Linking genetic, morphological, and behavioural divergence between inland island and mainland deer mice

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Running Title: Genomics of the island syndrome

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Abstract

The island syndrome hypothesis (ISH) stipulates that, as a result of local selection pressures and restricted gene flow, individuals from island populations should differ from individuals within mainland populations. Specifically, island populations are predicted to contain individuals that are larger, less aggressive, more sociable, and that invest more in their offspring. To date, tests of the ISH have mainly compared oceanic islands to continental sites, and rarely smaller spatial scales such as inland watersheds. Here, using a novel set of genome-wide SNP markers in wild deer mice (*Peromyscus maniculatus*) we conducted a genomic assessment of predictions underlying the ISH in an inland riverine island system: analysing island-mainland population structure, and quantifying heritability of phenotypes thought to underlie the ISH. We found clear genomic differentiation between island and mainland populations and moderate to high marker-based heritability estimates for overall variation in traits previously found to differ in line with the ISH between mainland and island locations. F_{ST} outlier analyses highlighted 12 loci associated with differentiation between mainland and island populations. Together these results suggest that the island populations examined are on independent evolutionary trajectories, the traits considered have a genetic basis (rather than phenotypic variation being solely due to phenotypic plasticity). Coupled with the previous results showing significant phenotypic differentiation between island and mainland groups in this system, this study suggests that the ISH can hold even on a small spatial scale.

Key Words: island syndrome, heritability, F_{ST} outlier, deer mice, ddRAD

Introduction

Islands are considered classical laboratories for the study of evolution (reviewed in Losos and Ricklefs 2009). In particular, the island theory of biogeography holds that the observed biodiversity on islands must have arisen from a combination of processes including immigration, extinction, and *in situ* speciation, all of which will be mitigated by the degree of insularity, the size, and the age of the island (MacArthur and Wilson 1963; Whittaker et al. 2017). However, it remains unclear as to which processes (e.g. plasticity, selection, or founder effects) exert the greatest influence on the divergence and diversification that occurs *in situ* on islands.

One hypothesis that emerged from this field is the 'island rule', which posits that smaller organisms increase in size, while larger organisms become smaller once they colonize islands (Van Valen 1973; Lomolino 1985). A similar pattern originally proposed to explain rodent diversification, known as the 'island syndrome', holds that in addition to changes in size, a suite of phenotypic and behavioural changes occur in island populations compared to their mainland counterparts (Adler and Levins 1994). Specifically, island populations are predicted to contain individuals that are larger, less aggressive, live at higher densities, and shift their reproductive strategies to produce fewer, larger offspring (Halpin and Sullivan 1978; Adler and Levins 1994; Goltsman et al. 2006). Both the 'island syndrome 'and 'island rule' have been examined in a variety of taxa including mammals (e.g. Michaux and 2002; Lister and Hall 2014), reptiles (e.g. Novosolov et al. 2012; Novosolov and Meiri 2013; Slavenko et al. 2015) and birds (e.g. Wang et al. 2009; Covas 2012; Ramos 2014). Furthermore, several large-scale meta-analyses have been undertaken to examine the generality of such phenotypic changes and what environmental or ecological factors underlie them (Benítez-López et al. 2021; Meiri et al. 2005, 2008; Raia and Meiri 2006; Lomolino et al. 2011, 2013). Major hypotheses to explain phenotypic changes in smaller species include: 1) colonization of islands releases small species from predators allowing them to become larger and more gregarious, 2) larger phenotypes arise from founder effects driven by a greater ability of larger individuals to survive the journey to the island, and 3) intraspecific competition on islands drives parents to invest resources in fewer, larger offspring. On the other hand, limited resources on islands may drive dwarfism in larger species. However, the combination, relative importance, and influence of such selective forces will be contextual (Benítez-López et al. 2021; Raia and Meiri 2006; Lomolino et al. 2011). In addition, the pervasiveness of these patterns is still debated (Benítez-López et al. 2021; Lokatis and Jeschke 2018).

Studies examining the island syndrome are generally done at large scales comparing continental and oceanic island populations (Benítez-López et al. 2021; Meiri et al. 2005, 2008; Raia and Meiri 2006; Lomolino et al. 2011, 2013). Therefore, the degree to which they extend to other insular systems, e.g. inland lakes or islands in river systems, is unknown. Knowledge of these systems, however, is necessary as the relative importance of local selection pressures and gene flow in such habitats is likely to be different from those in the more remote island systems studied so far. This knowledge would also be important to predict future phenotypic and genetic

changes in animal populations resulting from increased fragmentation of habitats in many ecosystems on earth.

In this study we examine population genetic structure and search for the genetic basis of phenotypes associated with the island syndrome among deer mice (*Peromyscus maniculatus*) residing on inland islands within the Winnipeg River Basin (Ontario, Canada). In a parallel study, Juette et al. (2020) found phenotypic differentiation for some traits that are likely linked to the island syndrome in this system. More specifically they reported that insular mice were less aggressive, more thorough in their exploration behaviours, and island males were bigger than mainland ones. They also found individuals had longer tails in island populations compared to mainland populations, but that the magnitude of this difference decreased between juvenile, subadult, and adult individuals. Together their results suggest that the island syndrome exists in this system. They note, however, that such differentiation may be less easily detected in inland island systems and naturally fragmented habitats because of the combination of multiple eco-evolutionary processes such dispersal and gene flow, dispersal syndrome and non-random colonization probability, intraspecific competition, and ecological release that are not found in remote, oceanic islands.

The widespread application as well as declining costs of high-throughput sequencing technologies mean that data sets with thousands of loci can now be generated for nearly any organism (Narum et al. 2013; Goodwin et al. 2016; Levy and Myers 2016). These large datasets open avenues of research including detecting fine scale genetic differentiation among populations (e.g. Viengkone et al. 2016), estimating migration among populations (Petkova et al. 2015), and finding the genetic basis of traits within wild populations (Santure and Garant 2018). In addition, methods to detect outlier loci can reveal targets of selection that are critical but not necessarily associated with phenotypes that can be measured (Ahrens et al. 2018).

Despite the availability of these methods, few studies have looked at the genetic basis of phenotypic differences between island and mainland populations (Gray et al. 2015; Parmenter et al. 2016; Trapanese et al. 2017; Baier and Hoekstra 2019). For instance, Trapanese et al. (2017) used transcriptomic analyses to look for differential gene expression between island and mainland populations of Italian wall lizards (*Podarcis siculus*). Several genes showed changes in expression patterns between the two populations, but additional work is required to link variation in expression level to changes in phenotypes between island and mainland individuals. Taking a

different approach, Gray et al. (2015) and Parmenter et al. (2016) used house mice (*Mus musculus domesticus*) from Gough Island, the largest known house mice, to examine the genetic basis of morphological characteristics such as body mass, growth rate, and skeletal features. Applying a quantitative trait locus (QTL) mapping approach, they found multiple QTL underlying all the traits considered, showing that there is a genetic basis to these phenotypes. Finally, Baier and Hoekstra (2019) used a controlled cross design to examine deer mice from the mainland and islands off British Columbia, Canada, and found a genetic basis to the large body size of the island mice. These same authors found that island-continent differences in behaviour, however, were not maintained in a controlled environment over generations and thus likely due to plastic responses (Baier and Hoekstra 2019). Here, rather than using controlled crosses, we examine the genetic basis of phenotypes directly measured in wild individuals, which could reveal novel associations (Slate et al. 2009; Santure and Garant 2018), though does not control for the effects of plasticity on phenotypic differentiation.

In this study we assess the genetic basis of traits linked to the island syndrome among deer mice residing on inland islands within the Winnipeg River Basin (Ontario, Canada). Given the results of Juette et al. (2020), we know that phenotypic differentiation exists for some traits that are likely linked to the island syndrome in this system: insular mice were less aggressive, more thorough in their exploration behaviours, and island males were bigger than mainland mice. Using a newly developed set of single nucleotide polymorphism (SNP) markers and a comprehensive set of morphological and behavioural traits directly measured in wild caught individuals, we test three predictions underlying the genetic basis of the island syndrome: i) island populations are genetically differentiated from adjacent mainland ones; ii) island populations show some connectivity despite genetic differentiation between each other; iii) phenotypic traits (morphological, physiological and behavioural) associated with the island syndrome show heritable variation. Furthermore, we also assess if specific loci are associated with these phenotypic traits and compare genetic diversity between the island and mainland populations, which may reveal other footprints of differential selection.

Methods and Materials

Sample collection and phenotypic measurements

The study area was located near Minaki (49°59′11″N 94°40′12″W), along the Winnipeg River system, north-western Ontario, Canada (Figure 1). The area is part of the boreal shield and dominated by conifers such as black and white spruce (*Picea mariana and P. glauca*), and jack pine (*Pinus banksiana*), as well as by deciduous trees such as trembling aspen (*Populus tremuloides*), and yellow birch (*Betulla alleghaniensis*). The islands in this system are granite formations from the Canadian Shield with the depth of the channels between our mainland and island sites varies between 5 and 30 m, with one exception (<2m between island A and I). Estimates based on sediment cores have shown that lake depths in the region have been relatively stable over the past 1000 years, with possible increases in depth within the past 100 years (Laird et al. 2011, 2012; Ma et al. 2012). Therefore, we believe our study sites are not ephemeral, and current connectivity levels would be reflective of those in the past.

We sampled three mainland sites on each river shore and 12 sites on 10 islands (Figure 1). Island area varied between 0.063 km^2 (Island C) and 3.164 km^2 (Island M) and isolation (i.e. distance from the closest island or river shore) varied between 14 m and 1779 m (Supplementary Table 1). On each site, we set up a 50 x 40 m trapping grid. The trapping grid contained 30 stations, each distanced 10 m apart with two traps deployed at each station for a total of 60 traps. We trapped for three nights at each site using Longworth and BioEcoSS traps. We baited traps with peanut butter, pieces of carrot, and dried oatmeal, as well as a ball of cotton for thermoregulation. Sampling dates between island and mainland sites were alternated to avoid seasonal bias on the effect of habitat types on phenotypes. Traps were checked around 6:30 a.m., and full traps were brought back to a processing area outside of the capture grid. Measurements and tests were done between 6:30 a.m. and 12:00 p.m. (Juette et al. 2020). We randomized the order in which we processed each mouse.

Using the phenotypic measurements of Juette et al. (2020), we focused on two morphological phenotypes (body mass and tail length) and three behavioural phenotypes (exploratory behaviour and two measures of handling aggression) that were shown to have significant differences between island and mainland populations. While not a “classical” island syndrome phenotype, tail length is generally assumed to have a role in swimming behaviour (Dagg and Windsor 1972) as well as arboreal lifestyle (Smartt and Lemen 1980), which may increase on islands because of increased intraspecific competition and ecological release.

Therefore, island mice should have longer tails because of the role of dispersal syndrome in the colonization of remote islands.

As detailed in Juette et al. (2020), over the course of four years (2013 - 2016) we captured 447 deer mice. Individuals were aged (juvenile, subadult, or adult) based on their coat colour pattern, and sexed based on ano-genital distance. Mice were then ear-punched for individual marking, and ear tissue was placed in an Eppendorf tube with 95% alcohol and stored at -20 °C for genetic analyses. Mice were weighted to the nearest mg, using micro-Line Pesola® scales (capacity of 60 g ± 0.18 g). Measures of tail length (in mm) were based on size-standardized pictures (Juette et al. 2020), and ImageJ version 1.50i (Schneider et al. 2012). We measured exploration in a novel environment using a classical open-field (OF) test (Walsh and Cummins 1976). The novel environment arena consisted of a 40 × 60 × 50 cm empty, plastic box covered with a Plexiglas sheet. After cleaning the arena with alcohol before each OF test, we placed each mouse in the arena and filmed its movements for three minutes. We measured the distance moved (in cm) during that period with the software EthoVision version 9.0.723 (Noldus ©). We measured handling aggression as an index of response to potential predators, in two ways (see details in Juette et al. 2020). "Handling aggression.1" was the individual's reactions during approximately 30 sec after being laid on the back of the manipulator's hand. Scores varied from 0 (no reaction or movement; very docile) to 3 (bites and struggles to escape; not docile). We measured "handling aggression.2" while individuals were held by the neck skin. During the first 10 seconds, the manipulator observed behaviours of the individual without any other intervention. Then, for the next 20 seconds, the manipulator gently approached his finger and made a ventral contact with the individual. Aggressiveness score varied from 0 (no reaction; not aggressive) to 7 (agitation, bites and attempts to escape; very aggressive). All trapping and handling procedures were approved by the Université du Québec À Montréal animal care committee (permit #783 to D. Réale).

Sequencing and SNP Discovery

Genomic DNA was extracted from 310 mice and prepared for double digest restriction-site associated DNA (ddRAD) sequencing (Peterson et al. 2012). Briefly, a salt-extraction protocol adapted from Aljanabi and Martinez (1997) was used. Sample quality and concentration were then checked on 1% agarose gels and with a NanoDrop 2000 spectrophotometer (Thermo

Scientific). Each individual's genomic DNA was normalized to 20 ng/µl in 10 µl (200ng total) using PicoGreen (Fluoroskan Ascent FL, Thermo Labsystems) in 96 well plates. The libraries were constructed at the Institut de Biologie Intégrative et des Systèmes (IBIS) at Université Laval (Québec, Canada), and sequenced on the Ion Torrent Proton platform following the protocol in Mascher et al. (2013). Specifically, restriction digest buffer (NEB4) and two restriction enzymes (*Pst*I and *Ms*pI) were added to each sample then digestion was completed by incubation at 37 °C for two hours before the enzymes were inactivated by incubation at 65 °C for 20 minutes. Two adapters (one unique to each sample and the second common) were added to each sample and ligation was performed using a ligation master mix followed by the addition of T4 ligase. The ligation reaction was completed at 22 °C for 2 hours followed by 65 °C for 20 minutes to inactivate the enzymes. Samples were pooled in two batches of 77 samples and two batches of 78 samples and cleaned-up using QIAquick PCR purification kits. The libraries were then amplified by PCR and sequenced on the Ion Torrent Proton P1v2 chips. Each library was sequenced once and reads per sample were counted. The libraries were then re-pooled to normalize the representation of each sample and each library was then re-sequenced on three further chips, for a total of 16 chips. Samples that had very low numbers of reads were removed at this step.

Raw reads were filtered with cutadapt (-e 0.2 -m 50) and demultiplexed by sample and trimmed to 80 bp within STACKS version 1.44 (Catchen et al. 2013) using the process_radtag program (-c -r -t 80 -q -s 0 --barcode_dist_1 2 -E phred33 --renz_1 pstI --renz_2 mspI). Reads that were shorter than 80bp were discarded. After filtering and demultiplexing, there were a total of 974 million reads, with an average (±SD) of 3.16 ±1.08 million reads per individual. The resulting reads were then processed using STACKS and custom scripts (https://github.com/enormandeau/stacks_workflow). Specifically, we used the genome assembly for *Peromyscus maniculatus* (GCA_000500345.1) as a reference to align the reads with bwa version 0.7.13 (Li and Durbin 2010; -k 19, -c 500, -O 0,0 -E 2,2 -T 0) and samtools view version 1.3 (Li et al. 2009; -Sb, -q 1, -F 4 -F 256 -F 2048). Within the stacks_workflow pipeline, we extracted stacks of loci with pstacks (-m 2, --model_type snp, --alpha 0.05), built the catalogue of stacks with cstacks (-n 2, -g) and sstacks (-g), and then did initial variant filtering and data exporting with populations (-r 0.5, -p 4, -m 4, -f p_value, -a 0.0, --p_value_cutoff 0.1, --vcf, --vcf_haplotypes) modules. Thirteen samples with 29% or more missing genotypes SNP data were

removed at this stage, leaving 296 samples (Table 1). The populations module was then re-run with only these 296 samples. The resulting list of SNPs was further filtered with the 05_filter_vcf.py script found in the stacks_workflow pipeline such that each locus had a minimum genotype read depth of 10, was present in at least 70% of individuals, had less than 60% heterozygosity in all populations, had a minimal global minor allele frequency (MAF) of 0.01 or a minimal MAF of 0.05 in at least one population, had a minimum and maximum F_{is} value of -0.3 and 0.3, respectively, and was in a locus with a maximum of 10 SNPs. Preliminary analyses of this dataset with PCA showed two outlier individuals that were removed from further analyses. With these individuals removed, the loci were re-filtered with PLINK to have a minimum minor allele frequency of 1%. This resulted in a dataset of 105,310 loci in 294 individuals.

Allelic diversity and genetic differentiation based on sampling location

Genetic diversity summary statistics among sampling locations was calculated using the R package *diveRsity* version 1.9.90 (Keenan et al. 2013) in R version 3.2.2 (R Core Team 2019). Note that for these estimates we removed two locations (G and O) where only a single individual was sampled. To determine pairwise genetic differentiation among sampling locations we calculated the θ estimator of F_{ST} values (Weir and Cockerham 1984).

Genetic structure

We examined genetic structure with three different methods. First, we built a UPGMA tree based on pair-wise genetic distances among individuals using the R packages *poppr* version 2.6.1 (Kamvar et al. 2014) and *ape* version 5.0 (Paradis et al. 2004). Second, admixture analyses were conducted using the snmf function within the *lea* package version 1.2.0 (Frichot and François 2015). This function provides least square estimates of individual ancestry proportions (Frichot et al. 2014) among genetic clusters (K) without *a priori* grouping of individuals. We chose this method as it is optimized for large SNP datasets and is robust to departures from traditional population genetic model assumptions used by programs such as STRUCTURE (Pritchard et al. 2000), including Hardy-Weinberg and linkage equilibrium (Frichot et al. 2014). We tested K=1 to 17 (the number of sampling locations with >1 individual captured) with 20 replicates of each K. We then examined cross-entropy (CE) scores to determine optimal K as the

point at which CE was minimized; CE scores were also used to select which replicate to use in visualization. The chosen ancestry matrices from *lea* were then processed with CLUMPAK (Kopelman et al. 2015). Finally, we examined genetic structure by conducting principal component analysis (PCA) with *lea*. The percent variation explained by each principal component was assessed with Tracy-Widom tests (Patterson et al. 2006).

Allelic diversity and genetic differentiation based on 'genetically differentiated groups'

Based on the results of the genetic structure analyses we regrouped samples into 'genetically differentiated groups 'corresponding to their position within the UPGMA tree. With these groups established, we recalculated the genetic diversity summary statistics and measures of population differentiation using the same procedures as above. We also re-visualized the clustering using discriminant analysis of principal components (DAPC). This method combines discriminant analysis with PCA to maximize differentiation among groups while minimizing within-group variation (in our case the genetic clusters; Jombart et al. 2010). We undertook DAPC analyses using the R package *adegenet* version 2.0.1 (Jombart 2008; Jombart and Ahmed 2011) and the optim.a.score function to determine the number of principal components to retain.

We then used an Analysis of Molecular Variance (AMOVA) to examine how variability is partitioned among groups of individuals. We implemented two sets of groupings: the first having sampling sites nested within either "island" or "mainland" categories, and the second having groupings reflect the 'genetically differentiated groups'. The AMOVA was carried out in GenoDive version 3.0 (Meirmans 2020) with 100 bootstrap replicates to assess significance.

Estimation of migration among sampling locations

We first examined the data for evidence of isolation by distance (IBD) using the *dartR* package (Gruber et al. 2018). Here a matrix of pairwise geographic distances among sampling locations was compared to pairwise genetic differences as measured by $F_{ST}/1\text{-}F_{ST}$ (Rousset 1997) using a Mantel test. The significance of the Mantel correlation was assessed using 999 permutations. However, given the geographic scale of the analysis, the sampling design, and the fact that we are dealing with an island-mainland system strict patterns of IBD as assessed via Mantel tests may not be applicable. Therefore, in addition, we tested for spatial patterns in the

genetic variation using Moran's Eigenvector Maps (MEM; Legendre and Fortin 2010) as implemented by the *MEMGENE* package (version 1.0; Galpern et al. 2014). MEM focuses on 'neighbourhood level' analysis of population structure (*sensu* Wagner and Fortin 2013) where variation among nodes (sampling locations) is placed in spatial context. Here a genetic distance matrix comprised of the proportion of shared alleles among individuals was compared to MEM eigenvectors (series of orthonormal variables produced from a principal coordinate analysis describing patterns of spatial autocorrelation), and the amount of genetic variation that can be explained by geographic patterns was calculated.

Estimates of heritability for morphology and behaviour

We implemented marker-based estimates of heritability, as no pedigree is available in this system to calculate values via traditional methods such as parent-offspring regression or an animal-model. A previous study by Perrier and (2018) showed that genome wide relatedness matrices and pedigree-based methods perform equally well, with a slight underestimation with the pedigree approach. For estimation of marker-based heritability we further screened loci using vcftools version v0.1.12b (Danecek et al. 2011) and selected markers that were biallelic and not within the same sequencing read to reduce the influence of linkage disequilibrium on the analyses. We used the subset of individuals from Juette et al. (2020) that had both genotypic data and phenotypic measures to examine each trait separately in an analysis that considered mainland and island mice simultaneously. Prior to the analyses, we determined if covariates should be included by fitting linear models containing all covariates and then using model simplification. The covariates considered were age (juvenile, subadult, adult), sex, habitat type (mainland or island), and year of capture (as a discrete variable). For morphological traits, we also included date of capture to account for changes over the season. Model simplification was done using the dredge function in the package *MuMIn* version 1.40.4 (Bartoń 2018) and assessing model differences with AICc (AIC values corrected for small sample sizes). Models were considered significantly different if they had ΔAICc scores greater than 2. When models did not differ by more than 2 AICc from the "top" model, we retained all variables contained in these models.

For each trait, the minimum adequate model found above was used as the base for heritability analysis with the *GenABEL* package version 1.8-0 (Karssen et al. 2016). Here, we

first used the 'ibs' function to calculate pairwise relatedness between all individuals from the SNP data, this kinship matrix was then added as an additional random effect to the model to correct for underlying population structure. We then used the polygenic_hglm function to calculate the marker-based heritability estimates for each trait (Lee and Nelder 1996; Rönnegård et al. 2010). Standard error for each heritability estimate was calculated based on the code from Silva et al. (2017). All modelling was performed in R version 2.4.3 (R Core Team 2019).

F_{ST} outlier analysis

To look for signals of selection between mainland and island populations we used an F_{ST} outlier approach as implemented in R package *OutFLANK* version 0.2 (Whitlock and Lotterhos 2015). For this analysis the input loci and individuals were the same as those used in the heritability analyses. However, to parameterize the model we first calculated a null distribution of F_{ST} values from a further pruned dataset generated in PLINK version 1.9 (Chang et al. 2015). Here we removed physically linked SNPs with variance inflation factors (VIF) greater than 2 in 100 kb sliding windows (flag --indep 100 10 2). Outliers were then determined using the mean F_{ST} from this null distribution, a q-threshold of 0.05, and a minimum heterozygosity of 0.1.

To complement these analyses, we calculated Tajima's D statistic (Tajima 1989) using the program VCF-kit version 0.2.9 (Cooke and Andersen 2017). Values were calculated separately for individuals from island or mainland locations using sliding windows 1Mb in length with a shift of 100kb between windows. We then compared values based on those windows with >1 SNP that were common to both groups of individuals (n = 10,948).

For associations resulting from the F_{ST} outlier analyses (see Results) we examined gene annotations in the deer mouse genome following the methods of Miller and (2018). Specifically, the genomic window within which to search was determined by estimating the 'half-length' of linkage disequilibrium (LD) for our marker set, i.e., the inter-marker distance at which LD decreased to half its maximal value (Reich et al. 2001). Half-length is thought to reflect the extent to which an association between genotypes at a given locus and a QTL can be detected (Reich et al. 2001). For this estimation we used PLINK to calculate pairwise values of r^2 between syntenic markers on all scaffolds (n = 2,169,116 pairwise comparisons). These estimates were then compared to inter-marker physical distance based on map positions from the

deer mouse genome, and half-length was determined using a custom script which calculated LD decay rate as in Appendix 2 of Hill and Weir (1988).

Results

Allelic diversity, genetic differentiation, and genetic structure

Diversity statistics among sampling sites are presented in Table 1. Average observed heterozygosity per lineage ranged from 0.128 in location C to 0.151 in location D (average±SD = 0.143±0.005). Interestingly, there was no difference in average observed heterozygosity between mainland and island populations. Pairwise F_{ST} values ranged from 0.038 between locations B and H, to 0.176 between locations C and I (Supplementary Table 2).

The UPGMA tree resolved seven groups of individuals (Figure 2A). Only sampling location Q formed a monophyletic group (Cluster 1; Figure 2A). The majority of the remaining groups contained either two (Cluster 2 and Cluster 4) or three sampling locations (Cluster 3, Cluster 5, Cluster 6, and Cluster 7). Clusters tended to be geographically structured (Figure 2B). Notably, the mainland sampling sites on the west (N, D, and H) side of the study area were genetically similar to one another and differentiated from the island locations; a similar pattern was seen with those sites from the east (X, B, and F) side with a small amount of shared variation observed at site B (Figure 2B). This pattern of differentiation is consistent with our prediction under the island syndrome that island populations would be genetically differentiated from adjacent mainland ones. It is noteworthy that within the islands there were a number of cases where individuals from the same sampling location belonged to different genetic clusters (e.g. A, P, M, J).

For the admixture analyses, cross entropy values were similar across K values (Supplementary Table 3). Examination of these plots showed that genetic clusters K=2 to K=4 sequentially differentiated island groups (Figure 3), and mainland sites from the western part of the study area (D, H, and N) grouping at K=5. Island site Q became differentiated at K=6. Across K values many individuals shared ancestry from a number of genetic groups, including those from the mainland sites along the eastern part of the study area (B, F, and X; Figure 3). Within the principal component analyses, principal components (PCs) 1 and 2 explained 2.7% and 2.2% of the variation in the dataset, respectively (Supplementary Figure 1A), while PCs 3 and 4 explained 2.1% and 1.9% of the variation (Supplementary Figure 1B). There was differentiation

of the sampling sites across these axes, which largely paralleled that seen in the UPGMA and admixture analyses. Specifically, locations I and L which were closely associated (Cluster 4) as well as location D, were separated across PCs 1 and 2, while PCs 3 and 4 showed distinct clustering of locations K, M, and Q.

Measures of population differentiation were higher when analysed according to 'genetically differentiated groups' (Table 2) than when considering sampling locations (Table 1). The range of pairwise F_{ST} values was narrower among genetic groups, ranging from 0.039 between Cluster 6 and Cluster 7 to 0.120 between Cluster 1 and Cluster 4. Based on the optim.a.score we retained 7 PCs for the DAPC analyses. Here, PCs 1 and 2 clearly differentiated the majority of the groups, with some minor overlap between Cluster 2, Cluster 3, and Cluster 5 (Supplementary Figure 2A). However, these groups were differentiated when considering PCs 3 and 4 (Supplementary Figure 2B).

Regardless of broad-scale groupings the AMOVA showed that the majority of variation (~83%) was within individuals, with an additional 6.6% of variation attributed to among individuals within sampling sites (Table 3). When sampling sites were grouped as either "mainland" or "island", we found that 9.8% of variation was due to populations within the groups and less than 1% attributed to among the groups. However, when sampling sites were grouped according to their 'genetically differentiated groups' 7.3% of variation was due to populations within the groups and approximately 3.0% was attributed to among the groups (Table 3).

Estimation of migration among sampling locations

There was no evidence for IBD among sampling locations (Mantel statistic $r = 0.121$, $p = 0.18$; Supplementary Figure 3). Similarly, the MEMGENE analyses revealed that a small amount of genetic variation was due to spatial autocorrelation among sampling sites ($R^2 = 0.058$).

Estimates of heritability for morphology and behaviour

A total of 291 mice had both genotypic and phenotype measures for at least one trait and were used in the subsequent analyses. Sample sizes and mean values per trait are listed in Table 3. As found in Juette et al. (2020) island mice were heavier and had longer tails than mainland mice (Table 4). Furthermore, island mice were less aggressive and slower explorers compared to mainland ones (Table 4).

Re-filtering the full dataset for only biallelic and "unlinked" loci resulted in a dataset of 40,399 SNPs. Covariates retained in the minimum adequate models are presented in Supplementary Table 4. Consistent with our prediction that traits associated with the island syndrome should show heritable variation, we found marker-based heritability estimates >0.30, with small associated standard errors, for all traits except for Docility (heritability = 0.05). These estimates range from 0.34 for exploratory behaviour, to 0.60 for tail length (Table 4).

F_{ST} outlier analysis

Comparison of island and mainland populations in OutFLANK showed 12 loci with F_{ST} values higher than expected (Supplementary Table 6; Supplementary Figure 4). These loci appear on 11 separate scaffolds, with two loci on scaffold 5 separated by 3,263 bp. Average Tajima's D was greater than 0 in both island and mainland locations (Supplementary Figure 5), though the magnitude was significantly larger in island (mean ± SE: 0.301 ± 0.006) sites compared to mainland (0.089 ± 0.006) ones (Welch two sample t-test: t = 23.978, df = 21,867, p-value < $2.2e^{-16}$).

Inter-marker LD showed a steep decline with physical distance along scaffolds (Supplementary Figure 6), with a half-length estimate of 1,902 bp. Therefore, we examined 2,000 bp up and downstream of variants when looking for annotations associated with candidate loci. Five of the 12 outlier loci were directly in genes (Supplementary Table 6), five were between 3,900 and 240,000 bp from annotations, and the remaining locus was on an unannotated scaffold. For the loci in genes we examined gene ontology (GO) terms to see if there was an over-representation of biological process terms. For this we used the enrichment analysis tool provided by the Gene Ontology Consortium website (http://www.geneontology.org/; accessed Aug 16, 2018) with the *Mus musculus* gene set as the background. No terms were found to be overrepresented.

Discussion

We investigated the genetic basis of overall variation in phenotypes thought to underlie the island syndrome in a meta-population of deer mice from the Winnipeg River system. To do so we developed a novel set of genomic markers to look at population structure and quantify heritability of phenotypes thought to underlie the ISH directly in wild individuals. This work

446 represents one of the few studies to use genomic data to test assumptions of the island syndrome
447 hypothesis, and the first to examine an inland island system.
448 Our new set of genome-wide SNP markers allowed for detection of genetic
449 differentiation among populations on a small spatial scale. In particular, mainland populations
450 were clearly differentiated from the island locations. Differentiation between island and
451 mainland populations may be more expected for previously examined cases of continental and
452 oceanic island populations (Gray et al. 2015; Parmenter et al. 2016; Trapanese et al. 2017; Baier
453 and Hoekstra 2019), where gene flow is more restricted. The observed differentiation suggests
454 that the island populations may be on an independent evolutionary trajectory, which would be
455 needed to evolve island syndrome phenotypes.
456 Pioneering work of Halpin and Sullivan (1978) and Adler and Levins (1994) among
457 others laid out a set of predictions for how phenotypes change between island and mainland
458 populations of rodents. Promisingly, the work of Juette et al. (2020) showed that there is
459 phenotypic differentiation for some of these island syndrome traits between island and mainland
460 populations in this system. However, multiple processes (e.g. plasticity, selection, or founder
461 effects) could hypothetically underlie such differences. Our finding that all but one trait shows
462 moderate to high levels of marker-based heritability suggests that the traits have a genetic basis
463 and overall variation is not simply a plastic response. The significant heritability of those traits
464 means that natural selection could potentially drive divergence between the island and mainland
465 populations, in line with expectations of the island syndrome. Previous examinations of the
466 genetic basis of body size differences of house mice (Gray et al. 2015; Parmenter et al. 2016) and
467 deer mice (Baier and Hoekstra 2019) have shown a genetic basis of such differences, and future
468 work in this system can aim to make such direct connections.
469 As a first step in this process, we implemented F_{ST} outlier tests to identify loci that were
470 divergent between mainland and island populations (Hoban et al. 2016; Ahrens et al. 2018).
471 These analyses are agnostic to any particular trait. By comparing differentiation between pools of
472 mainland populations and island populations, we found 12 novel candidate loci (Supplementary
473 Table 6). We found no obvious connections to island syndrome phenotypes among the loci near
474 or containing the associated SNPs or over-represented gene-ontology terms. Yet, candidate gene
475 association studies focusing on the regions highlighted here can help to elucidate if there is an
476 association between the genes in these regions and island syndrome phenotypes. Furthermore,

for both mainland and island populations mean Tajima's D values were positive, indicating possible balancing selection or recent population contraction. Though distinguishing demography from selection is challenging (MacManes and Eisen, 2014; Harris and Munshi-South, 2017).

Phenotypic changes associated with the ISH (both behavioural and morphological) are extremely complex, and environmental as well as ecological factors are likely to influence them (Benítez-López et al. 2021; Meiri et al. 2005, 2008; Raia and Meiri 2006; Lomolino et al. 2011, 2013). As a result, traits associated with the ISH are likely to have a polygenic basis as was seen with the multiple interacting QTL for body size and skeletal features in the analyses of the Gough Island house mice (Gray et al. 2015; Parmenter et al. 2016). Future work can also seek to directly link differentiation to environmental factors (e.g. Rellstab et al. 2015) to further elucidate how various selective forces influence the expression of the island syndrome (Benítez-López et al., 2021; Raia and Meiri 2006; Lomolino et al. 2011).

Going forward, association methods such as chromosome partitioning (Robinson et al. 2013; Yang et al. 2014) or haplotype analyses (Boleckova et al. 2012; Hayes 2013) may find additional candidates for the genomic basis of traits associated with the island syndrome. As sequencing costs continue to decline, it may also become economically feasible to generate low-coverage whole genome sequences for multiple individuals (Ellegren 2014; Fuentes-Pardo and Ruzzante 2017), and thereby provide a full inventory of genetic variation to base associations on. It will also be important to employ multiple, repeated genomic examinations of different island systems at varying spatial scales. Such studies would tease apart the genetic architecture of this suite of traits, assess the ubiquity of the associations found, and further show general evidence of the island syndrome.

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Competing Interests

The authors have no competing interests.

Data Accessibility

DNA sequencing reads are deposited on the short-read archive (SRA) BioProject ID: PRJNA759202. SNP genotypes along with phenotypic measurements have been deposited in Dryad (doi:10.5061/dryad.7m0cfxpw3).

Author Contributions

JMM conducted genomic analyses and drafted the initial manuscript. The original project was conceived by DR along with DG and LB. Field work was conducted by TJ and JWJ along with DR. Lab work and bioinformatic processing was conducted by EN. All authors contributed to and commented on the manuscript while it was being drafted.

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Figure 1: Map of the Minaki study area, north-western Ontario, Canada (red dot on small map, bottom right), and of the study sites (A to X). Red dots on the large map represent the sampled sites. Sites N, D, H, F, B, and X were located on the mainland and all of the other sites were located on islands. Islands that were sampled are in orange. Site M is located at the South end of a large island (3.164 km^2) that extends north. Map made with QGIS Girona 3.2.0.

Figure 2: Genetic structure of 294 deer mice from the Minaki study system. (A) UPGMA tree based on individual genetic distances; letters correspond to sampling locations as in Table 1. (B) Distribution of genetic clusters identified in the UPGMA tree on the landscape; wedges in pie charts are proportional to the number of samples in each cluster; letters correspond to sampling locations as in Table 1.

Figure 3: Ancestry bar plot from *lea* (Frichot & François 2015) for K=2-7. Each individual is represented as a vertical bar, with the proportion of colors representing their genetic assignment to a cluster.

Table 1: Sample information and summary statistics for each sampling location used in the study of the Minaki deer mice study system. Number of individuals (N), allelic richness (Ar), observed heterozygosity (Ho), and expected heterozygosity (He).

Table 2: Sample information and summary statistics for each genetic group found through UPGMA clustering, in the Minaki deer mice study system. Number of individuals (N), allelic richness (Ar), observed heterozygosity (Ho), and expected heterozygosity (He). Note that individuals from location P (N = 5) were distributed among several clusters and therefore are not included in the location column.

Table 3: Analysis of molecular variance for105,310 loci in 294 deer mice from Minaki area, northeastern Ontario, Canada. Statistics were calculated when samples were grouped either by location on islands or the mainland or based on genetic grouping.

778 Table 4: Average phenotypic values and marker based heritabilites (+/- SEs) of deer mice
779 morphological and behaviour traits from island (N = 10) and mainland (N = 6) sites in the
780 Minaki area, northeastern Ontario, Canada.


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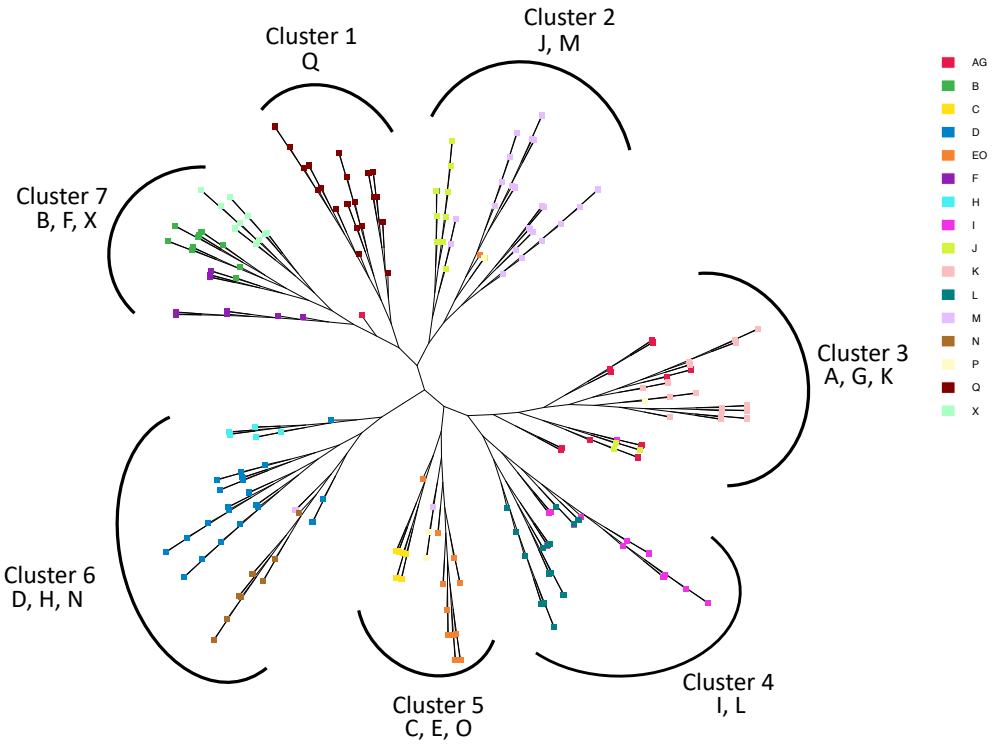
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Cluster 3
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Cluster 4
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Cluster 5
C, E, O
Cluster 6
D, H, N
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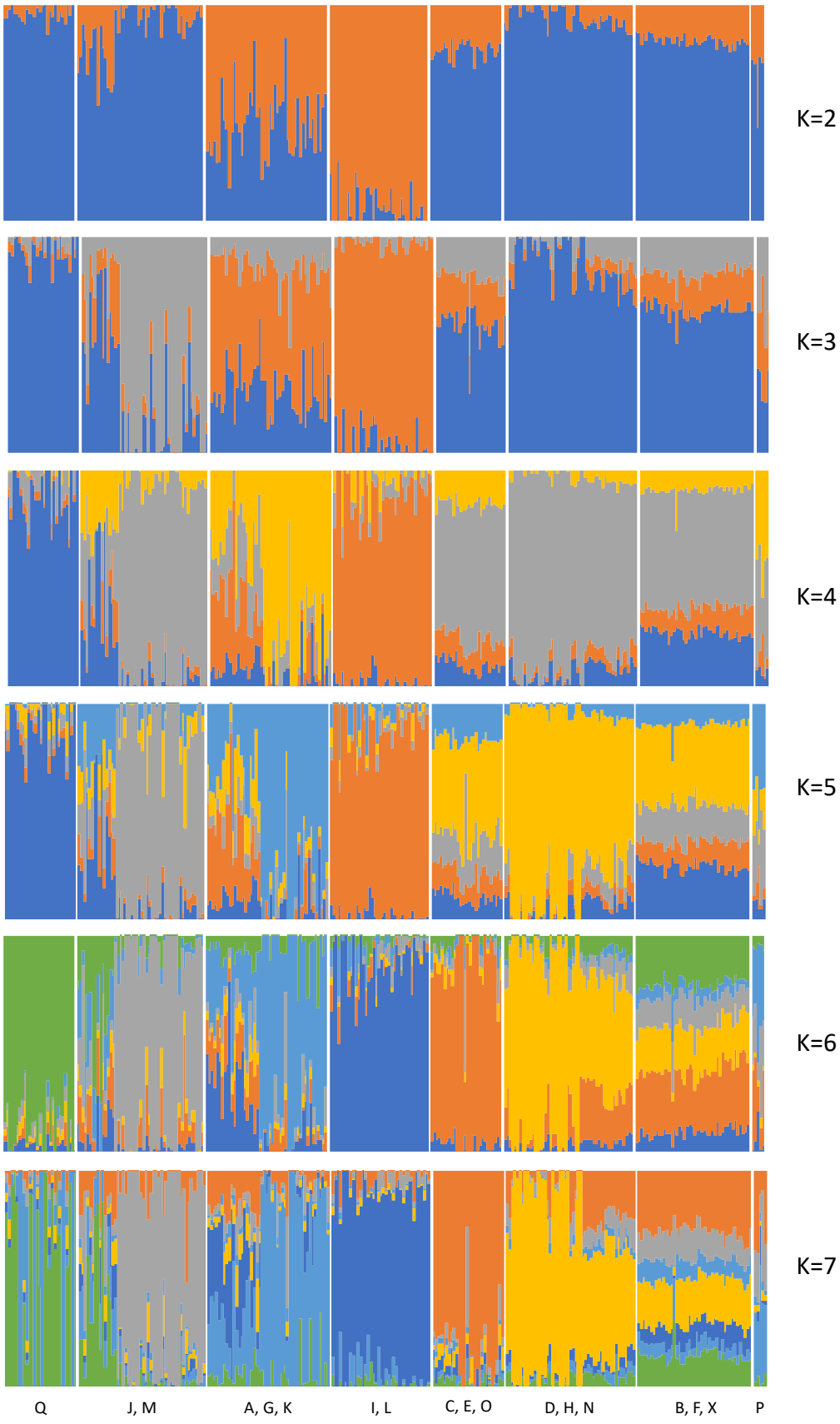
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Table 1: Sample information and summary statistics for each sampling location used in the study of the Minaki deer mice study system. Number of individuals (N), allelic richness (Ar), observed heterozygosity (Ho), and expected heterozygosity (He).

Location	**Habitat type**	**N**	**Ar**	**H_o**	**H_e**
A	Island	20	1.469	0.145	0.16
B	Mainland	17	1.461	0.145	0.157
C	Island	10	1.361	0.128	0.132
D	Mainland	30	1.458	0.151	0.16
E	Island	17	1.423	0.139	0.148
F	Mainland	14	1.471	0.144	0.157
G	Island	1	-	-	-
H	Mainland	9	1.407	0.141	0.142
I	Island	18	1.397	0.139	0.142
J	Island	16	1.445	0.149	0.153
K	Island	27	1.42	0.146	0.148
L	Island	21	1.406	0.146	0.148
M	Island	35	1.444	0.147	0.156
N	Mainland	12	1.411	0.146	0.145
O	Island	1	-	-	-
P	Island	5	1.405	0.141	0.131
Q	Island	28	1.421	0.141	0.15
X	Mainland	15	1.445	0.139	0.152

Table 2: Sample information and summary statistics for each genetic group found through UPGMA clustering, in the Minaki deer mice study system.
Number of individuals (N), allelic richness (Ar), observed heterozygosity (Ho), and expected heterozygosity (He). Note that individuals from location P (N = 5) were distributed among several clusters and therefore are not included in the location column.

Genetic Group	**Locations**	**N**	**Ar**	**H_o**	**H_e**
Cluster 1	Q	28	1.581	0.141	0.15
Cluster 2	J, M	46	1.694	0.147	0.161
Cluster 3	A, G, K	52	1.718	0.146	0.16
Cluster 4	I, L	39	1.595	0.143	0.152
Cluster 5	C, E, O	31	1.691	0.135	0.158
Cluster 6	D, H, N	53	1.752	0.148	0.166
Cluster 7	B, F, X	45	1.773	0.143	0.164

Table 3: Analysis of molecular variance for105,310 loci in 294 deer mice from Minaki area, northeastern Ontario, Canada.
Statistics were calculated when samples were grouped either by location on islands or the mainland or based on genetic grouping

Grouping	Source of Variation	Nested in	% var	F-stat	F-value	Std.Dev.	c.i.2.5%	c.i.97.5%	P-value
Island vs mainland									
	Within Individual	--	0.829	F_it	0.17145	0.00061	0.17025	0.17263	--
	Among Individual	Population	0.067	F_is	0.07435	0.0006	0.07315	0.0755	0.0099
	Among Population	Island vs mainland	0.098	F_sc	0.09825	0.00022	0.09783	0.09868	0.0099
	Island vs mainland	--	0.007	F_ct	0.00738	0.00015	0.00708	0.00768	0.0099
Genetically differentiated groups									
	Within Individual	--	0.83	F_it	0.17013	0.00061	0.16894	0.17132	--
	Among Individual	Population	0.067	F_is	0.07435	0.0006	0.0732	0.07553	0.0099
	Among Population	Genetic Groups	0.074	F_sc	0.07605	0.00022	0.07562	0.07649	0.0099
	Among Groups	--	0.03	F_ct	0.02967	0.00023	0.02922	0.03013	0.0099

Table 4: Average phenotypic values and marker based heritabilites (+/- SEs) of deer mice morphological and behaviour traits from island (N = 10) and mainland (N = 6) sites in the Minaki area, northeastern Ontario, Canada.

	Sample Size	Mean Value	Island Mean	Mainland Mean	Heritability
Body Mass	265	15.84 (3.47)	16.01 (3.43)	15.52 (3.54)	0.39 (0.15)
Tail Length	230	78.05 (7.52)	78.69 (7.31)	76.62 (8.19)	0.60 (0.14)
Exploratory Behaviour	256	1310.2 (663.51)	1228.69 (667.53)	1464.02 (631.26)	0.34 (0.15)
Handling aggression.1	266	1.42 (0.80)	1.32 (0.77)	1.64 (0.84)	0.05 (0.10)
Handling aggression.2	225	3.80 (1.69)	3.93 (1.66)	3.48 (1.72)	0.40 (0.16)