression early in small and declining populations is essential for management and conservation decisions. Correlations between heterozygosity and fitness (HFCs) provide a way to identify inbreeding depression without prior knowledge of kinship among individuals. In Northern Quebec and Labrador, the size of two herds of migratory caribou (Rivière-George; RG, and Rivière-aux-Feuilles; RAF) has declined by one to two orders of magnitude in the last three decades. This raises the question of a possible increase of inbreeding depression originating from, and possibly contributing to, the demographic decline in those populations. Here, we tested for the association of genome-wide heterozygosity (estimated with 22 073 SNPs) with body mass and survival in 400 caribou sampled in RG and RAF herds between 1996 and 2016. We found no association of individual heterozygosity with body mass or annual survival. Furthermore, individual heterozygosity remained stable over the period monitored. These results suggest that either our dataset could not detect HFCs due to low variance in inbreeding levels or that the rapid demographic decline of the herds, especially the RG herd that declined 99 %, did not cause inbreeding depression in those populations. Besides, a low but significant genetic structure was found between the two herds. Although we found no evidence for HFCs, the demographic decline could have caused an increase in genetic drift, a loss of genetic variation and an increase of population structure between herds. Further studies should focus on direct HFCs in those populations to understand how such processes could impair individual performance and limit evolutionary potential.

2.3 Introduction

Inbreeding depression, the reduced fitness of inbred individuals (Charlesworth and Charlesworth 1987), can contribute to genetic Allee effects, a positive correlation between population size and fitness caused by genetic factors (Luque et al. 2016, Wittmann et al. 2018), and increase the risk of extinction of small and/or declining populations. Because inbred individuals have an increased probability of expressing recessive, deleterious mutations or homozygous genotypes at over-dominant genes (Charlesworth and Charlesworth 1987), they usually have low survival (Cecchi et al. 2016, Norén et al. 2016) and reproduction (Norén et al. 2016), and/or poor body condition (Brommer et al. 2015, Gholizadeh and Ghafouri-Kesbi 2016). Lower values of life-history traits can in turn affect population growth (Gaillard et al. 2000b). In a conservation context, it is thus essential to identify inbreeding depression early in a population and to understand how it affects individual fitness to avoid extinction vortexes (Gilpin and Soulé 1986, Tanaka 1997, 1998, 2000).

Traditionally, inbreeding depression is studied with the help of exhaustive pedigrees, requiring long term monitoring of populations and extensive knowledge of kinship relations among individuals (Cecchi et al. 2016, Norén et al. 2016, Silió et al. 2016). Although this method allows directly assessing inbreeding, it is costly and often unrealistic to produce pedigrees for natural populations. Because the principal consequence of inbreeding is homozygosity (Wright 1977), it is frequently assumed that individual heterozygosity can be used as a proxy for inbreeding level (Balloux et al. 2004, Miller et al. 2014). Thus, heterozygosity-fitness correlations (HFCs) have become an appealing tool in conservation and evolutionary biology to indirectly detect inbreeding depression, without prior knowledge of kinship among individuals (Coltman and Slate 2003, Chapman et al. 2009, Hoffman et al. 2014). The rationale behind this approach is that a positive correlation between heterozygosity and fitness should appear when inbreeding depression affects a portion of the individuals in a population. In such cases, inbred individuals, with lower fitness, will also have reduced heterozygosity, whereas non-inbred individuals will present intermediate to high levels of heterozygosity and higher fitness as well (*general effect hypothesis*; David et al. 1995).

Studies have investigated the relationship between individual heterozygosity and fitness and found contrasted results (Chapman et al. 2009, Szulkin et al. 2010). In some cases, it has been shown that heterozygosity was positively associated with fitness or performance traits (Da Silva et al. 2009,

Herdegen et al. 2013, Hoffman et al. 2014, Brambilla et al. 2015) and those results were attributed to the general effect hypothesis (David et al. 1995). The general effect hypothesis posits that inbreeding is negatively correlated with (1) fitness and (2) genetic diversity (Slate et al. 2004, Szulkin et al. 2010). If the former assumption is broadly accepted (Charlesworth and Charlesworth 1987, Allendorf et al. 2013, Brommer et al. 2015, Norén et al. 2016), the later is still debated (Balloux et al. 2004, DeWoody and DeWoody 2005, Miller et al. 2014). Indeed, it is argued that marker-based estimates of genetic diversity should reflect genome-wide diversity and that it requires at the very least a few hundreds genetic markers to properly assess global diversity (Balloux et al. 2004, DeWoody and DeWoody 2005, Miller et al. 2014). Yet, HFC studies were until recently only based on a few microsatellite markers, usually between 5 and 15 loci (Coltman and Slate 2003), as a surrogate of genome-wide diversity (Da Silva et al. 2006, Herdegen et al. 2013, Velando et al. 2015). Balloux et al. (2004) suggested that most studies used too few microsatellite markers to adequately represent whole-genome diversity. Instead of demonstrating the effect of genome-wide diversity on fitness, those HFCs may result from the direct effect of diversity at functional loci or from local effect of fitness-related loci linked to the markers used to estimate diversity (*local* or *direct effect hypothesis;* David et al. 1995, David 1998). Besides, some studies on HFCs using microsatellites showed that the diversity of only a few markers was associated with performance traits, providing support to the local effect hypothesis rather than the general effect hypothesis (Lieutenant-Gosselin and Bernatchez 2006, Guinand et al. 2013, García-Navas et al. 2014). The advent of next generation sequencing techniques may facilitate the production of detailed studies on HFCs by giving access to many thousands of single nucleotide polymorphisms (*SNPs*) at a relatively low cost (Hoffman et al. 2014, Miller et al. 2014, Huisman et al. 2016, Kardos et al. 2016, Mitchell et al. 2017).

In Northern Quebec and Labrador (Canada), Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds of migratory caribou (*Rangifer tarandus*) have experienced a marked demographic decline starting in the 1990's and 2000's and still on-going (ministère des Forêts, de la Faune et des Parcs [MFFP], unpublished). Rivière-George herd decreased ca. 99 % in 23 years and Rivière-aux-Feuilles herd ca. 70 % in 15 years. Although the census sizes of RG and RAF herds are still relatively large compared to populations usually subjected to inbreeding depression (Blomqvist et al. 2010, Velando et al. 2015, Norén et al. 2016), certain mechanisms can reduce the effective size of a population (i.e. the true number of effective breeders; Wright 1931) and increase the risk of inbreeding. Namely, mating

systems that promote large bias in reproductive success among individuals or hidden population substructure can have such effect (Wright 1931, Balloux et al. 2004). Caribou are polygynous (L'Italien et al. 2012) and display large variations of reproductive success generated by environmental (Couturier et al. 2009b) and individual variation (Pachkowski et al. 2013). In addition, it has been shown in a reindeer population that females do not avoid inbreeding when choosing a mating partner (Holand et al. 2007). Because of the abrupt decline of RG and RAF herds and because caribou display characteristics that could increase inbreeding risk, it raises the question of whether inbreeding could have increased and hence affected individual fitness in those populations, through inbreeding depression, and contributed further to their decline.

Here, we tested for a potential association between genome-wide individual heterozygosity and two performance traits, body mass and annual survival, in two declining herds of migratory caribou. Multilocus heterozygosity was estimated using 22,073 SNPs obtained by double-digest Restriction-site Associated DNA Sequencing (ddRADSeq). We hypothesized that inbreeding depression could result in positive correlations between genome-wide heterozygosity and performance traits (body mass and survival). Nevertheless, since those herds are still large (>8000 individuals) and because unlike survival, condition is indirectly linked to fitness (Festa-bianchet et al. 1997, Beauplet and Guinet 2007, Gélin et al. 2016, Veiberg et al. 2017), we suspected that inbreeding depression could be low and would have a greater effect on condition than survival. Additionally, we investigated the genetic differentiation between the two herds to confirm with SNPs previous results obtained with 7 *microsatellites* showing no differentiation between herds (Boulet et al. 2007).

2.4 Methods

2.4.1 Study area and data collection

Both RG and RAF herds experienced large variations in population size over the last decades. In particular, RG herd increased from 61,842 individuals in 1963 (Des Meules and Brassard 1964) to 823,000 ± 104,000 individuals in 1993 (Couturier et al. 1996). Then, it declined to 8,900 ± 668 individuals in 2016 (MFFP, unpublished). For the RAF herd, it went from 56,000 individuals (Le Hénaff 1976) to more than 628,000 individuals (1,193,000 ± 565,482; Couturier et al. 2004) between 1975 and 2001. It then decreased to 199,000 (± 15,920) individuals in 2016 (MFFP; unpublished; see figure 2.S1 for details).

Using nets fired from helicopters, we captured and sampled 149 caribou of the RG herd (90 females; 59 males) between 2000 and 2014 and 251 caribou of the RAF herd (159 females; 92 males) between 1996 and 2016 following the guidelines from the Canadian Council on Animal Care. Individuals were captured up to 5 times over the study period, but most (85.5 %) were captured only once. We collected biopsies (n = 361), hairs (n = 27) or blood (n = 12) samples on each animal and froze all samples except for some biopsies (n = 150) that were stored in ethanol (≥ 70 %) at room temperature. We used EDTA to preserve blood samples and prevent them to coagulate. From the 400 individuals, 222 (≥ 2 years) were fitted with tracking collars using different satellite networks (Argos, Iridium, Globalstar), allowing the assessment of annual survival from capture date to 2017. Mortality was signalled by collar after 12-24 hours of inactivity and was then confirmed on the field based on visual cues when retrieving the collar. In addition, 253 caribou (≥ 1 year) were weighted to the nearest 0.1 kg using a hanging scale. Annual survival and body mass (collected in January, February, March, June, October or November) were considered as indices of individual performance and were included in our models as response variables. Moreover, 55 calves (0 year) were captured between 2007 and 2009 and included in the population structure analysis as well as to assess representativeness of our sample by comparing heterozygosity between calves and yearlings born in those years (n = 49).

2.4.2 DNA extractions

We used Qiagen's DNeasy Blood and Tissue kits (Qiagen, Inc., Valencia, CA, USA) to extract DNA from our samples. To digest hairs (~100 hairs), we used 1.3 X the recommended quantities of ATL lysis buffer and proteinase K and added 45 µl of dithiothreitol (DTT; 100 mg/ml). To maximise DNA yield, we eluted in 100 µl Elution Buffer for blood and hair samples and for all sample types, flow-through went through columns a second time at the final elution. We assessed DNA quality and checked for degradation on an agarose gel. Most samples of blood or biopsies led to high to moderate DNA quality (low to medium degradation), whereas hair samples led to lower DNA quality with higher levels of degradation. We quantified DNA concentrations with a Qubit 2.0 fluorometer (Life Technologies) and standardized all samples to 200 ng/µl before library construction.

2.4.3 ddRADSeq library construction and sequencing

We constructed ddRADSeq libraries from caribou's genomic DNA following the general protocol from Peterson et al. (2012) with some modifications. Following recommendations of Mastretta-Yanes et al. (2015), we replicated 70 (17,5 %) of our samples: 40 intra-library replicates and 30 inter-library replicates. We used a combination of 47 uniquely tagged P1 adaptors and 10 PCR indices to construct 10 libraries containing 47 DNA samples each. For each sample, 200 ng of DNA were digested with 20 units each of SbfI (CCTGCA/GG) and MspI (CC/GG). After ligation of P1 and P2 adaptors to SbfI and MspI cutsite overhang respectively, we pooled all samples from one library and purified it using Agencourt AMPure XP (Beckman Coulter) or NucleoMag (Macherey Nagel) systems with a DNA:magnetic beads solution ratio (v/v) of 1:1.8. We then selected fragments between 200 and 500 bp on agarose gels (1.6 %), using Promega Wizzard SV Gel and PCR Clean-Up System to perform gel extraction. Final amplification was achieved in 16 reactions of 20 µl containing 12.6 µl nanopure water, 4 µl Phusion © HF 5X Buffer (New England BioLabs), 0.4 µl dNTPs (10 mM), 0.4 µl each forward and reverse primers (10 µM; PCR1 and indexed PCR2), 0.2µl Taq Phusion HF (2000 U/ml) and 2 µl DNA template. After an initial denaturation step of 30 s at 98°C, 15 cycles of 10 s at 98°C, 10 s at 65°C and 30 s at 72°C were performed and followed by a final extension of 10 minutes at 72°C. All 16 PCRs from the same library were then pooled and purified using Promega Wizzard SV Gel and PCR Clean-Up System. The 10 pooled libraries were each sequenced on two lanes of Illumina HiSeq 4000 (with HiSeq 3000/4000 SBS Kit) by Fasteris (Switzerland).

2.4.4 ddRADSeq data processing

We used *Cutadapt* (v. 1.8.1; Martin 2011) to remove adapter sequences from sequence files and *FastQC* (v. 0.11.2; Andrews 2010) to assess data quality and detect adaptor contamination. Then, we used *Stacks* (v. 1.44; Catchen et al. 2011, 2013) to demultiplex data and build a *de novo* SNP catalog. As all libraries were sequenced two times, we obtained two files per sample (or four for replicated samples) after the *Process_radtags* component of *Stacks*. The next components (*Ustacks*, *Cstacks*, *Sstacks* and *Populations*) were run on combined files that included all replicates of each individual (intra- and inter-library and inter-run) to increase individual depth of coverage and genotype accuracy. We tested different sets of *Stacks* core parameters (-m (2-6), -M (2-6), -n (0-5) and -max_locus_stacks (2-6)) as suggested by Mastretta-Yanes et al. (2015) and chose the values that minimized error rates between replicates and maximized the amount of data recovered. To do so, we combined files from

the two sequencing runs to get only one file per replicate and a depth of coverage similar to that observed in our final set. First, using the default SNP calling model, we identified the optimal values of -m (3), -M (4), -N (6), -max_locus_stacks (3) and -n (3; figure 2.S2 and 2.S3). Then, to compare upper bound values for the bounded model to the default (SNP) model, we used those optimal values in combination with each value of upper bound tested (0.0125, 0.023, 0.5, 0.1, 0.15 and 1 (default SNP model)). In preliminary analysis, we found that standardized multi-locus heterozygosity (sMLH, see "Assessment of individual heterozygosity" for details) was strongly correlated, in a non-linear way, with the individual harmonic mean of locus depth of coverage (result not shown). We found that filtering data with lnl_lim = -10 (95 % of our loci had a log likelihood equal or higher than -10) in *Rxstacks* and eliminating individuals with more than 80 % missing data made the relationship linear. We did so and then chose the value of upper bound that led to the weakest and least-significant correlation between sMLH and individual harmonic mean of locus depth of coverage (figure 2.S4). The bounded model with an upper bound of 0.0125 or 0.023 both led to very weak and non-significant correlations; and we decided to use 0.023 as it also corresponded to the highest PhiX error rate reported for our sequencing runs. In all *Stacks* runs, including the one performed to produce our final set, we specified in the *Populations* component only one population and set -m (minimum stack depth required to call a genotype in an individual) to 8, -r (minimum percentage of individuals required to process a locus) to 0.5 and –min_maf (minimum minor allele frequency required to process a nucleotide site at a locus) to 0.01. The -m value seemed to offer the best trade-off between the number of retained loci and genotype quality. We were rather permissive for -r and -min_maf because our heterozygosity scores were standardized to account for missing data and mean observed heterozygosity at genotyped loci. This allowed retaining a maximum number of loci for subsequent analysis. To produce the final dataset, we set all parameters to their optimal values identified above.

2.4.5 Assessment of individual heterozygosity

We computed individual standardized multi-locus heterozygosity with the function 'sMLH' implemented in the package *inbreedR* (Stoffel et al. 2016) in *R* (v. 3.4.0; R Core Team 2017). sMLH accounts for differences in the identity of the loci genotyped in all individuals and corrects the heterozygosity score for the mean heterozygosity observed at the typed loci in the rest of the population. In addition, to ensure the reliability of our datasets to properly assess HFCs, we assessed identity disequilibrium (covariance of heterozygosity among loci of a given individual; Weir and Cockerham 1973, Szulkin et

al. 2010) by computing the heterozygosity-heterozygosity correlation (HHC; Balloux et al. 2004) and the g2 value (David et al. 2007, Szulkin et al. 2010). HHC is a measure of correlation of heterozygosity across loci. The stronger the correlation, the more multi-locus heterozygosity is representative of global genetic diversity (Balloux et al. 2004). The g2 value measures the variance in the degree of covariance of heterozygosity at the individual level. The higher the variance, the more likely it is we detect an HFC resulting from general effects in the sample if there is actually one in the population (David et al. 2007, Szulkin et al. 2010). HHC values were assessed using 100 iterations, and the g2 values, obtained using 100 permutations and 10 bootstraps, with the functions 'HHC' and 'g2_snps' of the *InbreedR* package (Stoffel et al. 2016). Because the datasets used to assess the effect of sMLH on body mass and annual survival did not include the same individuals, HHC and g2 were computed separately for both datasets. Although HHC and g_2 are useful tools to detect identity disequilibrium, it was shown that HFCs could still occur and be caused by inbreeding depression in populations in which identity disequilibrium could not be detected (Kardos et al. 2014, Miller and Coltman 2014).

2.4.6 Statistical analysis

We used linear mixed-effects models with the function 'lmer' implemented in the *R* package *lme4* (Bates et al. 2015) to assess the effect of multi-locus heterozygosity on body mass. Body mass was log-transformed to improve homogeneity of variances and sMLH was centered to improve convergence of models. To investigate the possible effect of heterozygosity in interaction with sex, age or herd, we adopted a model selection approach. We first considered a baseline model that included age, $\sqrt{}$age, sex, herd and month of capture as fixed-effect variables, as these factors are known to influence caribou body mass (Parker 1981, Couturier et al. 2009a, 2009b). We added year and individual identity as random factors to consider annual variation and repeated measures on individuals. To the baseline model, we added sMLH and $sMLH^2$ as fixed effect variables to build a simple model that included heterozygosity. Considering a potential quadratic effect of sMLH on performance, we accounted for a potential threshold above which an increase of heterozygosity would not have any effect on performance traits. Then, we derived competitive models including interactions between sMLH or $sMLH^2$ and sex, herd, age and $\sqrt{}$age. All models were compared using Akaike's Information Criterion for small sample sizes (AICc) and we selected the model with the lowest value of AICc. ΔAICc and AICc weights (ω_i) were computed with the *R* package *AICcmodavg* (Mazerolle 2017). We used the same approach to test for the effect of sMLH on annual survival in adults (>2 years). Annual survival

was coded as a binary variable with a value of 1 if the individual was alive during a given year and 0 if the individual was dead. Malfunctioning collars were censored starting from the point of malfunction. We fitted generalized mixed-effect models with a binomial distributed error (logit link function), using the 'glmer' function implemented in the package *lme4* (Bates et al. 2015). The baseline model included age, age^2, herd and sex (Loison et al. 1999, Couturier et al. 2010) as fixed-effect variables and year and individual identity as random factors. In the derived models, we added sMLH and sMLH^2 and their interaction with sex, herd, age and age^2.

2.4.7 Variation of heterozygosity through time and age

We suspected that the abrupt decline observed in the two herds could have caused a reduction of sMLH in more recent cohorts. We thus tested for variation of sMLH through time fitting a linear model with sMLH as the response variable and cohort (birth year; coded as a continuous variable), herd and the interaction between the two as predictor variables. Furthermore, the sample used for HFC analyses, including individuals captured at ages > 1 year, may not have been representative of individuals born during our sampling period if inbred individuals died early in life before we could sample them. We thus compared the sMLH scores of calves (0 year) born between 2007 and 2009 to that of individuals born in the same period, but captured for the first time as yearlings. To do so, we fitted a linear model with sMLH as a response variable and with age, herd and year, all coded as categorical variables, as fixed effect with the *R* function 'lm' (R Core Team 2017).

2.4.8 Populations structure

To assess the genetic differentiation between the two herds, we filtered the dataset described in "ddRADSeq data processing" by keeping only a SNP by locus and using r = 0.8 in *Populations* to avoid artificially increasing or decreasing differentiation between populations. This subset included 6,384 SNPs typed for 362 individuals (10.44 % missing data). Prior to analysis, input files were exported in the appropriate format using *radiator* (v. 0.0.5; Gosselin 2017). We removed from the data set known related individuals (e.g., female-calf pairs identified in the field) or related individuals detected with a Genetic Relatedness Matrix (GRM) obtained using the 'snpgdsGRM' function implemented in the *R SNPRelate* package (Zheng et al. 2012). This resulted in a dataset including 344 individuals. Genetic differentiation between herds was then investigated in *R* (v. 3.4.0; R Core Team 2017), using classical

F-statistics according to Weir and Cockerham (1984) implemented in *StAMPP* (Pembleton et al. 2013), Principal Component Analysis (PCA) and Discriminant Analysis of Principal Components (DAPC; Jombart et al. 2010) implemented in *adegenet* (Jombart 2008).

2.5 Results

2.5.1 Estimation of sMLH

At the end of our *de novo* SNP calling procedure, we obtained 22,073 SNPs distributed at 14,847 loci. Thirty-six out of 400 individuals were excluded from our analyses because they had more than 80 % missing data. We also eliminated 2 individuals who had very high sMLH scores as we suspected that they were contaminated during DNA extraction or library preparation. The median of individual locus depth of coverage was 22.08 reads/non-missing locus. Individuals scored between 4,408 and 21,713 SNPs with a mean of 16,983 ± 4,198 (sd). sMLH scores varied between 0.94 and 1.06 and were moderately correlated with (unstandardized) multi-locus heterozygosity (Pearson's r = 0.50; p<0.001). The average (unstandardized) observed heterozygosity was 0.23 ± 0.01 (sd) in both herds and ranged between 0.20 and 0.27. The HHC value was 0.30 ± 0.05 (sd) for the mass dataset and 0.33 ± 0.05 (sd) for the survival dataset. The g_2 values were $3.8 \times 10^{-4} \pm 4.4 \times 10^{-4}$ (se; $p(g^2 > 0) = 1$) for the mass dataset and $1.2 \times 10^{-4} \pm 3.8 \times 10^{-4}$ (se; $p(g^2 > 0) = 1$) for the survival dataset, respectively.

2.5.2 Variation of heterozygosity through time and age

sMLH did not vary significantly through the sampling period (figure 2.1) or between herds (table 2.S1). In addition, we found no significant difference in sMLH scores between calves and yearlings born between 2007 and 2009 (table 2.S2).

2.5.3 Population structure analysis

We observed a very low but significant genetic differentiation between the RG and RAF migratory herds (F_{ST}: 0.0027; 95 % CI: 0.0024 - 0.0029, p-value <0.001). In line with this estimation, two genetic clusters appeared clearly on principal component analysis (figure 2.2 a)). Cross-validation determined that the use of the first 10 principal components (PCs) for the discriminant analysis of principal components was optimal. Although the 10 PCs portrayed only 12 % of the variance, it gave the highest predictive success (87.2 %) and the lowest root mean squared error (13.4 %). Overall DAPC indicated

some genetic overlap between the two clusters, but 85.2 % caribou of the RG herd and 93.4 % caribou of the RAF herd were assigned to the correct herd (figure 2.2 b) and c)). In addition, the two multivariate analyses revealed admixture between the two herds as well as individual herd switching (figure 2.2). GRM did not reveal strong particular clustering (results not shown), but confirmed that relatedness between known related individuals (*i.e.*, mother–calf pairs of both herds; RG = 11 pairs and RAF = 5) was as expected ~0.5 (0.45 ± 0.04 (sd)).

2.5.4 Effect of sMLH on performance

2.5.4.1 Effect of sMLH on body mass

The baseline model for the body mass candidate set was the best model (ΔAICc = 0 and ω_i = 0.49; table 2.S3). Age, √age, herd, sex and some months of capture had a significant effect on body mass in the baseline model (table 2.1). Body mass increased from 1 to 2 years and plateaued after that age at around 90 kg for females and 106 kg for males. Individuals from the RG herd were on average 8.07 kg heavier than their RAF counterparts in June and all individuals were on average heavier in the Fall months and lighter at the beginning of Summer, compared to Winter months. Next candidate models did not include significant effects of sMLH or $sMLH^2$ and their interactions.

2.5.4.2 Effect of sMLH on survival

The baseline model was also selected as the best model for survival (ΔAICc = 0 and ω_i = 0.55; table 2.S4). However, only herd had a significant effect on survival (table 2.2), which was lower for individuals from the RG herd in comparison with RAF herd (Odds ratio = 0.46, 95 % CI = [0.27, 0.78]; figure 2.3). None of the next-ranked models included significant effect of sMLH or its interactions.

2.6 Discussion

2.6.1 Estimation of sMLH

It was suggested that SNP markers would greatly improve HFC studies by increasing representativeness of genome-wide diversity (Miller et al. 2014, Kardos et al. 2016). Accordingly, it was shown in harbor seal (*Phoca vitulina*) that the strength of an HFC detected in a natural population increased significantly when heterozygosity was estimated with 14,585 SNP markers rather than 27

microsatellites (Hoffman et al. 2014). Although we found higher values of HHC in our datasets than those usually reported in HFC studies using microsatellites (Guinand et al. 2013, Voegeli et al. 2013, Queirós et al. 2016), our values were somewhat small, indicating that our estimate of heterozygosity may not have been representative of inbreeding levels (Balloux et al. 2004). In preliminary analyses, we found that some of the filters we applied in Stacks to reconstruct SNPs helped reduce the unintended correlation between heterozygosity and depth of coverage, but also reduced dramatically the values of HHC (results not shown). HHC values should be high if markers heterozygosity was highly dependent on a third variable, like the depth of coverage. Thus, it was not surprising that HHC values drastically decreased as the relationship between sMLH and depth of coverage weakened. Further studies should explore the impact of bioinformatic filters on SNPs-based estimates of multi-locus heterozygosity and, in the context of HFC studies, determine the best approach to adopt for data filtering. Meanwhile, SNPs could still improve HFC studies because they provide a broader representation of general genetic diversity than microsatellites by covering coding- and non-coding regions (Balloux et al. 2004, Miller and Coltman 2014, Miller et al. 2014).

2.6.2 Population structure

Contrarily to what has been shown previously (Boulet et al. 2007, Yannic et al. 2016), we found a very low but significant differentiation between RG and RAF herds. The high number of SNPs used in our study likely improved the detection of differences compared to microsatellite markers (Liu et al. 2005, Gärke et al. 2011, Fischer et al. 2017). SNP markers also helped uncover patterns of population structure in American lobster (*Homarus americanus*) that had not been found previously with microsatellite markers (Benestan et al. 2015). In addition, it appears that the recent demographic decline of both populations has been accompanied by a reduction in their overlapping ranges (Le Corre 2016) that could have contributed to their differentiation by reducing gene flow. Nevertheless, the low level of differentiation we observed and the few cases of herd switching observed in our dataset are concordant with previous observations of high gene flow between RG and RAF herds (Boulet et al. 2007, Yannic et al. 2016).

2.6.3 Effect of sMLH on performance

We used HFCs to detect inbreeding depression in RG and RAF declining herds of migratory caribou. We studied the association of genome-wide, multi-locus heterozygosity inferred with 22,730 SNPs with

two performance traits: body mass and annual survival. The effects of non-genetic variables on body mass and survival were conform to what had been found previously (Parker 1981, Couturier et al. 2009a, 2009b) and we did not find any effect of heterozygosity on these traits. On the opposite, positive effects of heterozygosity on reproductive success and survival have been found in a population of European shag (*Phalacrocorax aristotelis*) that presented similar levels of demographic decline as the RAF herd (Velando et al. 2015). Although this population declined by 70 %, the decline occurred over a much shorter period of time (5 years). Thus, the loss of genetic variants may have been more intense than in RG and RAF herds and strengthened the effect of inbreeding on individual performance (Luque et al. 2016). In addition, high philopatry and small population size (a few hundreds) could also have increased the probability of inbreeding in this population (Velando et al. 2015). In our study, individual heterozygosity remained stable overtime. It is likely that the genetic diversity of the herds was shaped by the low population sizes preceding their augmentation in the 1970's and 1980's. Thus, it was expected that the return to similar sizes would not lead to decrease in population genetic diversity (e.g. in terms of number of alleles). Regarding individual genetic diversity, it was suggested that relaxed polygyny in caribou could attenuate the negative effect of population decline on genetic diversity as it increases the number of breeders in the population (Lovatt and Hoelzel 2014). The fact that we detected no substructure within the populations and their relatively large population size also contribute to reduce inbreeding risk.

We are confident that our marker set reflected reliable biological information, because we found estimations of relatedness level around 0.5 between individuals from known mother-calf pairs and individual discrimination between herds using PCA was high (figure 2.2). Nevertheless, the g2 values were not significantly different from 0, suggesting that the variance in inbreeding in our sample was too small to detect an HFC. In one hand, we cannot exclude the possibility that our sample was not representative of studied populations, especially if only a small proportion of the individuals were inbred in the populations. If so, it is expected that the effect size of sMLH on performance traits we estimated was lower than its true effect (Miller et al. 2014). On the other hand, we ensured that our sample was as representative as it could, *a priori*, with 400 individuals of both sexes, captured at different ages, in different months of 20 years and in the two herds. It is possible that inbred individuals died early in life (Blomqvist et al. 2010) and eliminated variance in the population before we could sample it in yearlings and older individuals. Nevertheless, we found no significant difference in sMLH scores between calves

and yearlings born the same years, suggesting that our sample was representative of the population, even though we did not capture calves. Consequently, small g2 values in our sample could also indicate that variance in inbreeding levels in RG and RAF herds was truly small. In our study, the average (unstandardized) observed heterozygosity was 0.23 ± 0.01 (sd) for the RG herd and 0.23 ± 0.01 (sd) for the RAF herd. In another study conducted on those populations, observed heterozygosity estimated at 7 microsatellite markers was 0.71 for the RG herd (n = 98) and 0.73 for the RAF herd (n = 114, Boulet et al. 2007). Because microsatellites are much more polymorphic than SNPs mostly because of their high mutation rate and their high number of alleles, it was expected to observe lower heterozygosity estimates with SNPs (Kaiser et al. 2017). Our values of heterozygosity for the two types of markers are comparable to estimates of heterozygosity made in a population of bighorn sheep (*Ovis canadensis*; n = 26) after a successful genetic rescue, where observed heterozygosity was 0.28 when estimated at 412 SNP markers and 0.64 when estimated with 200 microsatellite markers (Hogg et al. 2006, Miller et al. 2014). Thus, assuming that our sample was representative of studied populations, our results suggest that RG and RAF herds do not suffer from inbreeding. Unless there was sub-structure and non-random mating within RG and RAF herds, which is not supported by our data, or high genetic loads, which we did not address here, it is expected that relatively large populations with few to many thousand individuals would not suffer from inbreeding, nor from inbreeding depression.

The strong rate of decline observed in RG and RAF herds could have and could continue to increase levels of genetic drift and lead to the loss of genetic variants at the population level (Allendorf 1986, Taylor et al. 2012). It would be interesting to study HFCs at known functional loci in those populations to understand how the loss of allelic diversity could affect declining populations, especially in the face of climate change where genetic diversity would provide a selective advantage (Allendorf et al. 2013, Forcada and Hoffman 2014).

2.7 Acknowledgments

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2.8 Tables

Table 2.1 Estimates of the body mass baseline model for migratory caribou from the Ungava peninsula. Lower bounds and upper bounds of the 95% confidence intervals on estimates are provided in addition to the standard errors (Std. error) and t-values. Variables with 95 % CI that do not overlap 0 are considered to have a significant effect and are in bold. Levels of reference are *Female* for sex, *Rivière-aux-Feuilles* for herd and *January* for month of capture. Baseline model: log(mass) ~ age + √age + sex + herd + month of capture + (1|year) + (1|individual identity).

				95 % Confidence interval	
Variable	Estimate	Std. Error	t-value	Lower bound	Upper bound
Intercept	**2.91**	**0.08**	**37.4**	**2.76**	**3.06**
Age	**-0.24**	**0.02**	**-14.4**	**-0.27**	**-0.20**
√Age	**1.25**	**0.07**	**18.6**	**1.12**	**1.38**
Herd - Rivière-George	**0.10**	**0.02**	**5.7**	**0.07**	**0.14**
Sex - Male	**0.15**	**0.03**	**5.9**	**0.10**	**0.20**
Month of capture - February	**-0.09**	**0.04**	**-2.1**	**-0.17**	**-0.01**
Month of capture - March	0.01	0.04	0.3	-0.06	0.08
Month of capture - June	**-0.23**	**0.04**	**-5.3**	**-0.31**	**-0.14**
Month of capture - October	-0.01	0.04	-0.3	-0.10	0.07
Month of capture - November	0.06	0.05	1.2	-0.04	0.16

Table 2.2 Estimates of the survival baseline model for migratory caribou from the Ungava peninsula. Lower bounds and upper bounds of the 95% confidence intervals on estimates are provided in addition to the standard errors (Std. error), Z-values and p-values. Variables with 95 % CI that do not overlap 0 are considered to have a significant effect and are in bold. Levels of reference are *Female* for sex and *Rivière-aux-Feuilles* for herd. Baseline model: annual survival ~ age + age^2 + sex + herd + (1|year) + (1|individual identity).

					95 % Confidence interval	
Variable	Estimate	Std. Error	Z-value	p-value	Lower bound	Upper bound
Intercept	**2.44**	**0.33**	**7.5**	**0.00**	**1.80**	**3.07**
Centered age	-0.25	0.16	-1.6	0.10	-0.56	0.05
(Centered age) ^{2}	-0.07	0.07	-0.9	0.36	-0.21	0.08
Sex - Male	-0.17	0.26	-0.7	0.51	-0.69	0.34
Herd – Rivière-George	**-0.77**	**0.27**	**-2.8**	**0.01**	**-1.30**	**-0.24**

2.9 Figures

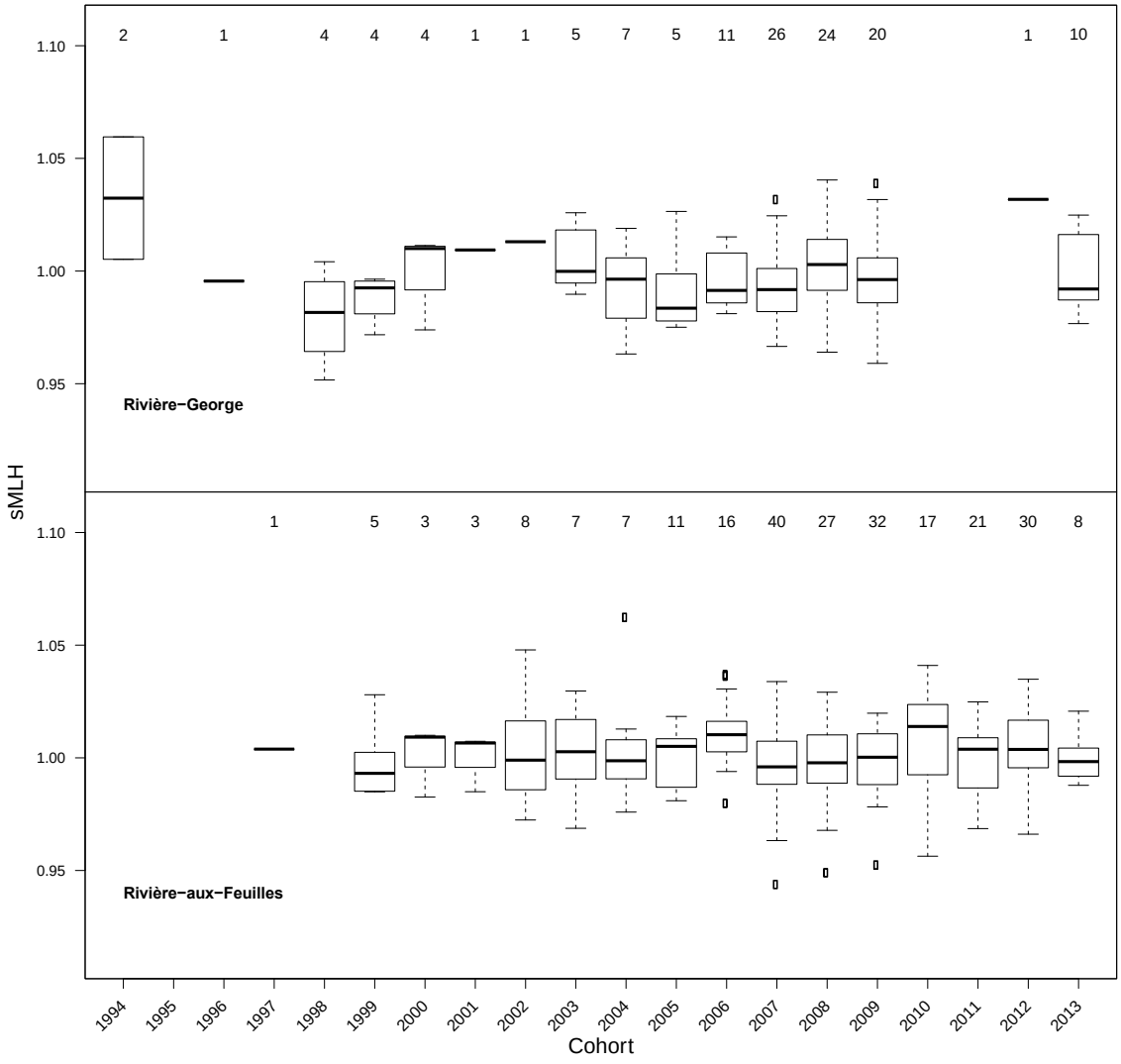

Figure 2.1 Distribution of standardized Multi-Locus Heterozygosity (sMLH) in the cohorts (defined by the birth year of individuals) sampled in our study of migratory caribou in the Ungava peninsula. The numbers in the upper part of the plots show the number of individuals sampled for each cohort.

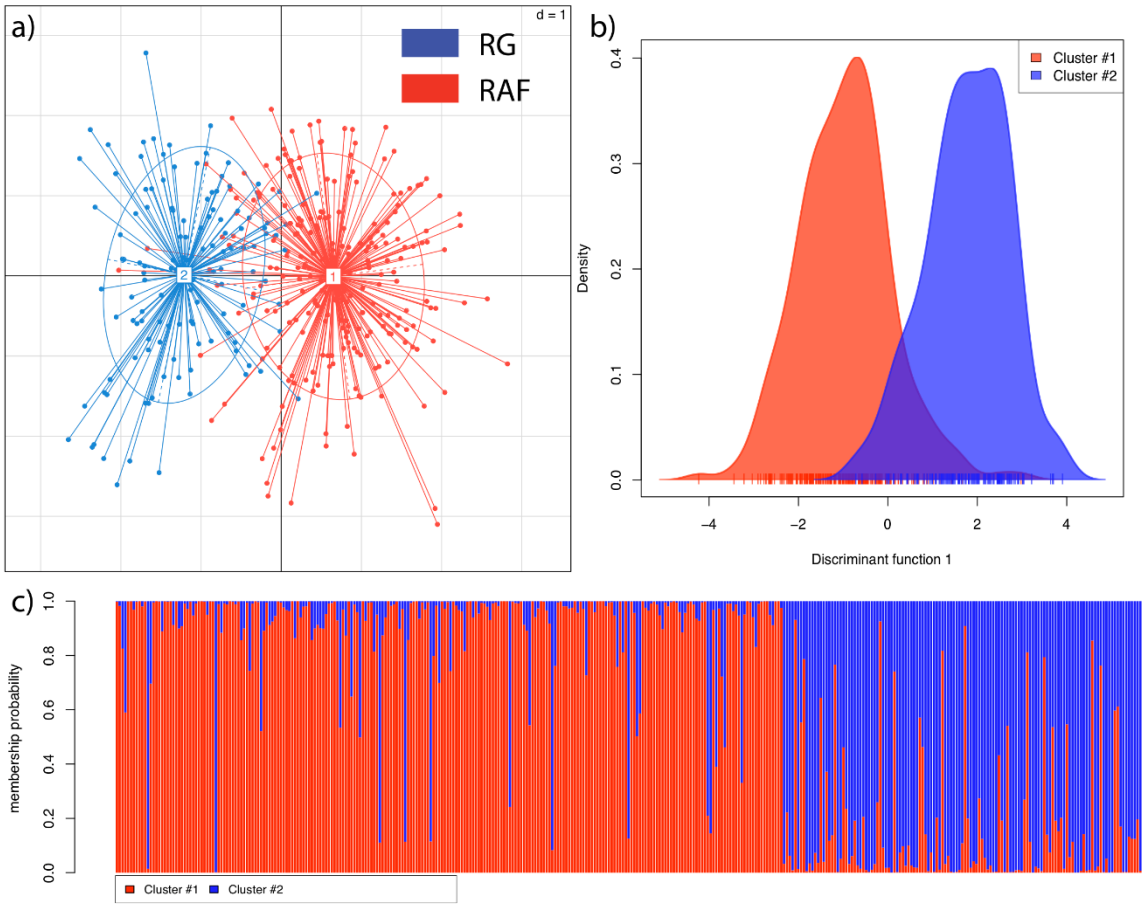

Figure 2.2 a) Principal Component Analysis (PCA) scatter plot (x-axis: 2.99 % y-axis: 2.15 %). Dots represent individuals, with colors denoting sampling origin and inclusion of 95 % inertia ellipses. b) individual density plot on the first discriminant function of the Discriminant Analysis of Principal Components (DAPC); and c) group assignment probability of individuals to the two clusters, drawn across 362 migratory caribou individuals. Cluster #1 corresponds mainly to individuals from the Rivières-aux-Feuilles herd (RAF; in red), and Cluster #2 to individuals from the Rivière-George herd (RG; in blue).

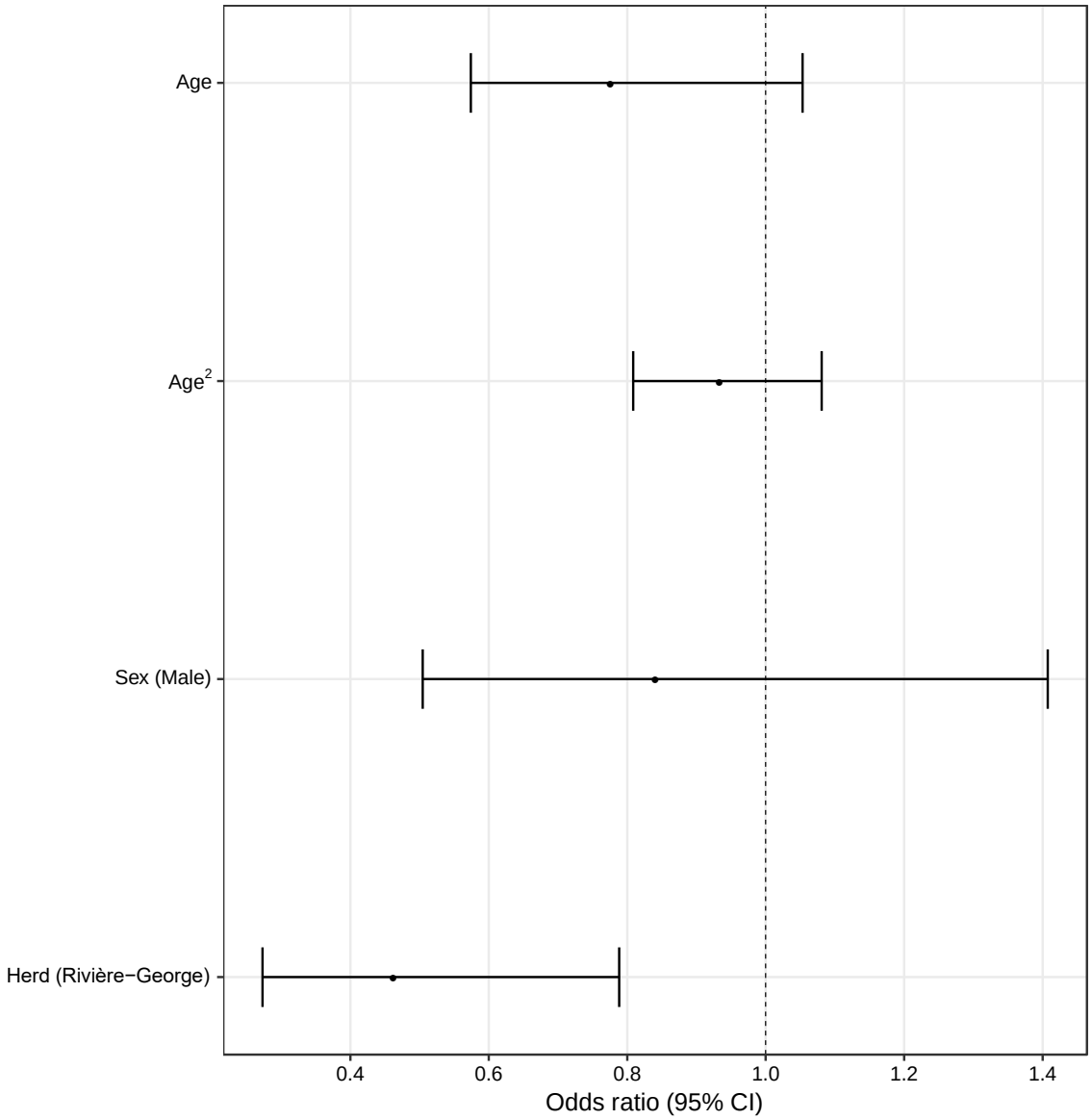

Figure 2.3 Odds ratios and 95 % confidence interval (CI) for the fixed-effect variables included in the baseline model of survival. Odds ratios with CI that do not overlap 1 are considered statistically significant. Levels of reference are *Female* for sex and *Rivière-aux-Feuilles* for herd. Baseline model: annual survival ~ age + age^2 + sex + herd + (1|year) + (1|individual identity).

2.10 Supplementary material

Table 2.S1 Estimates of the model of standardized Multi-Locus Heterozygosity (sMLH) through time for migratory caribou from the Ungava peninsula. Lower bounds and upper bounds of the 95% confidence intervals on estimates are provided in addition to the standard errors (Std. error), t-values and p-values. Time is expressed as cohort, i.e. year of birth. The level of reference for herd is *Rivière-aux-Feuilles*. No variable had a significant effect. Model: sMLH ~ cohort + herd + cohort : herd.

					95 % confidence interval	
Variable	Estimate	Std.error	t-value	p-value	Lower bound	Upper bound
Intercept	0.86	0.72	1.2	0.23	-0.54	2.27
Cohort	0.00	0.00	0.2	0.85	-0.00	0.00
Herd – Rivière-George	-0.10	1.10	-0.1	0.93	-2.26	2.06
Cohort : Herd – Rivière-George	0.000	0.001	0.1	0.93	-0.001	0.001

Table 2.S2 Estimates of the model comparing sMLH in calves and yearlings born between 2007 and 2009 for migratory caribou from the Ungava peninsula. Lower bounds and upper bounds of the 95% confidence intervals on estimates are provided in addition to the standard errors (Std. error), t-values and p-values. Cohort refers to the birth year of the individuals. Variables with 95 % CI that do not overlap 0 are considered to have a significant effect and are in bold. The levels of reference are *Rivière-aux-Feuilles* for herd, *calf* for age and *2007* for cohort. Model: sMLH ~ age + herd + cohort.

					95 % Confidence interval	
Variables	Estimate	Std.error	t-value	p-value	Lower bound	Upper bound
Intercept	**0.994**	**0.003**	**288.7**	**0.00**	**0.988**	**1.001**
Age - Yearling	0.002	0.004	0.7	0.51	-0.005	0.009
Herd – Rivière-George	-0.001	0.004	-0.2	0.81	-0.008	0.006
Cohort - 2008	**0.008**	**0.004**	**2.0**	**0.05**	**0.000**	**0.016**
Cohort - 2009	0.007	0.005	1.6	0.12	-0.002	0.016

Table 2.S3 Model selection for body mass of migratory caribou from the Ungava peninsula. sMLH models are derived from the baseline model and include standardized Multi-Locus Heterozygosity (sMLH) and $sMLH^2$ and their interactions (indicated in the model name) with age, √age, sex and herd in addition to the non-genetic variables from the baseline model (K parameters in total). In bold is the selected model. Baseline model: log(mass) ~ age + √age + sex + herd + month of capture + (1|year) + (1|individual identity).

Model	K	AICc	ΔAICc	ω_i
Baseline	**13**	**-401.8**	**0.0**	**0.49**
sMLH x Age	16	-398.7	3.1	0.11
sMLH x Age^2	16	-398.4	3.4	0.09
$sMLH^2$ x Herd	16	-398.4	3.4	0.09
sMLH	15	-397.9	3.9	0.07
$sMLH^2$ x Sex	16	-397.1	4.7	0.05
$sMLH^2$ x Age	16	-396.1	5.8	0.03
$sMLH^2$ x Age^2	16	-396.0	5.8	0.03
sMLH x Sex	16	-395.7	6.1	0.02
sMLH x Herd	16	-395.7	6.1	0.02

Table 2.S4 Model selection for annual survival of adult (> 2 years) migratory caribou from the Ungava peninsula. sMLH models are derived from the baseline model and include sMLH and $sMLH^2$ and their interactions with age, age^2, sex and herd in addition to the non-genetic variables (K parameters in total). In bold is the selected model. Baseline model: survival ~ age + age^2 + sex + herd + (1|year) + (1|individual identity).

Model	K	AICc	ΔAICc	ω_i
Baseline	**7**	**584.3**	**0.0**	**0.55**
sMLH	9	587.6	3.3	0.10
sMLH x Age	10	589.0	4.7	0.05
sMLH x Herd	10	589.0	4.8	0.05
sMLH x Sex	10	589.2	4.9	0.05
$sMLH^2$ x Sex	10	589.4	5.2	0.04
$sMLH^2$ x Herd	10	589.4	5.2	0.04
$sMLH^2$ x Age^2	10	589.5	5.2	0.04
sMLH x Age^2	10	589.6	5.3	0.04
$sMLH^2$ x Age	10	589.7	5.4	0.04

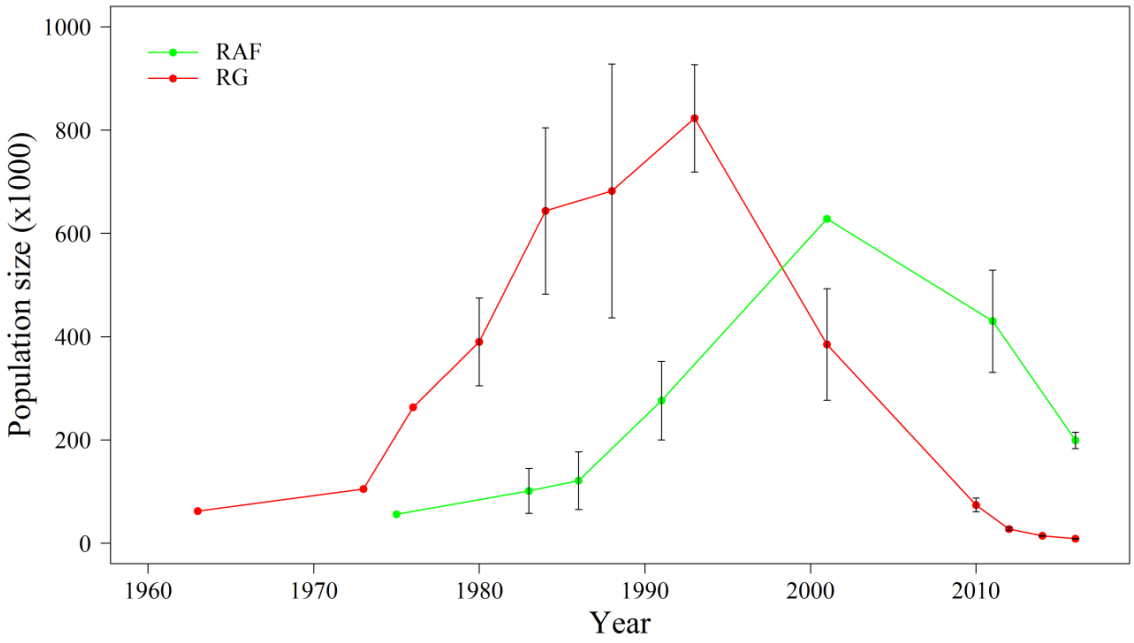

Figure 2.S1 Population sizes of Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds. Population sizes were estimated based on aerial surveys (MFFP). Their confidence intervals were computed using a number of parameters recorded during surveys and are inherent to the method.

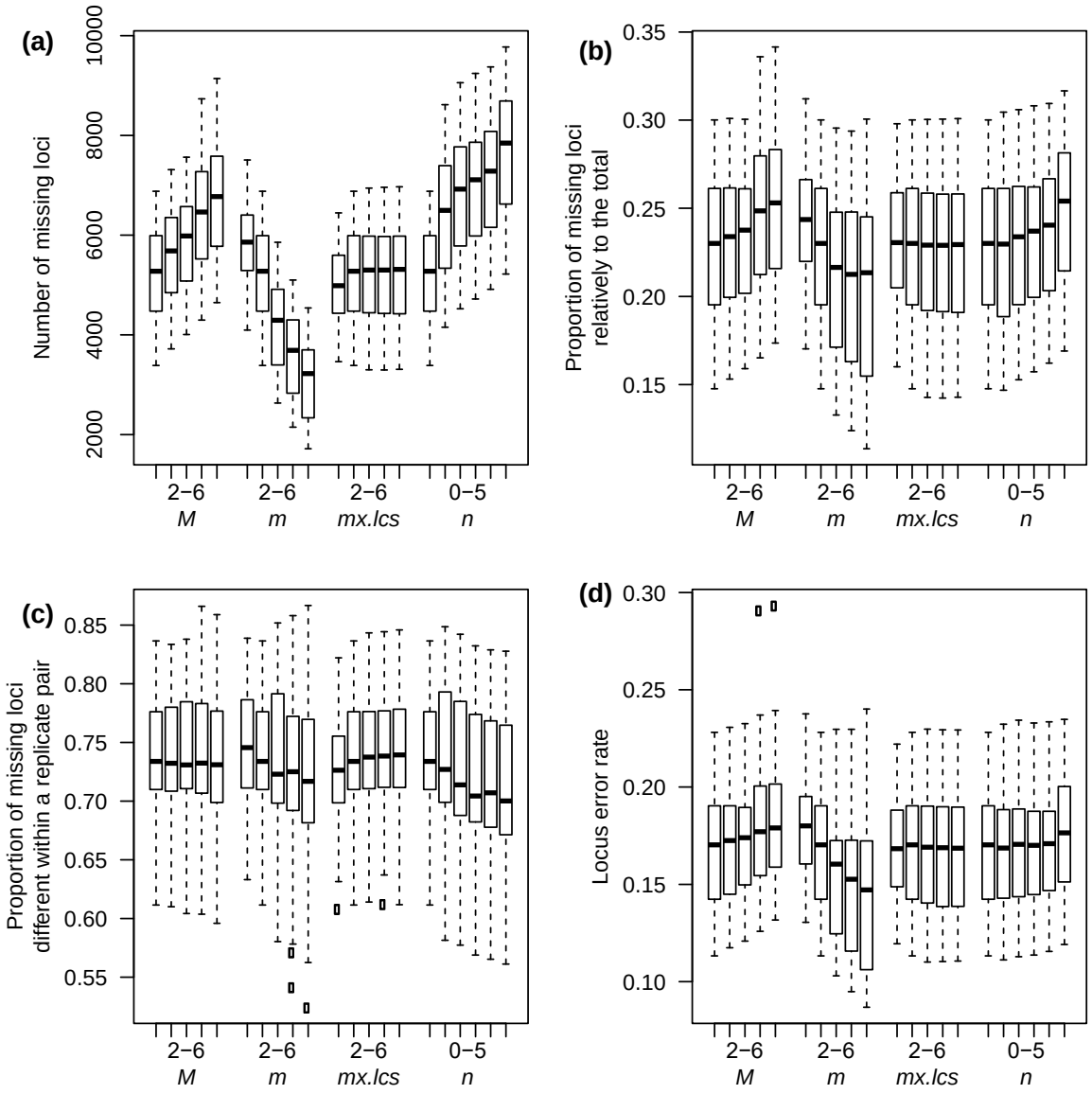

Figure 2.S2 Effect of the variation of different *Stacks* core parameters on missing data. Each box represents the result of a single *Stacks* run in which all parameters were set to their default values (m = 3, M = 2, N = M+2, n = 0, max_locus_stacks (mx.lcs) = 3, model = snp) except for one that varied (indicated on the x axis). (a) Total number of missing loci in a replicate pair, (b) proportion of missing loci relatively to the total number of loci, (c) proportion of missing loci that differed within a replicate pair, (d) locus error rate: proportion of missing loci in only one of the two replicates of a pair. See Mastretta-Yanes et al. (2015) for further discussion on parameters.

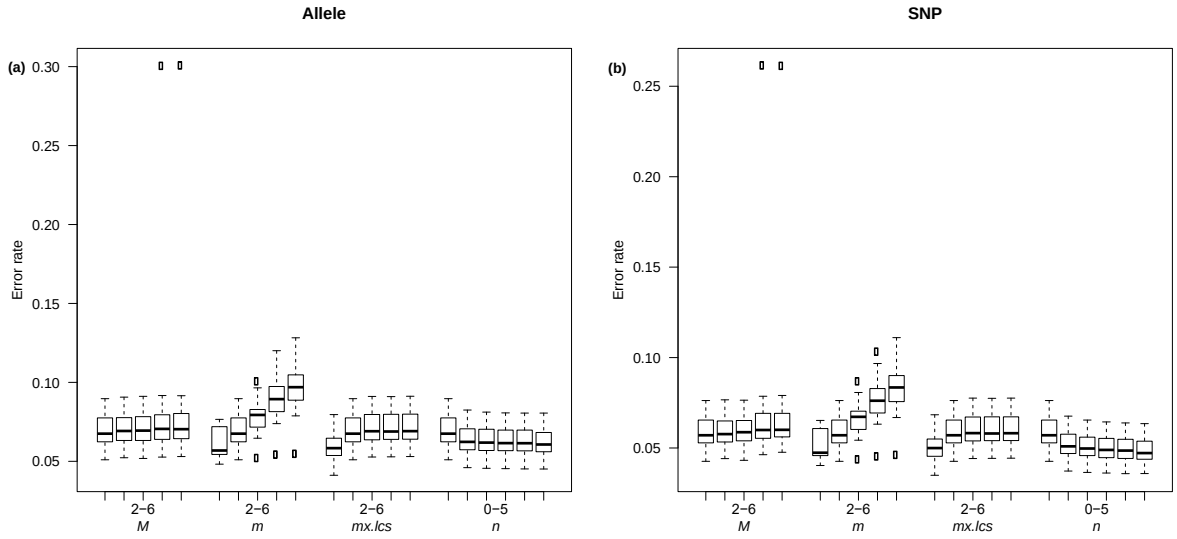

Figure 2.S3 Effect of the variation of different *Stacks* core parameters on missing data. Each box represents the result of a single *Stacks* run in which all parameters were set to their default values (m = 3, M = 2, N = M+2, n = 0, max_locus_stacks (mx.lcs) = 3, model = SNP) except for one that varied (indicated on the x axis). (a) Allele error rate (proportion of allele mismatch within a replicate pair), (b) SNP error rate (proportion of SNP mismatch within a replicate pair). See Mastretta-Yanes et al. (2015) for further discussion on parameters.

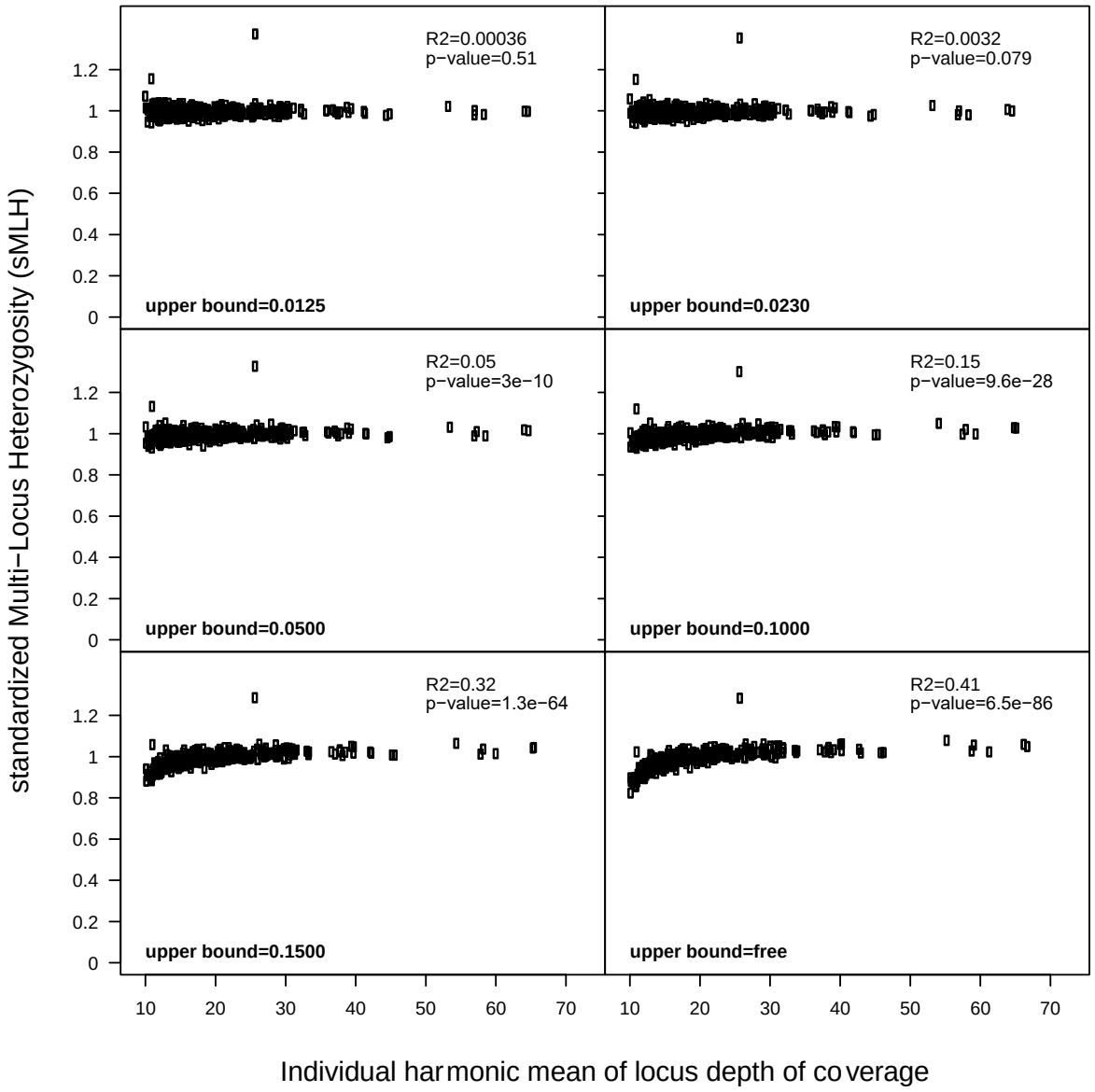

Figure 2.S4 Correlations between standardized Multi-Locus Heterozygosity and individual harmonic mean of locus depth of coverage in relation to different values of upper bound after filtration for *lnl_lim* -10 in *Rxstacks* and without individuals that had more than 80 % missing data. *Stacks* was run with optimal core parameter values identified by analysis of inter-replicates error rates (m = 3, M = 4, N = 6, max_locus_stacks = 3, n = 3). Free upper bound means that the model SNP was used in *Ustacks* instead of the bounded model.

3. Adult survival in migratory caribou is negatively associated with MHC functional diversity

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3.1 Résumé

La dynamique des populations est déterminée en partie par les variations individuelles de traits associés à la valeur adaptative tels que la survie et la reproduction. Les traits associés indirectement à la valeur adaptative, comme les fonctions immunitaires, peuvent aussi affecter la dynamique des populations à travers une interaction avec l'environnement. Chez les vertébrés, les gènes du complexe majeur d'histocompatibilité (CMH) sont impliqués dans l'immunité acquise et spécifique. Pourtant, seulement quelques études se sont intéressées aux conséquences de la diversité génétique du CMH sur la valeur adaptative dans des populations naturelles. Ici, nous avons testé l'association de la survie annuelle et de la masse corporelle avec la diversité de l'exon 2 du gène DRB du CMH (CMH-DRB) dans deux troupeaux en déclin de caribou migrateur entre 1996 et 2016. Pour distinguer les potentiels effets directs des effets généraux de la diversité génétique, nous avons comparé différents indices de diversité estimés à partir des génotype au CMH-DRB et traduisant un gradient allant d'une diversité purement génétique (séquences d'ADN) à une diversité fonctionnelle (séquences d'acides aminés). Nous avons trouvé que i) la masse corporelle n'était pas associée à la diversité au CMH-DRB, ii) que la survie n'était pas associée à la diversité purement génétique au CMH-DRB, mais iii) que les homozygotes fonctionnels au CMH-DRB avaient une probabilité de survie plus élevée que celle des hétérozygotes fonctionnels, peu importe l'allèle fonctionnels qu'ils portaient. De plus, nous n'avons trouvé aucune évidence de sélection fluctuante dans le temps sur le CMH-DRB. Nous pensons que la diversité fonctionnelle au CMH peut ne pas être avantageuse pour le caribou dans un environnement qui était jusqu'à présent stable et présentait une faible abondance et une faible diversité de pathogènes. De plus, des changements rapides des communautés de pathogènes dans le Nord causés par les changements climatiques pourraient causer un décalage entre la diversité et l'abondance de pathogènes et la diversité au CMH chez le caribou. Nos résultats suggèrent que la diversité génétique n'est pas toujours avantageuse, même chez des gènes qui sont censés être fortement affectés par la sélection balancée.

3.2 Abstract

Population dynamic is partly determined by variation in individual fitness traits such as survival and reproduction. Fitness-related traits such as immune functions can also alter population dynamics through their interaction with the environment. The genes of the major histocompatibility complex (MHC) are involved in acquired, specific immunity in vertebrates. Yet, only a few studies have investigated the fitness consequences of MHC gene diversity in wild populations. Here, we tested the association between annual survival and body mass and MHC-DRB exon 2 (MHC-DRB) genetic diversity in two declining migratory caribou herds sampled between 1996 and 2016. To disentangle the potential direct and general effects of genetic diversity, we compared different indices of genetic diversity estimated on MHC-DRB translating a gradient of genetic (DNA-sequence) to functional (amino acid sequences) diversity. We found that i) body mass was not related to MHC-DRB diversity, ii) that purely genetic diversity was not related to survival, but iii) that functional homozygotes at MHC-DRB had higher survival probability than functional heterozygotes, no matter which functional alleles they carried. In addition, we found no evidence of fluctuating selection in time on MHC-DRB gene. We suggest that functional diversity at MHC might not be beneficial for caribou in so far stable environments with low pathogen abundance and diversity. In addition, changes in pathogen communities in the North caused by global change may create a mismatch between pathogen abundance and diversity and MHC diversity for caribou. Our results suggest that genetic diversity is not always beneficial even in genes that are supposed to be strongly shaped by balancing selection.

3.3 Introduction

Population dynamic, *i.e.* changes in population growth rate, results in part from variations in individual fitness traits (Gaillard et al. 2000b). Fitness can be defined as the product of viability (probability of survival until sexual maturity) and fertility (number of offspring produced; Allendorf et al. 2013, but see Orr 2009). As some of the traits likely to influence fitness components (*e.g.* body mass) are sensitive to environmental variations, factors such as climate, predator abundance, disease prevalence or human activity can lead to variation in fitness traits and thus to changes in population dynamics (Gaillard et al. 2000b). In the current context of global climate and anthropogenic changes, it is crucial to understand how those changes could impact individual fitness to better predict potential changes in population dynamics and to adapt conservation and management policies.

An expected consequence of climate change is the modification of the pathogen communities and host-pathogen interactions (Kutz et al. 2005, Chen et al. 2011, Altizer et al. 2013, Dobson et al. 2015), especially in ecosystems where climate is limiting the development of pathogens (Callaghan et al. 2004), *e.g.* in Arctic environments (Kutz et al. 2009, Davidson et al. 2011). Similarly, anthropogenic activities could also lead to changes in pathogen communities through transport and introduction of new pathogens in ecosystems or switch of pathogens from domestic to wild host species (Peeler and Feist 2011). Because immune functions are costly, they often generate trade-offs between energy allocation to immunity and other functions, *e.g.* maintenance, growth or reproduction (Soler et al. 2003, Moreno-Rueda 2011, Souchay et al. 2013, Lo and Shaner 2015, Griesser et al. 2017). Thus, in many species, infected individuals are in poorer condition (Bize et al. 2010, Moreno-Rueda 2011), have lower reproductive success (Griesser et al. 2017) and/or lower survival in comparison with their healthy counterparts (Hanssen et al. 2003, Souchay et al. 2013). Because infection can greatly impact fitness, immune responsiveness is probably under strong stabilizing selection that tends to minimize both the costs associated with immune response and pathogen-induced damages (Lochmiller and Deerenberg 2000, Moret 2003). In a global change context, changes in pathogen communities could modify selective pressures on immunity (Kutz et al. 2005, Chen et al. 2011, Dobson et al. 2015) and lead to changes in population dynamics.

In vertebrates, the genes of the major histocompatibility complex (MHC) play a key role in pathogen recognition (van Bleek and Nathenson 1992). They code for cell-surface glycoproteins that present

antigens to T cells and allow a specific defence against recognized pathogens (Klein 1986). MHC genes are usually highly polymorphic (Klein 1986), especially in the regions that code for antigen binding sites, such that different alleles allow the recognition of different pathogen arrays (Lohm et al. 2002). It is then assumed that gene diversity (heterozygosity) at these loci is positively correlated to immunocompetence (*i.e.*, overdominance) and, as a result, to individual performance ("*heterozygote advantage hypothesis*"; Doherty and Zinkernagel 1975). It is especially likely to be true in populations exposed to multiple pathogens, where individuals carrying different MHC alleles benefit from recognizing a broad array of pathogens. In line with this hypothesis, Oliver et al. (2009) showed that MHC-heterozygote water voles (*Arvicola terrestris*) were co-infected by fewer parasite types than homozygotes. Similarly, de Assunçao-Franco et al. (2012) found that grey seals (*Halichoerus grypus*) with higher number of MHC alleles had higher survival until adulthood. Alternatively, specific alleles are sometimes associated with better or worse individual performance (Langefors et al. 2001, de Assunção-Franco et al. 2012). This could support the "*rare allele advantage hypothesis*" (Clarke and Kirby 1966, Takahata and Nei 1990) or the "*fluctuating selection hypothesis*" (Hill 1991). The former assumes that pathogens continuously try to escape the resistance of the most common host MHC alleles in the population, conferring an advantage to the rare MHC alleles (Clarke and Kirby 1966, Takahata and Nei 1990). The later suggests that if the pathogens regime faced by an organism fluctuates spatially and/or temporally, the direction of selection at MHC genes shall also fluctuate in space and/or in time (Hill 1991). Consequently, spatial and temporal heterogeneity in selective forces act to maintain pools of MHC alleles and, as a result, MHC diversity within populations. In both cases, individuals with higher MHC diversity are more likely to have higher relative fitness because they have more chances to carry a rare, advantageous allele, assuming pathogens diversity is high or fluctuates in the environment.

Because MHC diversity is sometimes correlated to genome-wide diversity, associations between genetic diversity at MHC and individual performance could result from 1) functional advantage of certain genotypes (the "*direct effect hypothesis*"; David 1998) or 2) from inbreeding or outbreeding depression (the "*general effect hypothesis*"; David et al. 1995, Hansson and Westerberg 2002). In several studies, the comparison of MHC diversity and neutral diversity allowed to discriminate the effects of MHC diversity ("*direct effect hypothesis*") from the effects of genome-wide diversity ("*general effect hypothesis*") on performance. For instance, Osborne et al. (2015) confirmed that local effects were

likely responsible for the correlation between genetic diversity at MHC and survival in New Zealand sea lion (*Phocarctos hookeri*) pups because no correlation was observed for neutral microsatellite loci. Charbonnel and Pemberton (2005) also used neutral markers to assess selection at MHC genes in Soay sheep (*Ovis aries*) and concluded that balancing selection was probably acting on MHC genes because genetic differentiation among subpopulations was lower for MHC genes in comparison with neutral loci. A limitation of those approaches is that it indirectly assumes that a small number of neutral markers (usually microsatellites) are reliable to assess genome-wide diversity, although it might not be the case (Balloux et al. 2004). Moreover, heterozygote advantage and fluctuating selection in time or space would both lead to evidences of positive selection such as an excess of heterozygotes and a more even distribution of alleles than expected under neutrality (Spurgin and Richardson 2010). The lack of long-term monitoring of populations often prevents the discrimination between those two options.

Most of the caribou and reindeer populations worldwide are currently declining (Vors and Boyce 2009). It was suggested that climate change and anthropogenic development were likely the main causes of this decline (Vors and Boyce 2009). Yet, only a few studies have investigated the role of pathogens and parasites on individual performance of caribou and reindeer (Stien et al. 2002, Ballesteros et al. 2012, Pachkowski et al. 2013) and only one has studied their potential impact on population dynamics (Albon et al. 2002). Those studies confirmed that parasites could reduce reproductive success and body condition and that these effects were sufficient to regulate caribou densities. Understanding how caribou respond to pathogens and parasites could help understand how host-pathogen dynamic should change over time in response to climate change. In addition, it could provide insight on the adaptive potential of the species or populations to changes in pathogen communities.

Here, we assessed the effect of genetic diversity of the MHC class II DRB exon 2 locus (hereafter MHC-DRB) on fitness components (body mass and survival) of two declining herds of migratory caribou (*Rangifer tarandus*). We hypothesised that MHC diversity should increase immunocompetence and predicted that performance traits would be positively associated with MHC diversity. We propose a comprehensive approach to discriminate between direct and general effects at MHC genes, comparing the relative influence of four MHC diversity indices on individual performance. These indices cover a gradient from purely genetic diversity, i.e. DNA sequence, to functional diversity in terms of protein

structure. We predicted that purely genetic diversity indices would present a stronger correlation with performance if general effects were involved, whereas the correlation would be stronger with functional indices if direct effects were involved.

3.4 Material and methods

3.4.1 Study area and data collection

We sampled individuals from two herds of migratory caribou in Northern Québec and Labrador (Canada): Rivière-George herd (RG) and Rivière-aux-Feuilles herd (RAF). Both herds experienced large fluctuations in population size with strong declines in recent years. Namely, RG herd population size was estimated at 61,842 individuals in 1963 (Des Meules and Brassard 1964). It increased to 823,000 (± 104,000) individuals in 1993 (Couturier et al. 1996) and declined to reach 8,900 ± 668 individuals in 2016 (ministère des Forêts, de la Faune et des Parcs [MFFP], unpublished). Regarding RAF herd, its size was estimated at 56,000 individuals in 1975 (Le Hénaff 1976) and at > 628,000 individuals in 2001 (1,193,000 ± 565,482; Couturier et al. 2004). It decreased to 199,000 (± 15,920) individuals in 2016 (MFFP; unpublished; see figure 3.S1 for details on population dynamics). Between 1996 and 2016, 152 caribou from RG (59 males and 93 females) and 252 from RAF herds (92 males and 160 females) were captured with a net gun fired from a helicopter following the guidelines from the Canadian Council on Animal Care and sampled for DNA (biopsies, hairs and/or blood samples). All samples were frozen, except for some biopsies that were conserved in ethanol (> 70 %). Blood samples were conserved with EDTA in vials. At capture, we estimated caribou's age from the wear pattern of their incisor teeth (Hamlin et al. 2000). We assessed annual survival of 254 adults (≥ 2 years; RG n = 69, RAF n = 185) who were fitted with tracking collars using different satellite devices (Argos, Globalstar or Iridium). Among all caribou marked, 253 animals (1 to 12.75 years; RG n = 82 and RAF n = 171) were also weighted at capture to the nearest 0.1 kg using a hanging scale. Annual survival (from capture to 2015) and body mass (collected in January, February, March, June, October, or November) were used as indices of individual performance and included in models as response variables. For allelic frequencies analysis (see "Assessing population structure and selection" below), we added 114 individuals that were either captured as calf (so their mass and survival were too dependant on maternal effects (Skogland 1990, Veiberg et al. 2017) to be included in our models, n = 109), dead by hunting and not weighted (so they were not included in neither survival nor mass models; n = 4) or of unknown age (n = 1).

3.4.2 DNA extractions

We isolated genomic DNA from muscle (n = 242), blood (n = 10) or hair (n = 29) samples using the DNeasy Blood & Tissue Kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturer's protocol, with minor modifications for hair and blood samples. For hairs (~100 hairs), we used 270 µl ATL lysis buffer, 30 µl proteinase K and added 45 µl of dithiothreitol (DTT; 100 mg/ml) for the digestion step; the elution volume was 100 µl of Elution buffer EB for blood and hair samples; and for all sample types, flow-through went through columns a second time to maximise DNA yield. Genomic DNA concentrations were quantified using a Qubit 2.0 fluorometer (Life Technologies) and all samples standardized at 200 ng/µl.

3.4.3 Illumina MiSeq library preparation

We used Illumina MiSeq technology to sequence a 250 bp fragment of the MHC-DRB gene for 404 caribou. We amplified MHC-DRB with LA31 and LA32 primer pair designed by Sigurdardottir et al. (1991) for bovine but successfully used in caribou from Alaska (Kennedy et al. 2010) and for Peary caribou (*Rangifer tarandus pearyi*; Taylor et al. 2012). Previous results suggest that this pair of primers amplifies only one copy of MHC-DRB in caribou (Kennedy et al. 2010). To multiplex individuals in the same final library, we modified forward and reverse primers by adding to the 5'-end of the sequence a unique barcode of 8 bp. We also added 2 to 4 random nucleotides (NN, NNN or NNNN) to the barcodes to circumvent the low sequence diversity issue in amplicon sequencing with Illumina technologies (Mitra et al. 2015). We designed a total of 24 forward barcodes and 40 reverse barcodes, resulting in 960 unique combinations. Two or three independent PCR were performed for each individual, using different barcode combinations. We used 130 barcode combinations as negative controls consisting of PCR mix without DNA template to estimate sequence-sample contamination (Gaigher et al. 2016). Each PCR reaction was performed in a total volume of 20 µl containing 16 µl of AmpliTaq Gold™ 360 Master Mix (ThermoFisher Scientific), 2 µl of a mix of two tagged primers (5 µM each), and 2 µl of template DNA (200 ng/µl). The thermocycling conditions were as follow: 10 minutes at 95°C; 35 cycles of 30 seconds at 95°C, 30 seconds at 55°C, 45 seconds at 72°C; 7 minutes at 72°C and hold at 4°C. All reaction products were pooled together and purified using Promega Wizzard® SV Gel and PCR Clean-Up System following the protocol provided with the kit. Final library was prepared using the

TruSeq Nano DNA HT Sample Prep Kit from Illumina using the MetaFast protocol (Fasteris SA; Geneva, Switzerland). Purified library was sequenced in a full run with a 250 bp paired-end MiSeq technology (Illumina).

3.4.4 Illumina MiSeq data processing

Raw reads were first processed with the package *OBITools* (Boyer et al. 2016): i) Forward and reverse reads were assembled together to reconstruct the full length amplicon and only assemblies having an alignment score of 40.0 (corresponding to an overlap of at least 10 bp associated to maximum base-calling qualities) or more were conserved (96.3 % of sequences kept); ii) sequences were then assigned to their sample according to the tag sequences contained in the amplicon and the MHC fragment reconstructed (*i.e.* primer and tags were removed; 80 % of sequences assigned); iii) MHC fragments were then dereplicated (100 % identity clustering). We used *R* (v. 3.4.0; R Core Team 2017) for the next steps in order to obtain genotypes for all individual replicates. Sequence variants shorter than 240 bp and those that were not associated with at least 20 reads in one sample were discarded. We discarded 46 of all samples sequenced because their coverage was inferior to 500 reads; this threshold seemed to discriminate sequencing artefacts from true alleles because the mean coverage was very high (13,220 ± 5,623 (sd) sequences per sample). After visual inspection of the coverage of all sequence variants in each sample, it clearly appeared that only one copy of the DRB locus was amplified in the samples, which was consistent with findings from previous study on caribou MHC-DRB (Kennedy et al. 2010). Indeed, for each sample, we identified a maximum of two sequences with coverage 10 times higher than the next most sequenced sequence observed in the same sample. We thus expected individuals to carry a maximum of two alleles and we assumed that genotypes that included more than 2 alleles included sequencing artefacts. We tested different depth of coverage thresholds to discriminate between sequencing artefacts and true alleles and chose to keep only sequences supported by 17 % or more of the reads in one sample as this threshold minimized both genotypes with 0 or more than 2 alleles. Finally, comparing the different replicates led to only one mismatch between the genotypes of a replicate pair caused by one replicate retaining 3 alleles. This individual was excluded from further analysis.

3.4.5 MHC Sequence analysis

We used *MEGA7* (v. 7.0.18; Kumar et al. 2016) to align sequences and to estimate genetic diversity indices based on individual genotypes. First, individual heterozygosity was coded as a binary variable describing whether MHC-DRB was heterozygous (coded as 1) or homozygous (coded as 0). Second, we calculated nucleotide diversity (π) as the number of nucleotide substitutions between the two alleles carried by an individual divided by total length of the DNA fragment (250 bp). Heterozygosity and nucleotide diversity were computed with *DNAsp* (v. 5.10.1; Librado and Rozas 2009). Third, we estimated amino acid (a.a.) diversity as the number of a.a. substitutions between the peptide sequences produced by the two alleles carried by an individual divided by the total length of the peptide (82 a.a.). We computed the number of a.a. substitutions using *MEGA7* (Kumar et al. 2016). Fourth, functional heterozygosity was coded as a binary variable describing whether an individual carried one (0) or two (1) different MHC-DRB supertypes.

We defined supertypes as functional groups of alleles and used the *R* package *adegenet* (v 2.0.1; Jombart 2008, Jombart and Ahmed 2011) to cluster alleles following the procedure described by Buczek et al. (2016). To do so, we identified 24 positively selected sites (PSS) using the BEB procedure in *PAML* (v. 4.8; Yang 2007). We assumed PSS corresponded to the antigen binding sites (ABS) because 13 of the 24 PSS identified corresponded to human ABS identified by Reche and Reinherz (2003) and 5 additional PSS were shifted one position with respect to the human ABS (table 3.S1). Thus, we clustered together alleles sharing similar physico-chemical properties (e.g. lipophilicity, steric bulk and polarity; Sandberg et al. 1998) of positively selected amino acids.

3.4.6 Statistical Analysis

We assessed the effect of genetic and functional diversity at MHC on body mass using linear mixed-effect models and the 'lmer' function implemented in the package *lme4* (Bates et al. 2015) of *R* (v. 3.4.0; R Core Team 2017). We log-transformed body mass to improve homogeneity of variances. For each diversity index, we compared a model including only relevant, non-genetic variables to three models derived from this baseline model (see below) with simple (fixed) effect of diversity or in interaction with sex or age. We determined that the baseline variables for body mass models should include age, $\sqrt{\text{age}}$, sex, herd and month of capture as fixed-effect variables because these factors are known to influence body mass in caribou (Parker 1981, Couturier et al. 2009a, 2009b). We also

included year of capture and individual identity as random factors to account for repeated measures and annual variation. Following the same approach, we assessed the effect of genetic and functional diversity of MHC on annual adult survival. Here we fitted generalized mixed-effect models with a binomial distributed error (logit link function), using the 'glmer' function implemented in the package *lme4* (Bates et al. 2015). Annual survival was binary coded as 1 if an animal was alive during a given year and as 0 the first complete year the animal was dead. The baseline variables for survival models included age, age^2, sex and herd as fixed-effect variables (Loison et al. 1999, Couturier et al. 2010) and individual identity and year as random effect variables. We centered the age variable on its mean in order to avoid high collinearity between age and age^2. We used Akaike's Information Criterion for small sample sizes (AICc) to rank models, and computed ΔAICc and AICc weights (ω_i) implemented within the *R* package *AICcmodavg* (Mazerolle 2017). We selected the model with the lowest value of AICc. We used the same approach to determine whether the combination of supertypes carried by an individual (i.e. its functional genotype) affected its body mass or survival, replacing the diversity indices by a categorical variable representing the functional genotype. If the selected model included functional genotype, we used the function 'lsmeans' implemented in the R package *lsmeans* (Lenth 2016) to compute pairwise comparisons between all the genotypes (n=6).

3.4.7 Assessing equilibrium and changes in allelic frequencies

We also tested for temporal changes in allelic and *supertypic* frequencies overtime, indicating potential fluctuating selection. We first defined cohort as a group of ≥ 5 individuals born the same year. Consequently, not all years were included in the analysis: 13 cohorts for RAF and 12 cohorts for RG herd. Then, *G*-tests implemented in the software *GenoDiv*e (v. 2.Ob27; Meirmans and Van Tienderen 2004) were used to test for temporal differentiation between cohorts within each herd in terms of allelic or *supertypic* frequencies. We adjusted p-values for multiple comparisons using the Benjamini and Yekutieli (2001) correction. We also computed observed and expected heterozygosities and functional heterozygosities, as well as F_{is} by cohort in *GenoDive*. In doing so, we assessed departure from Hardy-Weinberg Equilibrium (HWE). All tests mentioned here were based on 1,000 permutations. The analysis described in this section were conducted using all captured individuals for whom we had an estimated age, including the calves that were not included in previous models testing effect of MHC diversity on mass and survival.

3.5 Results

3.5.1 MHC Illumina Sequencing

Only 0.067 % of all reads were associated with negative control combinations of barcodes and no control sample had sufficient amount of reads to be retained after the filters. After processing sequence data, we obtained a genotype for 391 individuals out of 404 originally sequenced. All those individual genotypes were supported by at least two concordant replicates with a mean depth of coverage of 8,915 ± 3,072 reads (sd) per replicate. Only one pair of replicates that had passed the filters was not concordant and was eliminated. These results indicate that tag jumping and cross contamination have a negligible impact on the whole genotyping quality. We identified 20 allelic sequences (GenBank Accession numbers: MH171262 - MH171281) out of which 2 and 4 were private alleles to RG and RAF herds, respectively (tables 3.1 and 3.S2). Nine alleles had already been identified in Grant's Caribou (*Rangifer tarandus grantii*) in Alaska (Kennedy et al. 2010), in Peary Caribou (*Rangifer tarandus pearyi*) in Canadian Arctic (Taylor et al. 2012) and in other Reindeer populations (Mikko et al. 1999, Wei and Happ (unpublished; GenBank), Djakovic (unpublished; GenBank)). Eight alleles were variants (1-2 bp differences) or subtypes (3-4 bp differences) of those previously identified alleles and three were novel, unidentified alleles (table 3.S2). Among these alleles, 38 nucleotidic sites were polymorphic and all alleles coded for different peptide chains, with 25 variable amino acids. We clustered the alleles in three supertypes among which the alleles were distributed approximately equally, with 6, 6, and 8 alleles respectively included in supertypes 1, 2, and 3 (table 3.S2).

3.5.2 Effect of MHC diversity on body mass

For each diversity index set of models, the best model predicting body mass was the baseline model, *i.e.*, the one including age, √age, sex, herd and month of capture as fixed effect variables. Next-ranked models presented no significant effect of genetic variables (table 3.S3). For the functional genotype set of models, the model including functional genotype in addition to the baseline variables was the best model (ω_i =0.56; table 3.S4). In this model, age, $\sqrt{}$age, sex, herd had a significant effect on body mass and the functional genotype 3/3 had a marginally significant effect. For each increase of one year in age, log(mass) decreased by -0.19 to -0.27 and for each increase of one unity of $\sqrt{}$age, log(mass) increased by 1.08 to 1.36. Roughly, this translated into a steep increase in weight for the first years of

life and a stabilization of the weight after 4 years around 95 kg. Males were heavier than females and individuals from RG herd were heavier than RAF individuals. Although in the model functional genotype 3/3 appeared to be marginally significantly different from the 1/1 functional genotype (reference level; 95% CI on estimate = [0.00, 0.15), pairwise comparisons revealed no significant differences between functional genotypes (results not shown). Nevertheless, two functional genotypes (1/2 and 3/3) were nearly significantly different (adjusted p-value= 0.0537), with functional genotype 3/3 being associated with slightly higher weights than 1/2. Individuals were generally heavier in Fall months compared to Winter or (early) Summer months. Non-genetic variables followed the same trends in the baseline model, with similar statistical significance. All estimates of the mass baseline or functional genotype models are listed in tables 3.2 and 3.3, respectively.

3.5.3 Effect of MHC diversity on survival

The baseline model was selected for all sets of candidate survival models (MHC diversity and MHC functional genotypes), except for the model that included functional heterozygosity (tables 3.S5 and 3.S6). All next-ranked models did not present any significant effect of the genetic variables. The selected model in the functional heterozygosity set was the model including simple effects of functional heterozygosity in addition to the non-genetic variables (ω_i = 0.37; table 3.S5). In this model, herd and functional heterozygosity had a significant effect on survival, *i.e.*, negative heterozygosity-fitness correlation (*HFC*). The odd that a caribou from RG herd survived was 0,51 times lower than that of a caribou from the RAF herd (95 % CI odd ratio (OR) = [0.33, 0.79]; figure 3.1). In addition, functional heterozygosity was associated with an odd of surviving 0.67 times lower compared to functional homozygosity (95 % CI OR = [0.45, 0.99]; figure 3.1). Functional homozygotes had a survival probability on average 6.1 ± 2.5 % (sd) superior to that of functional heterozygotes (figure 3.2). Centered age and $(\text{centered age})^2$ had marginally significant negative effects on survival (p-value = 0.08 for centered age and p-value = 0.08 for $(\text{centered age})^2$). Sex did not affect survival (p-value = 0.90). Non-genetic variables followed the same trends in the baseline model, with similar statistical significance. All estimates of the survival baseline model and the survival functional heterozygosity model are listed in tables 3.4 and 3.5, respectively.

3.5.4 Equilibrium and changes in allelic frequencies

We did not detect departure from HWE for the MHC alleles and supertypes in any of the cohorts (all p-values > 0.05; table 3.S7). Multiple comparisons between cohorts of each herd revealed that there was no significant temporal change of allelic or *supertypic* frequencies over the ~ 20 years of sampling (G-test; $\alpha = 0.05$; all p-values equal to 1 after Benjamini and Yekutieli (2001) correction), indicating that there was no significant genetic differentiation overtime.

3.6 Discussion

We investigated the effect of immune gene variability (MHC-DRB) on performance-related traits in two declining migratory caribou populations. Body mass was not associated with genetic or functional MHC diversity, but showed a slightly significant positive association with one functional genotype. Surprisingly, we also found a strong negative effect of functional heterozygosity (or negative HFC) at MHC-DRB on both male and female survival; survival of functional homozygotes was ca. 7 % higher than that of functional heterozygotes.

3.6.1 Effects of MHC diversity on body mass

Diversity at MHC has previously be shown to be associated with body mass in male white-tailed deer (*Odocoileus virginianus*; Ditchkoff et al. 2001). More genetically diverse individuals were heavier and harboured larger antlers. It was suggested that those individuals could allocate more energy to growth because they were more efficient at obtaining food, had a more efficient metabolism or used other unidentified alternative mechanisms (Ditchkoff et al. 2001). In either case, the link between MHC genotype and body mass is indirect and may involve resource allocation (Derting and Compton 2003), behavioural response or extrinsic constraints (Moret and Schmid-Hempel 2000), all of which could vary seasonally. Ungulates usually present large variations of body mass over seasons (Parker et al. 1993, Chan-McLeod et al. 2000). Although we accounted for season in our analyses, the correlation design we used was less powerful than sampling individuals of the same sex-age class at the same time. In addition, we did not know the reproductive status of individuals, which could have also influenced body mass, especially for females (Chan-McLeod et al. 2000). Such seasonal fluctuations, in conjunction with variance in the reproductive status may thus have obscured the effect of MHC diversity or functional genotype on body mass.

The selected model for functional genotypes and body mass, as well as the *a posteriori* pairwise comparisons both suggest that individuals carrying the 3/3 functional genotype tended to be heavier than other individuals, although the differences were not significant. It could suggest that resistance to infections is recessive (i.e. that heterozygotes perform as badly as the worst corresponding homozygotes; Ilmonen et al. 2007), although in that case, 3/3 functional genotype should perform better than 1/3 and 2/3, which is not the case here. Here, the fact that the differences were marginally significant, that they were not the same according to the test used and that the result does not clearly support a biological hypothesis suggest that it could be a statistical artefact.

3.6.2 Effects of MHC diversity on survival

Our most striking finding is the negative association between functional heterozygosity and annual adult survival (or negative HFC). Few studies (Paterson et al. 1998, Brouwer et al. 2010, Worley et al. 2010, de Assunção-Franco et al. 2012, Sepil et al. 2013, Osborne et al. 2015) have investigated the effects of an immune-related gene on survival rate, which can be greatly impacted by pathogens or parasites (Hanssen et al. 2003, Lachish et al. 2007, Höner et al. 2012, Souchay et al. 2013). For instance, in a parasite-removal experiment conducted on greater snow goose (*Chen caerulescens atlantica*), Souchay et al. (2013) showed that treating sub-adults with anthelmintic drug increased survival probability by about 10 %. Here, we detected a relationship between MHC diversity and survival when including functional heterozygosity as an index of diversity. Because natural selection acts on phenotypes and not on genotypes (Endler 1986), functional heterozygosity, that reflects the diversity of physico-chemical properties of the antigen-binding site, is more likely to show an association with fitness-related traits because it is more closely related to the phenotype than pure heterozygosity or nucleotide diversity. In addition, this approach allowed disentangling the direct and general effects of diversity on performance without the need to compare diversity at MHC and global neutral diversity. Because survival was associated with functional diversity but not with purely genetic diversity indices, we assume that direct effects caused the observed relationship between survival and functional heterozygosity.

Surprisingly, the sign of the relationship between functional heterozygosity and survival was contrary to our predictions, because higher diversity at immunity-related genes is usually associated with higher performance (*e.g.* Oliver et al. 2009, de Assunção-Franco et al. 2012, Osborne et al. 2015), especially when general effects can be ruled out. Studies have however already shown that lower MHC diversity

can be beneficial to individuals. Ilmonen et al. (2007) observed recessiveness rather than dominance or overdominance of most MHC haplotypes for resistance to Salmonella (*Salmonella enterica*) in mice (*Mus musculus domesticus*), *i.e.* the heterozygote's resistance was as poor as the resistance of the worst homozygote. They suggested that there might be a gene-dose effect that conferred an advantage to homozygotes. Nevertheless, they also emphasized that their experiment was conducted with only one species of pathogens and that in the wild, multiple infections could lead to different patterns of selection. In addition, in this study, heterozygous individuals had a maximal level of heterozygosity for all classes I and II MHC genes, which could result in depleted T-cell repertory and thus to reduced immunocompetence. In the wild, this phenomenon is usually observed in species with a high number of copies of MHC genes (*e.g.* in three-spined stickleback (*Gasterosteus aculeatus*) with 2 - 9 alleles/individual; Wegner et al. 2003). In our sample as well as in another study (Kennedy et al. 2010), caribou appeared to have only one copy of MHC-DRB, with no variation in copy number. Then, it would be surprising that heterozygote caribou experienced decreased immunocompetence associated with depleted T-cell repertory. Moreover, our study was conducted on wild animals, and it is likely that caribou were spatially and temporally exposed to multiple species of pathogens (Simard et al. 2016). We do not definitely refute the possibility that our result support recessiveness for MHC-DRB, but for the abovementioned reasons, we considered this hypothesis unlikely. In this context, additional studies are required to investigate the underlying mechanism behind the higher survival of MHC functional homozygote vs. heterozygote caribou.

Homozygote advantage or underdominance could arise through two mechanisms: i) outbreeding depression, as a mechanism for disrupting co-adapted gene complexes (Freeland et al. 2011), or ii) good allele effect or allelic incompatibilities (McClelland et al. 2003). We exclude the latter mechanism because no functional genotype showed any association with higher or lower survival. If RG and RAF herds were subject to outbreeding depression, the negative association between MHC-DRB diversity and survival should have arisen from purely genetic diversity indices (heterozygosity or nucleotide diversity) rather than functional heterozygosity. In addition, outbreeding depression suggests massive gene flow from genetically differentiated neighbouring herds or genetic substructure within a herd. High gene flow between RG and RAF herds has been reported (Boulet et al. 2007, Gagnon et al. unpublished); and based on microsatellite data the herds were considered genetically similar (n = 7 loci; Boulet et al. 2007, n = 16 loci; Yannic et al. 2016) and slightly differentiated with SNPs (F_{ST}: 0.0027;

95 % CI: 0.0024-0.0029, p-value <0.001; Gagnon et al. unpublished). Thus outbreeding depression is unlikely and should not have a major impact on performance. Gene flow could also occur between migratory caribou and forest-dwelling caribou herds located further south in the continuous boreal forest (Boulet et al. 2007). Forest-dwelling caribou herds are small (orders of magnitude smaller in comparison with migratory caribou herds; Boulet et al. 2007) so it is unlikely that it would be sufficient to generate outbreeding depression within migratory caribou herds.

The mechanisms linking the host MHC-DRB genotype and performance still remain unclear, although results suggest they most likely originate from direct effects. Assuming that functional heterozygotes do have higher immunocompetence than functional homozygotes, we can hypothesize that they could be disadvantaged by higher costs of immunity. Acevedo-Whitehouse et al. (2018) discuss this hypothesis, arguing that if higher MHC diversity translated into better antigen-recognition and higher antibody production, then it should also increase the risks of immunopathology (*i.e.* damages caused by the host immunity to the host tissue). In this context, functional heterozygote caribou could benefit from the recognition of a broader array of pathogen but also suffer the costs associated with more frequent or more intense immune responses. This idea is in line with observations made in badgers (Sin et al. 2016) showing that total investment in immune functions (acquired and innate) was positively correlated with MHC diversity. If caribou showed the same pattern, then functional heterozygotes would indeed invest more in immunity, and as a consequence, could pay a higher cost for those functions. In addition, Moret (2003) suggests that optimal investment in immune functions should decrease as infection frequency increases. If climate change led to increased pathogen abundance, then it could have shifted the optimal investment further away from that of functional heterozygotes. This hypothesis could reconcile the fact that we observed a high MHC diversity even if the individuals whose diversity was high performed poorly.

It seems odd that MHC-DRB diversity was maintained through time, because it is detrimental to carry different supertypes. We were unable to show fluctuating selection or negative frequency-dependent selection in our dataset. It is worth noting that our sample size for each cohort may have been too small to detect rare alleles, as a minimum of 15 diploid individuals are required to have a 95 % chance of detecting an allele with a frequency of 0.1 (Allendorf and Luikart 2007). In addition, because not all individuals were captured at their first year of life, selection may have acted before our sampling,

making it more difficult to detect it afterward. The presence of positively selected sites (table 3.S1), the maintenance of 3 supertypes and the detection of 20 different alleles during 20 years of sampling (~5 generations) despite the intense demographic decline and the disadvantage conferred by functional heterozygosity, however, suggest that some kind of selection acts to maintain MHC-DRB allelic diversity in RG and RAF herds. Conversely, immune regulation genes may have undergone constant directional or stabilizing selection, for instance if infection intensity has remained stable overtime. While standing MHC diversity should allow for quick adaptation to new constraints associated with climate change and resulting changes in pathogen communities, immune regulation genes may be more limited in their potential adaptation due to probably lower diversity (Quéméré et al. 2015).

Finally, as survival is only one component of fitness, trade-offs between life-history traits such as survival and reproduction could conceal a more complex relationship between MHC diversity and fitness. For instance, Lo et al. (2015) showed that reproductive females of the Taiwan field mouse (*Apodemus semotus*) were more infected with parasites than non-reproductive ones, and that females treated with an anthelmintic drug had reduced survival. Although this result is counterintuitive, the authors suggested that treated females were in better condition and may have chosen to invest more in their current reproduction at the expense of their own survival and future reproduction. We cannot conclude that higher MHC functional diversity leads to lower fitness in caribou, as we do not have any information on reproductive success and how it correlates with MHC diversity.

We found a large effect of MHC functional heterozygosity on survival, which corroborates the conclusions of Albon et al. (2002) that pathogens could play an important role in population dynamics of caribou. If the advantage to functional homozygotes was caused by a poor regulation of the immune functions associated with recent changes in pathogen communities, it could also provide a mechanism by which global climate change is causing the decline of caribou and reindeer populations (Vors and Boyce 2009). Further studies are required to test this hypothesis. Moreover, our research highlights the importance of using appropriate indices of genetic diversity when investigating the effects of genetic diversity on performance, especially when direct effects are suspected. Here, we found an effect of MHC diversity on survival using functional heterozygosity as an index of phenotypic diversity on which selection directly acts. We showed that different indices of genetic diversity could lead to different results regarding association with individual performance. Therefore, we suggest that to account for

diverse mechanisms underlying HFC at MHC genes, further studies should consider different indices of genetic diversity. Our results open new perspectives for future researches on the genetic interaction between host survival, immunity and pathogens, and on the mechanisms that maintain genetic variability at MHC in natural declining populations.

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3.8 Tables

Table 3.1 Summary statistics of genetic diversity in Rivière-George and Rivière-aux-Feuilles herds for both sexes (F: Females; M: Males). For heterozygosity and functional heterozygosity, H_o and H_e express observed and expected heterozygosity, respectively. For quantitative indices (nucleotide and amino acid diversity), mean and standard deviation (sd) are indicated.

Herd	Sex	Number of individuals	Number of alleles	Number of supertypes	Heterozygosity		Nucleotide diversity		Amino acid diversity		Functional heterozygosity	
					H_o	H_e	Mean	sd	Mean	sd	H_o	H_e
TRAF	F	155	18	3	0.832	0.892	0.106	0.061	0.106	0.061	0.626	0.656
	M	90	15	3	0.900	0.894	0.118	0.056	0.118	0.056	0.722	0.659
	All	245	18	3	0.866	0.893	0.110	0.059	0.110	0.059	0.674	0.658
TRG	F	88	14	3	0.852	0.879	0.108	0.062	0.108	0.062	0.602	0.641
	M	58	15	3	0.862	0.892	0.110	0.060	0.110	0.060	0.552	0.646
	All	146	16	3	0.857	0.885	0.108	0.061	0.108	0.061	0.577	0.643
All		391	20	3	0.862	0.889	0.110	0.060	0.110	0.060	0.626	0.650

Table 3.2 Estimates of the body mass baseline model for migratory caribou from the Ungava peninsula. Lower bounds and upper bounds of the 95% confidence intervals on estimates are provided in addition to the standard errors (Std. error) and t-values. Variables with 95 % CI that do not overlap 0 are considered to have a significant effect and are in bold. Levels of reference are *Female* for sex, *Rivière-aux-Feuilles* for herd and *January* for month of capture. Baseline model: log(mass) ~ age + √age + sex + herd + month of capture + (1|identity).

				95% Confidence interval	
Variable	Estimate	Std. Error	t-value	Lower bound	Upper bound
Intercept	**2.95**	**0.08**	**39.2**	**2.79**	**3.11**
Age	**-0.23**	**0.02**	**-13.9**	**-0.27**	**-0.19**
√Age	**1.22**	**0.07**	**18.3**	**1.08**	**1.36**
Sex - Male	**0.14**	**0.03**	**5.4**	**0.08**	**0.20**
Herd - Rivière-George	**0.09**	**0.02**	**5.2**	**0.05**	**0.13**
Month of capture - February	-0.08	0.04	-1.9	-0.16	0.00
Month of capture - March	0.01	0.04	0.3	-0.07	0.09
Month of capture - June	**-0.24**	**0.04**	**-5.7**	**-0.32**	**-0.16**
Month of capture - October	-0.01	0.04	-0.3	-0.09	0.07
Month of capture - November	0.04	0.05	0.9	-0.06	0.14

Table 3.3 Estimates of the body mass functional genotypes model for migratory caribou from the Ungava peninsula. Lower bounds and upper bounds of the 95% confidence intervals on estimates are provided in addition to the standard errors (Std. error) and t-values. Variables with 95 % CI that do not overlap 0 are considered to have a significant effect and are in bold. Levels of reference are *Female* for sex, *Rivière-aux-Feuilles* for herd, 1/1 for (functional) genotype (numbers represent combinations of supertypes) and *January* for month of capture. Model: log(mass) ~ age + √age + sex + herd + functional genotype + month of capture + (1|identity).

				95% confidence interval	
Variable	Estimate	Std. Error	t-value	Lower bound	Upper bound
Intercept	**2.95**	**0.08**	**38.3**	**2.8**	**3.10**
Age	**-0.23**	**0.02**	**-14**	**-0.26**	**-0.19**
√Age	**1.22**	**0.07**	**18.4**	**1.09**	**1.35**
Sex - Male	**0.13**	**0.03**	**5.3**	**0.08**	**0.18**
Herd - Riviere-George	**0.09**	**0.02**	**5.5**	**0.06**	**0.12**
Genotype - 1/2	-0.02	0.02	-0.9	-0.07	0.03
Genotype - 1/3	0.03	0.03	1.3	-0.02	0.09
Genotype - 2/2	0.00	0.03	0.1	-0.05	0.05
Genotype - 2/3	-0.01	0.03	-0.3	-0.06	0.04
Genotype - 3/3	0.07	0.04	2.0	0.00	0.15
Month of capture - February	-0.07	0.04	-1.8	-0.15	0.01
Month of capture - March	0.01	0.04	0.4	-0.06	0.08
Month of capture - June	**-0.24**	**0.04**	**-5.8**	**-0.32**	**-0.16**
Month of capture - October	-0.02	0.04	-0.4	-0.10	0.07
Month of capture - November	0.05	0.05	1.0	-0.05	0.15

Table 3.4 Estimates of the annual adult (>2 years) survival baseline model for migratory caribou from the Ungava peninsula. Lower bounds and upper bounds of the 95% confidence intervals on estimates are provided in addition to the standard errors (Std. error), Z-values and p-values. Variables with 95 % CI that do not overlap 0 are considered to have a significant effect and are in bold. Levels of reference are *Female* for sex and *Rivière-aux-Feuilles* for herd. Baseline model: Survival ~ age + age^2 + herd + sex + (1|year) + (1|identity).

					95 % Confidence Interval	
Variable	Estimate	Std. error	Z-value	p-value	Lower bound	Upper bound
Intercept	**2.35**	**0.27**	**8.8**	**0.00**	**1.82**	**2.87**
Centered age	-0.06	0.05	-1.3	0.21	-0.16	0.04
(Centered age)^2	-0.02	0.01	-1.5	0.14	-0.04	0.01
Sex - Male	-0.11	0.24	-0.5	0.66	-0.57	0.36
Herd - RG	**-0.64**	**0.22**	**-2.9**	**0.00**	**-1.08**	**-0.21**

Table 3.5 Estimates of the functional heterozygosity model of annual adult (>2 years) survival for migratory caribou from the Ungava peninsula. Lower bounds and upper bounds of the 95% confidence intervals on estimates are provided in addition to the standard errors (Std. error), Z-values and p-values. Variables with 95 % CI that do not overlap 0 are considered to have a significant effect and are in bold. Levels of reference are *Female* for sex, *Rivière-aux-Feuilles* for herd and homozygote for functional heterozygosity. Model: Survival ~ age + age^2 + sex + herd + functional heterozygosity + (1|year) + (1|identity).

					95 % Confidence Interval	
Variable	Estimate	Std. error	Z-value	p-value	Lower bound	Upper bound
Intercept	**2.64**	**0.29**	**9.0**	**0.00**	**2.06**	**3.21**
Centered age	-0.09	0.05	-1.7	0.09	-0.18	0.01
(Centered age)^2	-0.02	0.01	-1.7	0.08	-0.04	0.00
Sex - Male	-0.03	0.25	-0.1	0.90	-0.51	0.45
Herd - RG	**-0.68**	**0.23**	**-3.0**	**0.00**	**-1.12**	**-0.24**
Functional heterozygosity - Heterozygote	**-0.40**	**0.20**	**-2.0**	**0.05**	**-0.80**	**-0.01**

3.9 Figures

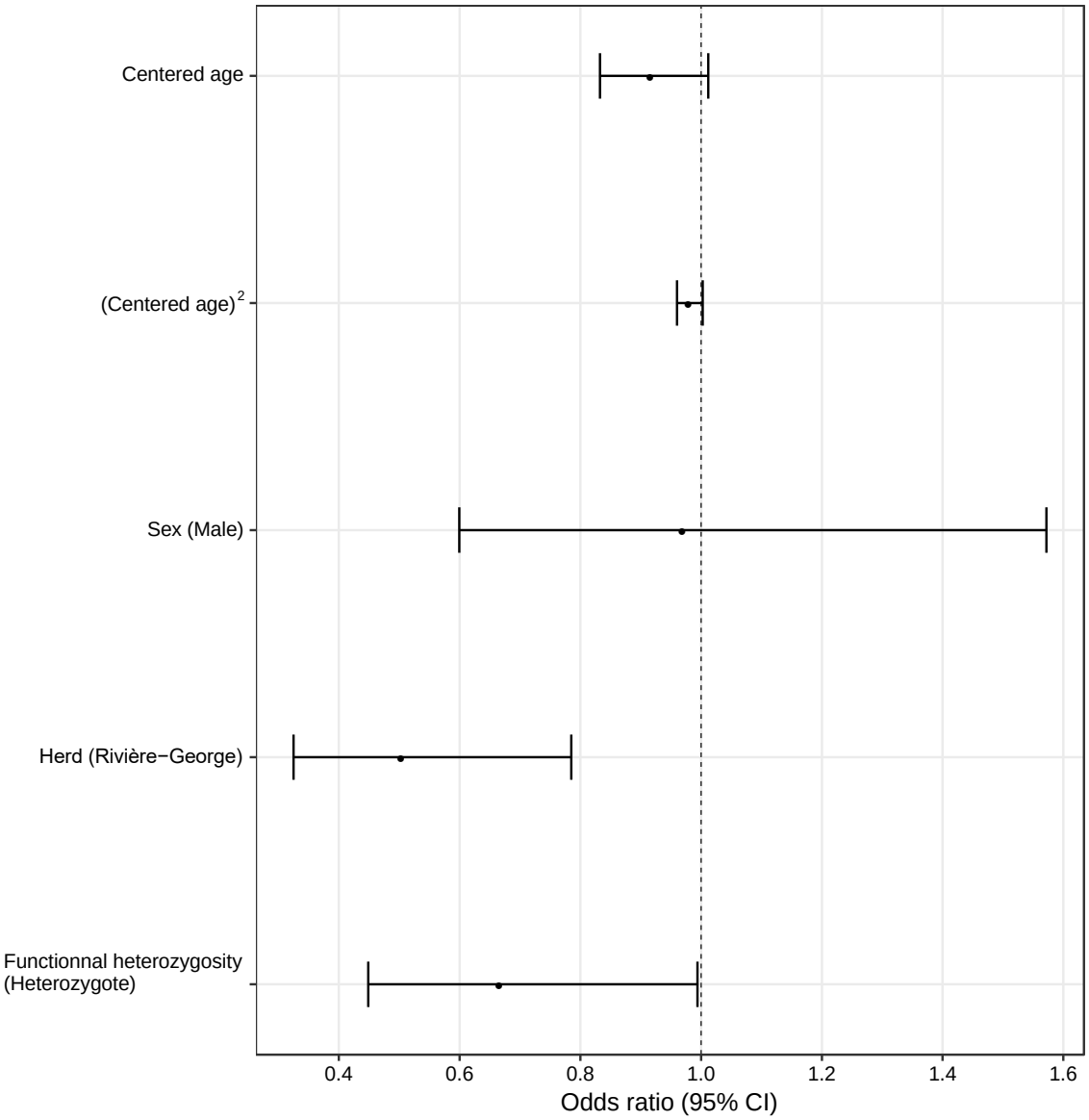

Figure 3.1 Odds ratios and 95 % confidence intervals (CI) of the fixed-effect variables of the functional heterozygosity model of survival. Odds ratios with CI that do not overlap 1 are considered statistically significant. Levels of reference are *Female* for sex, *Rivière-aux-Feuilles* for herd and *Homozygote* for functional heterozygosity. Functional heterozygosity model: Survival ~ age + age^2 + sex + herd + functional heterozygosity + (1|year) + (1|identity).

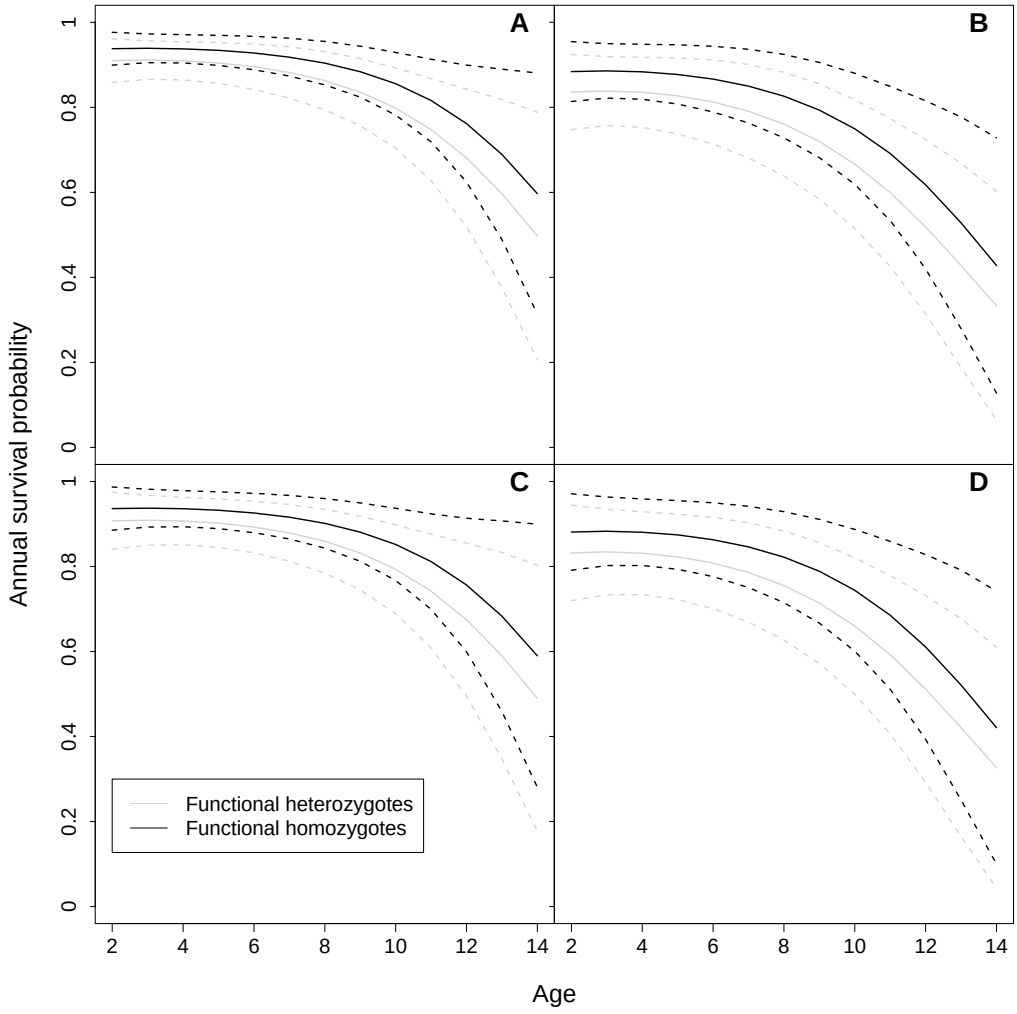

Figure 3.2 Annual survival probability of females (A-B) and males (C-D) from Rivière-George (A-C) and Rivière-aux-Feuilles (B-D) herds according to their age and degree of functional heterozygosity. In the best model ($\omega_i = 0.37$), functional heterozygosity (estimate ± se: -0.40 ± 0.20) and herd (estimate ± se: -0.68 ± 0.23) had significant effects, age and age^2 had marginally significant effects and sex did not have a significant effect.

3.10 Supplementary material

Table 3.S1 Alignment of antigen-binding sites (ABS) identified by Reche and Reinherz (2003) in human HLA-DRβ and positively selected sites (PSS) in caribou MHC-DRB. ABS and PSS are marked by a *. Dark grey sites are sites identified in human HLA-DRβ and caribou MHC-DRB. Light grey sites are sites identified in caribou MHC-DRB shifted by one position compared to human HLA-DRβ ABS. Amino acid positions are numbered in reference to the fragment we amplified in caribou.

Amino acid position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
HLA-DRβ ABS	*		*		*													*		*		*						
MHC-DRB PSS			*		*									*	*					*		*		*		*		

Amino acid position	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
HLA-DRβ ABS	*	*									*									*	*				*			
MHC-DRB PSS	*	*																		*	*			*				*

Amino acid position	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82
HLA-DRβ ABS			*				*			*				*				*		*	*	*			*	*
MHC-DRB PSS		*				*	*			*				*			*					*	*			*

Table 3.S2 Description of the MHC-DRB alleles detected in our study and allelic frequencies of those alleles in Rivière-George and Rivière-aux-Feuilles herds (1996-2016). Alleles differing from published alleles from 1-2 bp were considered variants (v) of those alleles and those that differed by 3-4 bp were considered subtypes (s). New alleles differing by >4 bp from published alleles were considered different types and were given a name following the nomenclature used by Taylor et al. (2012) and using a new number (ex: 1201, 1301, 1401). It should be noted that our alleles had slightly different length (250 bp) than previously published alleles (234 - 252 bp).

Allele number	Corresponding supertype	GenBank accession number	Correspondence with previously identified allele		Frequency	
			Name	Accession number	Rivière-George	Rivière-aux-Feuilles
1	2	MH171262	DRB1*1301	New	0.126	0.114
2	2	MH171263	DRB1*1201	New	0.198	0.183
3	1	MH171264	DRB1*0201	AF012719.1	0.055	0.058
4	1	MH171265	-	HQ245651.1 (v1)	0.171	0.181
5	2	MH171266	DRB1*0301	AF012720.1	0.072	0.051
6	1	MH171267	-	HQ245649.1 (v1)	0.051	0.048
7	3	MH171268	DRB1*0105	New	0.051	0.065
8	3	MH171269	DRB1*0102	AF012717.1	0.082	0.087
9	1	MH171270	-	HQ245651.1(v2)	0.075	0.048
10	3	MH171271	DRB1*0601	AF012723.1	0.027	0.022
11	3	MH171272	DRB1*0103	AF012718.1	0.044	0.051
12	3	MH171273	DRB1*0101	AF012716.1	0.000	0.007
13	3	MH171274	DRB1*0102 (v1)	AF012717.1 (v1)	0.010	0.000
14	2	MH171275	DRB1*1401	New	0.024	0.044
15	3	MH171276	-	HQ245647.1 (s)	0.003	0.012
16	2	MH171277	DRB1*0701 (v1)	AF012724.1 (v1)	0.003	0.000
17	3	MH171278	-	HQ245646.1	0.000	0.022
18	2	MH171279	DRB1*AK12	AF458950.1	0.007	0.003
19	1	MH171280	-	HQ245649.1 (s)	0.000	0.002
20	1	MH171281	DRB1*0202	KF294883.1	0.000	0.002

Table 3.S3 Model selection for body mass in migratory caribou from the Ungava peninsula using different indices of MHC diversity. Genetic diversity models are derived from the baseline model and include diversity indices in addition to the non-genetic variables (K parameters in total). In bold are the selected models for each index. Baseline model: log(mass) ~ age + √age + sex + herd + month of capture + (1|identity).

		Heterozygosity			Nucleotide Diversity			Amino Acid Diversity			Functional Heterozygosity		
Model	K	AICc	ΔAICc	ω_i	AICc	ΔAICc	ω_i	AICc	ΔAICc	ω_i	AICc	ΔAICc	ω_i
Baseline	12	**-408.0**	**0.0**	**0.58**	**-408.0**	**0.0**	**0.46**	**-408.0**	**0.0**	**0.45**	**-408.0**	**0.0**	**0.48**
Genetic diversity	13	-405.9	2.0	0.21	-407.1	0.9	0.30	-407.2	0.8	0.30	-406.9	1.1	0.28
Genetic diversity x age	14	-405.1	2.9	0.14	-404.9	3.1	0.10	-405.0	3.0	0.10	-404.7	3.3	0.09
Genetic diversity x sex	14	-403.8	4.2	0.07	-405.6	2.4	0.14	-405.8	2.2	0.15	-405.6	2.4	0.14

Table 3.S4 Model selection for body mass in migratory caribou from the Ungava peninsula using MHC functional genotypes. Functional genotype models are derived from the baseline model and include the functional genotype variable (representing all possible combinations of supertypes) in addition to the non-genetic variables (K parameters in total). In bold is the selected model. Baseline model: log(mass) ~ age + √age + sex + herd + month of capture + (1|identity).

Model	K	AICc	ΔAICc	ω_i
Functional genotype	**17**	**-408.6**	**0.0**	**0.56**
Baseline	12	-408.0	0.6	0.42
Functional genotype x sex	22	-401.8	6.7	0.02
Functional genotype x age	22	-399.5	9.1	0.01

Table 3.S5 Model selection for annual adult (>2 years) survival of migratory caribou from the Ungava peninsula using different indices of MHC diversity. Genetic diversity models are derived from the baseline model and include diversity indices in addition to the non-genetic variables (K parameters in total). In bold are the selected models for each index. Baseline model: survival ~ age + age^2 + herd + sex + (1|year) + (1|identity).

		Heterozygosity			Nucleotide Diversity			Amino Acid Diversity			Functional Heterozygosity		
Model	K	AICc	ΔAICc	ω_i	AICc	ΔAICc	ω_i	AICc	ΔAICc	ω_i	AICc	ΔAICc	ω_i
Baseline	**7**	**723.4**	**0.0**	**0.59**	**723.4**	**0.0**	**0.54**	**723.4**	**0.0**	**0.58**	723.4	2.0	0.14
Genetic diversity	8	725.3	2.0	0.22	725.2	1.9	0.21	725.4	2.0	0.21	**721.4**	**0.0**	**0.37**
Genetic diversity x age	9	726.7	3.4	0.11	725.8	2.5	0.16	726.3	2.9	0.13	721.5	0.2	0.34
Genetic diversity x sex	9	727.3	3.9	0.08	727.1	3.7	0.08	727.4	4.0	0.08	723.2	1.8	0.15

Table 3.S6 Model selection for annual adult (>2 years) survival in migratory caribou from the Ungava peninsula using MHC functional genotype. Functional genotypes models are derived from the baseline model and include the functional genotype variable (representing all possible combinations of supertypes) in addition to the non-genetic variables (K parameters in total). In bold is the selected model. Baseline model: survival ~ age + age^2 + herd + sex + (1|year) + (1|identity).

Model	K	AICc	ΔAICc	ω_i
Baseline	**7**	**723.4**	**0.0**	**0.85**
Genotype	12	727.2	3.8	0.13
Genotype x age	17	730.2	6.8	0.03
Genotype x sex	17	736.0	12.7	0.00

Table 3.S7 Levels of heterozygosity and functional heterozygosity, and departure from Hardy-Weinberg Equilibrium in cohorts of caribou from the Rivière-George and Rivière-aux-Feuilles herds. Each cohort is composed of N (≥ 5) individuals born the same year. H_o and H_e express observed and expected heterozygosity, respectively. The p-values are corrected with the Benjamini & Yekutieli (2001) correction and are associated to the F_{is} estimates.

			Heterozygosity				Functional heterozygosity			
Herd	Cohort	N	H_o	H_e	Fis	Adjusted p-value	H_o	H_e	Fis	Adjusted p-value
RAF	2002	6	0.67	0.87	0.23	1.00	0.33	0.70	0.52	1.00
	2003	8	0.88	0.92	0.05	1.00	0.63	0.71	0.13	1.00
	2004	9	0.67	0.90	0.26	1.00	0.33	0.71	0.53	0.97
	2005	11	1.00	0.90	-0.11	1.00	0.91	0.65	-0.41	1.00
	2006	16	0.81	0.85	0.04	1.00	0.50	0.64	0.22	1.00
	2007	39	0.87	0.89	0.02	1.00	0.74	0.64	-0.17	1.00
	2008	29	0.90	0.91	0.01	1.00	0.69	0.67	-0.03	1.00
	2009	32	0.97	0.91	-0.07	1.00	0.84	0.67	-0.26	0.97
	2010	17	0.94	0.90	-0.05	1.00	0.71	0.66	-0.06	1.00
	2011	22	0.82	0.89	0.08	1.00	0.68	0.68	-0.01	1.00
	2012	31	0.80	0.90	0.11	0.99	0.60	0.66	0.10	1.00
	2013	7	1.00	0.89	-0.12	1.00	0.71	0.52	-0.36	1.00
RG	1999	5	1.00	0.85	-0.18	1.00	0.40	0.53	0.24	1.00
	2000	5	1.00	0.88	-0.14	1.00	0.40	0.75	0.47	1.00
	2003	6	1.00	0.87	-0.15	1.00	0.67	0.53	-0.25	1.00
	2004	9	0.78	0.90	0.14	1.00	0.78	0.57	-0.37	1.00
	2005	5	0.80	0.65	-0.23	1.00	0.60	0.50	-0.20	1.00
	2006	12	0.83	0.93	0.10	1.00	0.75	0.68	-0.11	1.00
	2007	30	0.70	0.86	0.19	0.99	0.40	0.62	0.36	0.77
	2008	28	0.93	0.92	-0.01	1.00	0.68	0.67	-0.01	1.00
	2009	23	0.91	0.88	-0.03	1.00	0.61	0.65	0.07	1.00
	2012	7	0.86	0.93	0.08	1.00	0.57	0.62	0.08	1.00
	2013	8	1.00	0.95	-0.06	1.00	0.50	0.71	0.30	1.00

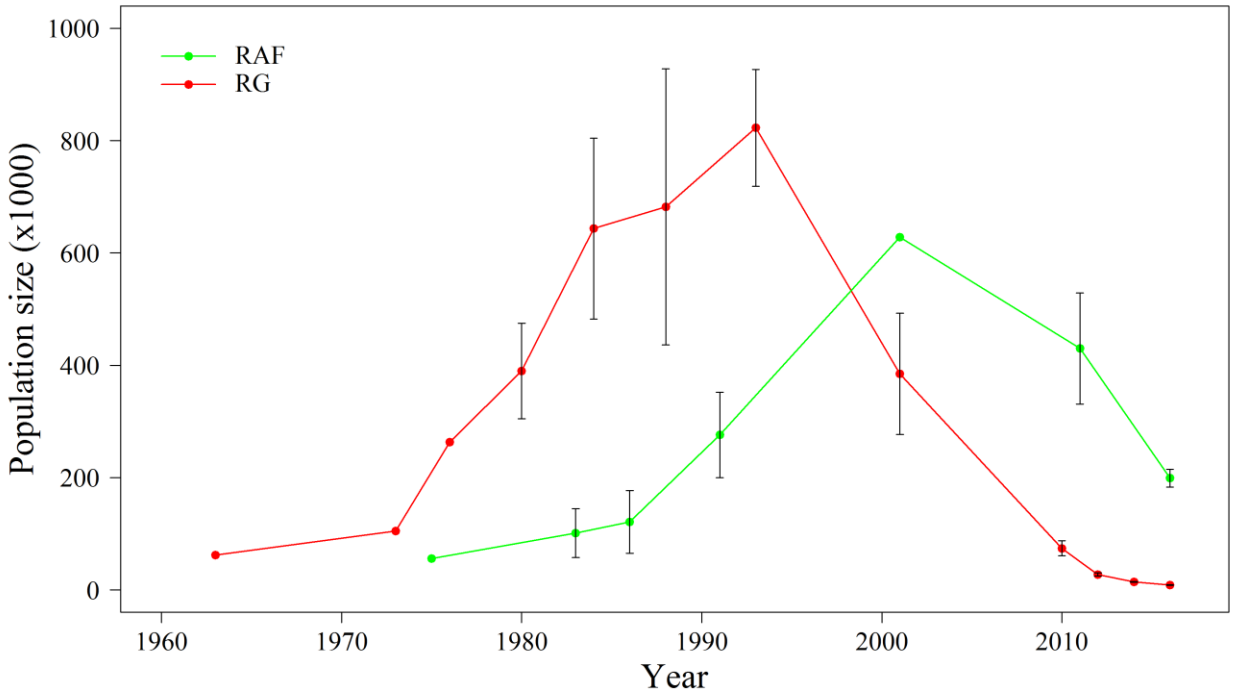

Figure 3.S1 Population sizes of Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds. Population sizes were estimated based on aerial surveys (MFFP). Their confidence intervals were computed using a number of parameters recorded during surveys and are inherent to the method.

4. Conclusion

Le déclin rapide des deux troupeaux de caribous migrateurs au Québec et au Labrador entre les années 1990 et aujourd'hui a fait émerger des questions sur les causes potentielles de ces déclins (Côté et al. 2012). Auparavant, peu d'attention avait été portée aux facteurs génétiques individuels potentiellement impliqués dans ces déclins. Mon projet visait donc à étudier les effets de la diversité génétique individuelle sur la performance des caribous. Plus précisément, nous avons contrasté les effets de la diversité génétique globale (effets généraux) des individus et ceux de leur diversité à l'échelle d'un locus fonctionnel impliqué dans l'immunité (effets directs).

4.1 Associations entre la diversité génétique et de la performance individuelle du caribou migrateur

Nos résultats suggèrent que jusqu'à présent, le déclin des troupeaux RG et RAF n'aurait pas entraîné de perte de diversité génétique globale à l'échelle individuelle. De plus, nos résultats supportent l'idée que les troupeaux ne souffriraient pas de dépression de consanguinité ou du moins, que cette dernière ne se traduirait pas par des effets sur la masse corporelle ou la survie annuelle des individus, tel que démontré par nos analyses. La variance du degré d'hétérozygotie observée dans notre jeu de données était non significativement différente de 0 et suggère qu'il était peut-être impossible de détecter une HFC dans notre échantillon étant donné la faible variance. Toutefois, nous avons tenté de créer un échantillon le plus représentatif possible des populations étudiées, en incluant des individus des deux sexes, capturés à différents moments de leur vie et pendant différentes années. Nos analyses suggèrent que notre échantillon n'était pas biaisé par l'absence de faons dans nos modèles puisqu'une comparaison de ceux-ci avec des individus d'un an nés la même année n'a pas montré de différence significative dans leur degré d'hétérozygotie. En somme, bien que nous ne puissions pas complètement exclure l'hypothèse que notre échantillon était biaisé et n'incluait pas d'individus consanguins présents dans les populations, nous avons confiance que la faible variance observée dans notre échantillon témoigne plutôt d'une absence d'individus consanguins dans les troupeaux RG et RAF. Par ailleurs, les valeurs d'hétérozygotie observées chez les caribous de notre échantillon sont plutôt élevées et sont comparables à des valeurs observées dans une population de mouflon canadien (*Ovis canadensis*) après une rescousse génétique réussie (Hogg et al. 2006, Miller et al. 2014). L'idée que les troupeaux ne seraient pas affectés par la dépression de consanguinité a du sens quand on

considère la taille des populations qui s'élève encore à plusieurs milliers d'individus (MFFP, non publié). À moins qu'il n'y ait eu de la sous-structure dans les populations, ce qui n'est pas supporté par nos analyses, le contraire aurait été surprenant. Toutefois, même si le déclin rapide des populations ne semble pas avoir causé une augmentation la consanguinité dans les troupeaux RAF et RG, il est possible que la dérive génétique ait augmenté et causé la perte de variants génétiques à l'échelle des populations (Taylor et al. 2012). Nous avons d'ailleurs détecté une faible différentiation génétique entre les deux troupeaux qui étaient considérés auparavant comme une seule métapopulation, au point de vue génétique (Boulet et al. 2007, Yannic et al. 2016). Nous pensons que l'utilisation des SNPs au lieu de marqueurs microsatellites peut avoir facilité la détection de cette structure génétique (Liu et al. 2005, Gärke et al. 2011, Fischer et al. 2017), mais il est aussi possible qu'une augmentation de la dérive génétique ait contribué à la différentiation entre les deux troupeaux (Lovatt and Hoelzel 2014). De plus, il a été observé que le déclin démographique des troupeaux RG et RAF s'est accompagné d'une réduction du chevauchement de leurs aires de rut dans les dernières décennies (Le Corre 2016), ce qui a probablement réduit le flux de gène entre les deux troupeaux.

Les résultats associés au deuxième objectif de mon projet suggèrent qu'entre 1996 et 2016, les fréquences alléliques et *supertypiques* au locus CMH-DRB seraient restées stables. Néanmoins, nous avons aussi montré que l'hétérozygotie fonctionnelle au locus CMH-DRB aurait un fort effet négatif sur la survie annuelle des adultes (~7 %). Nous sommes parmi les premiers à détecter une corrélation négative entre des traits de performance et la diversité du CMH. En outre, les hypothèses habituellement évoquées pour expliquer de telles corrélations n'ont que très peu d'appui dans le contexte de notre étude. En ceci, notre étude apporte de nouvelles questions quant aux pressions de sélection appliquées sur le CMH dans des populations comme celles des troupeaux RG et RAF. Nous suggérons plusieurs pistes de réflexion pour expliquer ces résultats, sans pouvoir pour le moment identifier les mécanismes sous-jacents. D'abord, nos analyses concernent exclusivement la survie et la masse corporelle comme indicateurs de la performance individuelle. Ces indicateurs sont souvent étudiés dans le cadre des HFCs (Chapman et al. 2009). Pourtant, la valeur adaptative ou la performance des individus dépend aussi en grande partie de leur reproduction, c'est-à-dire la capacité des individus à transmettre leurs allèles aux générations futures. Nous n'avons pas étudié ce paramètre ici. Il est possible que comme chez d'autres espèces (Lo and Shaner 2015), il existe un compromis entre la survie et la reproduction modulé par l'immunité chez le caribou. Ce compromis

laisserait supposer, en étudiant seulement la survie, que les hétérozygotes fonctionnels sont désavantagés même si leur reproduction pourrait être meilleure.

Chez l'humain, les haplotypes (combinaisons d'allèles à différents loci du CMH) les plus fréquents semblent être sélectionnés au profit des plus rares (Alter et al. 2017). Similairement, une sélection purificatrice contre les allèles rares chez le caribou pourrait générer une sélection apparente contre les hétérozygotes si les allèles rares sont toujours présents dans cet état. Ce genre de sélection pourrait agir par exemple dans un environnement stable où les allèles optimaux sont déjà présents dans la population. Dans ce contexte, de nouveaux variants n'apporteraient pas d'avantage supplémentaire et auraient donc plus de chances d'être délétères. Bien que cette hypothèse soit attrayante, il ne faut pas perdre de vue que dans notre étude, ce sont les individus hétérozygotes *fonctionnels* qui sont désavantagés et non pas les hétérozygotes au sens classique. Or, c'est l'hétérozygotie au sens classique qui devrait le mieux discriminer les individus porteurs d'allèles rares de ceux qui n'en portent pas. En effet, un homozygote fonctionnel pourrait quand même être hétérozygote (au sens classique) pour un allèle rare. De plus, les trois groupes fonctionnels ont une fréquence à peu près égale dans toutes les cohortes et aucun ne semble contenir une proportion plus élevée d'allèles rares (< 5 % ; donnée non présentée). Pour cette raison, nos résultats ne sont pas tout à fait compatibles avec cette hypothèse.

Une récente étude émet une nouvelle hypothèse pour expliquer une association négative entre la diversité du CMH et la performance (Acevedo-Whitehouse et al. 2018). Il est suggéré ici que les individus avec une diversité plus grande au CMH peuvent souffrir davantage des effets immunopathologiques des réponses immunitaires (dommages aux tissus de l'hôte par ses propres défenses immunitaires) parce que leurs réponses immunitaires sont plus intenses. Ainsi, même s'il peut être avantageux d'avoir une diversité au CMH plus élevée, car cela augmente l'immunocompétence, cela peut vraisemblablement s'accompagner de coûts plus élevés associés à l'immunité. Par ailleurs, il est possible que le caribou soit en phase d'adaptation à de nouveaux défis écologiques apportés par les changements rapides dans son écosystème, notamment en ce qui a trait aux communautés de pathogènes (Kutz et al. 2004, 2005). Il est suggéré que la diversité et l'abondance de pathogènes dans une communauté devraient contraindre l'évolution de l'investissement dans les fonctions immunitaires chez l'hôte (Moret 2003). De plus, nous savons que

chez certaines espèces, l'investissement dans les fonctions immunitaires est corrélé à la diversité du CMH (Sin et al. 2016). Par conséquent, si la balance entre les coûts et les bénéfices de l'immunité est déséquilibrée par des changements dans l'environnement, il est possible que les hétérozygotes fonctionnels souffrent davantage de ce déséquilibre s'ils investissent effectivement plus dans leurs fonctions immunitaires (Acevedo-Whitehouse et al. 2018).

Une façon de tester cette hypothèse chez le caribou serait d'abord de vérifier que l'immunocompétence des hétérozygotes fonctionnels est plus grande que celle des homozygotes fonctionnels. Par exemple, des tests d'agglutination microscopique (*en anglais : microscopic agglutination test*) permettent de quantifier la réponse immunitaire des individus et d'identifier les souches de pathogènes qu'ils peuvent reconnaître (Acevedo-Whitehouse et al. 2018). Ensuite, nous pourrions comparer le stress oxydant subi par les deux groupes en mesurant par exemple la capacité d'adaptation leucocytaire, une mesure du stress oxydant (*en anglais : leukocyte coping capacity*; Sin et al. 2016) ou l'acroléine liée aux protéines, un marqueur de dommage oxydatif aux protéines (Kurtz et al. 2006). Selon l'hypothèse avancée, les hétérozygotes fonctionnels devraient avoir une immunocompétence plus élevée, mais un degré de stress oxydant ou de dommage oxydatif également plus élevé que ceux des homozygotes fonctionnels. Des connaissances de base sur l'évolution des communautés de pathogènes pourraient aussi apporter du support à cette hypothèse si l'abondance de pathogènes avait augmenté dans les dernières décennies.

Notre étude a mis à profit l'utilisation de nouvelles technologies de séquençage pour obtenir un score d'hétérozygotie globale représentatif, tel que suggéré par Balloux (2004). Nous sommes parmi les premiers à utiliser un aussi grand nombre de marqueurs génétiques pour l'étude des HFCs (Hoffman et al. 2014, Bérénos et al. 2016, Head et al. 2017). Nous avons également élaboré une nouvelle méthode pour discriminer les effets directs des effets généraux dans nos analyses sur le CMH. Il s'agit de comparer l'association des traits de performance avec des indices de diversité *purement* génétique (hétérozygotie, diversité nucléotidique) et des indices de diversité fonctionnelle (diversité en acides aminés, hétérozygotie fonctionnelle). La diversité fonctionnelle devrait mieux refléter le phénotype d'un individu et, par conséquent, témoigner des effets directs plus fidèlement que le ferait la diversité purement génétique. Il pourrait s'agir d'un nouveau protocole permettant de discriminer les effets directs et les effets généraux au CMH sans nécessiter de marqueurs neutres. Bien que notre approche

n'ait pas été testée dans plusieurs populations, l'absence de HFC générale et de HFC au CMH avec les indices de diversité (purement) génétique (et non fonctionnelle) semble supporter notre idée que différents indices de diversité peuvent permettre de discriminer les effets généraux des effets directs.

4.2 Limites de l'étude

L'utilisation de la masse corporelle comme indice de performance individuelle est un couteau à double tranchant. D'une part, il s'agit d'un paramètre très intégrateur et il pourrait présenter des associations indirectes avec la diversité génétique. Par exemple, la diversité génétique du CMH pourrait être associée à la masse corporelle parce qu'elle influence l'intensité des infections par des pathogènes, ce qui influence à son tour la masse corporelle. D'autre part, la masse corporelle pourrait être tellement intégratrice qu'il soit impossible de détecter les effets de la diversité génétique parmi les autres effets intégrés. Par exemple, le climat (Couturier et al. 2009b) ou la densité de population (Couturier et al. 2010) pourraient avoir un effet sur la masse corporelle. De plus, notre jeu de données présente une limitation importante puisqu'il ne tient pas compte du statut reproducteur des individus. Nous savons pourtant que la masse des femelles, par exemple, est grandement influencée par la lactation, étant donné les coûts énergétiques imposés par ce processus (Bårdsen et al. 2009). Il est possible que des effets sur la masse corporelle n'aient pas été détectés puisqu'une grande partie de la variance, associée au statut reproducteur, n'était pas pris en compte dans nos analyses (65 % des femelles capturées en juin, pendant la lactation, le reste entre novembre et mai). Néanmoins, la concordance des résultats chez les femelles et les mâles (qui sont moins affectés par la reproduction ; Barboza et al. 2004) suggère qu'il n'y avait réellement pas d'association de la diversité génétique avec la masse corporelle dans notre étude.

L'utilisation d'un jeu de données récoltées sur deux décennies et dans deux troupeaux nous rend confiants en nos résultats, particulièrement en ce qui a trait à nos analyses de survie. En effet, la reproductibilité de nos résultats dans le temps et à travers les deux troupeaux suggère que nos résultats sont fiables. Pourtant l'association négative que l'on détecte entre la survie annuelle et la diversité fonctionnelle du CMH du caribou met en évidence que ces populations sont probablement très différentes de la majorité des populations préalablement étudiées. Notre étude s'oppose à de nombreuses études sur les HFCs au CMH qui ont détecté des effets positifs ou nuls de la diversité sur la performance (Oliver et al. 2009, de Assunção-Franco et al. 2012, Sepil et al. 2013, Osborne et al.

2015). Les quelques cas où des relations négatives existent s'expliquent par des mécanismes qui ne reçoivent que très peu d'appui dans notre étude. Une seule émet une hypothèse qui pourrait s'appliquer à nos populations: celle qu'une diversité plus élevée au CMH pourrait imposer les coûts immunopathologiques plus élevés (Acevedo-Whitehouse et al. 2018). Étant donné qu'on ne connaît pas le mécanisme qui génère une association négative entre la diversité fonctionnelle et la survie dans les populations de caribous à l'étude, il est difficile d'émettre des prédictions pour d'autres populations et d'autres espèces à partir de nos résultats.

Finalement, notre jeu de données n'était probablement pas optimal pour effectuer certaines analyses que nous avons effectuées *a posteriori*. Notamment, le nombre limité d'échantillons à notre disposition au début de la période couverte par cette étude peut être à l'origine de l'absence de détection de sélection fluctuante dans le temps sur le MHC. De plus, puisque les individus n'ont pas tous été capturés lors de leur première année de vie, il n'est pas impossible que la sélection ait agi avant notre échantillonnage, soit avant l'âge de 1 an. Par conséquent, les phénomènes de sélection pourraient être plus difficiles à mettre en évidence à partir d'un échantillonnage d'où sont exclus les individus contre-sélectionnés. En outre, l'échelle temporelle de notre étude, bien que grande pour une étude écologique, est peut-être encore trop réduite pour mettre en évidence des phénomènes qui se déroulent à l'échelle de temps évolutifs, considérant que le temps de génération du caribou est d'environ 4 à 6 ans (Couturier et al. 1990, Adams and Dale 1998). Ainsi, même si notre effort d'échantillonnage avait été constant et suffisant pendant toute la période de notre étude, il est possible que nous n'aurions pas été en mesure de détecter les effets de la sélection fluctuante de toute façon.

4.3 Perspectives de recherche

Nos résultats inattendus sur l'association négative entre la diversité fonctionnelle du CMH et la performance individuelle rappellent l'importance d'étudier les HFCs au CMH dans plusieurs populations, sous différentes conditions. Ils soulignent également que notre compréhension des HFCs au CMH n'est pas exhaustive et qu'il existe apparemment des mécanismes qu'on ne connaît pas encore pouvant générer des HFCs négatives. Pour généraliser nos résultats à d'autres populations, il faudrait identifier ce qui différencie les troupeaux RG et RAF des autres populations étudiées dans le cadre d'études sur la diversité du CMH. Il pourrait s'agir du comportement migrateur du caribou qui l'expose à une grande variété de conditions (Minias et al. 2017) et qui représente une contrainte

énergétique importante (Couturier et al. 2009b). Il pourrait aussi s'agir de caractéristiques de l'écosystème subarctique : des conditions climatiques contraignantes pour le développement des pathogènes et des changements rapides des conditions biotiques et abiotiques en réponse aux changements climatiques (Kutz et al. 2005). Nous soupçonnons que des modifications des communautés de pathogènes en réponse aux changements climatiques et anthropiques dans le nord du Québec et du Labrador pourraient être à l'origine de nos résultats. À cet effet, on observait déjà en 2009 des changements importants dans les interactions hôtes-pathogènes dans les écosystèmes arctiques et subarctiques de l'ouest du Canada, avec notamment l'expansion de l'aire de répartition de certains pathogènes vers le Nord (Kutz et al. 2009). Dans le futur, les changements climatiques devraient continuer de profiter aux pathogènes en relaxant les contraintes environnementales liées à leur développement (Kutz et al. 2009). Par conséquent, le besoin d'établir des connaissances de base sur les communautés de pathogènes dans les milieux nordiques se fait d'autant plus criant (Kutz et al. 2014, Dobson et al. 2015, Hoberg et al. 2017).

Il est difficile de prédire comment la diversité génétique observée chez le caribou influencera la dynamique des populations étudiées dans le futur. Les résultats de ce projet permettent quand même de tirer deux conclusions générales : (1) malgré le déclin rapide des troupeaux RG et RAF, la taille actuelle des troupeaux semble encore les protéger des risques de la dépression de consanguinité et (2) plus de la moitié des caribous échantillonnés ont un degré de diversité fonctionnelle au CMH qui réduit leur probabilité de survie d'environ 7 %. Si la première conclusion est encourageante, la deuxième se fait plus inquiétante. Dans l'avenir, il serait utile d'étudier le lien entre la prévalence des infections de pathogènes et la diversité fonctionnelle du locus CMH-DRB, de même que les coûts associés à l'immunité pour le caribou. Ce genre de connaissance nous permettrait probablement de comprendre comment la diversité du CMH est maintenue chez le caribou et de mieux anticiper la trajectoire des troupeaux RG et RAF en réponse aux changements climatiques et anthropiques auxquels ils sont confrontés.

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