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ARTICLE

Genetic association with boldness and maternal performance in a free-ranging population of grey seals (*Halichoerus grypus*)

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Abstract

Individual variation in quantitative traits clearly influence many ecological and evolutionary processes. Moderate to high heritability estimates of personality and life-history traits suggest some level of genetic control over these traits. Yet, we know very little of the underlying genetic architecture of phenotypic variation in the wild. In this study, we used a candidate gene approach to investigate the association of genetic variants with repeated measures of boldness and maternal performance traits (weaning mass and lactation duration) collected over an 11- and 28-year period, respectively, in a free-ranging population of grey seals on Sable Island National Park Reserve, Canada. We isolated and re-sequenced five genes: dopamine receptor D4 (*DRD4*), serotonin transporter (*SERT*), oxytocin receptor (*OXTR*), and melanocortin receptors 1 (*MC1R)* and 5 (*MC5R*). We discovered single nucleotide polymorphisms (SNPs) in each gene; and, after accounting for loci in linkage disequilibrium and filtering due to missing data, we were able to test for genotype-phenotype relationships at seven loci in three genes (*DRD4*, *SERT*, and *MC1R*). We tested for association between these loci and traits of 180 females having extreme shy-bold phenotypes using mixed-effects models. One locus within *SERT* was significantly associated with boldness (effect size = 0.189) and a second locus within *DRD4* with weaning mass (effect size = 0.232). Altogether, genotypes explained 6.52–13.66% of total trait variation. Our study substantiates *SERT* and *DRD4* as important determinants of behaviour, and provides unique insight into the molecular mechanisms underlying maternal performance variation in a marine predator.

Introduction

Understanding the origin and maintenance of variation in behavioural and life-history traits in the wild remains a challenging area of research (van Oers and Mueller 2010). In a recent meta-analysis on the heritability of behaviour in vertebrate and invertebrate species, Dochtermann et al. (2019) reported an average 24% heritable component for behavioural traits, comparable to an average 26% for life-history traits (Mousseau and Roff 1987). These heritability estimates suggest an appreciable genetic component underlying phenotypic variation. While estimating heritability is an important starting place in investigating the genetic basis of complex traits (Boake et al. 2002), quantitative genetic approaches cannot determine the genetic architecture (i.e., specific genes or pathways) underlying the heritable component of the variation we observe. Fortunately, advances in molecular genetic methodologies and analytical techniques now facilitate the exploration of genotype-phenotype relationships in natural systems of

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non-model organisms (Laine and van Oers 2017; Bengston et al. 2018). The goal of this study is to apply these methodologies to study the genetic basis of personality and life-history trait variation in a wild population.

The study of animal personality is a rapidly growing area of research, as evidenced by a large increase in the number of studies published in recent decades (Carere and Maestripieri 2013). Personality, defined as consistent individual differences in behavioural responses across time and contexts (Gosling 2001), influences ecological processes and is subject to selective pressures (Réale et al. 2007; van Oers 2008). With implications for wildlife conservation, management, and animal welfare, personality research plays a key role in our understanding of individual differences in other behavioural categories (e.g., dispersal, reproduction, parental care), as well as evolutionary changes in populations resulting from these differences (McDougall et al. 2006). For instance, individuals of a particular personality type may exhibit more or less care of offspring, potentially affecting reproductive success and productivity. Researchers are now attempting to address fundamental evolutionary questions by examining the proximate mechanisms underlying personality trait (i.e., a quantifiable and specific repeatable characteristic of an individual's behavioural repertoire) variation and the ultimate mechanisms responsible for maintaining variation within and among populations or species (Laine and van Oers 2017; Bubac et al. 2020).

Much research has been undertaken on five major axes of personality: activity, aggression, boldness, exploration, and sociability (Gosling 2001; Réale et al. 2007). These personality traits are often highly correlated forming behavioural syndromes, wherein some individuals are generally more bold, active, and aggressive than their counterparts (Sih et al. 2004). Boldness, widely regarded as an individual's propensity to take risks or an individual's response to a potentially risky situation (Sloan Wilson et al. 1994; Réale et al. 2007), including interactions with hetero-specifics, has been documented in taxonomically diverse groups such as cephalopod mollusks (Sinn et al. 2008), songbirds (Timm et al. 2018), and ungulates (Réale et al. 2000). Phenotypes along the shy-bold continuum have been associated with variation in survivorship, reproductive success, and dispersal—life-history characteristics with important ecological and evolutionary consequences. For example, in bighorn sheep (*Ovis canadensis*), among-individual variation in boldness was related to female reproductive investment and life-history traits, with bold ewes younger at primiparity and exhibiting higher offspring weaning success than shy females (Réale et al. 2000). Being bold and taking more risks may prove beneficial and adaptive, yet the optimal degree of boldness is ultimately dependent upon the context and/or situation, likely contributing to the maintenance of behavioural variation within populations.

One approach to discovering sources of variation in behavioural and life-history traits is a candidate gene study, wherein a gene of known function is screened for genetic variants to test for association with traits of interest. A favourable option to investigate the genetic basis of complex traits in organisms with limited genomic resources (Fitzpatrick et al. 2005), a candidate gene approach permits tracking allele frequencies of trait-associated genes within and among populations, providing insight into the adaptive selective processes operating on these traits. Currently, a few candidate genes have been associated with the shy-bold continuum in songbirds, primates, and rodents (Laine and van Oers 2017). The dopamine receptor region D4 (*DRD4*) gene has been extensively studied in its association with various personality traits (Savitz and Ramesar 2004). Identified by its relationship with neurological and psychiatric disorders in humans and laboratory organisms (Mitsuyasu et al. 2001; Kluger et al. 2002), *DRD4* has subsequently been associated with similar behavioural effects in domesticated and wild animals. In free-ranging populations of avian and non-human primate species, *DRD4* has been linked with interindividual variation in boldness, novelty seeking, and impulsivity (e.g., Fairbanks et al. 2012 and Riyahi et al. 2015).

Another gene widely studied in relation to personality trait variation is the serotonin transporter gene (*SERT*), a protein-coding gene involved in the uptake of serotonin (Savitz and Ramesar 2004). *SERT* has been associated with various behaviours, including anxiety, harm avoidance, aggression, and risky behaviour in humans and animals, alike (Lesch et al. 1996; Kim et al. 2006). For instance, Holtmann et al. (2016) found that wild female dunnocks (*Prunella modularis*) heterozygous at a *SERT* locus engaged in more risky behaviour when approached by researchers than their homozygous counterparts. Still other genes, many with pleiotropic effects, have been identified in explaining a proportion of personality trait variance, such as the oxytocin receptor (*OXTR*) gene and genes of the melanocortin system (Sala et al. 2013; Jacobs et al. 2016). Despite these promising gene-behaviour findings, investigations into the underlying genetic basis of personality in natural systems remains in its infancy (van Oers and Mueller 2010; Laine and van Oers 2017). Further, behavioural-related candidate gene studies focused on wild mammals have been limited in taxonomic breadth to predominantly non-human primates and rodents (Bubac et al. 2020).

Notwithstanding an increased number of personality studies, marine mammal personality research is currently underrepresented. Recent studies on free-ranging pinnipeds highlight the potential role that personality trait variation plays in shaping population dynamics of marine mammals, including detection of relationships between behavioural variation and fitness-related traits and coping strategies within changing and challenging environments (Twiss et al. 2020). Grey seals

(*Halichoerus grypus*) provide an excellent system to explore the association between genetics and behaviour, as they exhibit repeatable behavioural variation in the wild (e.g., Lidgard et al. 2012; Twiss et al. 2012; Bubac et al. 2018). The grey seal is a philopatric, colonial-breeding species where individuals haul out on land or ice annually to give birth and to mate (Mansfield and Beck 1977). With long reproductive lifespans (upwards of 35 years; Bowen et al. 2006), females give birth to a single pup on a nearly annual basis beginning at the age of 4–6 years and provide all parental care during a brief lactation period lasting approximately 16–18 days (Bowen et al. 1992). While grey seals have few natural land-based predators (e.g., canids and predatory/scavenging birds), pups can sustain life-threatening injuries from non-maternal females in aggressive acts and from males in mating attempts, acts of aggression, and during male-male battles for access to mates (Boness et al. 1982; Baker 1984; van Neer et al. 2019). Therefore, by showing an increased level of aggression towards conspecifics in defence of her pup (Boness et al. 1982), a female may improve the survival probability of her offspring in densely populated colonies (McCann 1982). When confronted with a novel object or exposed to a potentially risky situation during the lactation/parental care period, grey seal females respond consistently different from others in frequency of pup-checking rates and protection of offspring from a perceived threat (Twiss et al. 2012 and Bubac et al. 2018, respectively). High repeatability estimates, serving to set the upper bounds to heritability (but see Dohm 2002), of inter-individual behavioural responses provide evidence of personality signals in local grey seal colonies (Twiss et al. 2012; Bubac et al. 2018), and further indicate that these traits may have a genetic basis.

Patterns have emerged linking personality types with reproductive success in grey seals. Bubac et al. (2018) showed that bold females weaned pups that were on average heavier than those of shy moms, providing the pup with a slight early life advantage. In a separate breeding colony of grey seals, Twiss et al. (2012) found that reactive (e.g., behaviourally flexible and generally more docile) females weaned pups with more varied growth rates than proactive individuals. The reasons for these observations remain uncertain. Easily disturbed females may frequently interrupt essential suckling bouts, periods wherein a female must transfer enough milk energy to her pup before weaning occurs (Iverson et al. 1993), and/or exhibit shortened lactation durations. Still yet, a female's genetic makeup may influence reproductive success by directly or indirectly affecting her ability to deliver milk (e.g., daily milk output and milk composition) (Lang et al. 2009). Genes, such as *SERT* and *OXTR*, that have been linked with behavioural variation are also suspected to be associated with parental care phenotypes including offspring responsiveness (Bakermans-Kranenburg and van IJzendoorn 2008) and other reproductive parameters (Timm et al. 2018).

In this study, we used a candidate gene approach to explore the relationships between genetic variants and boldness and maternal performance under natural conditions in grey seals. Using a longitudinal database (11 and 28 years for boldness and maternal performance, respectively) and archived tissue samples from the Sable Island National Park Reserve breeding colony of grey seals, our primary objectives were to: a) determine whether seals in our study population show DNA sequence polymorphisms in the *DRD4*, *SERT*, *OXTR*, and melanocortin receptors 1 (*MC1R)* and 5 (*MC5R*) genes, genes previously related with behavioural variation; b) test the hypothesis that individual differences in boldness and life-history traits (pup weaning mass (PWM) and lactation duration) are affected by genetic polymorphisms in the aforementioned genes; and, c) extend our understanding of the relative importance of these candidate genes to phenotypic variation in a wider range of natural systems.

Materials and methods

Study site

Our field study was performed on Sable Island National Park Reserve (hereafter Sable Island), located 300 km east of Halifax, Nova Scotia in Canada (43°55′49″N, 60°00′67″ W) (Fig. 1). The island supports the world's largest breeding colony of grey seals (~370,000 individuals and an estimated 82,000 pups born annually) (Bowen et al. 2007; Hammill et al. 2017). The Sable Island population of grey seals has been the focus of long-term research dating back to the 1960s, with individual-based records obtained for a subset of the population. As weaned pups, individuals were selected randomly from the colony and permanently marked with a unique-character hot-iron brand applied to the lower back. Study animals are therefore of known age. The branding program occurs for two to three consecutive years about every ten years. Beginning annually in 1983, the entire island has been systematically searched for branded individuals. Each census is performed weekly from early December to late January, a period when females haul out to give birth and to mate. Subjects of our study were adult females born in the years 1974, 1985–1987, 1989, and 1998–2002 (Supplementary Table S1). Tissue samples were collected from a hind-flipper at the time of branding (i.e., as pups) for genetic analysis. For 33 females, tissue samples collected from the individual as a pup were lost, and thus required re-sampling as adults using a biopsy pole to obtain a skin scraping from the female's shoulder or hip area. All capture and handling techniques were in compliance with the Canadian Council on Animal Care and approved by Canada's Department of Fisheries and Oceans (DFO) and the University of Alberta's Animal Care Committees.

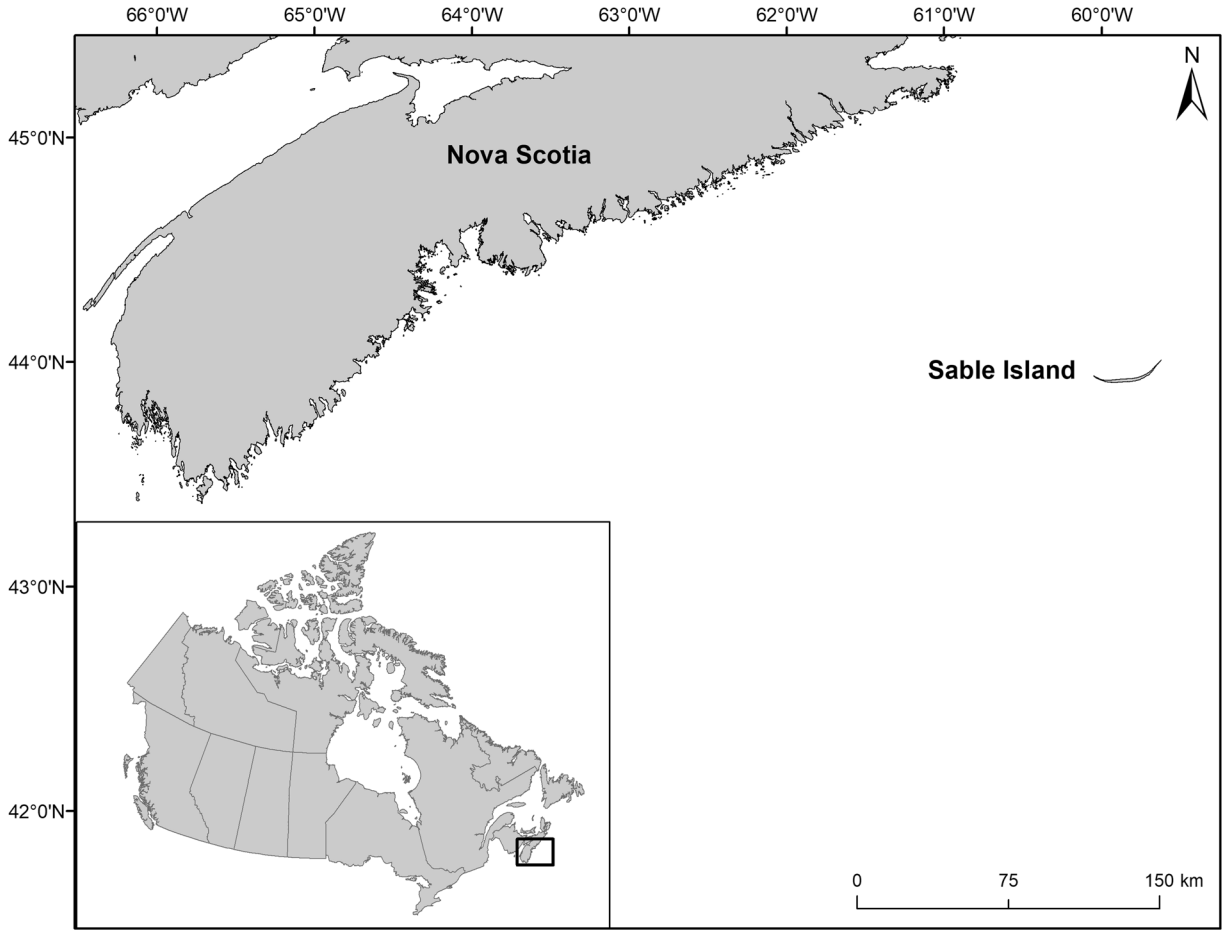

Fig. 1 Map showing the location of Sable Island National Park Reserve of Nova Scotia, Canada. Female grey seals of Sable Island have been monitored annually since the 1980s and behaviourally assayed from 2008 to 2018.

The research was performed under permits issued by DFO Canada.

Boldness measurements

Boldness data were collected in consecutive breeding seasons during 2008–2018. Here, boldness was determined according to a female's response to a potentially risky situation, specifically in defense of her offspring (see Bubac et al. 2018). Females were assigned a boldness score from one to six following the advancement of three researchers toward the focal female and handling of her pup. A score of one represented shy individuals and of six very bold individuals (Table 1). Interactions lasted approximately one minute, giving researchers enough time to sex the pup and apply a uniquely numbered tag to the webbing of the pup's hind-flipper for later identification. Each female was tested on day 3 postpartum or later to permit adequate bonding between mother and offspring. Boldness scores were determined for 525 females over the 11-year period. Repeatability of boldness was estimated for this dataset with the R package 'MCMCglmm' (Hadfield 2010) (see Bubac et al. 2018 for details). With evidence that boldness is highly repeatable in this population ($R = 0.581$ [CI: 0.543–0.624]; $N = 460$ females with two or more boldness scores), and for the purposes of the current study, we selected a subset of females with the criteria of having extreme shy-bold phenotypes along the normal distribution of boldness values, as determined by a female's scores averaged across years (Table 1). Extremes of the shy-bold continuum were phenotypes falling in the shyest (average boldness scores of ≤2.5) and boldest (average boldness scores of ≥4) ranges, yielding 188 total individuals for which genetic material was available. To confirm this subset was appropriate, we performed analyses on residuals from a GLM correcting for sources of variation on boldness using the entire dataset of females (see Supplementary Fig. S1). Upon selection of the shyest and boldest females, we considered repeated measures collected from the 2008 to 2018 sampling period.

Table 1 Boldness scores with descriptions of behaviour, and the associated number of observations collected for each score as well as the number of Sable Island grey seal females.

Score	Behaviour	Observations
1	Shy; flees, quickly moves >2 m away from researchers and pup	101
2	Shy; moves away <2 m away from researchers and pup	377
3	Intermediate; stays nearby and shows no boldness	178
4	Mild boldness; vocalizes and makes abrupt movements towards researchers	223
5	Moderate boldness; vocalizes, lunges towards researchers, displays open mouth threat	196
6	Extreme boldness; vocalizes, displays an open-mouth threat, lunges and attempts to bite	108

Of the 180 females with repeated measures obtained from 2008 to 2018, the average score for shy individuals was 1.789 (SE ± 0.187; $n = 95$ females with 478 observations), while that of the bold individuals was 4.782 (SE ± 0.0332; $n = 85$ females with 527 observations).

We additionally recorded a female's birth-site habitat selection, characterized by the microhabitat type where a female and her pup resided. Sable Island has various microhabitat features and the density of individuals is not uniformly distributed over the island, wherein sandy shoreline and inland areas are more densely populated than vegetated dunes. We recorded birth-site habitat selection to account for differences that microhabitat feature, here a proxy of density, might have on boldness scores (Supplementary Table S2).

Maternal performance traits

For the 188 females behaviourally assayed, records of maternal performance traits (PWM and lactation duration) were available for up to 28 years (1991–2018). After locating branded females in the breeding colony during the weekly island-wide censuses as described above, study females were monitored daily to assess her reproductive status. A female's parturition date could be estimated from her pup's developmental stage, such that newborn pups (i.e., 'stage one' lasting approximately 1–2 days after birth) are characterized by having loose skin folds and yellowish pelage that is still wet from birth fluids (Bowen et al. 2003). We could not determine the parturition date for females first located with pups beyond stage one. Female-pup pairs were monitored daily, and from a distance following the behavioural assay/tagging of the pup to reduce disturbance to the pair. Weaning and cessation of parental care are abrupt in the grey seal, with females abandoning her pup to return to the ocean. Daily monitoring, therefore, permitted identification of the weaning date, giving an estimate of lactation duration for females in which parturition date was known. Upon weaning, researchers identified the pup by its hind-flipper tag to confirm a focal female's pup. At this time, the pup was weighed to the nearest 0.5 kg and its sex confirmed. Phenotypic values for each trait (boldness, PWM, and lactation duration) were plotted and visually examined to ensure normality.

Candidate gene sequencing

Total genomic DNA was extracted from tissue using Qiagen DNeasy Blood and Tissue Kits according to the manufacturer's protocol (Qiagen, Valencia, California, USA). As no sequence information for the candidate genes of interest existed for grey seals, we designed several *de novo* forward/reverse primer pairs spanning each gene (i.e., *DRD4*, *SERT*, *OXTR*, *MC1R*, and *MC5R*) using homologous sequences from a closely related species, the Weddell seal (*Leptonychotes weddellii*) (*DRD4*: GenBank: XM_006741797.1; *SERT*: GenBank: XM_006734532.1; *OXTR*: GenBank: XM_006736571.1; *MC1R*: GenBank: XM_006746161.1; and, *MC5R*: GenBank: XM_006744833.1) in Geneious v. 11.1.5 (http://www.geneious.com, Kearse et al. 2012) (Supplementary Table S3). These primer sets were used to sequence both intron and exon gene structures of each candidate gene in grey seals (Fig. 2).

To test the efficacy of primers and search for putative polymorphisms, we initially sequenced 24 individuals (12 very bold and 12 very shy females) for each gene. Fragments of each candidate gene were amplified using touchdown polymerase chain reactions (PCRs) in 20 µl reactions consisting of 10–100 ng genomic DNA, 10X PCR buffer, 25 mM $MgCl_2$, 0.2 mM forward and reverse primers, 2 mM dNTPs, and 0.05 U TopTaq DNA polymerase (Qiagen). Thermocycling parameters consisted of denaturation at 94 °C for 4 min, followed by 24 cycles of 94 °C × 1 min, 60 °C × 30 s (successively decreased by 0.5 °C with each cycle), 72 °C × 2 min, then 15 cycles of 94 °C × 1 min, 48 °C × 30 s, 72 °C × 2 min, and a final extension of 72 °C × 10 min and 4 °C soak. PCR products were visualized on a 1% agarose gel after ethidium bromide staining to ensure amplification of anticipated fragment size. Products were purified with Exonuclease and Shrimp Alkaline Phosphatase (ExoSap) enzymatic reactions (USB, Cambridge, Massachusetts, USA), and subsequently cycle-sequenced in both directions using amplification primers and ABI Big Dye Terminator (Applied Biosystems, Inc.,

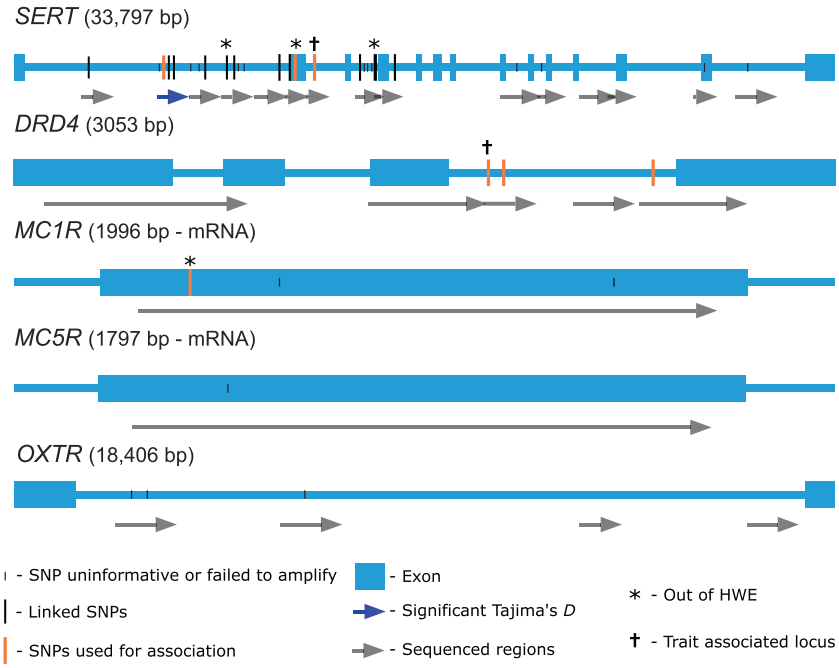

Fig. 2 Schematics of gene structure for five candidate genes. These genes [serotonin transporter (SERT), dopamine receptor D4 (DRD4), melanocortin receptors 1 and 5 (MC1R and MC5R), and oxytocin receptor (OXTR)] were used to assess the relationship between genotype and boldness and maternal performancevariation in female grey seals of Sable Island, Nova Scotia.

Foster City, California, USA). Cycle-sequencing conditions included the following: denaturation at 96 °C for 3 min, and 45 cycles of 96 °C for 10 s, 50 °C for 05 s, and 60 °C for 2 min. Reactions were purified using a standard ethanol precipitation to remove unincorporated dye terminators and electrophoresed on an ABI 3730 genetic analyzer (Applied Biosystems, Inc.). Sequences were aligned and visually examined for single nucleotide polymorphisms (SNPs) using Geneious. We used the National Center for Biotechnology Information's BLAST search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the standard nucleotide BLAST (blastn) against the entire nucleotide database on all resulting candidate gene sequences to confirm gene identity, and align SNPs within coding/non-coding regions.

Genotyping

PCR and ExoSap reactions were performed for all 188 females according to methods described above. Following gel electrophoresis and PCR purification, we used SNaPshot reactions to genotype each individual at every known SNP location as identified from the initial panel of 24 females (Applied Biosystems, Inc.). SNP primers were created using Geneious, such that primers were immediately adjacent to the identified SNP, and were designed to contain a repeating T tail of varying lengths to ensure accurate differentiation and genotyping at each locus. PCR products were pooled to include groups of sequences that had compatible SNP primers (i.e., primers with differing lengths of T tails). SNaPshot reactions consisted of 4 µl pooled PCR products, 1 µl pooled forward primers (diluted to 2 uM), and 5 µl SNaPshot Multiplex Ready Reaction Mix (Applied Biosystems, Inc.). Reactions were amplified with the following protocol: 96 °C for 3 min, and 25 cycles of 96 °C × 10 s, 50 °C × 5 s, and 60 °C × 30 s. We performed ExoSap reactions to remove unincorporated ddNTPs. Finally, 3 µl of reaction products were combined with 8 µl of Hi-Di Formamide/Liz-120 bp size standard mix, denatured at 95 °C for 2 min and electrophoresed on an ABI 3730 genetic analyzer (Applied Biosystems, Inc.). Genotypes for each individual were scored using GeneMapper 4.0 (Applied Biosystems, Inc.).

Genotype results

We used the online version of Genepop 4.7 (Raymond and Rousset 1995; Rousset 2008) to check each locus for deviations from Hardy-Weinberg equilibrium (HWE) and to test for pairwise linkage disequilibrium between variants. For each test, we raised the Markov chain default parameters to 10,000 dememorizations, 1000 batches, and 10,000 iterations. To account for multiple testing, significance levels were adjusted for false discovery rate (FDR) using the Benjamini–Hochberg method (Benjamini and Hochberg 1995) with the p.adjust function in R v3.6.1 (R Core Team 2019). As population stratification from genetic structure and relatedness may cause false positives in association tests (Laird and Lange 2011), we considered both population structure and relatedness. We completed a principal component analysis (see Supplementary Fig. S2)

to confirm our dataset was unstructured; however, we recognize our limited number (seven) of unlinked SNPs do not have sufficient marker resolution to estimate stratification. Previous studies on the genetics of Northwest Atlantic grey seals, including seals from our study population, found no evidence of population structure using microsatellites (Wood et al. 2011), mtDNA sequence data (Cammen et al. 2018a), and a large SNP panel (8700 loci; Cammen et al. 2018b). The nature of the longitudinal dataset (individual-based records for branded females) highlighted six mom-daughter pairs; therefore, mom or daughter were removed from statistical analyses. The female with less missing genotypic data across the five genes was retained for analysis.

Genetic diversity and neutrality tests

Genetic diversity indices and Tajima's D neutrality tests (Tajima 1989) were estimated using DnaSP v6.12 (Rozas et al. 2017) for the sequence data. Positive Tajima's D values are indicative of balancing selection and/or a decrease in the overall size of a population whereas negative values infer positive selection and/or population expansion. For each SNP locus, we calculated expected and observed heterozygosities using Genepop.

Association tests

Generalized linear mixed-effects models (GLMMs) were used to test for the effects of genotypes on boldness, PWM, and lactation duration. Models were run using the package 'lme4' (Bates et al. 2015) in R, with ML and Satterthwaite's t-test method implemented in the 'afex' package (Singmann et al. 2019). We used the additive model (each additional copy of the variant allele increases the response), and the overdominance model (genotype categories coded as heterozygotes versus homozygotes). For all models, genotypes were fitted as fixed factors in separate, gene-specific models using only loci that passed quality control filters (i.e., unlinked and polymorphic loci).

Boldness was scored on an ordinal scale, but effectively represents an axis of continuous values along a shy-bold continuum of behavioural variation. Therefore, boldness was fitted as a continuous response variable. We included year and female identity as random factors to account for interannual variation and repeated measures on the same individual taken through time, respectively. In addition to SNP genotype(s), we fitted maternal age as a fixed factor, as it has been shown that older grey seal females are on average bolder than younger individuals (Bubac et al. 2018). Age was grouped into biologically relevant, five-year interval bins, with the oldest females aged ≥ 26 years pooled into one group. This resulted in a 5-level factor: bin 1 = ≤ 10 years (females are young and less experienced); bin 2 = 11–15 years (females are young with more experience); bin 3 = 16–20 years (females in prime reproductive years); bin 4 = 21–25 years (females in prime reproductive years); bin 5 = ≥ 26 years (females are old with some evidence of senescence) (Bowen et al. 2006). We also fitted birth-site habitat selection as a 3-level fixed factor (Supplementary Table S2), given that microhabitat features account for boldness variation in the Sable Island population (Bubac et al. 2018).

It has been found that bold females generally wean heavier pups (Bubac et al. 2018), a relationship supported with our reduced dataset ($t = 2.999$; $P = 0.004$, $r = 0.10$; $n = 176$ females). In addition, a relationship exists between boldness and lactation duration ($t = 2.559$; $P = 0.011$, $r = 0.18$; $n = 118$ females) (Supplementary Figs. S3, 4), substantiating the investigation of gene-PWM/lactation duration associations to explore whether the same underlying mechanisms are at play. In models assessing the genetic effects on life-history traits, PWM and lactation duration were fitted as continuous response variables in individual models, with year and identity included as random factors. As maternal age is expected to affect reproductive performance (Bowen et al. 2006), we fitted age again as a 5-level fixed factor (see above). In the model including PWM as the response variable, we further fitted pup sex as a 2-level fixed factor to account for previous findings that male pups are often heavier than female pups at weaning (Bowen et al. 2006; Bubac et al. 2018).

The importance of variables was assessed based on both estimates of significance and whether the SNP confidence interval included zero. Model selection was performed using Akaike's information criterion values (Supplementary Table S4). We further estimated effect size (r) for each SNP using the equation $r = \text{sqrt}(t\text{^}2/(t\text{^}2 + df))$; however, these values should be interpreted with caution when using GLMMs (Jaeger et al. 2016). The biological significance of effect sizes were evaluated following suggestions by Cohen (1988), wherein a small effect is represented by $r = 0.1$, a medium effect by $r = 0.3$, and a large effect by $r = 0.5$ (Møller and Jennions 2002). Lastly, to estimate the proportion of trait variation explained by each gene and the other fixed factors, as well as all loci combined, we calculated marginal R^2 ($R^2_{\text{GLMM(m)}}$) according to Nakagawa and Schielzeth (2013).

Results

We obtained quality genotypic data for 185 grey seal females; however, removing individuals to account for relatedness left 180 females for association analyses. The age of females with corresponding behavioural and maternal performance records ranged from 4 to 37 years, with an

Table 2 Allele and genotype frequencies for both linked and unlinked loci detected between three candidate genes (*DRD4*, *SERT*, and *MC1R*) in 180 female grey seals of Sable Island, Nova Scotia (Canada).

Locus	Major/minor allele	Location	MAF	H_{Obs}	H_{Exp}	HW *P* value	Genotype (frequency)			Protein coding
DRD4										
*DRD4*_1363[a]	C/T	Intron 3	0.116	0.17	0.21	0.168	CC (0.8)	CT (0.17)	TT (0.032)	–
*DRD4*_1496[a]	G/A	Intron 3	0.389	0.48	0.48	1.000	GG (0.37)	AG (0.47)	AA (0.15)	–
*DRD4*_1853[a]	C/T	Intron 3	0.331	0.37	0.44	0.204	CC (0.48)	CT (0.37)	TT (0.14)	–
SERT										
*SERT*_2946	G/C	Intron 1	0.364	0.41	0.46	0.246	GG (0.43)	GC (0.41)	CC (0.16)	–
*SERT*_5987[a]	T/C	Intron 1	0.393	0.42	0.48	0.246	TT (0.4)	TC (0.42)	CC (0.18)	–
*SERT*_6056	G/T	Intron 1	0.356	0.44	0.46	0.700	GG (0.42)	GT (0.44)	TT (0.14)	–
*SERT*_6147	A/G	Intron 1	0.396	0.46	0.48	0.700	AA (0.38)	AG (0.46)	GG (0.17)	–
*SERT*_7235	A/T	Intron 1	0.261	–	–	–	AA (0.00)	AT (0.52)	TT (0.48)	–
*SERT*_7535	G/A	Intron 1	0.328	0.39	0.41	0.246	GG (0.48)	GA (0.39)	AA (0.13)	–
*SERT*_7540	A/G	Intron 1	0.210	0.19	0.33	0.000	GG (0.12)	GA (0.18)	AA (0.70)	–
*SERT*_11432	T/G	Intron 1	0.125	0.19	0.22	0.146	TT (0.78)	TG (0.18)	GG (0.03)	–
*SERT*_11673	C/G	Exon 2	0.138	0.25	0.24	0.700	CC (0.74)	CG (0.25)	GG (0.011)	Nonsynonymous
*SERT*_11689	C/T	Exon 2	0.478	0.92	0.50	0.000	CC (0.06)	CT (0.92)	TT (0.016)	Nonsynonymous
*SERT*_12472[a]	G/A	Intron 2	0.0819	0.13	0.15	0.204	GG (0.85)	AG (0.13)	AA (0.017)	–
*SERT*_15549	G/A	Intron 3	0.409	0.43	0.48	0.270	GG (0.38)	AG (0.43)	AA (0.19)	–
*SERT*_15636[a]	G/A	Intron 3	0.308	0.53	0.43	0.003	GG (0.43)	AG (0.53)	AA (0.04)	–
*SERT*_16657	C/G	Intron 4	0.286	0.41	0.41	1.000	CC (0.51)	CG (0.41)	GG (0.082)	
MC1R										
*MC1R*_126[a]	T/C	Exon 1	0.451	0.68	0.50	0.001	CC (0.11)	CT (0.68)	TT (0.21)	Synonymous
*MC1R*_740	A/T	Exon 1	0.348	–	–	–	AA (0.31)	AT (0.69)	TT (0.00)	Nonsynonymous

Only unlinked loci were included in association analyses.

[a]Unlinked loci that were included in association analyses.

average age of 14 years (Supplementary Table S1). A total of 1183 behavioural scores along the shy-bold continuum were recorded for this subset of females over an 11-year study period (Table 1). Measures of PWM and lactation duration were available from 1991–2018 (28 years) for 177 females (1496 observations) and 152 females (544 observations), respectively. Two records of lactation duration from two females were exceptionally low (1 and 4 days), resulting from storms in 2002 and 2012 that caused premature female-pup separation. As such, these outliers were removed from analyses, leaving 542 observations from 151 females.

From the 24 females initially sequenced (GenBank accession numbers MW864572-MW864597), we detected a total of 36 SNPs. We sequenced the majority of the *DRD4* gene (86% of gene sequenced) and detected three SNP variants in intron 3 (Fig. 2). A total of 26 SNPs (23 intronic and 3 exonic) were identified in *SERT* (45% sequenced), three intronic SNPs in *OXTR* (13% sequenced), three exonic SNPs in *MC1R* (61% sequenced), and one exonic SNP in the *MC5R* gene (47% sequenced). Overall, the sequenced gene regions had low nucleotide diversity ($\pi = 0$ to 0.00628, with an overall mean of $\pi = 0.001$) (Supplementary Table S5). In the analysis of Tajima's *D*, we found eight regions (*MC5R*, one segment in *DRD4*, two in *OXTR*, and four in *SERT*) to be under positive selection according to the sign of Tajima's *D*. The other ten regions were indicative of balancing selection (*MC1R*, one in *DRD4*, and eight in *SERT*), wherein one sequenced region of *SERT* was statistically significant ($P < 0.05$) (Fig. 2; Supplementary Table S5).

Out of the total 36 SNPs detected and assayed between the five genes in all 185 individuals, three variants from *DRD4*, 13 from *SERT*, and one variant from *MC1R* were retained for analysis (Table 2), as the other loci were effectively monomorphic in our study subset [10 SNPs; minor allele frequency threshold < 0.01], or failed to amplify using the SNaPshot protocol (9 SNPs). This failure rate is comparable to those reported in other studies using SNaPshot (Pati et al. 2004). Five loci deviated significantly from HWE ($P < 0.05$) (*MC1R*_126, *SERT*_11432, *SERT*_15636, *SERT*_7540, and *SERT*_11689), which reduced to four following correction for multiple testing (Table 2), indicating that some genotypes were absent or under-represented. This may be due to sequencing errors; yet, these SNPs are potentially associated with the fitness-related traits, and therefore may be under selection and

expected to deviate from HWE. An extreme excess proportion of heterozygotes at one of the deviating *SERT* loci, *SERT*_11689, was suggestive of a paralog, so was excluded from further analysis. We retained the remaining HWE deviating SNPs for analysis.

Following correction for multiple testing, 35 locus-pairs were significantly linked ($P < 0.05$), and all linked loci were within *SERT* (Supplementary Table S6), potentially indicating a hitchhiking effect. Among the *SERT* SNPs, we observed two that were not linked while the remainder represented one linkage group and therefore we retained one SNP from this group. This yielded a total of seven unlinked-SNPs between three genes (*DRD4*, *SERT*, and *MC1R*) for association analyses. Removal of linked loci in turn removed two of the loci out of HWE (*SERT*_11432 and *SERT*_7540). Genotype and allele frequencies for each SNP retained are presented in Table 2.

Association tests

We found a significant additive allele effect ($P < 0.05$), and a second locus that showed a marginally non-significant association ($P < 0.1$) between boldness and SNPs in the *SERT* gene (S*ERT*_12472 and *SERT*_15636, respectively) (Table 3; Supplementary Table S7). At locus *SERT*_12472 (intron two), shy female seals lacked the major allele (G). In the suggestive link between *SERT*_15636 (exon four) and boldness (Fig. 3; Supplementary Fig. S5), the homozygote minor allele genotype (AA) was associated with the bold phenotype. The estimated effect size of *SERT*_12472 was 0.189 and of *SERT*_15636 was 0.137, suggesting moderate genetic effects. Variance in boldness accounted for by *MC1R* ($R^2_{\mathrm{GLMM(m)}} = 9.48 \times 10^{-5}$) was low, whereas *DRD4* ($R^2_{\mathrm{GLMM(m)}} = 0.0199$) and *SERT* ($R^2_{\mathrm{GLMM(m)}} = 0.0345$) explained more. Altogether, genotypes of the three genes explained 13.66% of boldness variation. Age and habitat further explained 2.85 and 0.57% variance in boldness, respectively (Supplementary Table S8).

For maternal performance, we found one SNP within *DRD4* (*DRD4*_1363 in intron three) that was marginally non-significantly associated with PWM ($P = 0.051$; $r = 0.232$) (Table 3), with homozygote minor allele (TT) individuals weaning pups on average 3.2 kg more than homozygote major allele (CC) conspecifics (Fig. 4). While not significant, trends existed at locus *SERT*_12472 and *SERT*_15636, such that females with the minor allele (A) weaned lighter pups than GG females (Supplementary Fig. S6). Overall, 6.52% of total variation in PWM was explained by genetic effects of the seven loci. *MC1R* and *SERT* explained very little PWM variance ($R^2_{\mathrm{GLMM(m)}} = 0.00125$ and 0.00648, respectively), while *DRD4* ($R^2_{\mathrm{GLMM(m)}} = 0.0178$) had a more appreciable genetic effect. *SERT*_12472 also showed a trend with lactation duration ($r = 0.127$) (Table 3; Fig. 4), with individuals having the minor allele (A) exhibiting shorter lactation durations (Supplementary Fig. S7). Much less total variance in lactation duration was explained by *MC1R* ($R^2_{\mathrm{GLMM(m)}} = 0.00014$), *DRD4* ($R^2_{\mathrm{GLMM(m)}} = 0.00977$), and *SERT* ($R^2_{\mathrm{GLMM(m)}} = 0.00677$) (overall gene effect: $R^2_{\mathrm{GLMM(m)}} = 0.106$) (Supplementary Table S8).

We found three marginally non-significant associations based on overdominance models for all three phenotypic traits examined (Supplementary Tables S9–S11). *SERT*_12472 had moderate overdominant genetic effects on boldness ($r = 0.148$) and lactation duration ($r = 0.188$), and *DRD4*_1853 had a moderate effect on PWM ($r = 0.204$). To ensure that other variants within the *SERT* linkage group did not have a greater effect or higher level of association with the traits examined, we re-ran models including alternate SNPs linked with locus *SERT*_5987. These alternate loci demonstrated similar effect sizes to those presented for association between *SERT*_5987 and boldness, PWM, and lactation duration (Supplementary Table S12). Lastly, to validate that rare genotypes were not driving the relationships observed, we re-fitted rare genotypes at the *DRD4*_1363, *SERT*_12472, and *SERT*_15636 loci, grouping the minor allele homozygote genotype with the heterozygote genotype and also eliminating the rare genotype from analyses altogether (Supplementary Figs. S8–10) (Fig. 5).

Discussion

Exploring the genetic basis of quantitative traits in free-ranging populations is challenging given the simultaneous influence of environmental variables and state-dependent factors (e.g., size, body condition, and energy reserves). Nevertheless, advances in molecular genetics and analytical techniques can provide insights on genotype-phenotype links. Here, we used long-term boldness and life-history data collected on grey seals to examine relationships between phenotype and genetic variation of five candidate genes in an effort to shed light on the mechanisms behind the coexistence of different phenotypes within a free-ranging pinniped population. We used genomic resources from a closely related species, the Weddell seal, to detect SNPs in grey seal orthologous sequences of the following candidate genes: *DRD4, SERT, OXTR*, *MC1R*, and *MC5R*. Association analyses revealed a relationship between *SERT* and shy-bold phenotypes. Furthermore, we found evidence that *DRD4* and *SERT* likely play a role in maternal performance traits. To the best of our knowledge, our association study is the first to explore the association between genetics and behavioural variation and maternal performance characteristics in a wild marine mammal population, extending the candidate gene literature to include more taxonomic diversity.

Table 3 Additive model results for assessing the genetic association of three candidate genes (*DRD4, MC1R*, and *SERT*) with boldness, pup weaning mass (PWM), and lactation duration in female grey seals of Sable Island, Nova Scotia (Canada).

Fixed effects	Estimate	SE	df	*t*	*P* value	2.5% CI	97.5% CI	Effect size (r)
Boldness ~ SNP(s) + Age + Habitat; 180 females, 1183 total observations								
MC1R								
(Intercept)	**2.978**	**0.288**	**117.929**	**10.345**	**<0.0001**	**2.408**	**3.551**	**0.690**
*MC1R*_126	−0.193	0.226	97.983	−0.856	0.394	−0.641	0.254	0.086
DRD4								
(Intercept)	**3.040**	**0.422**	**88.629**	**7.204**	**<0.0001**	**2.205**	**3.877**	**0.608**
*DRD4*_1853	−0.197	0.202	75.629	−0.975	0.333	−0.599	0.203	0.111
*DRD4*_1496	−0.073	0.255	77.416	−0.285	0.776	−0.579	0.435	0.0324
*DRD4*_1363	0.255	0.307	74.517	0.830	0.409	−0.355	0.864	0.0957
SERT								
(Intercept)	**1.979**	**0.558**	**156.060**	**3.544**	**<0.0001**	**0.878**	**3.081**	**0.273**
*SERT*_12472	**0.626**	**0.267**	**149.329**	**2.346**	**0.020**	**0.100**	**1.152**	**0.189**
*SERT*_5987	0.049	0.138	151.509	0.357	0.722	−0.222	0.320	0.029
SERT_15636	*−0.303*	*0.180*	*149.401*	*−1.688*	*0.093*	*−0.657*	*0.051*	*0.137*
PWM ~ SNP(s) + Age + Sex; 177 females, 1496 total observations								
MC1R								
(Intercept)	**47.503**	**1.480**	**117.636**	**32.089**	**<0.0001**	**44.560**	**50.417**	**0.947**
*MC1R*_126	0.381	1.062	90.016	0.359	0.721	−1.716	2.498	0.038
DRD4								
(Intercept)	**49.416**	**1.578**	**86.415**	**31.307**	**<0.0001**	**46.275**	**52.572**	**0.959**
*DRD4*_1853	−0.726	0.730	65.650	−0.995	0.323	−2.178	0.726	0.122
*DRD4*_1496	−0.984	0.924	68.000	−1.065	0.291	−2.828	0.857	0.128
*DRD4*_1363	*2.274*	*1.146*	*69.383*	*1.985*	*0.051*	*0.00399*	*4.561*	*0.232*
SERT								
(Intercept)	**45.347**	**2.533**	**144.528**	**17.902**	**<0.0001**	**40.350**	**50.349**	**0.830**
*SERT*_12472	0.177	1.196	133.524	0.148	0.883	−2.184	2.538	0.013
*SERT*_5987	0.722	0.620	133.945	1.164	0.246	−0.502	1.945	0.100
*SERT*_15636	1.002	0.802	131.035	1.249	0.214	−0.587	2.583	0.108
Lactation duration ~ SNP(s) + Age; 151 females, 542 total observations								
MC1R								
(Intercept)	**15.629**	**0.408**	**65.749**	**38.293**	**<0.0001**	**14.823**	**16.473**	**0.978**
*MC1R*_126	−0.028	0.291	65.567	−0.097	0.923	−0.615	0.545	0.012
DRD4								
(Intercept)	**16.498**	**0.493**	**67.510**	**33.476**	**<0.0001**	**15.525**	**17.491**	**0.971**
*DRD4*_1853	0.043	0.233	55.322	0.186	0.853	−0.420	0.510	0.025
*DRD4*_1496	−0.267	0.308	70.740	−0.867	0.389	−0.878	0.346	0.103
*DRD4*_1363	0.053	0.401	78.421	0.133	0.894	−0.744	0.850	0.015
SERT								
(Intercept)	**14.688**	**0.701**	**133.107**	**20.956**	**<0.0001**	**13.310**	**16.088**	**0.876**
*SERT*_12472	0.469	0.337	118.074	1.391	0.167	−0.205	1.131	0.127
*SERT*_5987	0.133	0.172	117.297	0.772	0.442	−0.207	0.477	0.071
*SERT*_15636	0.015	0.224	116.217	0.069	0.945	−0.430	0.456	0.006

Bolded values indicate statistical significance ($P < 0.05$). Italicized values are marginally non-significant ($P < 0.10$).

Full model results with additional fixed factors (age, habitat, and pup sex) are provided in Supplementary Table S7. Female identity and year were included in models as random factors.

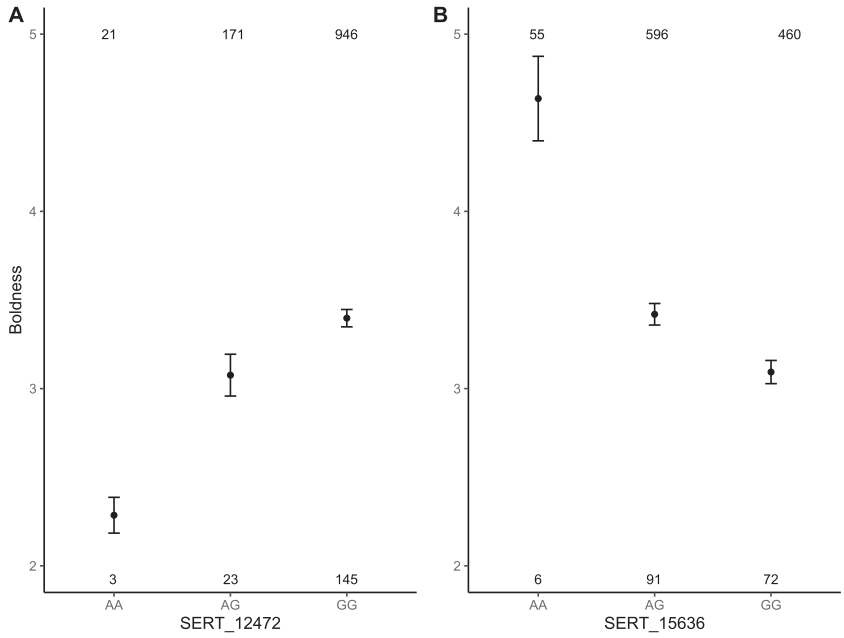

Fig. 3 Boldness of female grey seals and the additive allele effect of the serotonin transporter (*SERT*) gene. Boldness showed associations with genotypes at: **A** the *SERT*_15636 locus; and **B** the *SERT*_12472 locus. Sample size of females used for analyses are included above each genotype and number of behavioural observations are above the genotype mean. Error bars represent standard error of the genotype mean. The corresponding test statistics are provided in Table 3.

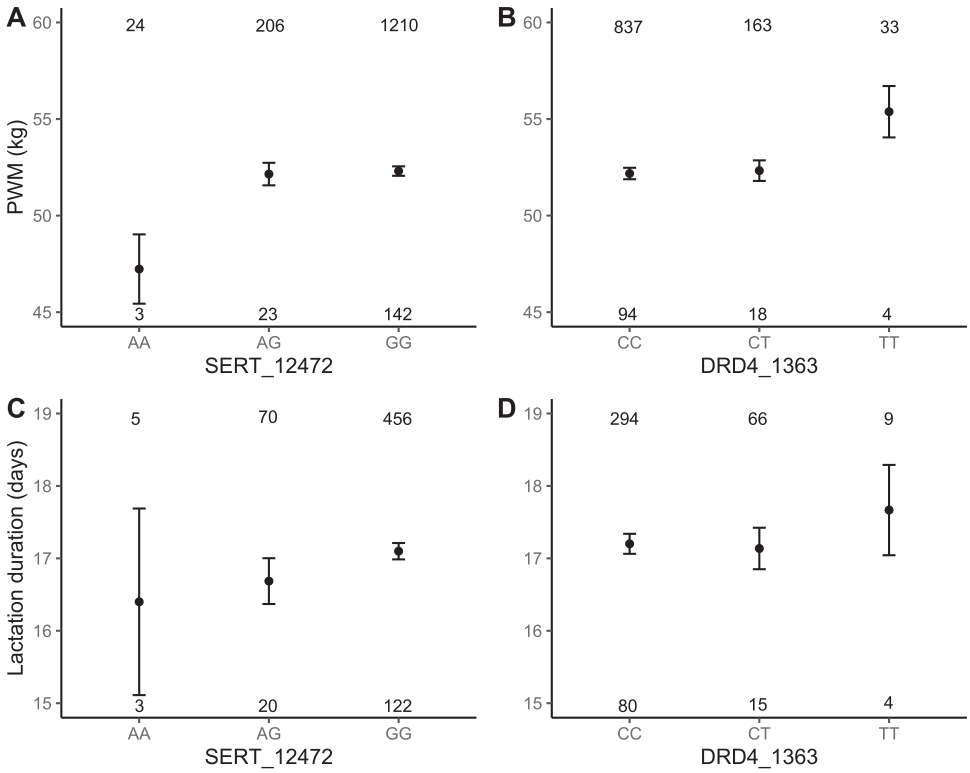

Fig. 4 Relationship between the additive allele effect and grey seal maternal performance at loci of two candidate genes [serotonin transporter (SERT) and dopamine receptor D4 (DRD4)]. SERT_12472 and DRD4_1363 genotypes showed trends with pup weaning mass (PWM) (**A**, **B**) and lactation duration (**C**, **D**). Sample size of females used for analyses are included above each genotype and number of behavioural observations are above the genotype mean. Error bars represent standard error of the genotype mean. The corresponding test statistics are provided in Table 3.

Genotype-phenotype associations

We found a relationship between *SERT* and risk-taking behaviour in female grey seals, adding to a growing list of studies documenting the general role and conserved function that *SERT* has on the expression of behavioural traits (e.g., great tits; Riyahi et al. 2015 and Timm et al. 2018). Boldness was linked to a SNP genotype at *SERT*_12472 in intron two, such that individuals with the minor allele (A) were generally shyer than major allele homozygotes (GG). Polymorphisms in intron two of *SERT* have been linked with changes in transcription and alternative splicing, as well as the regulation and efficiency of gene expression, affecting various

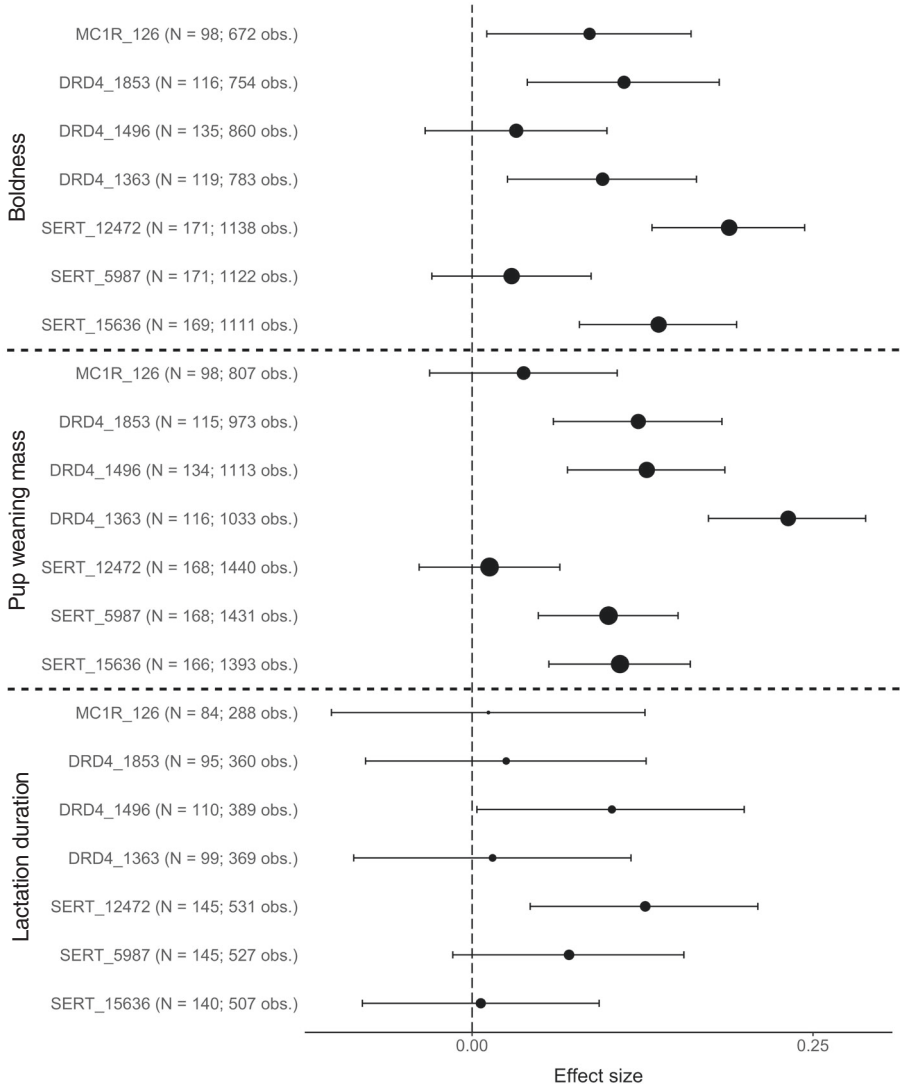

Fig. 5 Forest plots of effect size estimates (±95% confidence intervals) for the association of seven loci across three candidate genes with boldness, pup weaning mass, and lactation duration in female grey seals of Sable Island, Nova Scotia (Canada). Size of the point is proportional to the number of observations used in association tests.

behaviours in other mammalian species, including humans (MacKenzie and Quinn 1999; Battersby et al. 1996). Holtmann et al. (2016) similarly discovered a number of significant associations between loci in intronic regions and boldness in their study on a wild bird population, and highlight the importance of targeting various gene regions for candidate gene studies.

The test we used to assess boldness stimulated a natural response of offspring protection in seals assayed, effectively measuring a component of parental care. Therefore, the genotype-boldness relationship discovered may provide insight into the behavioural functional link between personality and other behaviours, including parental care (Réale et al. 2007). In support of this, associations between boldness and weaning mass and lactation duration have been established in the grey seal, with bolder females generally exhibiting longer lactation durations and weaning heavier pups than more timid individuals (Bubac et al. 2018). The biological significance of this is nontrivial. Grey seal pup survival is dependent upon acquiring sufficient mass (i.e., blubber) from maternal milk energy transfer during the lactation period (Iverson et al. 1993; Mellish et al. 1999). Upon weaning, the percent fat gained supports the pup through a post-weaning fast that lasts until the individual is capable of foraging independently (on average ~3 weeks; Noren et al. 2008). As such, body condition and size at weaning increases the pup's probability of survival and recruitment to the population (Hall et al. 2001; Bowen et al. 2015). That a significant relationship exists between boldness, PWM, and lactation duration does not necessarily indicate that the same underlying molecular mechanisms are at play. For instance, while female grey seals exhibit consistent individual differences in behaviour, they also vary in their physiological capacity to deliver milk (Lang et al. 2009); thus, creating an impetus to examine specific genotype-phenotype relationships.

We discovered a suggestive link between variations in *SERT* (*SERT*_12472) and lactation duration, a finding consistent with other patterns that we detected at this locus wherein the rare type is generally associated with shyness and lower weaning mass. Serotonin affects affiliative responses towards offspring by its influence on an individual's disposition and behavioural decisions (Emiliano et al. 2007; Bakermans-Kranenburg and van IJzendoorn 2008), and other studies have similarly reported a correlative relationship between serotonin genes and fitness-related traits in wild vertebrate populations (e.g., see Prasad et al. 2015; Timm et al. 2018). We further detected a correlation between *DRD4* genotypes and PWM. The dopaminergic system has been a system commonly studied for the role it plays in behavioural, cognitive, and locomotive variation; yet, the definitive function of *DRD4* remains uncertain and its biological significance varied in different systems and environments (Oak et al. 2000; Korsten et al. 2010; Riyahi et al. 2017). While the fitness-related traits examined in this study may be partly modulated by behavioural decisions via *SERT* and *DRD4*, it is also possible that results observed may be due to the influence of the serotoninergic and dopaminergic systems on the regulation of other hormones (Emiliano et al. 2007).

The serotonergic system, for example, mediates the release of oxytocin (Jorgensen et al. 2003), a neuropeptide hormone that plays a crucial role in promoting parturition and lactation as well as developing mother-offspring social bonds (Kendrick 2000; Lim and Young 2006). Natural and experimental studies performed on another population of grey seals have established a link between levels of oxytocin in females and the likelihood of pup separation, aggressive acts towards conspecifics, and strength of maternal bonds with offspring (Robinson et al. 2015; Robinson et al. 2017); thus, making *OXTR* an interesting gene with which to explore its genetic effect on maternal phenotypic variation. Unfortunately, we only sequenced ~13% of *OXTR* given difficulties encountered designing primers from Weddell seal genomic resources and non-specific amplification, preventing complete interrogation of the *OXTR* gene and detection of genetic variants with which to perform association analyses. Nevertheless, the continued and rapid development of genomic resources, as recently reported among marine mammals (Cammen et al. 2016), show promising potential to provide a complete and annotated reference genome for the grey seal, or closely related species, that will permit further unravelling the relationship between *SERT, OXTR*, and maternal performance traits.

Which behavioural traits are favoured is likely a product of specific spatial, temporal, and contextual influences (Wolf and Weissing 2010), such that, for example, shy or bold behaviours may be selected against in one context or situation but favourable in other instances (e.g., foraging, reproduction, anthropogenic conflict) (Sih et al. 2004; Wolf et al. 2007). It is therefore likely that fluctuating selection pressures dependent upon particular contexts are driving the behavioural variation that we observed. Tajima's *D* values and excess sequence variation (notably at *SERT*) support the occurrence of balancing selection, and thus, have consequences for the underlying genetic composition of this population. Prior studies have reported similar patterns of selection on behaviour-related genes, including those underlying dopaminergic and serotonergic function (Howell et al. 2007; Chakraborty et al. 2010). In blackbirds, (*Turdus merula)*, for instance, harm avoidance traits found to be associated with a *SERT* polymorphism were subject to selection pressures in a novel environment, where rare alleles had a selective advantage in a population undergoing an urbanization event (Mueller et al. 2013). However, our results indicating balancing selection should be interpreted with caution. Demographic processes, including bottleneck events as experienced by Northwest Atlantic grey seals (Cammen et al. 2018b), can yield similar neutrality test values (Maruyama and Fuerst 1985). Given our preliminary results, it will be interesting to track changes in the genetic composition of the Sable Island population over time, as well as to test for these genetic effects in other populations of grey seals.

Limitations

Like many marine mammals, Northwest Atlantic grey seals have undergone a severe bottleneck with its genetic signature evident today (Cammen et al. 2018b). We discovered SNPs that were infrequent in sequences of females examined as to be effectively uninformative in association analyses, which is consistent with the expectation of many rare genetic variants from rapid growth and expansion during recovery after a bottleneck event (Nei and Li 1976; Maruyama and Fuerst 1985). Furthermore, molecular diversity indices as estimated across the sequenced regions revealed low variation, a result not unexpected given the demographic history of the grey seal. The nature of the genetic structures (i.e., coding versus non-coding) sequenced may contribute to low diversity values. Though *SERT* had an appreciable amount of intronic regions sequenced, much of the other sequenced regions of the remaining genes were primarily exonic where less variation is typically detected (Fig. 2). As such, *SERT* was more diverse than the other genes, possibly explained by different selection pressures between exons and introns. Still yet, without an annotated reference genome for the grey seal, we relied on primers designed from a closely related species that diverged approximately 15 million years ago (Fulton and Strobeck 2010), likely reducing primer specificity and gene coverage. This, combined with long repetitive regions in certain genes

sequenced, contributed to difficulty in obtaining high-quality sequencing data, an issue frequently encountered among non-model organisms (Garvin et al. 2010; Helyar et al. 2011).

While it is possible that we may not have had enough power to resolve certain gene-phenotype relationships, other candidate gene studies have also been met with heterogeneous results, demonstrating the complex relationship between genes and quantitative traits (e.g., Edwards et al. 2015; Korsten et al. 2010). Many quantitative traits are polygenic, wherein detection of significant associations may be biased towards loci of larger effect size (Göring et al. 2001; Wellenreuther and Hansson 2016). The amount of variation in boldness, PWM, and lactation duration accounted for by *SERT* and *DRD4* likely explains an important fraction of the moderate-to-high repeatability measures of these traits ($R = 0.48$-0.61) (Lang et al. 2009; Bubac et al. 2018), and of what heritability might be, in the Sable Island population of grey seals. Yet, with the expectation that personality traits are controlled by many genes of small effect (Laine and van Oers 2017), other genes [e.g., arginine vasopressin receptor 1 A (*AVPR1A*) and monoamine oxidase A (*MAO-A*)] not explored herein could be contributing to the behavioural variance observed, and the unaccounted variability in the observed grey seal phenotypes deserves further attention. Although candidate genes offer important preliminary information, a genome-wide association analysis should be done in an effort to further resolve the molecular genetic basis of these fitness-related traits.

Conclusion

Despite the difficulties, it is important to study the genetic basis of complex traits across a variety of species in an effort to determine whether particular molecular mechanisms underlying these traits are evolutionarily conserved (Bengston et al. 2018). Only by examining the genetic basis of boldness and other fitness-related traits across taxonomically diverse species will we be able to discern whether such mechanisms are common to all taxa (Fidler 2011), or are unique to specific populations of a particular species. This research may also provide insight into the processes shaping and maintaining individual phenotypic variation in wild populations, thereby allowing researchers to assess the capacity of populations to adapt in response to changing environmental conditions and other selection pressures. Individual-based data records and archived tissue samples from a longitudinal study enabled us to test for a link between fitness-related traits and genotype in a species of marine mammal. This underscores the importance of pre-existing long-term studies for unravelling the molecular genetic basis of quantitative traits in free-ranging species.

Data availability

The genotype and phenotype information used for this study is available on the University of Alberta Dataverse repository (https://doi.org/10.7939/DVN/OPAOMU). Sequence data have been submitted to GenBank (accession numbers MW864572-MW864597).

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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