INVASIVE RAT COLONIZATION HISTORY AND MOVEMENT DYNAMICS IN THE HAIDA GWAII ARCHIPELAGO

by

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ABSTRACT

Brown rats (*Rattus norvegicus*) and black rats (*R. rattus*) are among the most invasive animals on the planet with global distributions. These species are found on >80% of all oceanic islands with devastating impacts on native fauna. Nesting seabirds are particularly affected by rat invasions, as they have evolved ground-nesting strategies in the absence of a terrestrial predator. It is estimated that 60% of all seabird extinctions are due to invasive rats. On the archipelago of Haida Gwaii, BC, invasive rats are having strong detrimental effects on native seabird populations, causing several extirpations of large breeding colonies and population declines in six of the twelve seabird species present. To promote seabird recovery, Parks Canada began initiating whole-island rat eradications in 1997. Since then, there have been seven successful eradications; however, brown rats have reappeared on the Bischof Islands after two eradication attempts. To better inform future management, we investigated population history and movement dynamics of invasive rats in Haida Gwaii. 606 rats were sampled from 2008-2018, including pre- and post-eradication samples from the Bischof Islands. We used double-digest restriction-site associated DNA sequencing to genotype individuals at approximately 28 000 single nucleotide polymorphisms identified in each species. We used population genetic and spatially-explicit analyses to determine the source of re-established populations and to quantify the extent and direction of dispersal and infer levels of gene flow throughout the system. We showed that populations were largely structured based on island sampled, and we identified groups of islands that should be eradicated simultaneously to prevent reinvasion. We determined that the source of brown rats on the Bischofs was due to reinvasion from neighbouring Lyell Island and not from bait failure, and that recent invasions onto Faraday and Murchison Islands (with subsequent introduction to Hotspring Island) were also from Lyell Island. Using a previously compiled database, we identified a likely western European origin for brown rats in Haida Gwaii

consistent with anecdotal evidence. These results will help facilitate future eradications and provide useful insights to prevent the further spread within the system.

LAY SUMMARY

In this study, we aimed to inform management of invasive rat populations in Haida Gwaii to promote seabird recovery. Since their introduction, invasive rats have contributed to population declines in half of the Haida Gwaii seabird species. To plan effective eradications with the lowest risk of re-invasion, we used population genomic analyses to identify the most suitable islands for eradication. Additionally, we identified the source of four recent island invasions. Furthermore, we were able to use a globally-sourced dataset to identity a western European origin for brown rats in Haida Gwaii. This work highlights the utility of genetics for invasive species management, not only in Haida Gwaii, but in other systems throughout the world.

PREFACE

I have worked in collaboration with many individuals for both of Chapters Two and Three. Manuscripts for these chapters will be co-authored when submitted for publication.

Dr. Michael Russello and I were responsible for study design of Chapter 2, with contributions from Dr. Emily Puckett. Parks Canada employees Goox Beaton, Chris Ashurst, Richard Kennedy, and Charlotte Houston were responsible for collecting samples from Haida Gwaii, and Dr. Puckett and Dr. Jason Munshi-South supplied the global reference samples. I was responsible for all data collection and analysis, and manuscript preparation. Dr. Puckett helped with data analysis, and all coauthors contributed feedback for manuscript preparation. This chapter is currently being drafted as a manuscript for submission to *Heredity*.

For Chapter 3, Dr. Russello, Dr. Robyn Irvine, and I were responsible for study design, with contributions from Dr. Adam Ford. Samples were collected by Parks Canada employees Goox Beaton, Chris Ashurst, Richard Kennedy, and Charlotte Houston. Dr. Irvine and Gregg Howald provided significant background and insights on the history of invasive rats in Haida Gwaii. I was responsible for all data collection and analysis, and manuscript preparation. All coauthors contributed feedback for manuscript preparation. This chapter is currently being drafted as a manuscript for submission to *Molecular Ecology*.

Throughout both chapters, Dr. Russello provided feedback and guidance across all aspects of the projects.

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I must thank all my family for their continued emotional support, especially my fiancée Theresa, you kept me sane throughout this whole process. Honestly, without your support, I would have never made it this far.

DEDICATION

To Donna. You would have loved to see this day. Love you more than a million purple smarties

Chapter 1: INTRODUCTION

1.1 Invasive species

An invasive species can be defined as an organism that has spread from its non-native range to occupy novel environments with negative consequences on the ecosystem (Clout, 2002; Facon *et al.*, 2006; Prentis *et al.*, 2008; Richardson & Pyšek, 2006). Historically, physical barriers (such as mountain ranges and oceans) have limited the dispersal capacity (and thus, the invasion potential) of most species; however, as humans began to disperse throughout the world, we both inadvertently and intentionally transported alien species with us, often without knowledge of the potential consequences (Mooney & Cleland, 2001). Invasive species represent a significant threat to global biodiversity, second only to habitat loss (Allendorf & Lundquist, 2003; Clavero & García-Berthou, 2005; Walker & Steffen, 1997). In fact, 52% of extinctions chronicled by the International Union for the Conservation of Nature (IUCN) attribute invasive species as a leading cause, with 20% of those extinctions having invasive species listed as the only cause. Furthermore, 27% of vertebrate species listed by the IUCN are considered threatened by invasive species (Bellard *et al.*, 2016). Invasive species threaten the stability and persistence of native flora and fauna through: (1) direct and indirect competition for resources; (2) compositional changes to local ecosystems; (3) additive levels of herbivory and/or predation; (4) introduction of novel pathogens and parasites; and (5) evolutionary impacts caused by introgression and hybridization (Gurevitch & Padilla, 2004; Mooney & Cleland, 2001).

In addition to ecological consequences, invasive species can also come with substantial economic costs. Pimentel (2011) estimated the yearly cost of damage and losses for invasive species was approximately $220 billion in the United States alone. These costs can be attributed to management of invasive populations, restoration of habitat and agricultural land, and losses to

crop yields and domestic livestock (Pimentel *et al.*, 2005). Due to the financial burden associated with invasive species, identification and development of effective strategies is vital to the successful management of the species.

1.2 Rats as invasive species

Brown rats (*Rattus norvegicus*) and black rats (*R. rattus*) are among the most invasive mammals worldwide, with global distributions that include every inhabited continent (Clout, 2002). They are found in nearly every habitat type ranging from hot, humid environments, such as tropical islands, to cold, arid environments, such as subarctic tundra (Jones *et al.*, 2008). Invasive rats are among a group of taxa that are thought to be responsible for most of invasive species-caused extinctions and are directly implicated as the main factor in at least 50 species extinctions (Gurevitch & Padilla, 2004; Howald *et al.*, 2007).

Several biological traits are responsible for the incredible invasive potential of brown and black rats. First, both species are generalist omnivores and can adapt to a wide variety of diet items. For example, rats have been reported to consume marine and terrestrial plants, alga, and animal species across all major phyla including aquatic and terrestrial invertebrates, and rarely consume only a single food source (Clark, 1982; Major *et al.*, 2007; St Clair, 2011). Moreover, their diet is relatively plastic, allowing the rats to adapt to low abundance of their primary diet item; during these times, shifting to a new food source has been observed (Caut *et al.*, 2008). In addition, both species have high reproduction rates. Litter sizes range between 3-11 pups, and a short gestation period of 20-24 days as well as almost year-round breeding (based on food availability) mean that a single female can potentially have many (>10) litters in any given year (Global Invasive Species Database, 2019b, 2019a; Woodside *et al.*, 1981). Furthermore, offspring are quick to reach sexual maturity and can reproduce in as little as six weeks (Clark &

Price, 1981). These feeding and reproductive abilities allow black and brown rats to adapt quickly and proliferate in a vast array of novel ecosystems (Jones *et al.*, 2008).

Once an invasive population is established, rats quickly begin to have detrimental impacts on the native flora and fauna. Rats are effective competitors and often outcompete native species both through indirect (*e.g.*, use of a shared resource) and direct (*e.g.*, predation) conflicts (Harris & Macdonald, 2007). Competition with native species can have several negative effects on ecosystems. On a species level, competition with invasive rats has led to population declines, range contractions, niche displacement, local extirpation, and even entire species extinctions (Atkinson, 1985; Clout, 2002). Rats can also have impacts on the ecosystem level. For example, Mulder *et al.* (2009) found that invasive rats on islands in New Zealand directly reduced seabird burrow densities which resulted in significant shifts in soil chemistry, ultimately resulting in reduced primary productivity. Also in New Zealand, the presence of invasive rats has led to whole community shifts in below-ground invertebrate populations, causing reduced soil fertility and a reduction in primary productivity (Fukami *et al.*, 2006). In extreme cases, indirect conflicts between invasive rats and invertebrate communities have led to entire regime shifts and forest collapse (Harper & Bunbury, 2015; Towns *et al.*, 2009). In most cases, rats have multiple negative impacts on invaded ecosystems with a net consequence of reduced native biodiversity (Clout, 2002).

1.3 Rats, islands, and seabirds

Not all ecosystems respond equally to a rat invasion. Ecosystems with a native rat species appear to be robust to rat invasions, likely due to the adaptation to a rodent predator by the endemic fauna; for the same reason, the presence of native predatory land crabs seems to similarly mitigate the effects of a rat invasion (Atkinson, 1985; Jones *et al.*, 2008). However,

ecosystems that have evolved in the absence of rats (or most other mammalian, terrestrial predators for that matter) respond poorly to the addition of invasive rats (Harper & Bunbury, 2015). Islands are particularly sensitive to invasive rats; native fauna are poorly adapted to cope with terrestrial predators, which are frequently limited or missing from the island environment (Harper & Bunbury, 2015).

Nesting seabirds are disproportionally affected by rat invasions, especially pelagic feeders, which leave their nests unguarded for prolonged periods of time while feeding in the open ocean (Atkinson, 1985; Jones *et al.*, 2008). While the adults are away feeding, rats will consume both eggs and chicks. For smaller-bodied seabirds, guarding the nest is not effective, as rats can predate upon the adults as well (Atkinson, 1985). Differences in prey behaviour also affect rat predation, where less aggressive bird species are prone to higher predation rates (Atkinson, 1985; Kepler, 1967). Indeed, invasive rats have been directly implicated in 60% of all seabird extinctions, with the majority occurring on islands (Howald *et al.*, 2007). Many seabirds have also developed ground-nesting or burrowing strategies due to the absence of terrestrial predators, exposing them further to invasive rat predation. As seabirds are significant vectors for nutrient transmission to the otherwise isolated insular ecosystems, invasive rats pose a serious risk not only to the seabirds themselves but also to ecosystem stability (Fukami *et al.*, 2006; Mulder *et al.*, 2009; Towns *et al.*, 2009).

1.4 Management of invasive rats

Management of highly fecund species, such as brown and black rats, can be acutely challenging, particularly with species with density-dependent population growth rates (Moe *et al.*, 2002; Pardini *et al.*, 2009; Zipkin *et al.*, 2009, 2008). Both brown and black rats have density-dependent growth rates and recover rapidly following severe reductions in population

size (Efford *et al.*, 2006; Emlen *et al.*, 1948). Management through exclusion systems and permanent trap fixtures, such as trap-crops and trap-barrier systems, can mitigate the damages caused by rats on commercial crops (Singleton *et al.*, 1998; Wang *et al.*, 2017); despite the success of such methods in agricultural settings, implementation of these methods in natural systems is an impractical solution. Long-term poison programs aimed at reducing rather than removing invasive rat populations are ineffective due to an increase in reproduction at low densities as well as the evolution bait-resistance or neophobia (Damin-Pernik *et al.*, 2017; Parsons *et al.*, 2017; Takács *et al.*, 2016). In most cases, complete removal through eradication (often using rodenticide) is the only viable method for managing invasive rat populations (Stenseth *et al.*, 2002). Fortunately, eradication of rats can often lead to species and full ecosystem recovery in a relatively short time period (Jones, 2010; Le Corre *et al.*, 2015). Considering the devastating impacts of invasive rats on island ecosystems, complete removal should always be the highest priority to best conserve native biodiversity (Fukami *et al.*, 2006; Mulder *et al.*, 2009; Towns *et al.*, 2009).

1.5 Haida Gwaii, British Columbia

Haida Gwaii is an archipelago consisting of approximately 100 smaller islands and two, main larger islands located 100 km off the northern coast of British Columbia. It is often likened to the Galápagos Islands due to high levels of unique biodiversity, rare species, and endemism (Gaston *et al.*, 2008; Golumbia, 1999). Haida Gwaii likely acted as a glacial refugium during the Pleistocene era that allowed for these endemic species to evolve, though this hypothesis has been met with debate (Byun *et al.*, 1997; Demboski *et al.*, 1999). Dozens of species are listed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as vulnerable to endangered (Golumbia, 1999). In addition, Haida Gwaii represents a significant breeding site for

1.5 million seabirds across twelve species, in some cases representing large proportions of the total species' population (Harfenist, 2003); for example, it is estimated that 50% of the global breeding population of ancient murrelet (*Synthliboramphus antiquus*), and one-fifth of the breeding population of Cassin's aucklet (*Ptychoramphus aleuticus*), are located within Haida Gwaii. As such, any threats to these breeding colonies are not only a local issue, but one of species existence. Not surprisingly then, biological invasions have been identified as one of the top threats to biodiversity in Haida Gwaii, and both proactive and responsive steps have been taken to mitigate the potential impact of invasive species within the archipelago (Gaston *et al.*, 2008).

Both black and brown rats have invaded Haida Gwaii. Black rats are thought to have first invaded Haida Gwaii from European ships in the 1700s with the first recorded occurrence in 1908 (Gaston *et al.*, 2008; Harrison, 1925). Brown rats arrived sometime in the late 1800s to early 1900s, though the first confirmed record did not occur until 1981 (Bertram & Nagorsen, 1995; Gaston *et al.*, 2008). As seen on many other oceanic islands, brown rats have begun to displace black rats in Haida Gwaii, a phenomenon particularly noticeable on the islands of Kunghit and Lyell (Atkinson, 1985; Bertram & Nagorsen, 1995). This displacement likely stems from the larger body size of the brown rats compared to the black rats; a competitive advantage is positively correlated with increasing body size in small mammals (Brannon, 2000; Fox & Kirkland, 1992; Harper *et al.*, 2005). In addition, brown rats are better adapted than black rats to cold, wet environments, another benefit of larger body size (Harper *et al.*, 2005). Brown rats are almost exclusively terrestrial animals and will come into more frequent contact with seabird nests and burrows than the semi-arboreal black rats, allowing them to be the stronger competitors over the seabird-related food supply (Thorsen *et al.*, 2000).

Since their arrival, rats from both species have had devastating impacts on native sea bird populations in Haida Gwaii and have contributed to population declines for the ancient murrelet, Cassin's auklet, fork-tailed storm petrel (*Oceanodroma furcate*), Leach's storm petrel (*O. leucorhoa*), rhinoceros auklet (*Cerorhinca monocerata*), and tufted puffin (*Fratercula cirrhata*) (Gaston *et al.*, 2008; Harfenist, 2003). These include extirpations of historically-large and successful breeding sites; for example, Langara Island, located at the northern most tip of the archipelago, used to host one of the largest multi-species seabird colonies in British Columbia: since the introduction of both brown and black rats, many seabird species have been completely eliminated from island, and those that remain exist at a fraction of historical population numbers (Harfenist, 2003). Langara Island is not exceptional in this regard; large breeding colonies have also been reported as extirpated or severely declined on as many as eight islands throughout the archipelago (Harfenist, 2003). In addition to seabirds, invasive rats are suspected to have caused the extirpation of a native deer mouse (*Peromyscus maniculatus*) from Langara Island, and have also been implicated in population declines in several native shrews and other bird species (Gaston *et al.*, 2008; Kaiser, 1997). On the Bischof Islands, populations of deer mice have been extirpated, and a sharp decline in the native dusky shrew (*Sorex monitcolus*) has been observed (Howald, 2012). Due to the significant and pervasive impacts of invasive rats on native species, active management of rat populations has become necessary to preserve ecosystem health.

1.6 Genomic analysis as a tool for invasive species management

With the advent of next-generation sequencing, genomic analysis has never been more powerful and more accessible for the management of invasive species. A quick search of the keywords *invasive species genomics* on Web of Science show there has been a twenty-fold increase in citations between the years 2008 and 2018. Genomic analyses have many utilities

within invasive species management ranging in scale from population- and individual-level investigations to range-wide and whole-species research (Rius *et al.*, 2015). Genomic and genetic analyses have been used to address single population concerns, such as determining the cause of a failed eradication (Abdelkrim *et al.*, 2007; Amos *et al.*, 2016; Russell *et al.*, 2010; Savidge *et al.*, 2012). Knowing the cause of an eradication failure helps create more successful eradication attempts in the future. Genomics and genetics have also been used to describe patterns of gene flow among invasive populations (Abdelkrim *et al.*, 2005; Robertson & Gemmell, 2004; Savidge *et al.*, 2012). An understanding of both the degree and direction of migration among populations gives managers information to safeguard against re-invasion post-eradication. Similarly, correlations between gene flow and environmental variables can help elucidate potential barriers or even vectors of gene flow, thereby expanding our knowledge on the spread and invasive potential of non-native species through novel environments (Arim *et al.*, 2006; Patton *et al.*, 1975; Prentis *et al.*, 2008). On a broader level, genomics can give us information on the historical origins (*i.e.* the source) of an invasive population, again providing insights into the factors associated with dispersal of an invasive species (Aplin *et al.*, 2011; Matisoo-Smith & Robins, 2009; Puckett *et al.*, 2016). In most cases, genomics offers valuable information on invasive species biology that can lead to more effective management.

1.7 Study Objectives

Currently, little is known about invasive rat movements throughout Haida Gwaii. This represents a key knowledge gap needed to make effective management decisions concerning the control of invasive rats. Consequently, this study looks to investigate these movement dynamics by:

1. Investigating patterns of population connectivity and inferring levels and direction of gene flow among invasive rat populations in Haida Gwaii.

2. Identifying the source of a re-encountered population on the Bischof Islands following a putative island-wide eradication, testing whether these animals are re-established by a neighbouring population (*i.e.* re-invasion), a re-emergence of individuals that survived the eradication attempt (*i.e.* survivors), or a combination of both. Additionally, we aim to identify the source of novel brown rat invasions on Faraday, Murchison, and Hotspring Islands, which historically have been free of brown rats.

3. Determining the historical origin(s) of brown rats in Haida Gwaii using a global population genomic database.

Chapter 2: GLOBAL ORIGIN(S) OF BROWN RATS (*RATTUS NORVEGICUS*) IN THE HAIDA GWAII ARCHIPELAGO

2.1 Background

Though brown rats (*Rattus norvegicus*) are now a cosmopolitan species, their ancestral range has long been speculated. Based on L1 retrotransposon DNA, brown rats likely evolved approximately 0.5 million years ago, and fossil evidence points to a northern China and Mongolia origin (Smith *et al.*, 2010; Verneau *et al.*, 1998). Recent genomic studies best support a Chinese origin, though other studies indicate a southeast Asia source (Puckett & Munshi-South, 2018; Zeng *et al.*, 2018). From their ancestral range, brown rats spread commensally with humans throughout eastern and southeastern Asia and upwards into Russia ~700 years ago (ya) (Puckett & Munshi-South, 2018; Puckett *et al.*, 2016). Further reconstructions suggest they were transported through trade routes across the Pacific Ocean to the Aleutian Islands and western North America (~20,000 ya), as well as westward through the Middle East and later into western Europe (~550 ya). The western European populations expanded upwards into northern Europe (~535 ya). North America and South America were subsequently colonized (~500 ya) as European nations voyaged across the Atlantic Ocean and colonized the Americas.

The spread of brown rats throughout the world includes a large proportion of oceanic island systems; it is estimated that brown rats are now established on 80% of these archipelagos, often with devastating consequences on the native inhabitants (Atkinson, 1985; Harper & Bunbury, 2015). Anecdotal evidence suggest that brown rats invaded Haida Gwaii in the late 19th or early 20th century, though the first confirmed record of brown rats in Haida Gwaii wasn't until 1981 (Bertram & Nagorsen, 1995; Gaston *et al.*, 2008). Since their arrival, brown rats have caused multiple extirpations of large, multi-species breeding colonies, and this has led to

population declines in six of the twelve seabird species present, including the ancient murrelet and Cassin's auklet (Gaston *et al.*, 2008; Harfenist, 2003).

As rats are having such an extreme impact on seabird populations in Haida Gwaii, these invaders must be actively managed to achieve seabird species recovery. Part of this management includes preventing invasion of brown rats to novel parts of the archipelago. Since invasion has been shown to be a regulated process, knowing the factors that limit the spread of invasive rats in other ecosystems could lead to effective management strategies (Arim *et al.*, 2006). In this way, knowing the global source population(s) of the invasive rats in Haida Gwaii could be used to develop biosecurity measures to prevent invasion to new islands, effectively creating "safe havens" for seabirds to reproduce and recover. Additionally, an understanding of the shared genetic diversity among source and invasive populations could be used to develop predictors of invasion so that further spread can be prevented (Prentis *et al.*, 2008). In the case of Haida Gwaii, identification of routes of continued introduction can be used to develop biosecurity protocols, which are currently absent with respect to air and boat travel to and from mainland BC. It is only after these routes have been identified that targeted preventative management can be developed and implemented. Using genomic tools, managers can potentially elucidate the historical source of brown rats in Haida Gwaii and elsewhere, and they can use this information to manage invasive rat populations better, as well as gain new insights on invasive rat dispersal through a novel ecosystem (Aplin *et al.*, 2011; Matisoo-Smith & Robins, 2009; Puckett *et al.*, 2016).

This study aimed to identify the global origin(s) of brown rats in Haida Gwaii using population genomic analyses. A previous study by Puckett *et al.* (2016) examined the global genetic diversity of brown rats across their distribution, including a subset of samples (*n*=17)

from Haida Gwaii. In this case, all samples from Haida Gwaii consistently clustered into a single, unique genetic unit across analyses, suggesting a single source population; however, the small sample size and limited sample distribution, precluded the identification of the historical source of brown rats in Haida Gwaii.

Here, we used double-digest restriction site-associated DNA sequencing (Peterson *et al.*, 2012) to identify and genotype single nucleotide polymorphisms (SNPs) for brown rats sampled in Haida Gwaii, BC. We combined our genotype data with a globally compiled brown rat dataset to identify the putative origin of brown rats in Haida Gwaii using both traditional methods and approximate Bayesian computation to compare competing invasion models.

2.2 Methods

2.2.1 Study site and sample collection

All sample collection was performed by Parks Canada staff from 2008-2018. A total of n=288 brown rats were sampled across 12 islands throughout Haida Gwaii, and one sample was collected from Prince Rupert, the closest port on mainland BC (Figure 2.1). Most individuals were live trapped using Tomahawk collapsible traps, with some individuals collected during island-wide carcass searches following an eradication attempt. Sample locations were also chosen to be proximate to nesting seabird habitat. Traps were deployed along the shoreline at approximately 30-m intervals above the high-tide mark and partially concealed with moss and bark to prevent tampering by predatory birds (*e.g.*, ravens, eagles) and to keep rats dry once captured. As rats are primarily nocturnal, the traps were set in the late afternoon to evening period and baited with a combination of canned sardines and the commercially available rodent attractant Provoke (Bell Laboratories, Inc.) and checked the following morning. This process was repeated for up to 3 days for each sampling excursion at each location. Upon successful

capture, rats <250g were first anesthetized using isoflurane, then euthanized via cervical dislocation; rats >250g were euthanized with a strong dose of isoflurane. Whole ears were removed for downstream genomic analysis. Sample collection was performed in accordance with the internal Parks Canada Animal Care Guidelines. Additionally, genotypic data for *n*=11 Haida Gwaii samples sequenced by Puckett *et al.* (2016) were downloaded from the Dryad Digital Repository and included in these analyses.

2.2.2 DNA extraction and library construction

Whole genomic DNA (gDNA) was extracted from 10-20 mg of dried ear tissue using the Qiagen DNeasy® Blood and Tissue Kit and treated with RNase A (5PRIME) following the manufacturer's protocol. Double-digest restriction enzyme associated DNA sequencing (ddRAD) libraries were constructed using a modified protocol described by Puckett *et al.* (2016; see also Peterson *et al.* 2012). Approximately 1µg of gDNA was digested from each individual using the restriction enzymes MluCI and SphI-HF (New England Biolabs® Inc.), and a unique combinatorial barcode and index (New England Biolabs® Inc) was ligated onto the 5' and 3' ends of the resultant fragments, respectively. Barcoded individuals were pooled in equimolar concentrations into libraries (*n*= 96 individuals/library); subsets of these samples were replicated both within (*n*=7) and among (*n*=9) libraries to assess genotyping error. Approximately 400bp fragments were size-selected using a Pippin Prep™ (Sage Science), and the size selected libraries were PCR amplified for 12 cycles using Phusion PCR reagents (New England Biolabs® Inc). Final libraries were then sequenced on the Illumina HiSeq 2500 PE125 platform (125bp, paired-end).

2.2.3 Data processing and SNP genotyping

Reads were demultiplexed and assigned to individuals using the command *process_radtags* in the program STACKS v.1.48 (Catchen *et al.* 2013); during this process, barcodes and indices were removed, and the reads were trimmed to 100bp to remove low-quality bases at the 3' ends. Processed reads were then aligned to the brown rat reference genome (Rnor_6.0, GenBank assembly accession: GCA_000001895.4) using the software Bowtie 2 v.2.2.9 (Langmead & Salzberg, 2012). A minimum of 40% alignment to the reference genome was employed as a form of quality control, and individuals failing to meet this threshold (*e.g.*, due to poor sequence quality, failed adapter ligation, or DNA amplification from a non-target species such as bacteria) were removed from downstream analysis.

To connect our dataset with the global dataset (and to infer the historical origin(s) of brown rats in Haida Gwaii), we compiled a position list of 31,878 single nucleotide polymorphisms (SNPs) identified by Puckett *et al.* (2016; 2018) and used them to define evolutionary relationships among global brown rat populations. Genotypes for these SNPs were extracted from aligned sequence data using the *mpileup* command of SAMtools v.1.9 (Li, 2011; Li *et al.*, 2009). Variants that were genotyped in fewer than 80% of all individuals or had a minor allele frequency less than 1% were removed using VCFtools v0.1.14 (Danecek *et al.*, 2011), resulting in a final dataset of 12 433 SNPs. Mean depth of coverage across loci per individual as well as mean missing data was assessed using VCFtools; individuals with a mean depth <6x were removed from downstream analysis.

2.2.4 Reference populations and validation of SNP dataset

To develop reference populations and to validate the SNP dataset, we used genotypic data collected from global rat samples used in previous studies on global brown rat phylogeny

(Puckett *et al.*, 2018, 2016). We removed populations sampled from Africa, Galápagos Islands, New Zealand, and Lincoln City, IN, as these populations were highly drifted and represented a low probability as a source population for brown rats in Haida Gwaii. Additionally, rats sampled from Ketchikan, AK and Pigeon Forge, TN were removed, as each of these populations had an n=1, and thus were likely not representative of the genetic diversity of those areas. Rats sampled from Coastal Alaska (less those from Sitka and the Aleutian Islands) were removed, as these samples were identified as lab specimens and not wild rats (E. Puckett, pers. comm.). The remaining samples were then grouped based on geography (which, in this case, also mimicked genetic ancestry; see Puckett *et al.* 2016; Puckett & Munshi-South, 2018) for a final reference set containing n=330 individuals grouped into the following ten populations: China (CHI), southeast Asia (SEA), Aleutian Islands (ALN), western Europe (WER), northern Europe (NER), western North America (WNA), eastern North America (ENA), South America (SAM), San Diego, USA (SDN), and Vancouver, Canada (VAN) (Appendix A).

To ensure that our subset of 12,433 SNPs had the same explanatory power as the full dataset in Puckett *et al.* (2016; 2018), we compared standard measures of genetic diversity across datasets using GenoDive v.2.0b27 (Meirmans & Tienderen, 2004). We also compared model-based clustering results using the program ADMIXTURE v.1.3.0 (Alexander *et al.*, 2009) across datasets to ensure that population structure remained equivalent across datasets at k=10 genetic clusters.

2.2.5 Clustering-based assessment to infer global origin(s)

We used several different clustering-based techniques to identify the putative origin of brown rats in Haida Gwaii. First, we used the R statistical package SNPRelate (Zheng *et al.*, 2012) to conduct principle component analysis (PCA) based on genotypic data. We also conducted a PCA

using the *smartpca* function in the software package EIGENSOFT v.6.1.4 (Galinsky *et al.*, 2016; Patterson *et al.*, 2006; Price *et al.*, 2006) in which we first defined the parameter space using only the reference samples, then projected the Haida Gwaii samples onto this parameter space. This process ensured that the Haida Gwaii samples would cluster within the reference populations and provide a clearer picture of global origin(s).

We used ADMIXTURE to assess coancestry within a model-based Bayesian clustering framework. We initially assessed *a priori* population structure for number of genetic clusters (*k*) ranging from 1-50, identifying the optimal number of clusters *k* by minimizing the mean cross-validation error for each *k* over 10 iterations. We summarized these 10 independent runs using *CLUMPP* v1.1.2 (Jakobsson & Rosenberg, 2007). For this analysis, only a subset of Haida Gwaii samples (*n*=150) were used to avoid over-structuring of Haida Gwaii populations relative to the reference dataset. Additionally, we used the supervised mode (using all samples from Haida Gwaii, as the number of clusters is pre-defined), which allowed us to pre-define the 10 reference populations (Puckett *et al.*, 2018, 2016) and then estimated coancestry of the Haida Gwaii samples with those populations averaged over 20 iterations.

We also used the snmf() function from the R-package *LEA* v2.0 (Frichot & François, 2015) to estimate population structure. This function uses sparse non-negative matrix factorization to estimate individual ancestry coefficients from a genotypic matrix. We examined *k*=1-30 with 20 iterations for each *k.* We plotted the cross-entropy criteria for each *k* and identified the "elbow" as the optimal number of clusters and summarized iterations using *CLUMPP*.

2.2.6 Coalescent modelling of global origin(s)

In addition to clustering-based approaches, we also used coalescent modelling to infer the global origin(s) of brown rats in Haida Gwaii using the software DIYABC v2.1.0 (Cornuet *et al.*, 2014). This approach uses approximate Bayesian computation to model competing historical demographic scenarios based on current allele frequencies. We identified three distinct genetic clusters within Haida Gwaii; as such, we used a single representative population from each cluster for computation efficiency and to prevent biased model results. We used the Tlell population to represent the northern cluster, the Lyell population to represent the central cluster, and the Kunghit population to represent the southern cluster, as these showed admixture with all surrounding populations. We considered each of these representative subsets as discrete populations within Haida Gwaii for all demographic models. We used the WNA, VAN, and WER reference populations as putative sources, as upstream analysis indicated these to be the most likely origins of rats in Haida Gwaii.

In total, twelve models were compared in this analysis (Appendix A). We evaluated each reference population contributing to independent invasions of each Haida Gwaii cluster. We also considered a single invasion event (from each putative source) with subsequent colonization of the remainder of the archipelago. For these models, we varied the initial point of invasion among the three Haida Gwaii clusters. We constrained the timing of all Haida Gwaii invasion events to be between 100-1000 generations ago. Previous research has shown that brown rats colonized western North America ~500 years ago from western Europe, with subsequent colonization of Vancouver, BC, Canada from western North America (Puckett & Munshi-South, 2018).

Consequently, we used this scenario for all model comparisons. Maximum-likelihoods across scenarios were compared to determine the best supported scenario.

2.3 Results

2.3.1 Dataset quality

Fifteen individuals were removed during depth and alignment filtering, resulting in a final dataset of *n*=298 high quality samples. Mean sequencing depth across individuals was 19.7x (SD = 11.7) and mean percent missing data was 3.04% (SD = 1.86%). The estimated genotyping error rates within and among libraries were 3.92% (SD = 2.59%) and 4.80% (SD = 2.96%), respectively. Genetic diversity estimates for the subset dataset (12 433 SNPs) were slightly higher than the full dataset (31,878 SNPs), though there was minimal to no change in the genetic clusters inferred at *k*=10 (Appendix A). As such, we proceeded with the 12,433 SNP dataset for all downstream analyses.

2.3.2 Principle component analyses

PCA using SNPRelate clustered Haida Gwaii samples discretely from reference populations, with the first two components largely describing within-archipelago variation rather than variation compared to the reference populations (Figure 2.2a). Additionally, three distinct regional clusters (north, central, and south) were detected within Haida Gwaii (Figure 2.2b). Rats sampled from islands in the southern tip of the archipelago (*i.e.*, Kunghit, Ellen, and Rainy) formed one of the clusters; rats sampled from islands on the east-central coast of the archipelago formed another genetic unit (*i.e.*, Lyell and surrounding islands); and finally, rats sampled from Tlell on Graham island as well as the rat sampled from Prince Rupert on mainland BC formed their own genetic cluster. The PCA using the reference-defined parameter space detected a single

Haida Gwaii cluster distributed among North American, South American and western European reference populations (Figure 2.3).

2.3.3 Clustering-based approaches

Unsupervised analysis using ADMIXTURE best supported a k=35 (Appendix A). All the Haida Gwaii populations clustered separately from all reference populations; additionally, within-Haida Gwaii structure was detected, with populations largely clustering by island (Figure 2.4). We had similar results using *LEA*, though we identified a much lower optimal k=12, with support for k=10 through 15 (Appendix A). Haida Gwaii populations again clustered separately from the reference populations, and we were able to detect the same regional populations as identified in the first PCA (Figure 2.5; see Figure 2.2 for PCA).

For the supervised ADMIXTURE analysis, we also detected regional populations within Haida Gwaii with varying coancestry to reference populations (Figure 2.6). The Tlell-Prince Rupert population showed signs of ancestry primarily with western North America, with smaller signals from eastern North America, South America, and western Europe. The central population (Lyell and surrounding islands) primarily pointed to an introduction from San Diego, though there was some evidence of admixture with the Aleutian Islands and Vancouver populations. Finally, the southern cluster (Kunghit, Rainy, and Ellen Islands), showed the most admixture with the Vancouver reference population, with smaller degrees of ancestry with the Aleutian Islands and South America. Examination of individual iterations revealed that the regional populations were not admixed among reference populations, and rather showed an "all or none" pattern of ancestry to any one population, though individuals within a regional population always clustered together across runs.

2.3.4 Coalescent modelling

Coalescent modelling indicated that three independent invasions from western European origin was best supported, and there was near equal support for a single invasion from western Europe into northern Haida Gwaii with subsequent local colonization (Figure 2.7). There was also moderate support for a western European invasion to central Haida Gwaii with subsequent dispersal throughout the archipelago.

2.4 Discussion

Genomic tools are increasingly used to inform invasive species management practices. One such application is to identify the global origin of the invaders, which can be useful in several ways. First, identification of the historical source could identify a need for biosecurity at a specific location; for example, identifying a seaport associated with trade among nations as a point of invasion would allow for better screening of incoming shipments to and from that location (Brouat *et al.*, 2014). Second, identifying the number of invasions can also be useful, as each discrete invasion adds to propagule pressure and contributes to an increased invasiveness of the new species (Arim *et al.*, 2006; Guillemaud *et al.*, 2010; Prentis *et al.*, 2008). Invasions from multiple sources also leads to an increase in the rate of the range expansion of invasive species' (Wagner *et al.*, 2017). Finally, understanding the dynamics of past invasions can be used to evaluate risks of invasion and develop better strategies to prevent future invasions (Hulme, 2009).

For this study, we were unable to identify a source population for brown rats in Haida Gwaii using both standard PCA and Bayesian clustering methods (Figure 2.2; Figure 2.4-8). The supervised clustering analysis (Figure 2.6) did indicate substantial coancestry with North and South American populations, as well as some ancestry with western Europe. With this method

though, the unknown samples must assign to at least one of the reference populations (*i.e.*, there is no option to cluster independently from the reference populations). This method inherently assumes that the true ancestral population is included in the reference dataset. While it appears there is admixture from multiple source populations, inspection of individual runs shows an "all or none" pattern of assignment; for example, one iteration of the analysis indicated complete coancestry of rats sampled from Tlell and Prince Rupert to eastern North America, but when coancestry was averaged across all twenty iterations, Tlell and Prince Rupert only showed ~5% coancestry to eastern North America. The inconsistency of assignment across iterations is more likely an indication of poor assignment to any of the reference populations using this method.

The appropriateness of traditional population genetic analyses to identify historical origins of invasive species (as implemented here) has recently been called into question based on several limitations (Brouat *et al.*, 2014; Guillemaud *et al.*, 2010; Lombaert *et al.*, 2011). These methods do require that the true ancestral population be sampled, which is difficult, if not impossible, to assess *a priori*. While putative source populations can be hypothesized based on anecdotal evidence, there is no guarantee that these hypotheses are reflective of the actual invasion event. Even if the true source population has been sampled, the number of samples may be too low to accurately reflect the total genetic diversity within that population. This unsampled diversity could lead to the historical and invaded populations to seem genetically distinct and could even lead to erroneous assignment to a putative source. Invasions usually only occur by a small number of individuals as well, which can lead to founder effects and leave the newly established population sensitive to the genetic consequences of small population sizes, including drift (Mayr, 1963; Nei *et al.*, 1975). Invasion from multiple source populations can also lead to novel genetic structure to such an extent that the newly established population is radically

dissimilar from any source population, especially when compounded with the consequences of drift (Keller *et al.*, 2012). Lastly, these methods offer limited comparison among competing invasion scenarios; thus, distinguishing real from artificial historical events can be problematic.

Despite the short-comings of traditional methods, we were able to consistently identify structure among three regions within Haida Gwaii. These three regional populations largely corresponded with geography; rats sampled from the southern islands of Kunghit, Ellen, and Rainy consistently formed a cluster, those sampled from islands on the east-central coast formed a second cluster, and finally, rats sampled from Tlell on Graham Island and Prince Rupert on mainland BC formed a northern cluster. Each of these clusters are geographically distant from one another: the North and Central regional populations are separated by a Euclidean distance of approximately 100 km and the Central and South populations by approximately 70 km. Most of this distance is covered by ocean; moreover, the shorelines along which rats could travel (namely, Moresby and Graham Islands) are quite jagged, greatly increasing the total terrestrial distance among these regions. Both pathways pose significant challenges to unaided rat dispersal and migration, and any movements among these regions likely would have to be human-facilitated (*i.e.*, via boats). Though we found support for low levels of gene flow in the coalescent models, these likely are signatures of rats transported by boat traffic along the coast and represent very minimal physical relocations of rats among regions. While these three regional populations may represent three discrete introductions to Haida Gwaii, they also could be the product of local processes, such as isolation-by-distance, genetic drift, or bottleneck and founder effects; for the reasons mentioned previously, inferences about global origin based upon these traditional analyses may have limited support.

More complex invasion scenarios can be examined using coalescent modelling (Keller *et al.*, 2012). With this approach, scenarios that incorporate multiple introductions (either serial or independent), changes in population size, admixture of multiple sources, and gene flow among populations can all be modelled, and these models can be directly compared, allowing for explicit evaluation of competing scenarios (Excoffier *et al.*, 2013). We modelled various invasion scenarios for brown rats in Haida Gwaii using the methods implemented by DIYABC v2.1.0 (Cornuet *et al.*, 2014). We found that the best supported models indicated that the brown rat population in Haida Gwaii was most likely founded from a western European source population (Figure 2.7). Additionally, we found evidence for a single introduction into Haida Gwaii followed by dispersal. This result contrasted with the traditional methods we used, in which three distinct regional clusters within Haida Gwaii were consistently indicated with a western North American origin; however, the American populations were founded with the expansion out of Europe and remain genetically similar to the western European population (Puckett *et al.*, 2016; Puckett & Munshi-South, 2018), possibly explaining the support for both European and American invasions. Additionally, isolation among the three regional clusters coupled with strong founder effects could have led to the formation of genetically distinct clusters within Haida Gwaii. More investigation of within-island dynamics as well as exploration of more varied invasion scenarios is needed to better elucidate the history of these populations.

2.5 Tables & Figures

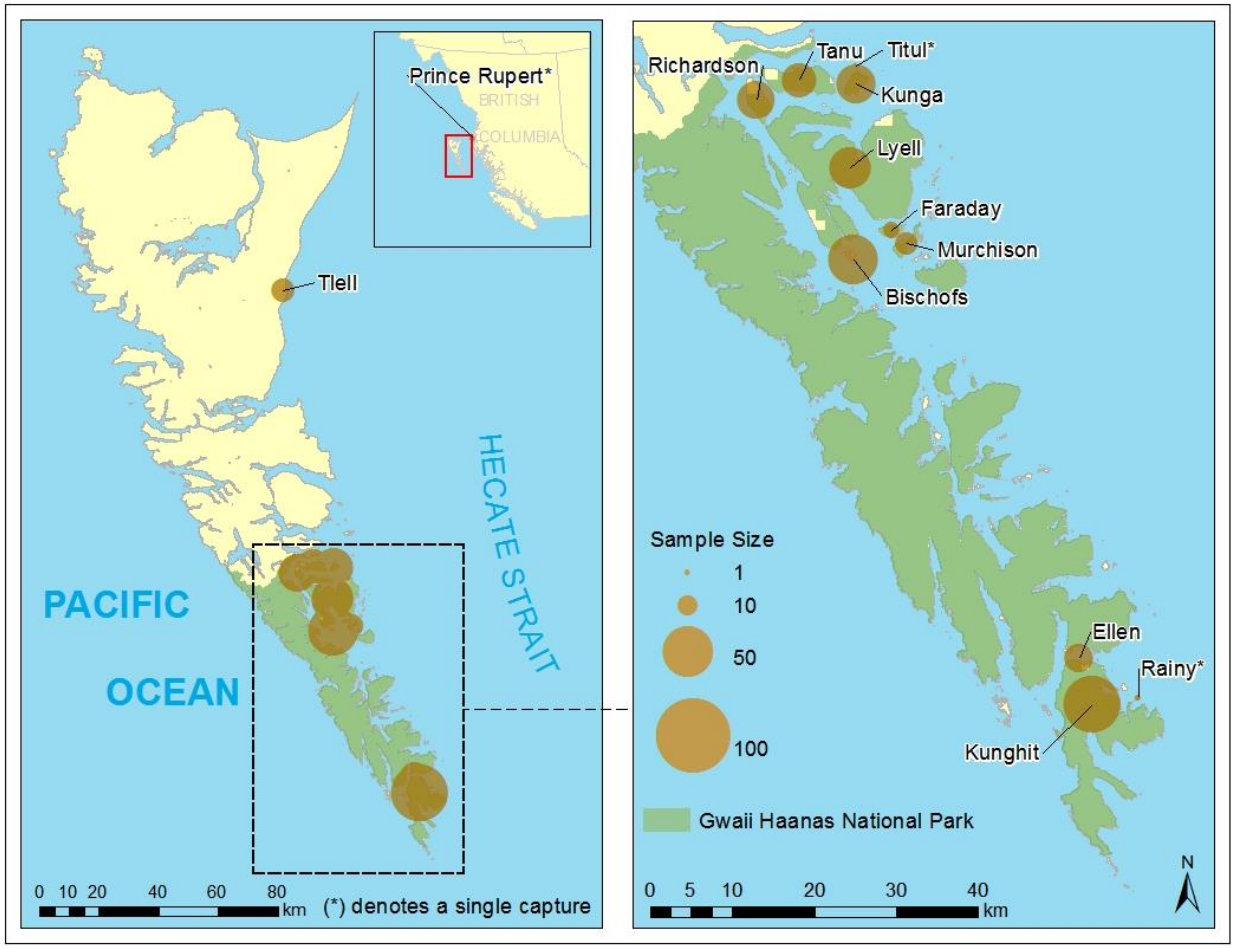

Figure 2.1. Sample sizes and location of brown rats (*R. norvegicus*) collected across Haida Gwaii, BC. All rats were lethally sampled and used in later genomic analysis.

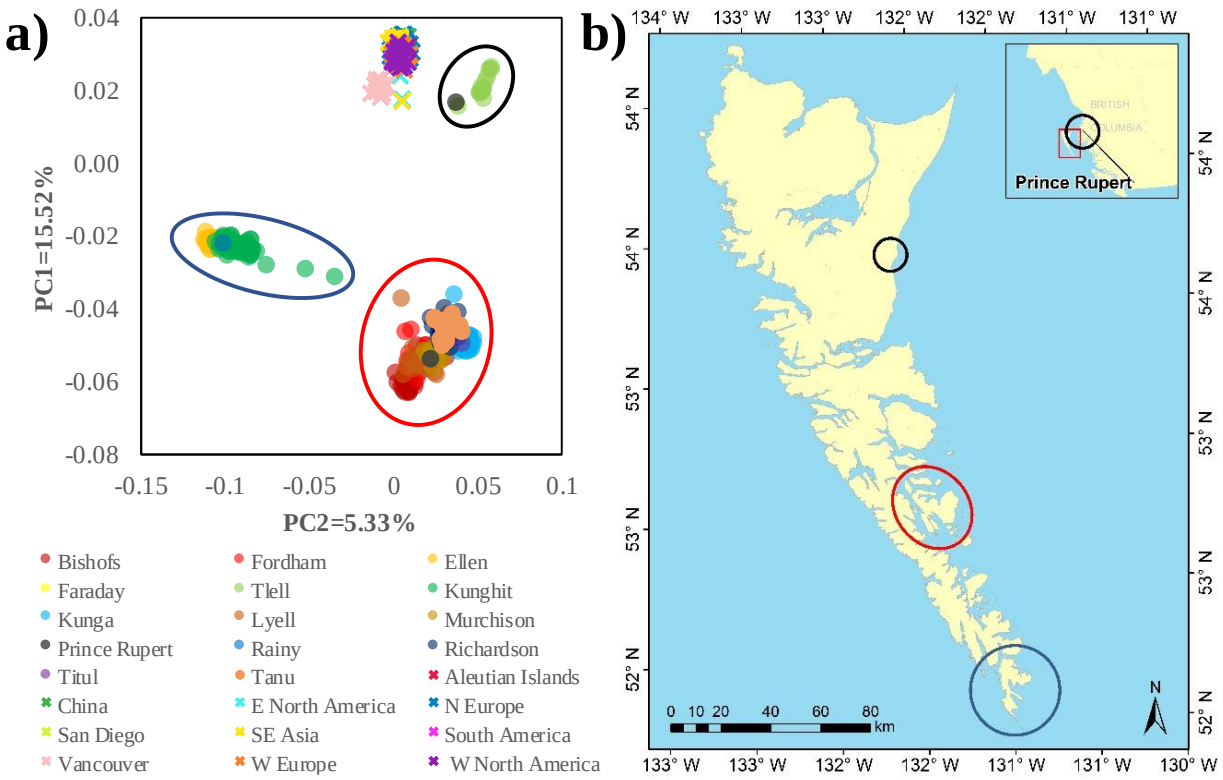

Figure 2.2. Principle component analysis (a) for globally-sampled brown rats. PCA was carried out using the R-package *SNPRelate* (Zheng *et al.*, 2012) using genotypic data from *n*=12 433 single nucleotide polymorphism nuclear markers. Filled circles represent samples collected within Haida Gwaii, BC (*n*=299), and crosses indicate samples sourced from a global reference dataset (Puckett, 2018a, 2016; *n*=330). Three regionally-distinct populations were identified within Haida Gwaii (b), indicated by the blue (southern), red (central), and black (northern) ovals.

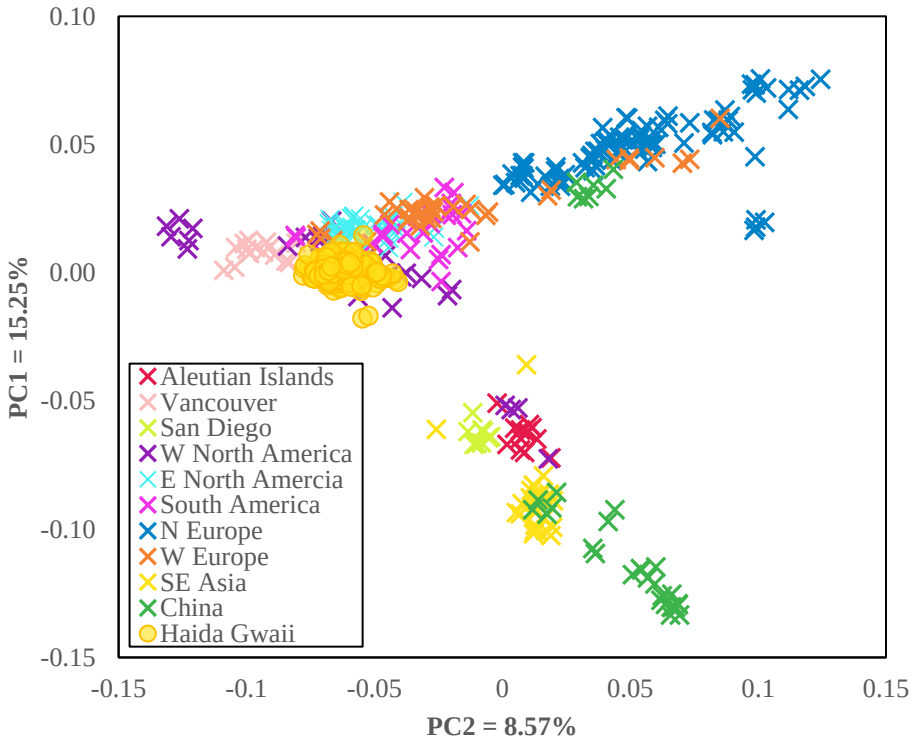

Figure 2.3. Projected principle component analysis for globally-sampled brown rats. PCA was carried out with EIGENSOFT v.6.1.4 (Galinsky *et al.*, 2016; Patterson *et al.*, 2006; Price *et al.*, 2006) using genotypic data from *n*=12 433 single nucleotide polymorphism nuclear markers. Filled circles represent samples collected within Haida Gwaii, BC (*n*=299), and crosses indicate samples sourced from a global reference dataset (Puckett, 2018a, 2016; *n*=330). For this analysis, the parameter space was first defined using only the reference dataset, then the Haida Gwaii samples were projected onto the parameter space.

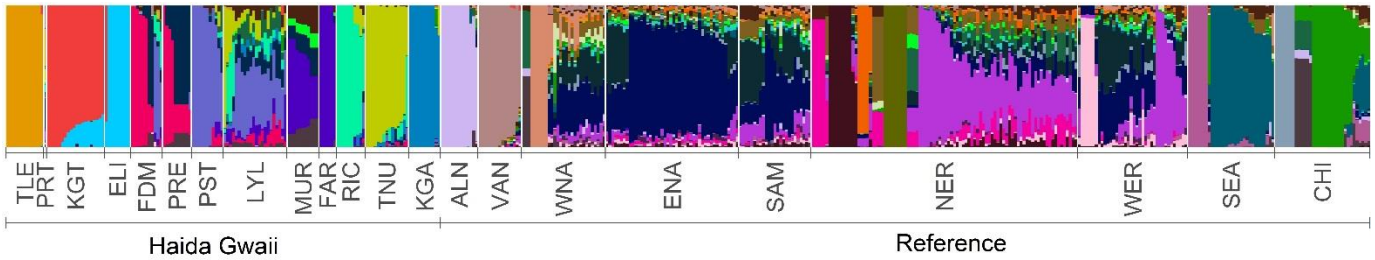

Figure 2.4. Unsupervised Bayesian clustering analysis, implemented by the software ADMIXTURE (Alexander *et al.*, 2009), for a globally-sampled brown rat dataset (*n*=480), sampled either from Haida Gwaii, BC (*n*=150) or collected from a previously compiled reference dataset (Puckett, 2018a, 2016; *n*=330). Genotypic data for *n*=12 433 single nucleotide polymorphism nuclear markers were used as inputs. An optimal *k*=35 was identified. Each vertical bar represents an individual and each colour represents a unique genetic cluster. The degree of colour for each individual represents the proportion of shared ancestry with that genetic cluster. Abbreviations for the "Haida Gwaii" samples indicate individual sample islands or sample locations and are defined as follows: (TLE) Tlell, BC; (PRT) Prince Rupert, BC; (KGT) Kunghit Island; (ELI) Ellen Island; (RNY) Rainy Island; (FDH) Fordham (sourced from Puckett *et al.* 2016); (PRE) Bischof Islands, pre-eradication; (PST) Bischof Islands, post-eradication; (LYL) Lyell Island; (FAR) Faraday Island; (MUR) Murchison Island; (RIC) Richardson Island; (TNU) Tanu Island; (KGA) Kunga Island; and (TTL) Titul Island. See Appendix A for "Reference" abbreviation definitions.

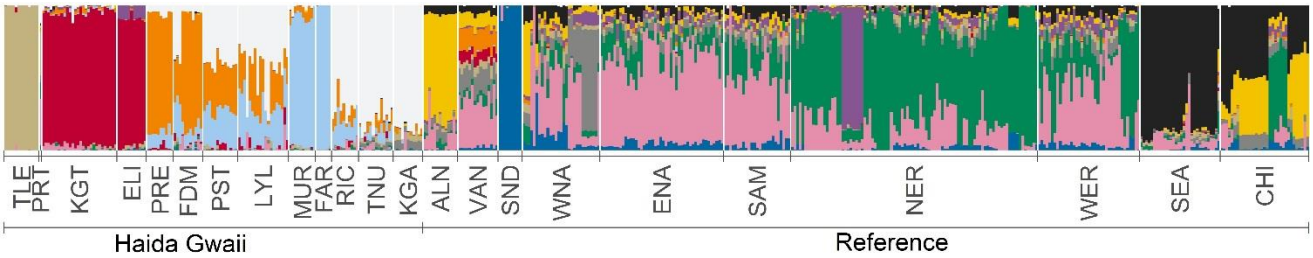

Figure 2.5. Shared ancestry coefficients for a global dataset of brown rats sampled either from Haida Gwaii, BC (*n*=150) or collected from a previously compiled reference dataset (Puckett, 2018a, 2016; *n*=330). Ancestry coefficients were estimated using sparse non-negative matrix factorization implemented by the R-package *LEA* (Frichot & François, 2015). Genotypic data for *n*=12 433 single nucleotide polymorphism nuclear marks were used as inputs. An optimal *k*=12 was identified. Each vertical bar represents an individual and each colour represents a unique genetic cluster. The degree of colour for each individual represents the proportion of shared ancestry with that genetic cluster. See Appendix A and Figure 2.4 for abbreviations.

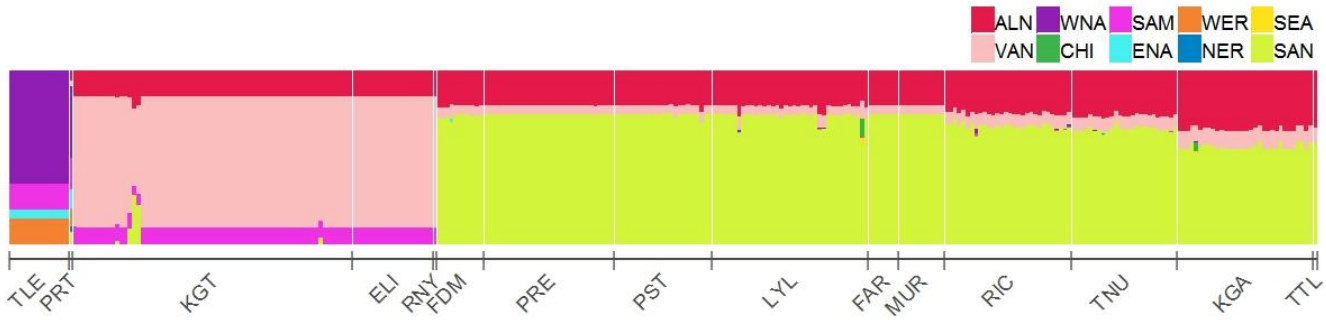

Figure 2.6. Estimated coancestry via supervised Bayesian clustering analysis implemented, by the software ADMIXTURE (Alexander *et al.*, 2009), for brown rats sampled from Haida Gwaii, BC (*n*=299). Ten reference populations were first pre-defined using a previously compiled reference dataset (Puckett, 2018a, 2016; *n*=330), then Haida Gwaii samples were compared against these populations to infer historical ancestry. Genotypic data for *n*=12 433 single nucleotide polymorphism nuclear markers were used as inputs, and coancestry estimates were averaged across *n*=20 iterations. Legend labels indicate reference populations, and bottom labels indicate island or sample location of Haida Gwaii samples (see Appendix A and Figure 2.4 for abbreviation definitions).

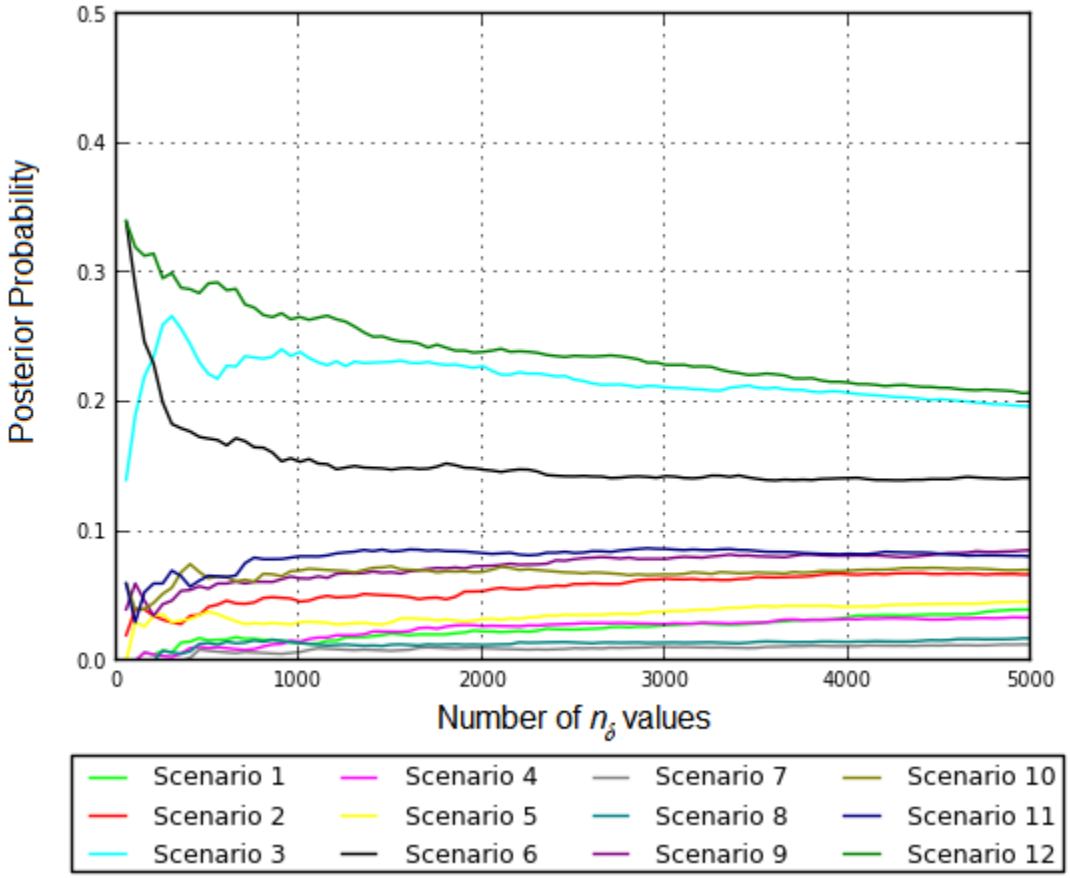

Figure 2.7. Posterior probabilities of twelve invasion scenarios for *n*=57 brown rats (*Rattus norvegicus*) sampled in Haida Gwaii, BC. A reference dataset containing individuals from Vancouver (*n*=15), western North America (*n*=29), and western Europe (*n*=38) were used to infer the global origin of brown rats in Haida Gwaii. Approximate Bayesian computation analyses were performed using DIYABC v2.1.0 (Cornuet *et al.*, 2014). For scenario topology, see Appendix A.

Chapter 3: Patterns of dispersal and inferred Gene flow among invasive rat populations in Haida Gwaii

3.1 Background

Understanding the dispersal patterns (*i.e.* gene flow) of an invasive species is critical for developing effective management strategies. One of the key factors associated with eradication failure is re-invasion from a nearby population (Holmes *et al.*, 2015). By describing the patterns of gene flow prior to eradication, managers can maximize the success of an eradication as well as plan for the prevention of re-invasion (Abdelkrim *et al.*, 2005). However, it can be difficult to elucidate patterns of gene flow by traditional observational methods such as visual- or telemetry-based measures. Dispersal patterns are dictated by multiple biotic and abiotic factors, and these factors are not always measurable or readily apparent. Invasive rats in island systems provide one such example; it is difficult, if not impossible to monitor intervening ocean passages for individuals swimming from island to island (Russell & Clout, 2004). Also, rats that disperse via human-mediated means (*i.e.*, boats) are often cryptic. Even if dispersers are detected, the origin of these individuals may remain unknown if the vessel docked at multiple ports. Additionally, fine-scale environmental data that influence dispersal (such as velocity of ocean currents through a passage) simply may not exist, and even well-documented natural barriers to gene flow may be rendered insignificant due to the commensal spread of invasive rats.

Island biogeography theory can be used to predict dispersal of individuals in island systems (MacArthur & Wilson, 1967). While the original theory describes a theoretical framework for colonization of islands by multiple species, the general predictors can also be used on an individual level, where dispersal is greater to: 1) large islands than small islands and 2) islands more proximate to a source population than those that are more distant (Simberloff,

1974; Vellend & Orrock, 2009). In fact, island biogeography theory has shown to be an effective predictor of mammalian distributions across multiple species and archipelagos worldwide, including invasive rats (Adler & Wilson, 1985; Lomolino, 1982, 1984; Ruffino *et al.*, 2009; Russell & Clout, 2004). While this theory can be useful for inferring historical migration from present distributions, empirical evaluation of current rates of dispersal, migration and gene flow still poses a challenge using traditional observational methods.

Population genetics and genomics can be used to infer gene flow in an efficient manner and without the need to track the movement of individual dispersers. To demonstrate the utility of genetics to track gene flow among populations, Abdelkrim *et al.* (2005) sampled brown rats across numerous islands located off the coast of Brittany, France. They showed that there was extensive gene flow among proximate island populations and suggested that populations within a few hundred metres of each other should be considered a single population. Robertson and Gemmell (2004) coined these connected populations as an "eradication unit" and recommended that all populations within the unit must be eradicated simultaneously to prevent failure by re-invasion. Though lacking pre-eradication samples, Savidge *et al.* (2012) used estimates of shared allelic diversity, low F_{ST} among populations, detection of gene flow, and spatial analyses to determine that a failed eradication on Congo Cay in the US Virgin Islands was likely due to re-invasion from the neighbouring Lovango Cay, demonstrating the importance of defining units prior to eradication. They were also able to identify potential abiotic factors that limited rat dispersal, in this case, island juxtaposition and ocean currents. Such investigations are important, as complete eradication of rats can prove to be challenging due to their high adaptability and fecundity (Holmes *et al.*, 2015; Howald *et al.*, 2007; Russell *et al.*, 2010; Simberloff, 2003). On islands throughout the world, eradication attempts have resulted in approximately a 90% success

rate in the complete removal of rats from the island, with eradication failure resulting in the re-occurrence of invasive rats (Howald *et al.*, 2007). Because of the costly nature of eradications financially, logistically and socially, identifying the cause of failure is paramount for success before attempting another eradication.

There are two prevailing hypotheses associated with rodent eradication failure. First, the Bait Failure Hypothesis argues that eradications failed because of inadequate distribution or toxicity of poisonous baits designed to kill rats. Bait containing rodenticide is distributed either by broadcasting by hand or helicopter, or by deployment of bait stations in strategic locations (Howald *et al.*, 2007). If the poison bait is inadequately spread throughout the population's distribution, some individuals may never encounter the bait and thus survive the attempt. Alternatively, a poison bait regime may fail due to insufficient toxicity or a development of resistance in the invasive population (Amos *et al.*, 2016; Holmes *et al.*, 2015). In both cases, this could result in a small handful of individuals surviving the eradication process to re-establish the invasive population.

The Recolonization Hypothesis argues that eradications fail due to post-eradication dispersal from a nearby source population. Unfortunately, this scenario can be particularly difficult to investigate without sufficient pre- and post-eradication genetic samples to capture the extent of genetic variation in either population (Abdelkrim *et al.*, 2007; Howald *et al.*, 2007; Savidge *et al.*, 2012). Often, it is the lack of pre-eradication samples that acts as a barrier to elucidating the origin of a population, as these are necessary to properly characterize the historical genetic composition; for this reason, it is essential for those performing an eradication to collect these samples in case of an eradication failure (Abdelkrim *et al.*, 2005, 2007). With the

appropriate genetic data, the origins of a re-encountered population can be explicitly evaluated and determined.

To meet management objectives of protecting ecological and cultural integrity (Parks Canada Agency, 2018), Parks Canada and other agencies are taking steps to eliminate the negative effects of invasive rats on seabirds in Haida Gwaii. Eradication has been the primary tool used to date. Both species of rats were successfully eradicated from the northern islands of Langara, Lucy, and Cox (1997), the southern St. James Island (1998), and the east-central island of Arichika (2011) (Gaston *et al.*, 2008; Gill *et al.*, 2014; Golumbia, 1999; Kaiser, 1997; see Figure 3.1 for island locations). Black rats were successfully eradicated from Faraday and Murchison Islands (2013) but have since been invaded by brown rats; additionally, brown rats have now invaded House and Hotspring Islands, which have historically never had invasive rats. Two eradications have been attempted on the Bischof Islands (2003, 2011), a small group of islets on the east-central coast of Haida Gwaii but have resulted in subsequent detection of brown rats via camera traps post-eradication. In all cases, the source(s) of the current invasive populations are unknown.

Historically, the Bischof Islands have hosted significant breeding colonies of both ancient murrelets (*Synthliboramphus antiquus*) and fork-tailed storm petrels (*Oceanodroma furcate*). Since the arrival of brown rats on these islands, the storm petrel population has experienced a 99% decrease in population size, and the ancient murrelet population was thought to have been completely extirpated, though acoustic recordings have confirmed that a small population of murrelets persists (C. Bergman, pers. comm., Howald 2012). Knowing the origin of the modern rat population on the Bischof Islands is important for future management. If the current population was established via re-colonization, a larger eradication area or perhaps the

implementation of preventative strategies may need to be considered. Conversely, eradication failure due to ineffective methodologies will require a modification of the techniques used historically.

Little is known about connectivity among rat populations in Haida Gwaii. This lack of knowledge, supported by two failed eradication attempts on the Bischof Islands, as well as the recent invasions on Faraday, Murchison, House, and Hotspring Islands, highlight the importance of understanding invasive rat population connectivity to develop the best eradication strategies possible. Additional eradications are currently being explored with the goal of supporting the recovery of the Haida Gwaii seabird populations.

Here, we paired archipelago-wide sampling of black and brown rats with genotyping-by-sequencing to investigate patterns of population connectivity and infer levels/direction of gene flow among invasive rat populations in Haida Gwaii. We used this information to identify candidate islands and define eradication units that present the lowest risk of re-invasion. Lastly, we investigated the source(s) of recent invasions, including an explicit testing of re-emergence vs. recent colonization hypotheses in the Bischofs, to evaluate the existing eradication methodology and inform biosecurity measures.

3.2 Methods

3.2.1 Sample collection, DNA extraction and library construction

Samples were collected as outlined in Chapter Two. In addition to the *n*=288 brown rat samples, *n*=315 black rats were sampled across nine islands throughout Haida Gwaii (Figure 3.1). One additional brown rat was collected from a recent invasion to Hotspring Island. Two rats collected from Graham Island could not be identified to species, likely due to DNA degradation and/or bacterial or fungal contamination and were not used in downstream analysis. DNA extraction

and ddRAD library construction was performed as outlined in Chapter Two. In total, seven libraries (*n*=96 individuals/library) were constructed, with samples replicated within (*n*=10) and among (*n*=6) libraries to evaluate genotyping error rates. Libraries were sequenced on the Illumina HiSeq 2500 PE125 platform (125bp, paired-end). As the Hotspring Island individual was collected after these libraries were sequenced, a separate ddRAD library was constructed from just the Hotspring sample and was sequenced in a series of spikes on the Illumina Mi-Seq PE150 platform (150bp, paired-end).

3.2.2 Demultiplexing and species determination

Raw sequence reads were demultiplexed to individuals using the *process_radtags* command in Stacks v2.0 (Catchen *et al.*, 2013) ; during this process, barcodes and indices were removed, and the reads trimmed to 100bp to remove low-quality bases at the 3' ends. Processed reads were then aligned to the brown rat reference genome (Rnor_6.0, GenBank assembly accession: GCA_000001895.4) using the software Bowtie 2 v2.2.9 (Langmead & Salzberg, 2012). A minimum of 40% alignment to the reference genome was used as a form of quality control, and individuals failing to meet this threshold (*e.g.*, due to poor sequence quality, failed adapter ligation, or DNA amplification from a non-target species such as bacteria) were removed from downstream analysis.

Putative SNP loci were first identified and genotyped across all individuals using the *gstacks* and *populations* programs in STACKS where a locus must be genotyped in at least 90% of the individuals (r=0.90) and the minor allele frequency must exceed 5% (min_maf=0.05). This initial dataset was used to designate individuals to species, as morphological identification can be difficult with juveniles as well as for some phenotypes. To assign individuals to species, genotypic data were analyzed using ADMIXTURE v1.3.0 (Alexander *et al.*, 2009) with the number

of clusters set to *k*=2. These results were verified using the R-package *SNPRelate* v1.8.0 (Zheng *et al.*, 2012) to generate a principle component analysis (PCA) plot with individuals coloured based on the species identified with ADMIXTURE. Some individuals ambiguously assigned to species; for those, we used a discriminant analysis of principle components, as implemented by the R-package *adegenet* v2.1.1 (Jombart & Ahmed, 2011), which first applies principle component analysis to identify genetic clusters, and then uses discriminant analysis to maximize the variation among these clusters while minimizing within-cluster variation (Jombart *et al.*, 2010). Furthermore, DAPC allows for posterior probabilistic assignment of individuals to the inferred clusters to add another degree of support for individuals that poorly assign based on other methods. The results of these analyses were summarized, and individuals were separated into species-specific datasets. Samples with inconsistent species assignment across analyses were removed from downstream analyses.

For the Hotspring Island sample, reads were first aligned to the brown rat reference genome (Rnor_6.0, GenBank assembly accession: GCA_000001895.4) with Bowtie2 (Langmead & Salzberg, 2012). During alignment, paired reads were merged and trimmed to 100 bp to remove low quality reads on the 3' ends. To maximize compatibility with previous libraries, loci were identified and genotyped using the *mpileup* command found in SAMtools v1.9 (Li *et al.*, 2009) using a whitelist of single nucleotide polymorphisms (SNPs) identified in the complete brown rat dataset. SNPs with a read depth of <6x or missing data were removed with VCFtools v0.1.14 (Danecek *et al.*, 2011).

3.2.3 SNP genotyping and filtering

Each species-specific dataset was independently run through the *gstacks* program in STACKS. Due to the relatively high frequency of sequencing errors associated with the Illumina platform

(see Pfeiffer *et al.*, 2018), a sensitivity analysis was performed to ensure robust and accurate genotypes through the *populations* program in STACKS. We varied the proportion of genotyped individuals (-r) to call a SNP from 0.7-0.95 and the minimum minor allele frequency (--min_maf) from 0.01-0.05 to identify optimum parameters. Only a single SNP per RADtag was retained (--write_single_snp) to minimize the potential for linkage disequilibrium, and the maximum observed heterozygosity (--max_obs_het) was set to 0.5 for all filtering iterations. Individual mean depth, missing data per individual, number of SNPs, and number of individuals with ≥6x depth of coverage were calculated for each filtered dataset using VCFtools v0.1.15 (Danecek et al., 2011). Once final filtering parameters were chosen, low coverage (<6x) individuals were removed, and each dataset was passed a final time through *populations* using the identified optimal filtering parameters. SNPs located on the X-chromosome were removed using PLINK v1.90b5 (Purcell *et al.*, 2007). F_{ST} outliers were detected using the Beaumont and Balding method (2004) as implemented by BayeScan v2.1 (Foll & Gaggiotti, 2008) across 100,000 iterations with a 50,000 iteration burn-in and a prior odds value of 10. Outlier loci were defined as having a mean *q*-value of 0.20 over five runs and were removed from downstream analysis. Each dataset was broken into putative populations structured by island, and then all loci were assessed for significant (α=0.05) deviation from Hardy-Weinberg equilibrium (HWE) using VCFtools. A locus was removed if it significantly deviated from HWE in at least 50% of populations with a minimum of *n*=2 individuals. Mean individual depth and percent missing data were reassessed using VCFtools for each dataset. Genotyping error rate was estimated by calculating the rate of discordance among replicate samples both within and across sequencing libraries. Standard measures of genetic diversity were assessed across populations within each dataset using GenoDive v2.0b27 (Meirmans & Tienderen, 2004).

3.2.4 Assessment of population structure

Several approaches were used to detect population structure. First, we used PCA to infer genetic clusters within each species and across all populations using the R-package *SNPRelate* (Zheng *et al.*, 2012). We then examined regional clusters of islands individually to detect even finer-scale structure. We estimated population differentiation among identified clusters by calculating θ (Weir & Cockerham, 1984) for all pairwise observations over 1000 permutations as implemented by Genetix v4.05.2 (Belkhir *et al.*, 2004). We chose θ over other measures, such as pairwise F_{ST}, as it is an unbiased estimate for population differentiation when sample sizes are unequal. For larger island populations, we also estimated pairwise θ among all within-island sample sites; if no differentiation was detected, all sample sites within the island were considered a single population. We estimated admixture coefficients among populations using model-based Bayesian clustering as implemented in ADMIXTURE (Alexander *et al.*, 2009). We examined genetic clusters *k* 1-30 over 10 iterations of each *k*, each run with a unique random seed. We used a cross-validation (CV) threshold of 10 and used the mean CV to identify the optimal number of clusters. All runs of the optimal *k* were summarized using CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007) and visualized with the R-package *pophelper* v2.2.3 (Francis, 2017). Admixture coefficients were also estimated using the snmf() function in the R-package *LEA* v2.0 (Frichot & François, 2015). This function uses sparse non-negative matrix factorization to estimate individual ancestry coefficients from a genotypic matrix. Additionally, the analysis is robust to deviation from Hardy-Weinberg equilibrium as well as unequal sample sizes among groups. For this analysis, we examined *k* 1-20 over 10 iterations of each *k*. We plotted the cross-entropy criteria for each *k* and identified the "elbow" as the optimal number of clusters. All runs for the optimal *k* were summarized using CLUMPP and visualized using *pophelper*.

To examine if the interior of Kunghit Island was acting a barrier to dispersal, we calculated the shortest distance between each pair of populations, allowing for movement up to 1 km from the coastline, as this has been previously proposed to be the maximum extent of rat movement inwards on islands (Harper, 2006; Pye & Bonner, 1980; Pye *et al.*, 1999). We tested a hypothesis of isolation-by-distance using Mantel tests where Euclidean distance and shoreline distance were considered separately as predictors of genetic distance (measure as $\theta/1-\theta$).

3.2.5 Directional migration rates among populations

We evaluated rates of recent migration among populations using a Bayesian framework as implemented by the software BayesAss v3.0.4 (Wilson & Rannala, 2003). This analysis uses multilocus genotypes to infer recent migration and estimates both the rate as well as the direction of migration. For each species, we used 10 million iterations and a burn-in period of 1 million steps, sampling at 100 iteration intervals. The migration rates, allele frequencies, and inbreeding coefficient mixing parameters were adjusted to achieve acceptance rates between 0.2 and 0.6 as recommended by the user manual. This analysis was completed five times for each species, with each run initialized by a different random seed, to assess chain convergence. We constructed 95% credible sets using the mean migration rate across runs minus 1.96 times the mean standard deviation. Migration rates were considered significant if the credible set did not include 0.

3.2.6 Identifying source population(s) for the Bischof Islands and novel invasions

A series of population assignment tests were used to identify the source population for the failed eradication on the Bischof Islands as well as the novel brown rat invasions on Faraday, Murchison, and Hotspring Island. We used a projected PCA using the *smartpca* function in the software package EIGENSOFT v6.1.4 (Galinsky *et al.*, 2016; Patterson *et al.*, 2006; Price *et al.*, 2006), which first defines the parameter space using only the reference samples, and then

projects samples of unknown origin onto this space. For the Bischof Islands, we used Lyell and Richardson Islands as putative source populations, as these are the most proximate islands with brown rat populations (*N.B.* Faraday and Murchison Islands were invaded after the Bischof Islands population re-appeared). Additionally, the pre-eradication population was considered as a reference population under the survivor hypothesis. For the Faraday and Murchison Islands invasions, Lyell Island, Richardson Island, the post-eradication Bischof Islands population, and Tlell were examined as the putative source. These populations were again chosen for their proximity, except in the case of Tlell, which was considered because recent lumber shipments from Tlell to Faraday Island posed a potential introductory pathway. For Hotspring Island, populations from Faraday Island, Murchison Island, post-eradication Bischof Islands, Lyell Island, and Richardson Island were examined as potential sources. We also used supervised Bayesian clustering, as implemented by ADMIXTURE (Alexander *et al.*, 2009), in which genetic clusters (*i.e.* reference populations) were first pre-defined, then memberships to these clusters for all individuals of unknown origin were estimated. This analysis was run over 20 iterations for each invasion, and mean admixture coefficients to each reference population were calculated. We also examined putative source populations using two different population assignment tests. We used discriminant analysis of principle components (DAPC), as implemented by the R-package *adegenet* (Jombart & Ahmed, 2011), which first applies PCA to identify genetic clusters, and then uses discriminant analysis to maximize the variation among these clusters while minimizing within-cluster variation (Jombart *et al.*, 2010). Additionally, DAPC allowed us to estimate the posterior probability of assignment to each of the identified genetic clusters. The second population assignment test followed the methods outlined by Rannala and Mountain (1997) and implemented in GeneClass2.0 (Piry *et al.*, 2004), which detects immigration by

estimating population allele frequencies using Bayesian methods, then calculates the probability of a genotype arising from each defined population. We used the Paetkau *et al.* (2004) Monte Carlo resampling algorithm with 100,000 simulated individuals to determine the probabilistic assignment of unknown samples to their respective reference populations. Due to computation limitations associated with GeneClass2.0, these analyses were based on a random subset of 2000 SNPs.

3.3 Results

3.3.1 Species determination and dataset quality

DNA sequencing resulted in 215 to 260 million high-quality reads per library and 622,865 reads for the Hotspring individual (Appendix B). Following demultiplexing and reference alignment, we identified $n = 294$ brown rats and $n = 299$ black rats based on the genetic analysis; $n = 10$ samples were ambiguous in their species assignment and were removed from downstream analyses (Appendix B). We retained loci genotyped in at least 80% of individuals that had a minimum minor allele frequency of 5% based on the results of the sensitivity analysis (Appendix B), parameters that were also consistent with a previous brown rat population genomic study (Puckett *et al.*, 2016). The filtering procedures resulted in 27,686 SNPs across 284 unique individuals ($n = 297$ with replicates) in the brown rat dataset and 28,818 SNPs across 245 unique individuals ($n = 251$ with replicates) in the black rat dataset (Appendix B; Table 3.1). Mean within and among genotyping error rates were 2% and 2.6%, respectively, for the brown rats and 13.4% and 13.1% for the black rats (Table 3.1). Using the brown rat SNP dataset as a whitelist, we were able to identify and genotype 557 polymorphic loci with a depth of coverage ≥6x for the Hotspring individual.

3.3.2 Genetic diversity and population differentiation

Genetic diversity was high across all brown rat populations except for the Tlell population, which had both relatively lower levels of heterozygosity and effective number of alleles, and high levels of inbreeding (Table 3.2). Genetic diversity across black rat populations was also high, though the Graham and Faraday Islands populations did show low levels of inbreeding (Table 3.3).

We detected low, but significant levels of differentiation among proximate brown rat populations (Figure 3.2). The Tlell population was highly divergent from all populations (pairwise $\theta > 0.50$ for all comparisons). Differentiation among sample sites on Lyell Island was low (pairwise $\theta < 0.07$ for all comparison; Table 3.4); as such, all samples sites on the island were grouped into a single Lyell population. Brown rats sampled from Arnold Point (KAP), Bowles Point (KBP), and Gilbert Bay (KGB) on Kunghit Island were not significantly differentiated, and rats from Hornby Point (KHP) displayed low, but significant differentiation from these sites (pairwise $\theta < 0.06$ for all comparisons); consequently, these sites were grouped into a single "NW-Kunghit" population (Figure 3.3). There was significant, albeit low, differentiation among the east Kunghit sample sites of Marshall Island (KMI) and Keeweenah Bay (KKB); as such, these sites were grouped into a single "E-Kunghit" population. The Luxana Bay (KLB) population displayed low to moderate differentiation from both the NW- and E-Kunghit populations and was grouped with the E-Kunghit population due to geography; additionally, the Rainy Islands (RIA) population was not significantly differentiated from any of the Kunghit sites and was also grouped with the E-Kunghit population due to geography.

Overall, pairwise differentiation among black rat populations was relatively higher than among brown rat populations (Figure 3.4). Proximate populations showed low to moderate

population differentiation. The Graham Island sample sites grouped into northern and southern populations and were moderately differentiated (Figure 3.5a; pairwise $\theta = 0.139$ between north and south group). There was low to moderate differentiation (pairwise $\theta = 0.07$-0.12) between the Faraday Passage (LFP) and all other Lyell Island populations, but low differentiation (pairwise $\theta < 0.06$) among the remaining Lyell Island sample sites (Figure 3.5b).

3.3.3 Principle component analysis

We found three distinct clusters using PCA in the brown rats, which segregated based on geography (Figure 3.6). Central populations formed a discrete cluster, as did southern populations, and rats collected from Tlell formed their own unique population. The single mainland sample from Prince Rupert did not cluster with any region, counter to what was discovered in Chapter 2. PCA of the central cluster indicated some substructure among islands (Figure 3.7a). Lyell Island and the post-eradication Bischof Islands populations formed a single cluster, as did Faraday and Murchison Islands. Tanu and Richardson Islands appeared to share some common ancestry, but still formed discrete clusters. Both the Kunga Island and pre-eradication Bischof Islands populations appeared to be quite divergent from all other populations. We also detected substructure among the southern cluster (Figure 3.7b). We detected some divergence between northwest and eastern population on Kunghit Island. The Ellen Island population also appeared to be divergent from those on Kunghit Island.

We found similar regional clustering among black rat populations; however, each cluster was less discrete than the three brown rat clusters (Figure 3.8). The northern cluster consisted of populations on Graham and Moresby Islands. We detected divergence between northern and southern sample sites within Graham Island, and some separation between Graham and Moresby Islands (Figure 3.9a). We found three subclusters within the central group: Faraday and

Murchison Islands; Shuttle and Huxley Islands; and the two Lyell Island populations (Figure 3.9b). There was some divergence seen between the Faraday Passage ("Lyell-FP") and southwest Lyell ("Lyell-SW") populations, which corresponded well with the pairwise θ estimates (Figure 3.5).

3.3.4 Bayesian clustering analysis of population structure

We found an optimal number of genetic clusters $k = 12$ for the brown rats (Appendix B) and $k = 9$ for the black rats (Appendix B) based on results from ADMIXTURE. The brown rats clustered by island, with Faraday and Murchison Islands representing a single unit (Figure 3.10). We also found the northwest and east Kunghit Island populations formed somewhat discrete clusters with some admixture between the two populations (Figure 3.10; Figure 3.11). The black rats, again, followed a similar pattern with genetic clusters largely segregated by island (Figure 3.12). The north and south Graham Island populations each represented a unique cluster. The southwest subpopulations on Lyell Island ("Lyell-SP", "Lyell-RP", and "Lyell-W") formed a mostly distinct cluster, though there was some admixture with the "Lyell-FP" subpopulation. In 8 of the 10 iterations, Faraday Island formed its own cluster, while in the other two iterations it formed a single group with Murchison Island.

The results from *LEA* indicated an optimal $k = 8$ for the brown rats and $k = 9$ for the black rats (Appendix B). For the brown rats, most clusters were structured the same as with the previous ADMIXTURE analysis, although with some differences (Figure 3.13). Tanu Island no longer represented a unique cluster and was grouped with Richardson Island. The two subpopulations on Kunghit Island formed a single cluster, though there was substantial admixture within the northwest subpopulation. Brown rats on Lyell Island were highly admixed with several proximate populations. The structure of clusters within the black rats was near-

identical to the ADMIXTURE analysis, though Faraday Island was always identified as its own cluster across all iterations (Figure 3.14).

3.3.5 Isolation-by-distance on Kunghit Island

We found significant isolation-by-distance patterns using both Euclidean distance (Mantel's r = 0.790; $p < 0.001$) and shoreline distance (Mantel's r = 0.802; $p < 0.01$) among Kunghit Island sample sites. Shoreline distance was more strongly correlated with genetic distance than Euclidean distance, though the difference was marginal.

3.3.6 Directional migration

We detected no significant migration rates among brown rat populations, though we were able to identify $n = 6$ first generation migrants consistently across runs (Table 3.5; Table 3.6). These detected migrants were in congruence with the clustering-based approaches. Furthermore, they support the grouping of the Titul Island individual with the Kunga Island population, and the Rainy Islands individual with the E Kunghit Island population, further indicating that these group of islands are acting as single genetic populations.

Similarly, we did not detect significant migration among black rat populations except for two populations on Lyell Island; we detected significant bi-directional migration between the Sedgewick Point (Lyell-SP) and Richardson Point (Lyell-RP) populations, though higher rates of migration were seen in the Sedgewick to Richardson direction (Table 3.7). We also detected $n = 16$ first generation migrants, with many of these individuals migrating from Sedgewick Point to Richardson Point (Table 3.8).

3.3.7 Source(s) of novel invasions

The post-eradication Bischof Islands population clustered closely with the Lyell Island population using the projected PCA, though one sample did cluster with the Richardson Island

population (Figure 3.15a). Supervised ADMIXTURE analysis also inferred ancestry to the Lyell Island population, with the same single sample assigning to Richardson Island (Figure 3.15c). DAPC identified three genetic clusters (Lyell Island, Richardson Island, and pre-Bischof Islands), and all samples had 100% posterior assignment to the Lyell Island population except for the single sample, which had 100% assignment to the Richardson Island population (Figure 3.15b). The same pattern was seen using the assignment implemented in GeneClass2.0, though all individuals had a high probability of assignment to either of the Richardson or Lyell Islands populations following resampling (Table 3.9). Across all analyses, a pre-eradication origin was poorly supported.

Both Faraday and Murchison Islands clustered with Lyell Island using the projected PCA (Figure 3.16a). Additionally, both populations had perfect assignment using both the supervised ADMIXTURE and DAPC analyses (Figure 3.16b, c). The population assignment test implemented in GeneClass identified a Lyell Island origin, though resampling indicated a high probability of assignment to either of the Richardson or Lyell Islands populations (Table 3.10). There was low support for a Bischof Islands origin, and zero support for a Tlell origin.

The projected PCA clustered the Hotspring Island individual with the Murchison Island population (Figure 3.17). Results from the ADMIXTURE analysis indicated primarily a Murchison Island origin, though there was some admixture detected with Richardson and Faraday Islands (Table 3.11). For the DAPC, we identified four genetic clusters among all individuals, with Faraday, Murchison, and Hotspring Islands representing a single genetic cluster; all other islands formed their own genetic cluster. Probabilistic posterior assignment of the Hotspring Island sample indicated complete assignment to the Faraday-Murchison Islands cluster, with zero percent assignment to Lyell Island, Bischof Islands, and Richardson Island (Table 3.11).

Probabilistic population assignment under the Rannala and Mountain (1997) criterion again identified Murchison Island as the most likely source of the population, though there was some support for a Richardson or Lyell Islands origin (Table 3.11).

3.4 Discussion

3.4.1 Brown rat population connectivity

Managing invasive species is a global problem (Buckley, 2008; Hobbs *et al.*, 2006). In the case of islands, eradication is the primary tool used for managing invasive species, as the impacts on native species from these invaders can be particularly severe (Glen *et al.*, 2013; Sax & Gaines, 2008; Simberloff *et al.*, 2011). To maximize success, Buckley (2008) highlighted the need for increased research when informing eradications, especially in terms of defining eradication units (Buckley, 2008). Genetic information can be particularly informative in this context, providing insights on population connectivity to assist managers in defining eradication units (Abdelkrim *et al.*, 2007; Dawson *et al.*, 2015; Robertson & Gemmell, 2004; Russell *et al.*, 2010).

Here, we paired archipelago-wide sampling of black and brown rats with genotypic data at >27,000 SNPs to reconstruct patterns of population connectivity and infer levels/direction of gene flow among invasive rat populations in Haida Gwaii. We found that proximate populations, for the most part, were more related than those that were more distant, though isolation-by-distance was only explicitly measured for the Kunghit Island populations. Additionally, populations on the larger islands, namely Kunghit and Lyell, appeared to have great connectivity with neighbouring populations than some of the smaller islands. Both of these observations are consistent with predictions from island biogeography theory, which states that larger, more

proximate islands will share higher levels of migrants than smaller, more distant islands (Johnson *et al.*, 2000; MacArthur & Wilson, 1967).

We detected three regional clusters among brown rat populations within the archipelago (Figure 3.6). For the brown rats, the northern population in Tlell formed its own cluster and showed low levels of genetic diversity (Table 3.2). This population is substantially isolated from all other brown rat populations within Haida Gwaii, likely leading to its substantial population differentiation ($\theta > 0.50$ in all pairwise comparisons). Lyell and surrounding islands formed a centrally-located cluster with some differentiation among island populations detected. We found low levels of differentiation among these island populations, suggesting that there is some gene flow among populations. Interestingly, Kunga Island was strongly differentiated from even the proximate Tanu Island (~1 km apart; pairwise $\theta = 0.152$) and represented a unique genetic cluster across all analyses, despite the fact that significant migration rates have been recorded between brown rat populations at this distance over open ocean in the Falkland Islands (Tabak *et al.*, 2015a). However in another study, Tabak *et al.* (2015b) did note that 97% of islands >1 km from an existing rat population remained rat free and suggested that this distance may be a threshold for brown rat dispersal over water. As such, continued introduction to Kunga Island is unlikely, and the population was likely established during a single invasion event.

The southern cluster of brown rats consisted of the populations on Kunghit and Ellen Islands. The pair were significantly differentiated, and Ellen Island consistently formed a distinct genetic cluster across analyses. This finding was also surprising, as Ellen Island is only ~130 m from Kunghit Island, and the nearest rat population that was sampled was ~1 km away, with ~850 m of that distance over land. Brown rats have been shown to have significant gene flow between populations >10 km apart, and all sample sites along the northwest side of Kunghit

Island exhibited little to no differentiation, indicating that the terrestrial distance is not acting as a barrier. One possible explanation for the divergence between the two islands could be due to a strong tidal current, which have been shown to inhibit dispersal over such short distances (Savidge *et al.*, 2012). However, we did identify two first generation migrants from the NW Kunghit population within the Ellen population, so further investigation is needed to understand the dynamics between these two populations.

In addition to differentiation between Ellen and Kunghit Islands, we also detected moderate levels of divergence among the northwest and eastern Kunghit Island populations. This divergence may be due to the topography of the Kunghit shoreline. While the east and west coastlines of the island are only separated by a Euclidean distance of approximately 10 km, brown rats rarely venture further than 1 km from the shoreline (Pye & Bonner, 1980), so movement through the island interior is unlikely. If the interior is acting as a barrier to gene flow, rats would have to disperse along the shoreline, which greatly increases the distance between populations. We recovered a significant IBD pattern using shoreline distance as a predictor; however, we also found a similar pattern of IBD using straight Euclidean distance, albeit with a marginally smaller effect. A more explicit landscape genetic analysis may better identify which environmental factors are affecting dispersal, and therefore gene flow, on Kunghit Island (Manel *et al.*, 2003; McRae, 2006).

3.4.2 Black rat population connectivity and interspecific interactions with brown rats

Black rats also segregated into northern, central, and southern clusters (Figure 3.8). The northern cluster was formed by populations located on Graham Island and a single population from Sandspit, BC. The northern and southern sample sites were substantially differentiated from each other (pairwise $\theta > 0.15$), and this is likely due to geographic isolation, as these populations are

>50 km apart, well beyond dispersal limits for rat species (Tabak *et al.*, 2015a). Though the Sandspit population was more genetically similar to this northern cluster than the other clusters, it was still discrete from the Graham Island population, as the ocean distance is substantial between Graham and Moresby Island across Alliford Bay (>3 km). A regular ferry passes between the two islands and is likely the only source of gene flow between the Graham and Sandspit populations.

The central cluster of black rats contained Lyell and surrounding islands as well as Huxley Island. In general, we found more population differentiation among island populations in this cluster than we saw among brown rat populations (Figure 3.2; Figure 3.4). These increases in differentiation likely arise from differences in body size. Brown rats are larger bodied than black rats which allows them to be better adapted to colder temperatures, and thus, makes them more efficient dispersers over ocean waters (Harper *et al.*, 2005). In fact, among island rat populations in the Mediterranean and in New Zealand, ocean distances from 2 m to 70 km were significantly negatively correlated with black rat presence on islands, but there was no correlation with brown rat presence (Ruffino *et al.*, 2009; Russell & Clout, 2004). This difference in dispersal ability can be further illustrated with the Faraday, Murchison, and Bischof Islands. Both Faraday and Murchison Islands had historical black rat populations that were successfully eradicated in 2013 with no subsequent detection; however, these islands have recently been invaded by brown rats, most likely from the neighbouring Lyell Island (Table 3.10; Figure 3.16). In this case, a difference in the dispersal ability between the species could explain why brown rats invaded Faraday Island, and subsequently, Murchison Island, but the black rats have not re-invaded. Furthermore, we have also shown that brown rats have re-invaded the Bischof Islands following a 2011 eradication, which is approximately the same distance from

Lyell Island (~550 m) as is Faraday Island (~700 m); black rats have never invaded the Bischof Islands, though the nearest Lyell Island population is just as proximate (Burles, 2006). In addition to differences in dispersal ability, there has been recent anecdotal observation of an increase in the population size of brown rats on Lyell (R. Irvine, pers. comm.), which could be driving these rats to expand into territories with less competition (Matthysen, 2005). Once established, the brown rats can then outcompete any new invaders (*e.g.*, black rats) by rapidly expanding their population numbers; in fact, the eradication of black rats on Faraday and Murchison Islands may have even facilitated the brown rat invasion by removing a source of competition (Fraser *et al.*, 2015; Russell *et al.*, 2010) or it simply could be due to the increased dispersal abilities of the brown rats and their increased dominance through time on adjacent Lyell Island. Additionally, historical presence of invasive rats can leave systems more prone to future invasions (Banks *et al.*, 2018; Witmer *et al.*, 2007). These interspecific interactions must be considered when planning future eradications to ensure their success.

The southern cluster of black rats consisted of only a single population from Kunghit Island. Kunghit Island historically supported a large black rat population (Bertram & Nagorsen, 1995). Brown rats invaded Kunghit Island later than the black rats, and since their arrival, black rat populations have diminished. This pattern of black rat displacement by brown rats has been recorded in many systems around the world and also likely stems from the larger body size of brown rats (Atkinson, 1985; Bertram & Nagorsen, 1995), a pattern observed in other small mammals (Brannon, 2000; Fox & Kirkland, 1992; Harper *et al.*, 2005). Moreover, brown rats are almost exclusively terrestrial animals and will come into more frequent contact with seabird nests and burrows than the semi-arboreal black rats, allowing them to be the stronger competitors for the seabird-related food supply (Thorsen *et al.*, 2000).

3.4.3 Source(s) of recent brown rat invasions

We were able to confidently identify the source of the current Bischof Islands brown rat population as well as the invasions to Faraday, Murchison, and Hotspring Islands. In the case of the Bischof Islands, brown rats re-invaded from Lyell Island and the current population was not founded by eradication survivors. This result is both positive and negative for management of brown rats on the Bischof Islands. Because of the short distance separating these two islands (~550 m), dispersal from Lyell Island to the Bischof Islands likely occurred without human intervention (*i.e.* brown rats swam across rather than commensally spreading as stowaways on vessels). Furthermore, identification of the source as re-invaders rather than survivors indicates that the eradication methodology was effective and highlights the need for increased biosecurity measures.

Lyell Island was also the source population for the recent invasion onto Faraday and Murchison Islands. From there, brown rats have now spread to Hotspring Island, which has never had any species invasive rats (R. Irvine, pers. comm.). These populations represent a significant biosecurity threat. Ramsay Island is situated 900-1000 m from House and Hotspring Islands and hosts a large seabird colony. It also has never had a rat population, and an invasion could have a devastating impact on the resident seabirds.

3.4.4 Management implications

We identified several genetically-isolated islands that would be suitable targets for rat eradication. The Kunga Island population was significantly isolated from all other populations and is a strong candidate for eradication, as it presents a low risk of re-invasion. The several smaller islets surrounding Kunga Island, such as Titul Island, should be eradicated as a single unit to prevent re-invasion to Kunga Island from these populations. Ellen Island appeared to be

isolated as well and may represent a suitable candidate for eradication, though a more thorough understanding of the barriers to gene flow between Ellen Island and Kunghit Island is needed before action should be taken. The Shuttle and Huxley Islands populations were identified as discrete genetic units using model-based approaches (Figure 3.12; Figure 3.14) and were moderately and significantly differentiated from all other populations (Figure 3.4), yet PCA grouped these populations into a single unit (Figure 3.9). Regardless, these islands could be eradication candidates, especially Huxley Island considering its relative isolation, though the risk of re-invasion of Shuttle Island from Lyell Island should be explicitly considered. Kunghit Island appeared to be sufficiently isolated from the north-central populations and could also be considered as a suitable candidate for eradication. Surrounding islets should also be examined for rat populations, and these islands would need to be simultaneously eradicated to prevent re-invasion.

Additional recommendations for eradication units include Tanu and Richardson Islands, as well as Faraday, Murchison, and Hotspring Islands, though these islands remain highly connected to Lyell Island, so re-invasion is probable. In order to successfully remove brown rats from these islands, the Lyell Island population needs to be removed to prevent eradication failure via re-invasion; an eradication on this scale, however, would be ambitious. Refinement of methods has led to eradications on increasingly large target areas; for example, South Georgia Island (>100 000 ha) was recently declared rat-free following a multi-year eradication effort (Piertney *et al.*, 2016; Russell & Broome, 2016). Still, a Lyell Island eradication would represent one of the largest on record (Glen *et al.*, 2013; Russell & Broome, 2016; Springer, 2016; Towns & Broome, 2003). Though the re-invasion risk from Lyell Island remains high, eradication of

brown rats from Faraday, Murchison, and Hotspring Islands should be viewed as a priority in order to protect the Ramsay Island seabird populations.

Here, we have described patterns of dispersal and inferred levels of gene flow among invasive rat populations in the Haida Gwaii archipelago. We have identified several candidate islands for eradication of rats as well as evaluated biosecurity risks to seabird populations of concern. These results also highlight the importance of targeted research prior to conducting eradications, both to decrease the risk of re-invasions as well as to promote recovery of at-risk species. Invasive rats occupy most oceanic islands around the world; the population genomic approach we demonstrated here could be used as a framework for guiding management of invasive rats in other island systems.

3.5 Tables & Figures

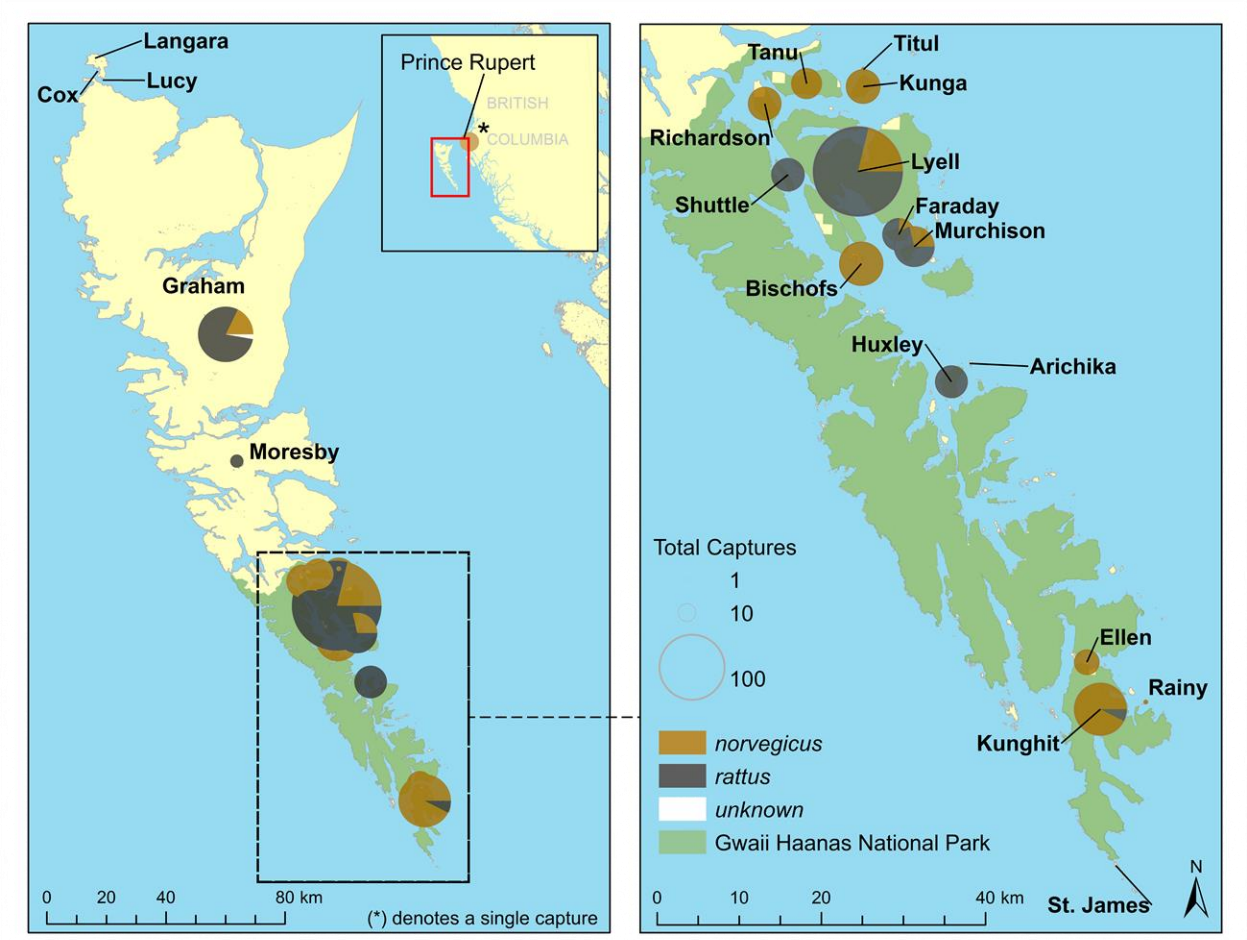

Figure 3.1. Distribution and sample size of brown (*Rattus norvegicus*) and black rats (*R. rattus*) collected in Haida Gwaii, BC. Rats were collected from 2008-2018 by Parks Canada staff.

Table 3.1. Summary statistics for the filtered brown rat and black rat datasets. Mean depth and missingness were calculated using VCFtools v0.1.15 (Danecek *et al.*, 2011). Mean genotyping error was calculated as percent discordance in genotypes between replicate individuals and was calculated both with and among sequencing libraries.

						Genotyping Error (%)	
Dataset	N	N_{UNIQUE}	N_{SNP}	Mean Depth	Mean Miss. (%)	Within	Among
Brown	297	284	27 686	16.1x	7.7	2.00	2.57
Black	251	245	28 818	14.2x	9.4	13.4	13.1

N = number of total individuals (with replicates)

N_{UNIQUE} = number of unique individuals (no replicates)

N_{SNP} = number of SNPs

Mean Depth = mean depth of coverage across all individuals

Mean Miss. = mean percent missing genotypes across all individuals

Table 3.2. Sample size and genetic diversity estimates for 12 brown rat (*Rattus norvegicus*) populations in Haida Gwaii, BC. (*) The Tlell population also includes *n* = 1 rat from Prince Rupert, BC. (†) The E-Kunghit population also includes *n* =1 rat from Rainy Islands. (‡) The Kunga population also includes *n* =1 rat from Titul Island.

Population	*N*	N_A	N_E	H_o	H_s	G_{is}
Post-Bischofs	23	1.581	1.280	0.176	0.173	-0.018
Pre-Bischofs	31	1.480	1.200	0.123	0.123	-0.004
Ellen	19	1.652	1.353	0.211	0.215	0.019
Faraday	7	1.326	1.217	0.172	0.131	-0.313
Tlell*	15	1.491	1.099	0.047	0.082	0.429
NW-Kunghit	40	1.821	1.428	0.264	0.257	-0.027
E-Kunghit†	27	1.720	1.418	0.254	0.248	-0.028
Kunga‡	33	1.543	1.275	0.170	0.165	-0.029
Lyell	35	1.728	1.320	0.197	0.202	0.025
Murchison	11	1.396	1.241	0.164	0.148	-0.106
Richardson	30	1.720	1.354	0.232	0.217	-0.071
Tanu	26	1.680	1.367	0.226	0.223	-0.010
Total	297	2.000	1.232	0.186	0.182	-0.022

N = number of individuals
N_A = mean number of alleles per locus
N_E = mean number of effective alleles per locus
H_o = mean observed heterozygosity
H_s = mean heterozygosity within population
G_{is} = inbreeding coefficient

Table 3.3. Sample size and genetic diversity estimates for 12 black rat (*Rattus rattus*) populations in Haida Gwaii, BC.

Population	N	N_A	N_E	H_o	H_s	G_{is}
Faraday	16	1.540	1.283	0.155	0.175	0.117
S-Graham	23	1.693	1.353	0.185	0.217	0.146
N-Graham	25	1.654	1.329	0.177	0.199	0.110
Huxley	15	1.547	1.263	0.161	0.166	0.028
Kunghit	4	1.387	1.223	0.149	0.161	0.074
Lyell-FP	52	1.777	1.298	0.188	0.186	-0.010
Lyell-W	7	1.530	1.291	0.189	0.190	0.001
Lyell-RP	38	1.775	1.323	0.197	0.203	0.027
Lyell-SP	18	1.723	1.324	0.218	0.207	-0.053
Murchison	22	1.686	1.340	0.202	0.209	0.031
Sandspit	5	1.406	1.242	0.178	0.167	-0.066
Shuttle	25	1.637	1.290	0.180	0.179	-0.006
Total	251	2.000	1.224	0.184	0.189	0.030

N = number of individuals
N_A = mean number of alleles per locus
N_E = mean number of effective alleles per locus
H_o = mean observed heterozygosity
H_s = mean heterozygosity within population
G_{is} = inbreeding coefficient

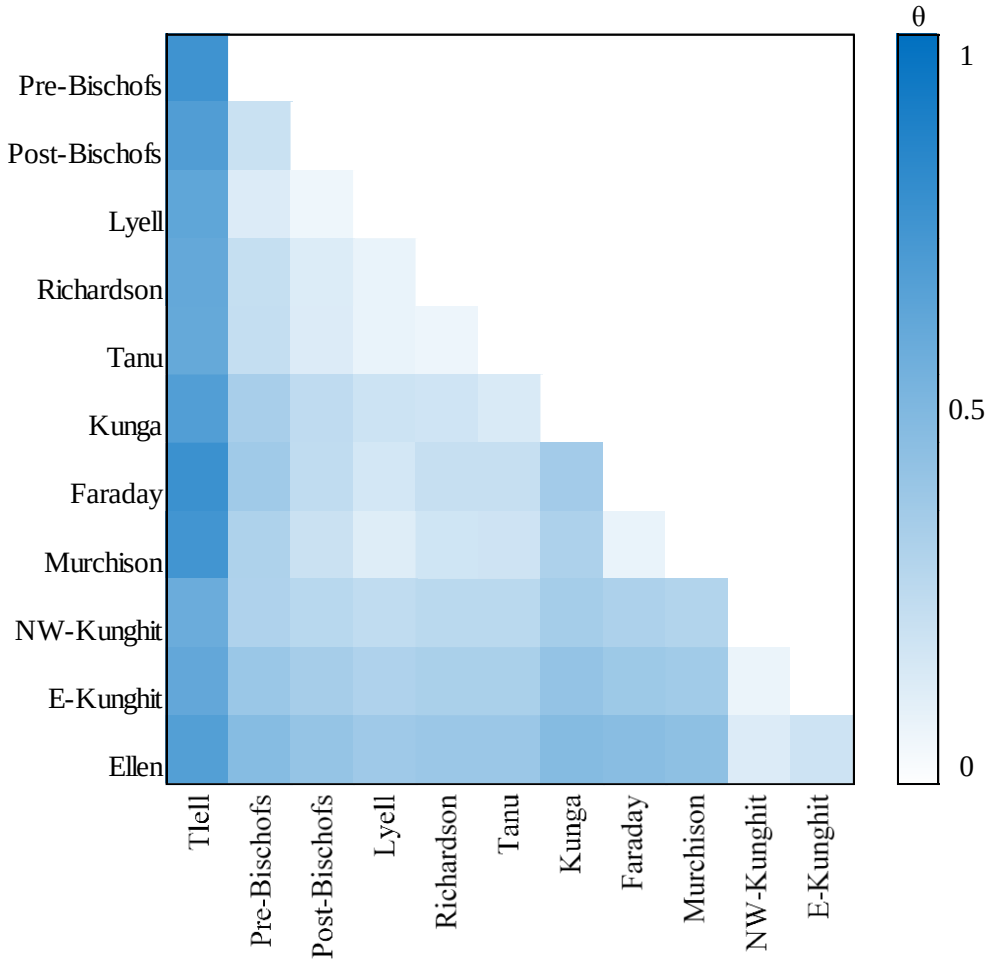

Figure 3.2. Heatmap of pairwise population differentiation (Weir & Cockerham θ (1984)) for n = 325 brown rats (*Rattus norvegicus*) collected across Haida Gwaii, BC. All pairwise comparisons were significant over 1000 permutations ($p < 0.05$).

Table 3.4. Pairwise population differentiation (Weir & Cockerham θ (1984)) of brown rats (*Rattus norvegicus*) among sample sites on Lyell Island, Haida Gwaii. All pairwise comparisons were significant over 1000 permutations ($p < 0.05$).

	Faraday Passage	Richardson Point
Richardson Point	0.018	-
Sedgwick Point	0.070	0.044

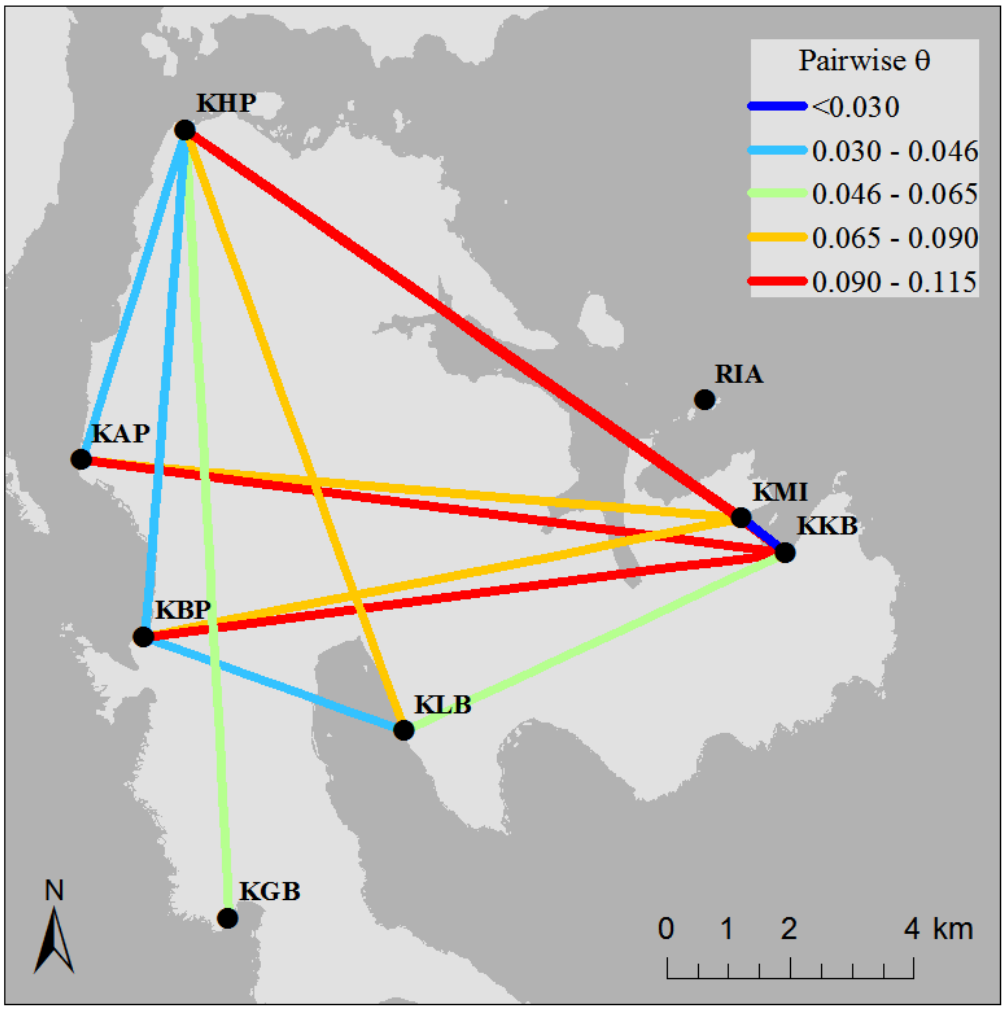

Figure 3.3. Pairwise population differentiation (Weir & Cockerham θ (1984)) of brown rats (*Rattus norvegicus*) among sample sites on Kunghit Island, Haida Gwaii. Only significant pairwise comparisons over 1000 permutations are shown ($p < 0.05$). Abbreviation definitions are as follows: (KAP) Arnold Point; (KBP) Bowles Point; (KGB) Gilbert Bay; (KHB) Hornby Point; (KKB) Keeweenah Bay; (KLB) Luxana Bay; (KMI) Marshall Island; and (RIA) Rainy Islands.

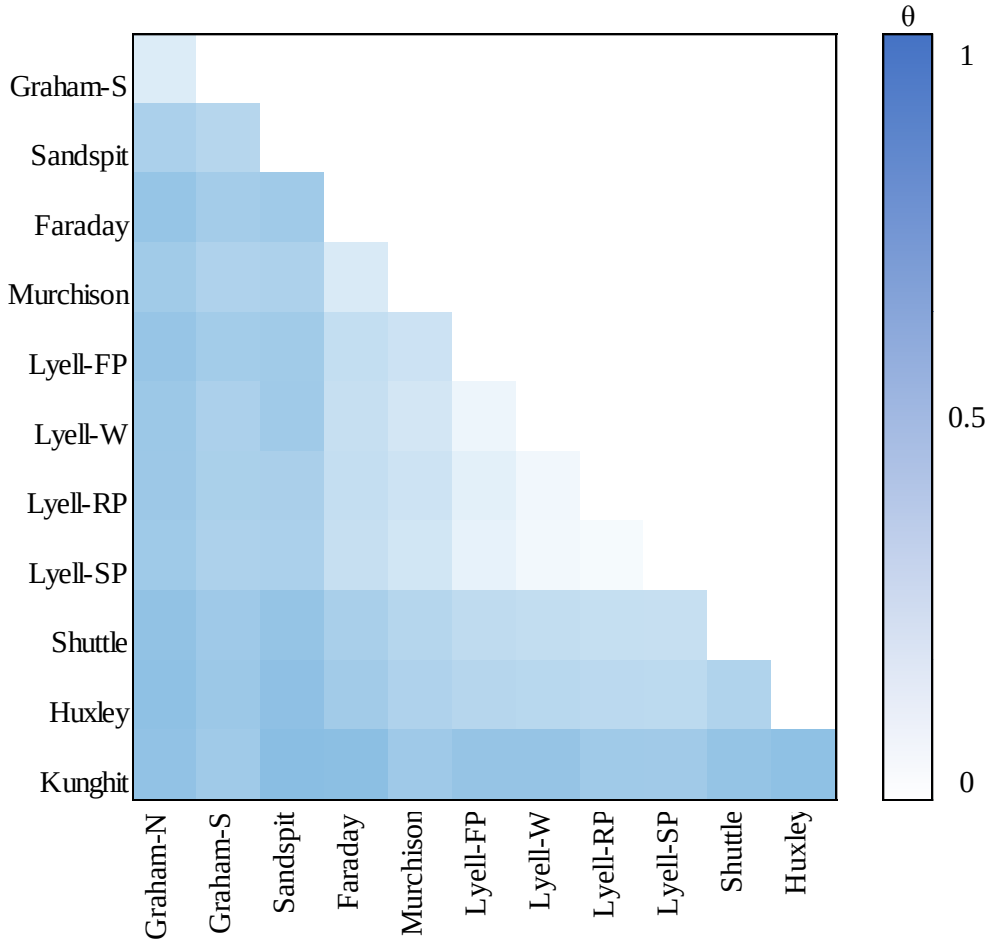

Figure 3.4. Heatmap of pairwise population differentiation (Weir & Cockerham θ (1984)) for n = 251 black rats (*Rattus rattus*) collected across Haida Gwaii, BC. (ns) Pairwise comparison was not significant. All remaining comparisons were significant over 1000 permutations ($p < 0.05$).

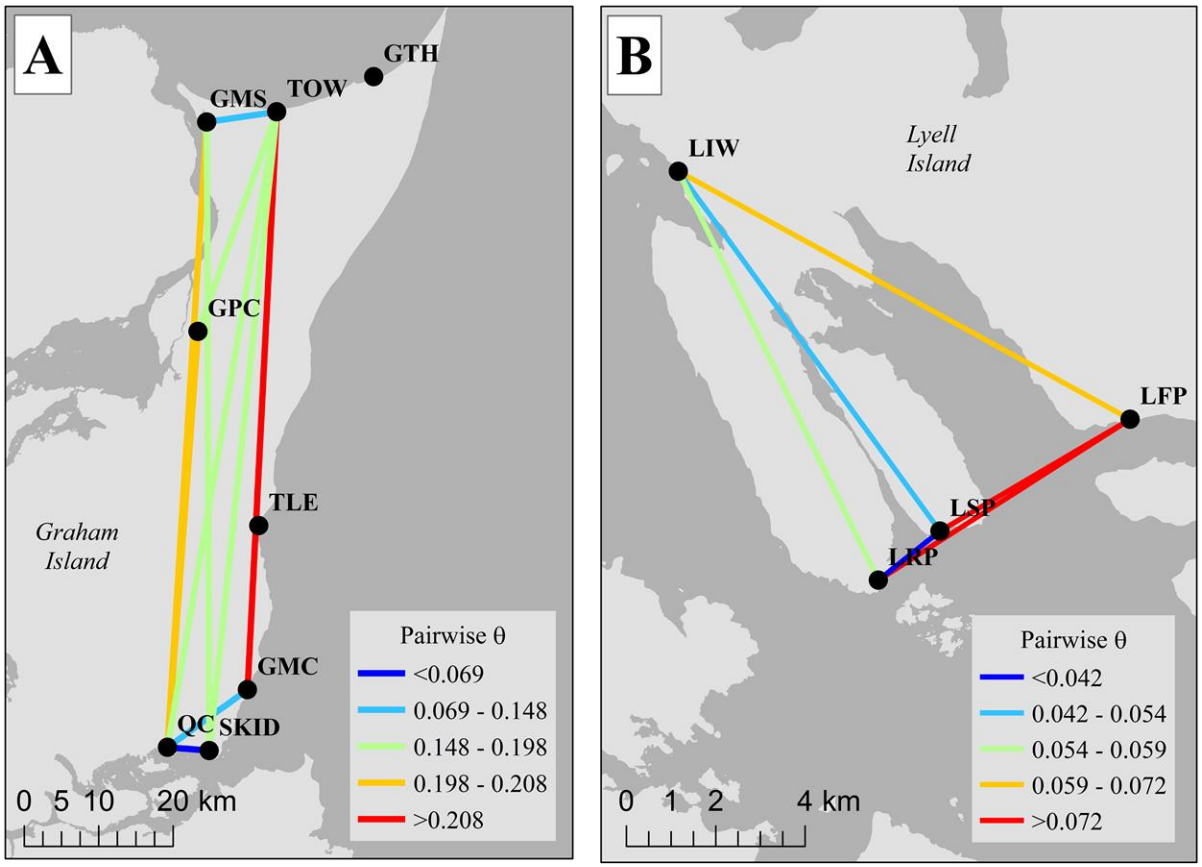

Figure 3.5. Pairwise population differentiation (Weir & Cockerham θ (1984)) of black rats (*Rattus rattus*) among sample sites on Graham Island (a) and Lyell Island (b) in Haida Gwaii. Only significant pairwise comparisons over 1000 permutations are shown ($p < 0.05$). Abbreviation definitions are as follows: (GMC) Miller Creek; (GMS) Masset; (GPC) Parks Canada dump; (GTH) Tow Hill; (LFP) Faraday Passage; (LIW) Lyell West; (LRP) Richardson Point; (LSP) Sedgewick Point; (QC) Queen Charlotte; (SKID) Skidegate; (TLE) Tlell; and (TOW) Tow Hill.

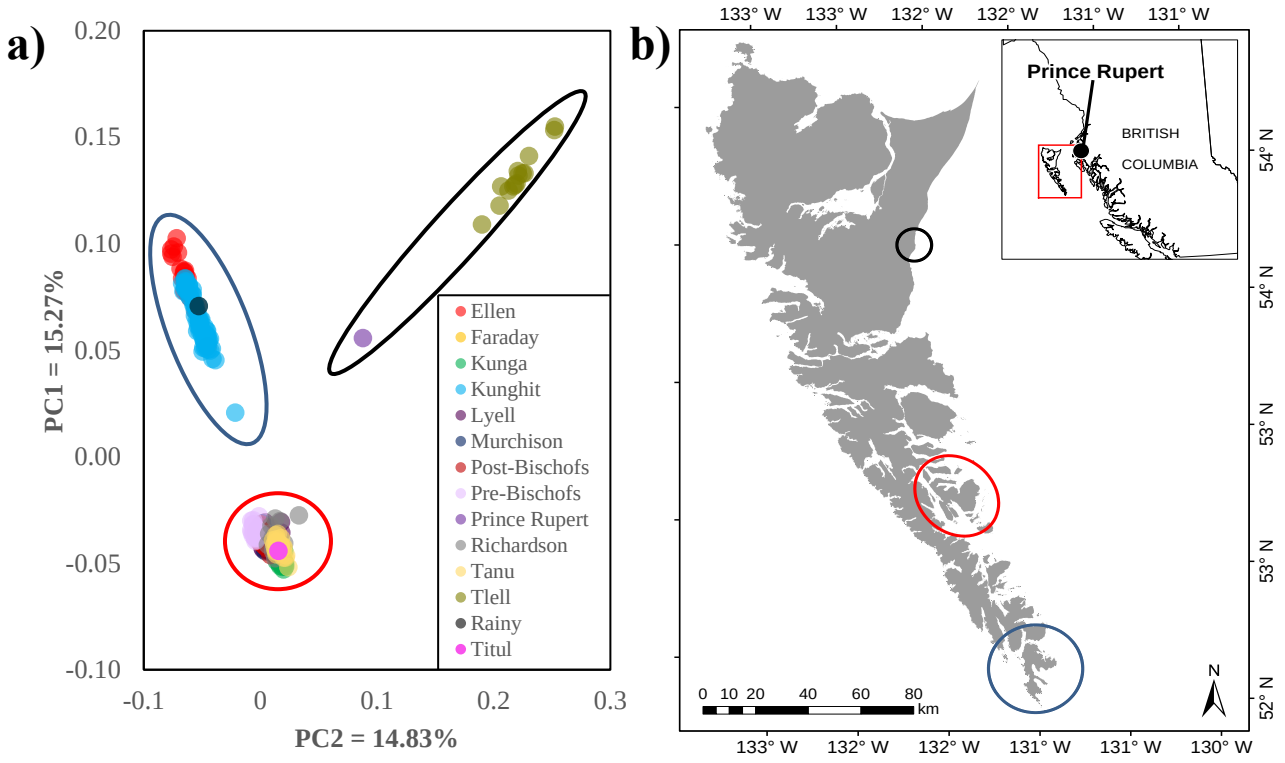

Figure 3.6. Principle component analyses (PCA; left) for *n* = 325 brown rats (*Rattus norvegicus*) collected in Haida Gwaii, BC (right). Three regionally-distinct populations were identified within Haida Gwaii, indicated by the blue, red, and black ovals.

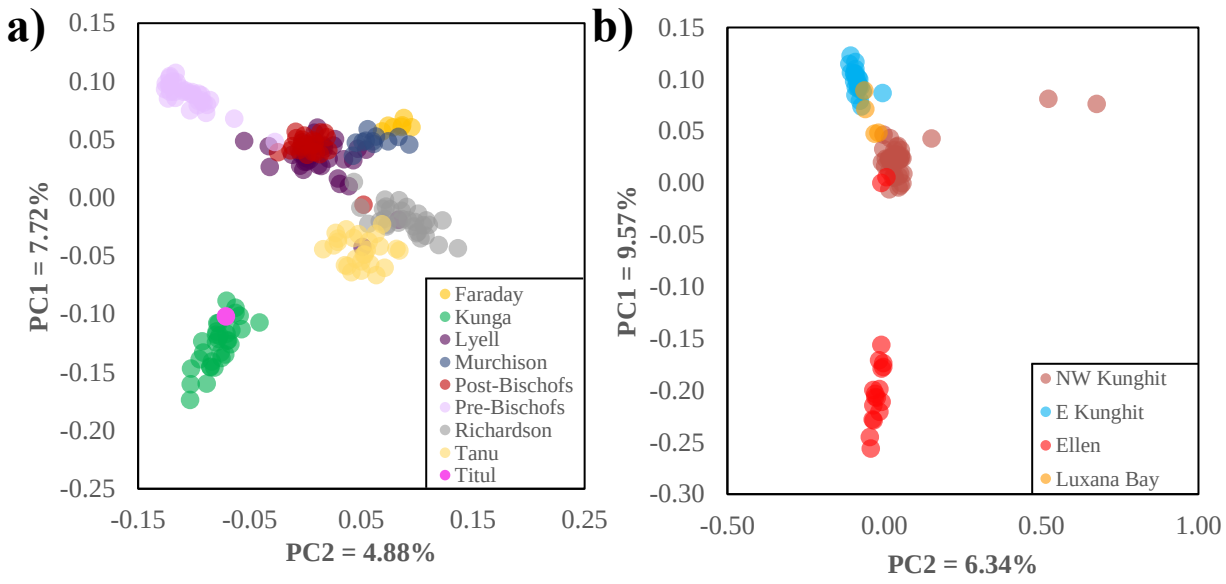

Figure 3.7. Principle component analyses of brown rats (*Rattus norvegicus*) collected from centrally located islands (left, *n* = 196) and southerly located islands (right, *n* = 86) within the Haida Gwaii archipelago. The "NW Kunghit", "E Kunghit", and "Luxana Bay" populations were all collected from Kunghit island.

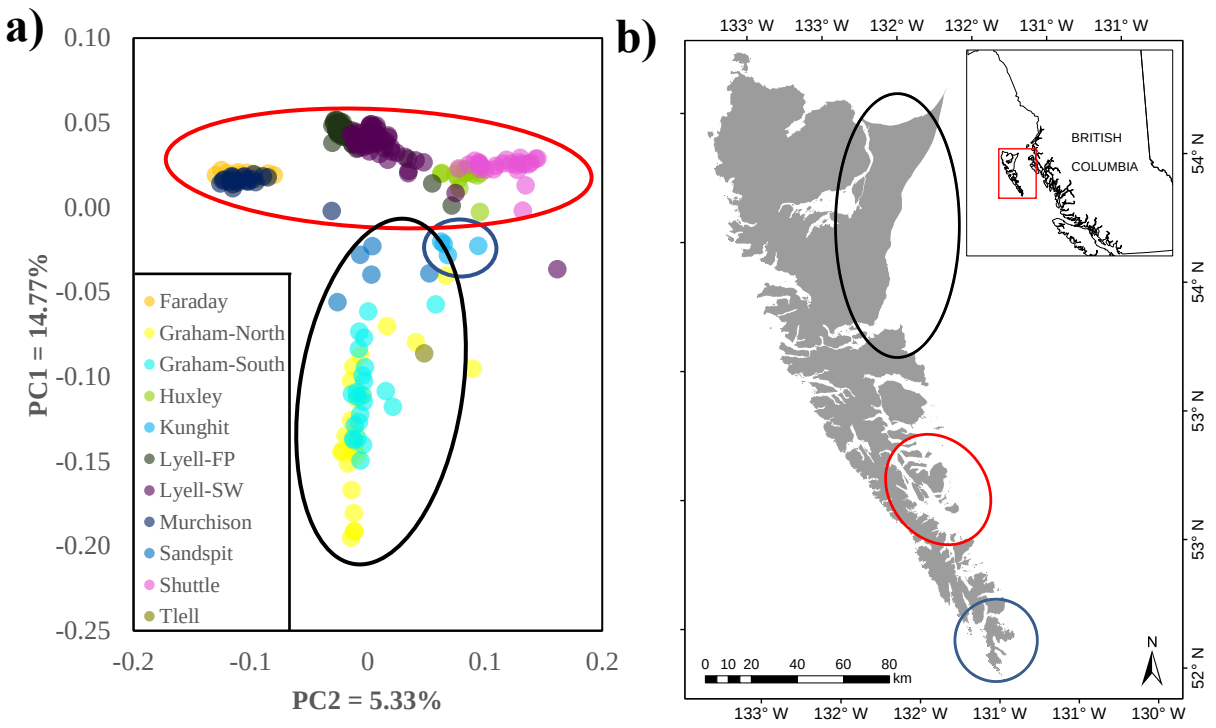

Figure 3.8. Principle component analyses (PCA; left) for *n* = 251 black rats (*Rattus rattus*) collected in Haida Gwaii, BC (right). Three regional populations were identified within Haida Gwaii, indicated by the blue, red, and black ovals.

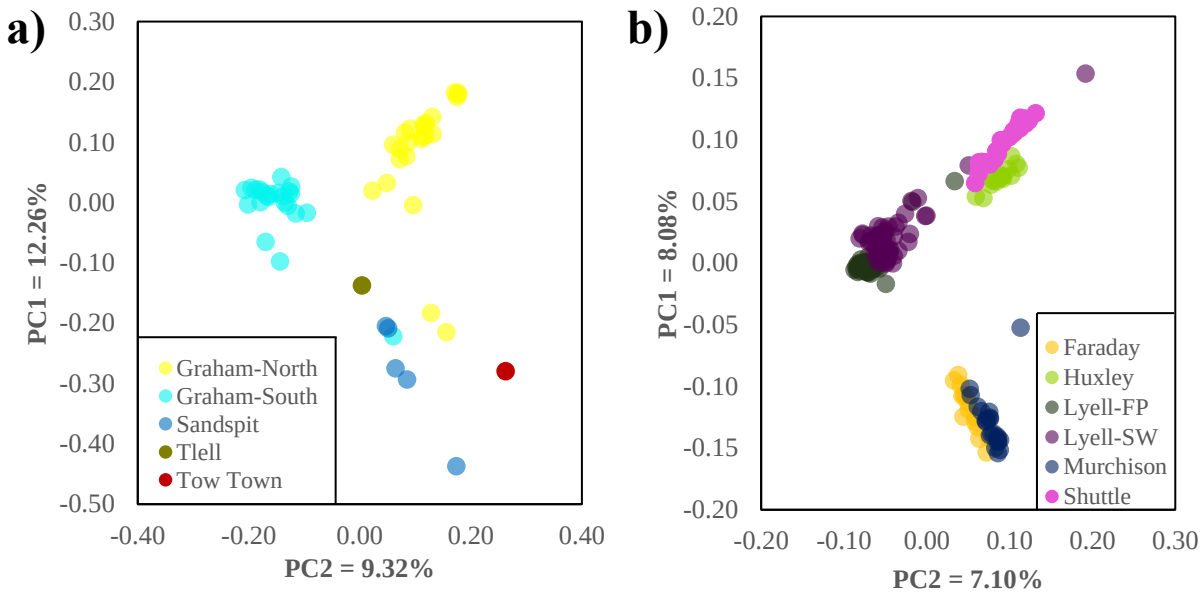

Figure 3.9. Principle component analyses of black rats (*Rattus rattus*) collected from northerly located islands (left, *n* = 54) and centrally located islands (right, *n* = 193) within the Haida Gwaii archipelago. The "Graham-North", "Graham-South", and "Tlell" populations were all collected from Graham Island, and the "Lyell-FP" and "Lyell-SW" populations were collected from Lyell Island.

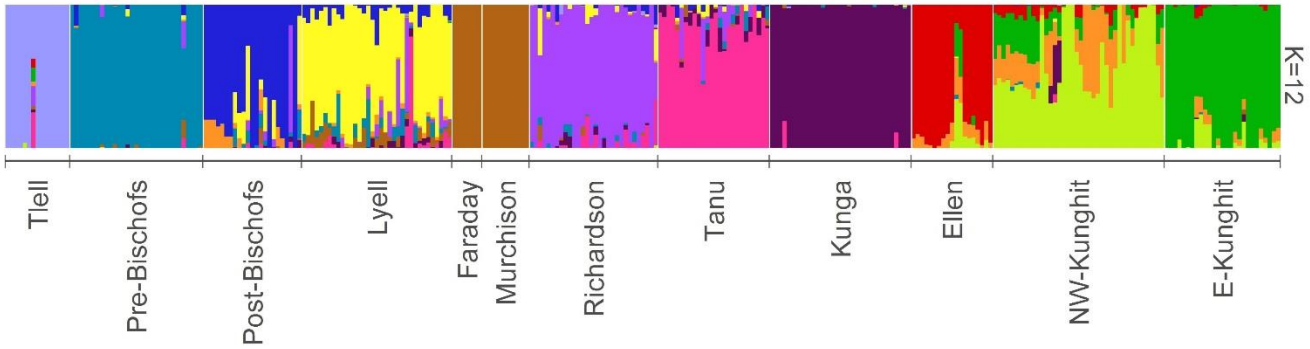

Figure 3.10. ADMIXTURE plot showing shared ancestry among *n* = 325 brown rats (*Rattus norvegicus*) collected in Haida Gwaii, BC. Ancestry was averaged over 10 iterations, and the optimal *k* = 12 was identified as the *k*-value with the lowest mean cross-validation error.

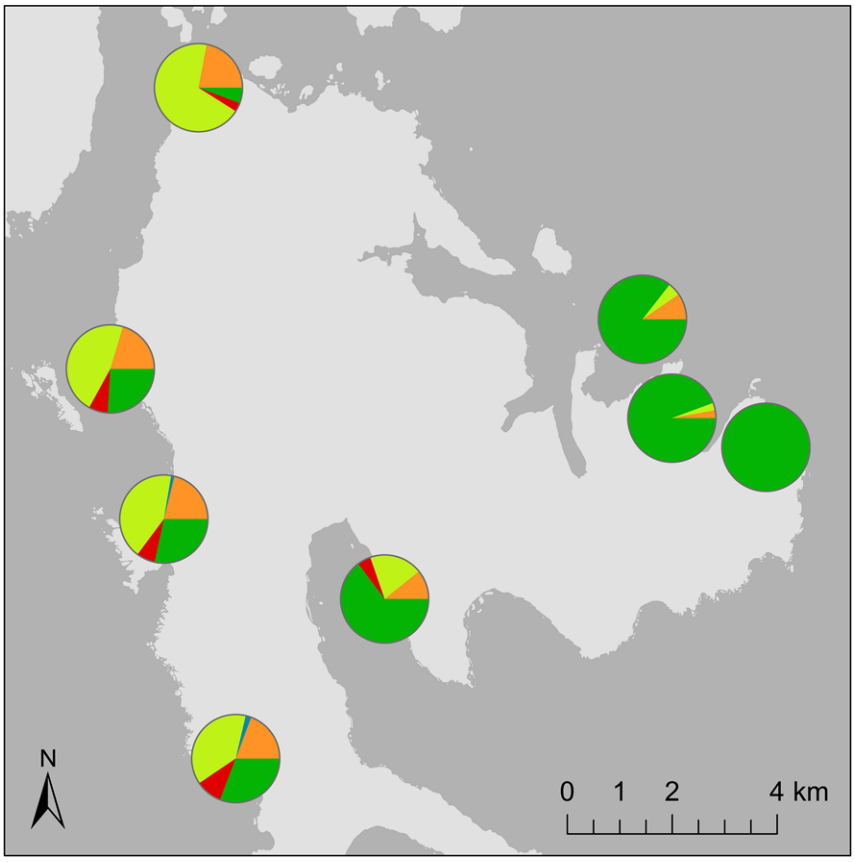

Figure 3.11. Mean admixture proportions of *n* = 67 brown rats (*Rattus norvegicus*) collected on Kunghit Island, Haida Gwaii. Individual admixture proportions were estimated using ADMIXTURE (Alexander *et al.*, 2009) and averaged for each sample site.

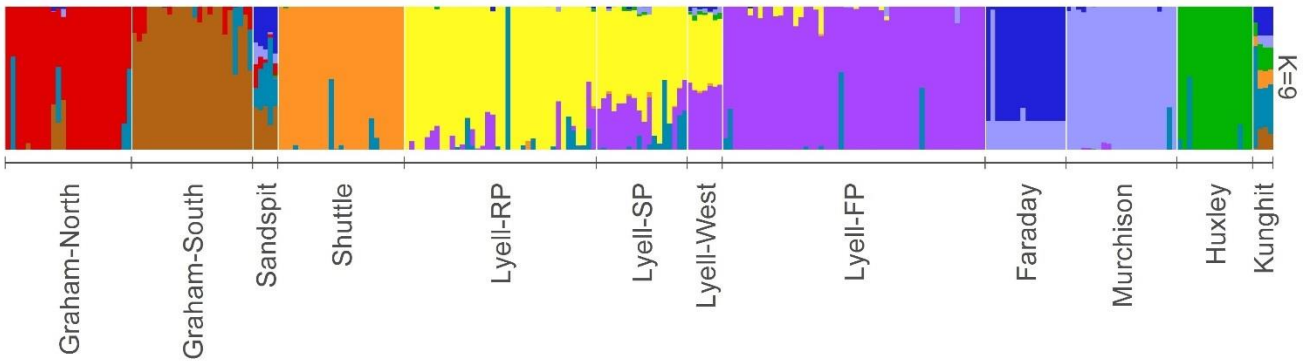

Figure 3.12. ADMIXTURE plot showing shared ancestry among *n* = 251 black rats (*Rattus rattus*) collected in Haida Gwaii, BC. Ancestry was averaged over 10 iterations, and the optimal *k* = 9 was identified as the *k*-value with the lowest mean cross-validation error.

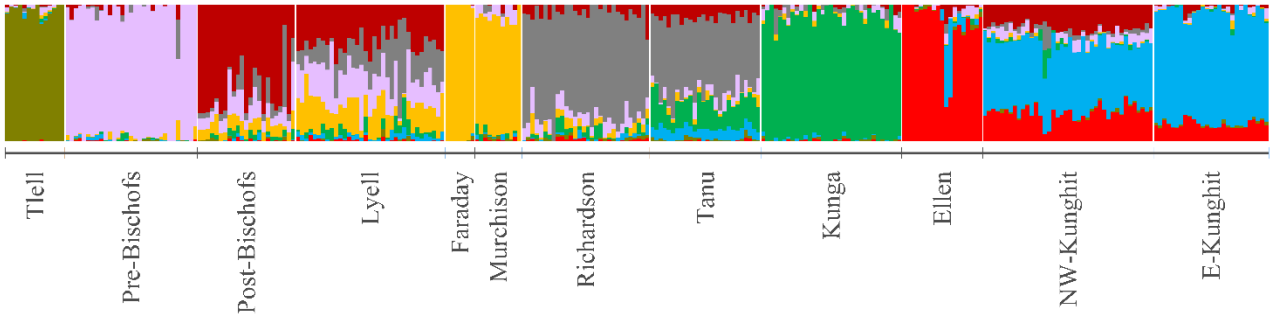

Figure 3.13. Shared ancestry coefficients for *n*=325 brown rats (*Rattus norvegicus*) sampled from Haida Gwaii, BC. Ancestry coefficients were estimated using sparse non-negative matrix factorization implemented by the R-package *LEA* (Frichot & François, 2015). An optimal *k*=8 was identified.

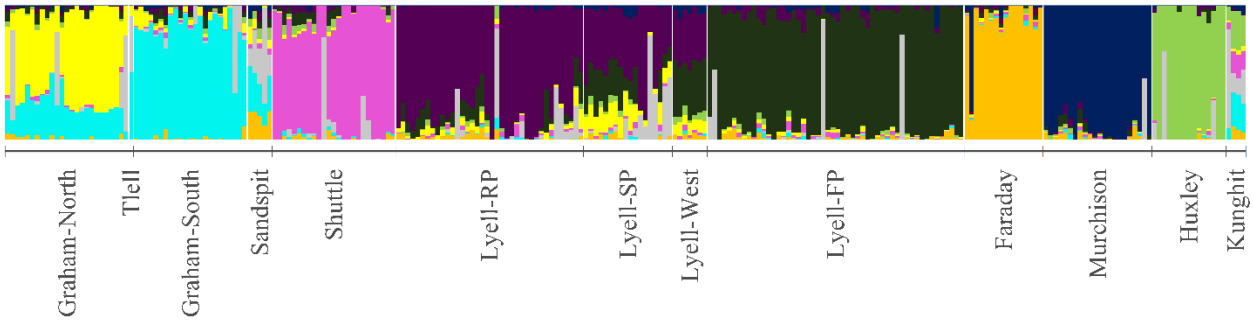

Figure 3.14. Shared ancestry coefficients for *n*=251 black rats (*Rattus rattus*) sampled from Haida Gwaii, BC. Ancestry coefficients were estimated using sparse non-negative matrix factorization implemented by the R-package *LEA* (Frichot & François, 2015). An optimal *k*=9 was identified.

Table 3.5. Mean directional migration rates for *n*=325 brown rats (*Rattus norvegicus*) sampled within Haida Gwaii, BC. Source populations are arranged in rows and sink populations are arranged in columns. Migration rates were averaged across five iterations and calculated using the software BayesAss v3.0.4 (Wilson & Rannala, 2003). 95% credible sets were calculated as mean standard deviation * 1.96; migration rates were deemed significant if the credible set did not include zero (shaded grey). Abbreviation definitions are as follows: (POST) post-Bischofs; (PRE) pre-Bischofs; (ELI) Ellen; (FAR) Faraday; (TLE) Tlell; (NKGT) NW Kunghit; (EKGT) E Kunghit; (KGA) Kunga; (LYL) Lyell; (MUR) Murchison; (RIC) Richardson; and (TNU) Tanu.

Source:SINK	POST	PRE	ELI	FAR	TLE	NKGT	EKGT	KGA	LYL	MUR	RIC	TNU
Post-Bischofs	**0.872**	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.010	0.020	0.010
	(0.054)	(0.019)	(0.019)	(0.019)	(0.019)	(0.019)	(0.019)	(0.019)	(0.027)	(0.019)	(0.026)	(0.019)
Pre-Bischofs	0.008	**0.902**	0.008	0.008	0.008	0.008	0.008	0.008	0.023	0.008	0.008	0.008
	(0.014)	**(0.044)**	(0.015)	(0.014)	(0.014)	(0.015)	(0.015)	(0.014)	(0.025)	(0.014)	(0.014)	(0.014)
Ellen	0.011	0.011	**0.860**	0.011	0.011	0.032	0.011	0.011	0.011	0.011	0.011	0.011
	(0.020)	(0.021)	**(0.057)**	(0.020)	(0.021)	(0.034)	(0.020)	(0.020)	(0.020)	(0.021)	(0.021)	(0.020)
Faraday	0.018	0.018	0.018	**0.807**	0.018	0.018	0.018	0.018	0.018	0.018	0.017	0.018
	(0.032)	(0.032)	(0.033)	**(0.072)**	(0.032)	(0.033)	(0.033)	(0.033)	(0.033)	(0.033)	(0.033)	(0.033)
Tlell	0.012	0.013	0.012	0.012	**0.840**	0.012	0.025	0.012	0.012	0.012	0.013	0.025
	(0.023)	(0.023)	(0.023)	(0.023)	**(0.062)**	(0.023)	(0.032)	(0.023)	(0.023)	(0.023)	(0.024)	(0.032)
NE-Kunghit	0.006	0.006	0.006	0.006	0.006	**0.917**	0.006	0.019	0.006	0.007	0.006	0.007
	(0.012)	(0.012)	(0.012)	(0.012)	(0.012)	**(0.039)**	(0.012)	(0.021)	(0.012)	(0.012)	(0.012)	(0.012)
W-Kunghit	0.009	0.009	0.009	0.009	0.009	0.030	**0.883**	0.009	0.009	0.009	0.009	0.009
	(0.017)	(0.017)	(0.017)	(0.017)	(0.017)	(0.031)	**(0.051)**	(0.017)	(0.017)	(0.017)	(0.017)	(0.017)
Kunga	0.008	0.008	0.008	0.008	0.008	0.008	0.008	**0.917**	0.008	0.008	0.008	0.008
	(0.015)	(0.015)	(0.014)	(0.015)	(0.015)	(0.014)	(0.015)	**(0.042)**	(0.014)	(0.014)	(0.015)	(0.015)
Lyell	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	**0.901**	0.007	0.021	0.014
	(0.013)	(0.014)	(0.014)	(0.013)	(0.013)	(0.014)	(0.014)	(0.014)	**(0.043)**	(0.014)	(0.023)	(0.019)
Murchison	0.014	0.014	0.014	0.028	0.014	0.014	0.014	0.014	0.014	**0.833**	0.014	0.014
	(0.026)	(0.026)	(0.026)	(0.052)	(0.026)	(0.026)	(0.026)	(0.026)	(0.026)	**(0.075)**	(0.026)	(0.026)
Richardson	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.016	0.016	0.008	**0.892**	0.016
	(0.015)	(0.015)	(0.015)	(0.015)	(0.015)	(0.015)	(0.015)	(0.021)	(0.021)	(0.015)	**(0.046)**	(0.021)
Tanu	0.009	0.009	0.009	0.009	0.018	0.009	0.009	0.009	0.009	0.009	0.026	**0.878**
	(0.017)	(0.017)	(0.017)	(0.017)	(0.023)	(0.017)	(0.017)	(0.017)	(0.017)	(0.016)	(0.028)	**(0.050)**

Table 3.6. First generation migrants detected in a directional migration analysis of *n*=325 brown rats (*Rattus norvegicus*) within Haida Gwaii, BC. Posterior probabilities for individual ancestry were estimated over five iterations using the software BayesAss v3.0.4 (Wilson & Rannala, 2003). Standard deviation is shown in parentheses.

Sample ID	Sampled Population	Source Population	Mean Posterior Probability
BIS-012	Post-Bischofs	Richardson	0.933 (0.045)
BIS-13-001	Post-Bischofs	Lyell	0.986 (0.029)
EIC-16-011	Ellen	NW Kunghit	1.000 (0.000)
EIN-16-001	Ellen	NW Kunghit	1.000 (0.000)
RIA-16-001	Rainy	E Kunghit	1.000 (0.000)
TIE-16-001	Titul	Kunga	0.843 (0.031)

Table 3.7. Mean directional migration rates for *n*=251 black rats (*Rattus rattus*) sampled within Haida Gwaii, BC. Source populations are arranged in rows and sink populations are arranged in columns. Migration rates were averaged across five iterations and calculated using the software BayesAss v3.0.4 (Wilson & Rannala, 2003). 95% credible sets were calculated as mean standard deviation * 1.96; migration rates were deemed significant if the credible set did not include zero (shaded grey). Abbreviation definitions are as follows: (FAR) Faraday; (SGRM) Graham-South; (NGRM) Graham-North; (HUX) Huxley; (KGT) Kunghit; (LFP) Lyell-FP; (LIW) Lyell-West; (LRP) Lyell-RP; (LSP) Lyell-SP; (MUR) Murchison; (SAND) Sandspit; (SHTL) Shuttle; and (TLE) Tlell.

Source:SINK	FAR	SGRM	NGRM	HUX	KGT	LFP	LIW	LRP	LSP	MUR	SAND	SHTL	TLE
Faraday	**0.851**	0.012	0.012	0.011	0.011	0.012	0.012	0.012	0.011	0.023	0.011	0.012	0.011
	(0.059)	(0.022)	(0.022)	(0.022)	(0.022)	(0.022)	(0.022)	(0.022)	(0.022)	(0.030)	(0.022)	(0.022)	(0.022)
Graham-South	0.009	**0.857**	0.028	0.009	0.010	0.010	0.010	0.010	0.014	0.010	0.014	0.010	0.009
	(0.018)	**(0.054)**	(0.030)	(0.018)	(0.018)	(0.018)	(0.018)	(0.018)	(0.024)	(0.018)	(0.024)	(0.018)	(0.018)
Graham-North	0.009	0.026	**0.852**	0.009	0.009	0.009	0.009	0.009	0.024	0.009	0.019	0.009	0.009
	(0.017)	(0.028)	**(0.052)**	(0.017)	(0.017)	(0.017)	(0.017)	(0.017)	(0.028)	(0.017)	(0.025)	(0.017)	(0.017)
Huxley	0.012	0.012	0.012	**0.845**	0.012	0.012	0.012	0.012	0.024	0.012	0.012	0.012	0.012
	(0.023)	(0.022)	(0.023)	**(0.060)**	(0.023)	(0.022)	(0.022)	(0.023)	(0.031)	(0.022)	(0.023)	(0.023)	(0.023)
Kunghit	0.020	0.020	0.019	0.020	**0.754**	0.020	0.020	0.020	0.031	0.020	0.020	0.020	0.020
	(0.036)	(0.036)	(0.036)	(0.036)	**(0.070)**	(0.036)	(0.036)	(0.036)	(0.048)	(0.036)	(0.037)	(0.036)	(0.036)
Lyell-FP	0.005	0.005	0.005	0.005	0.005	**0.923**	0.005	0.010	0.015	0.005	0.005	0.005	0.005
	(0.010)	(0.010)	(0.010)	(0.010)	(0.010)	**(0.034)**	(0.010)	(0.014)	(0.017)	(0.010)	(0.010)	(0.010)	(0.010)
Lyell-West	0.016	0.017	0.017	0.017	0.017	0.096	**0.709**	0.029	0.017	0.017	0.017	0.017	0.017
	(0.031)	(0.031)	(0.031)	(0.031)	(0.031)	(0.096)	**(0.097)**	(0.042)	(0.031)	(0.031)	(0.031)	(0.031)	(0.031)
Lyell-RP	0.007	0.007	0.006	0.007	0.007	0.013	0.007	**0.895**	**0.026**	0.007	0.007	0.007	0.007
	(0.013)	(0.013)	(0.012)	(0.013)	(0.013)	(0.018)	(0.013)	**(0.042)**	**(0.024)**	(0.013)	(0.013)	(0.013)	(0.013)
Lyell-SP	0.011	0.011	0.011	0.011	0.011	0.030	0.011	**0.182**	**0.679**	0.011	0.011	0.011	0.011
	(0.020)	(0.020)	(0.020)	(0.021)	(0.021)	(0.041)	(0.020)	**(0.067)**	**(0.031)**	(0.021)	(0.021)	(0.021)	(0.020)
Murchison	0.009	0.010	0.010	0.010	0.009	0.009	0.010	0.010	0.019	**0.867**	0.019	0.010	0.010
	(0.018)	(0.018)	(0.018)	(0.018)	(0.018)	(0.018)	(0.018)	(0.018)	(0.025)	**(0.053)**	(0.025)	(0.018)	(0.018)
Sandspit	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.018	0.019	0.037	**0.759**	0.018	0.018
	(0.034)	(0.034)	(0.034)	(0.035)	(0.034)	(0.034)	(0.035)	(0.034)	(0.034)	(0.047)	**(0.067)**	(0.034)	(0.034)
Shuttle	0.009	0.017	0.009	0.009	0.009	0.009	0.009	0.009	0.017	0.009	0.009	**0.880**	0.009
	(0.016)	(0.023)	(0.016)	(0.016)	(0.016)	(0.016)	(0.016)	(0.016)	(0.023)	(0.016)	(0.016)	**(0.049)**	(0.016)
Tlell	0.024	0.040	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	**0.698**
	(0.043)	(0.058)	(0.044)	(0.043)	(0.043)	(0.044)	(0.044)	(0.043)	(0.043)	(0.044)	(0.043)	(0.043)	**(0.053)**

Table 3.8. First generation migrants detected in a directional migration analysis of *n*=251 black rats (*Rattus rattus*) within Haida Gwaii, BC. Posterior probabilities for individual ancestry were estimated over five iterations using the software BayesAss v3.0.4 (Wilson & Rannala, 2003). Standard deviation is shown in parentheses.

Sample ID	Sampled Population	Source Population	Mean Posterior Probability
FAR-13-001_2	Faraday	Murchison	1.000 (0.000)
LRP-13_RA-002_7	Lyell-RP	Lyell-SP	1.000 (0.000)
LSP-002	Lyell-SP	Lyell-RP	0.873 (0.053)
LSP-007	Lyell-SP	Lyell-RP	0.708 (0.090)
LSP-008	Lyell-SP	Lyell-RP	0.706 (0.090)
LSP-009	Lyell-SP	Lyell-RP	0.792 (0.074)
LSP-010	Lyell-SP	Lyell-RP	0.824 (0.061)
LSP-011	Lyell-SP	Lyell-RP	0.726 (0.089)
LSP-012	Lyell-SP	Lyell-RP	0.677 (0.100)
LSP-013	Lyell-SP	Lyell-RP	0.797 (0.072)
LSP-14-003_6.2	Lyell-SP	Lyell-RP	0.671 (0.099)
LSP-14-004	Lyell-SP	Lyell-RP	0.587 (0.109)
LYE-18-01a	Lyell-FP	Lyell-RP	1.000 (0.000)
LYE-18-04a	Lyell-SP	Lyell-RP	1.000 (0.000)
LYE-18-10a	Lyell-SP	Lyell-RP	0.636 (0.104)
LYE-18-11a	Lyell-SP	Lyell-RP	0.742 (0.097)

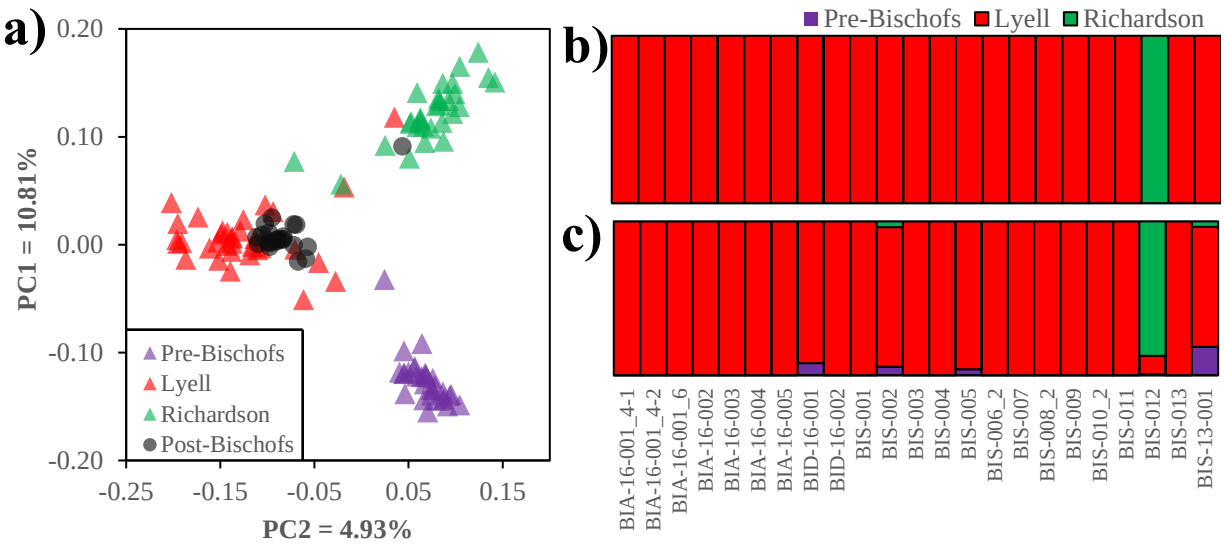

Figure 3.15. Population assignment of *n*=23 brown rats (*Rattus norvegicus*) collected from the Bischofs Islands, Haida Gwaii, following a failed eradication. Putative source populations included Lyell and Richardson Island, while the Pre-Bischofs population were samples collected prior to the failed eradication. We used a projected principle component analysis (a) where the parameter space was defined using only samples collected from the putative source populations and then the post-eradication samples were projected onto this space to identify its genetic origin. The analysis was performed using the *smartpca* function from the EIGENSOFT v6.1.4 software package (Galinsky *et al.*, 2016; Patterson *et al.*, 2006; Price *et al.*, 2006). We also calculated the posterior probability of assignment (b) to each of the three reference populations following a discriminant analysis of components using the R-package *adegenet* (Jombart & Ahmed, 2011). We also estimated coancestry via supervised Bayesian clustering analysis (c) as implemented by the software ADMIXTURE (Alexander *et al.*, 2009), where the reference populations were first pre-defined, then the post-eradication samples were compared against these populations to infer genetic ancestry.

Table 3.9. Population assignment test for *n*=23 brown rats (*Rattus norvegicus*) collected from the Bischof Islands, Haida Gwaii following a failed eradication. Putative source populations included Lyell and Richardson Island, while the Pre-Bischofs population were samples collected prior to the failed eradication. Population assignments were carried out under the Rannala & Mountain (1997), and posterior probability of assignment was calculated using 100 000 simulated individuals using the Paetkau *et al.* (2004) resampling algorithm as implemented in GeneClass (Piry *et al.*, 2004).

	Population assignment w/o simulations (%)			Posterior-Probability		
Sample ID	Pre-Bischofs	Lyell	Richardson	Pre-Bischofs	Lyell	Richardson
BIA-16-001_4-1	0	100	0	0.772	1.000	1.000
BIA-16-001_4-2	0	100	0	0.761	1.000	1.000
BIA-16-001_6	0	100	0	0.734	1.000	1.000
BIA-16-002	0	100	0	0.818	1.000	1.000
BIA-16-003	0	100	0	0.808	1.000	1.000
BIA-16-004	0	100	0	0.773	1.000	1.000
BIA-16-005	0	100	0	0.785	1.000	1.000
BID-16-001	0	100	0	0.909	1.000	1.000
BID-16-002	0	100	0	0.883	1.000	1.000
BIS-001	0	100	0	0.816	1.000	1.000
BIS-002	0	100	0	0.824	1.000	1.000
BIS-003	0	100	0	0.714	1.000	1.000
BIS-004	0	100	0	0.882	1.000	1.000
BIS-005	0	100	0	0.859	1.000	1.000
BIS-006_2	0	100	0	0.748	1.000	1.000
BIS-007	0	100	0	0.826	1.000	1.000
BIS-008_2	0	100	0	0.749	1.000	1.000
BIS-009	0	100	0	0.784	1.000	1.000
BIS-010_2	0	100	0	0.776	1.000	1.000
BIS-011	0	100	0	0.768	1.000	1.000
BIS-012	0	0	100	0.527	0.982	1.000
BIS-013	0	100	0	0.753	1.000	1.000
BIS-13-001	0	100	0	0.856	1.000	1.000

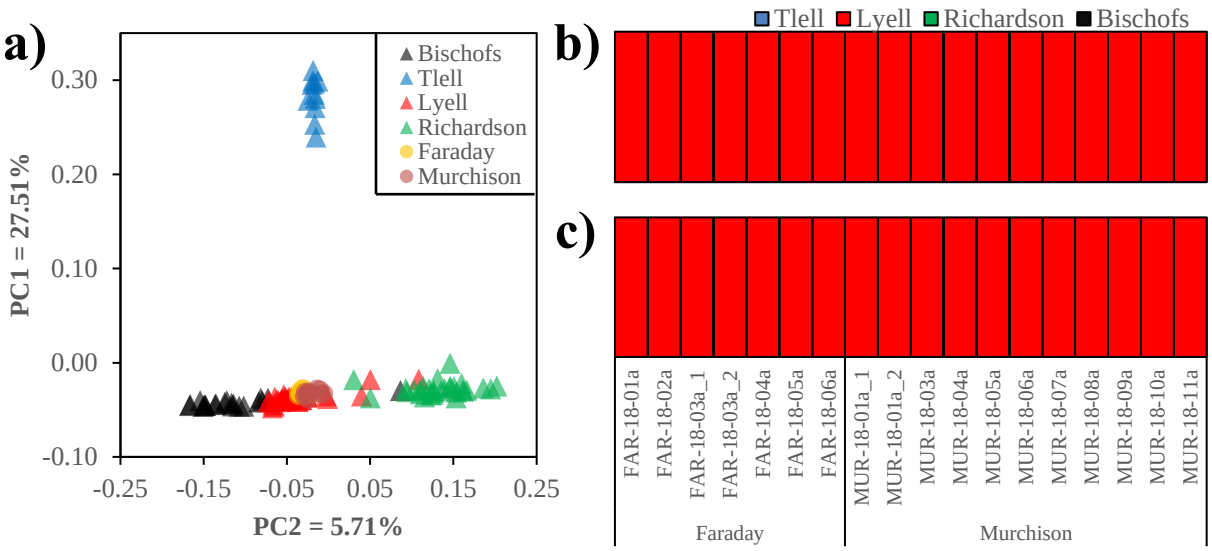

Figure 3.16. Population assignment of brown rats (*Rattus norvegicus*) collected from Faraday Island (*n*=7) and Murchison Island (*n*=11), Haida Gwaii. Putative source populations included Lyell, Richardson, and the Bischof Islands as well as Tlell, BC. We used a projected principle component analysis (a) where the parameter space was defined using only samples collected from the putative source populations and then unknown samples were projected onto this space to identify their genetic origin. The analysis was performed using the *smartpca* function from the EIGENSOFT v6.1.4 software package (Galinsky *et al.*, 2016; Patterson *et al.*, 2006; Price *et al.*, 2006). We also calculated the posterior probability of assignment (b) to each of the four reference populations following a discriminant analysis of components using the R-package *adegenet* (Jombart & Ahmed, 2011). We also estimated coancestry via supervised Bayesian clustering analysis (c) as implemented by the software ADMIXTURE (Alexander *et al.*, 2009), where the reference populations were first pre-defined, then the unknown samples were compared against these populations to infer genetic ancestry.

Table 3.10. Population assignment test for brown rats (*Rattus norvegicus*) collected from Faraday Island (*n*=7) and Murchison Island (*n*=11), Haida Gwaii. Putative source populations included Lyell, Richardson, and the Bischof Islands, as well as Tlell, BC. Population assignments were carried out under the Rannala & Mountain (1997), and posterior probability of assignment was calculated using 100 000 simulated individuals using the Paetkau *et al.* (2004) resampling algorithm as implemented in GeneClass (Piry *et al.*, 2004).

	Population Assignment w/o simulations (%)				Posterior probability			
Sample ID	Bischofs	Tlell	Lyell	Richardson	Bischofs	Tlell	Lyell	Richardson
FAR-18-01a	0	0	100	0	0.886	0.001	1.000	1.000
FAR-18-02a	0	0	100	0	0.878	0.001	1.000	1.000
FAR-18-03a_1	0	0	100	0	0.903	0.001	1.000	1.000
FAR-18-03a_2	0	0	100	0	0.861	0.001	1.000	1.000
FAR-18-04a	0	0	100	0	0.899	0.001	1.000	1.000
FAR-18-05a	0	0	100	0	0.804	0.001	1.000	1.000
FAR-18-06a	0	0	100	0	0.823	0.001	0.999	1.000
MUR-18-01a_1	0	0	100	0	0.824	0.001	1.000	1.000
MUR-18-01a_2	0	0	100	0	0.898	0.001	1.000	1.000
MUR-18-03a	0	0	100	0	0.814	0.001	0.998	1.000
MUR-18-04a	0	0	100	0	0.793	0.001	0.999	1.000
MUR-18-05a	0	0	100	0	0.854	0.001	1.000	1.000
MUR-18-06a	0	0	100	0	0.851	0.001	1.000	1.000
MUR-18-07a	0	0	100	0	0.889	0.001	1.000	1.000
MUR-18-08a	0	0	100	0	0.738	0.001	0.998	1.000
MUR-18-09a	0	0	100	0	0.936	0.001	1.000	1.000
MUR-18-10a	0	0	100	0	0.930	0.001	1.000	1.000
MUR-18-11a	0	0	100	0	0.834	0.001	1.000	1.000

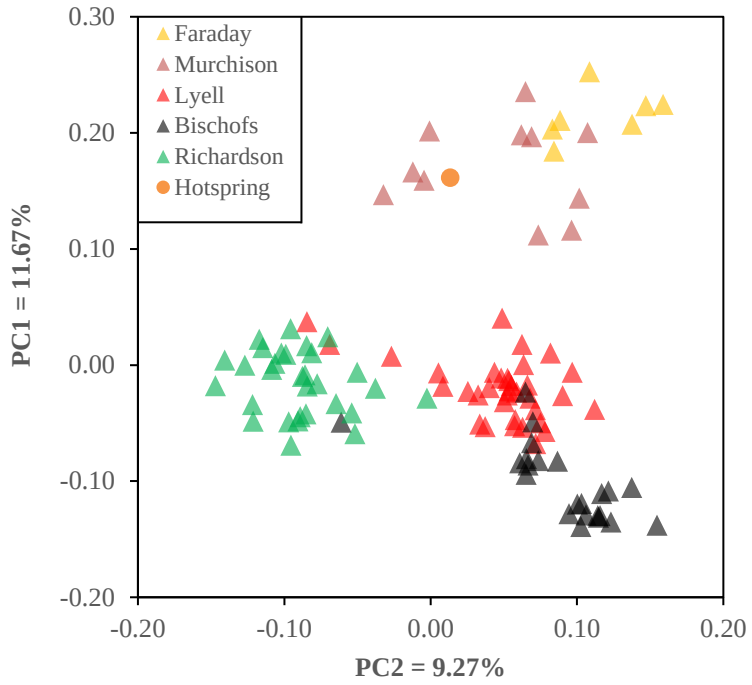

Figure 3.17. Projected principle component analysis for invasive brown rats (*Rattus norvegicus*) collected in Haida Gwaii, BC. The parameter space was defined using only samples collected from Faraday, Murchison, Lyell, Bischofs, and Richardson Island; one sample collected from Hotspring Island was then projected onto this space to identify its historical origin. The analysis was performed using the *smartpca* function from the EIGENSOFT v6.1.4 software package (Galinsky *et al.*, 2016; Patterson *et al.*, 2006; Price *et al.*, 2006).

Table 3.11. Identification of source population for a single brown rat (*Rattus norvegicus*) collected on Hotspring Island, BC. The discriminant analysis of components (DAPC) was implemented using the R-package *adegenet* (Jombart & Ahmed, 2011). Population assignment using the Rannala & Mountain (1997) methods were implemented using GeneClass2.0.h (Piry *et al.*, 2004). Supervised Bayesian clustering was implemented using ADMIXTURE v1.3.0 (Alexander *et al.*, 2009).

	Assignment				
Method	Faraday	Murchison	Bischofs	Lyell	Richardson
DAPC*†	1.000	1.000	0.000	0.000	0.000
Rannala & Mountain 1997†	0.007	0.990	0.000	0.343	0.618
Supervised Bayesian clustering‡	0.162	0.487	0.033	0.000	0.318

(*) Faraday and Murchison were identified as a single clustering for this analysis
(†) Assignment values reflect posterior probability
(‡) Assignment values reflect admixture coefficients

Chapter 4: Conclusions

4.1 Research findings and significance

This study represents the first use of population genomic data for analyzing and managing invasive rat movements in the Haida Gwaii archipelago. Previously, the connectivity among rat populations on these islands was unknown, with information on rat populations limited to presence/absence data. With this study, we were able to infer patterns of connectivity among invasive black and brown rat populations, thereby producing a more complete picture of how these animals have spread through the Haida Gwaii ecosystem. Traditional observational studies, such as mark-recapture or camera trapping, can be ineffective at describing cryptic relationships, though these relationships can often be described using genetic data (Westneat, 1987). Both brown and black rats are naturally reclusive species, and detection of their movements can be challenging via visual observation (Abdelkrim *et al.*, 2005, 2007; Russell & Clout, 2004). Using genomic analysis, we successfully identified key interactions among island populations and were able to make inferences on elements shaping these interactions.

We found that proximate populations, for the most part, were more related than those that were more distant. Additionally, populations on the larger islands, namely Kunghit and Lyell, appeared to have more connectivity with neighbouring populations than did populations on some of the smaller islands likely due to isolation-by-distance. Both of these observations agree with island biogeography theory, which states that larger, more proximate islands will share higher levels of migrants than smaller, more distant islands (MacArthur & Wilson, 1967). The extent of parallel shoreline between islands also seems to affect rat movements among populations. For example, the Kunga Island population was genetically distinct from all other brown rat populations, regardless of the relatively proximity of Tanu Island. In contrast, Faraday Island is comparably distant from its nearest source population (Lyell Island), and we detected shared

coancestry between these two populations. At their nearest point, Kunga and Tanu Islands share ~600 m of parallel coastline, whereas nearly the entire length of Faraday Island (~3km) parallels Lyell Island, potentially explaining the disparity in rates of dispersal. In a similar system, differences in island juxtaposition did impact rat migration rates, where islands that had a larger distance of parallel coastline had more genetic connectivity between populations (Savidge *et al.*, 2012).

We also found that the island interior may act as a barrier to dispersal for brown rats on Kunghit Island. Rats rarely venture too far inland from the shoreline, primarily due to food abundance (Pye & Bonner, 1980). Along the intertidal zone, rats have access to numerous diet items such as marine animals and algae; furthermore, seabirds frequently nest along the coastline and serve as another food source for invasive rats (Caut *et al.*, 2008; Clark, 1982; Major *et al.*, 2007). With a variety of diet options available along the coast and minimal pressure from predation, there is little incentive for rats to disperse inwards except to alleviate competition with conspecifics. Ocean currents may also be affecting patterns of gene flow in this system. We found significant differentiation between Kunghit and Ellen Islands though these islands are relatively proximate (~150 m). One explanation could be the presence of a strong tidal current that may inhibit the ability for rats to swim across this stretch of ocean. A similar effect could be occurring between Kunga and Tanu Islands, and, coupled with the limited parallel coastline, may explain the strong divergence between these populations.

We identified that the Bischof Islands were recolonized by the Lyell Island brown rat population following a 2011 eradication. We were also able to confidently exclude the pre-eradication population as the source population, indicating that bait failure did not occur and that all rats were successfully eliminated from the Bischof Islands. While this result is positive in that

it confirms eradication efficacy, it does highlight a need for either a larger eradication target area or increased biosecurity to prevent re-invasion. Similarly, we found that Faraday and Murchison Islands were also invaded from Lyell Island, providing more evidence that Lyell Island acts as a source population for neighbouring islands. Recently, brown rats were detected on Hotspring Island which was historically rat-free, and we showed that these rats arrived from Murchison Island. The establishment of a rat population on Hotspring is particularly concerning due to its proximity to Ramsay Island, a rat-free island which also hosts a large seabird colony (R. Irvine, pers. comm.). Eradication of the Faraday-Murchison-Hotspring Islands rat population is essential to protect this colony, as history suggests that an invasion to Ramsay will have devastating consequences on significant seabird populations. Eradication of rats from these islands should be considered a priority to maintain the health of this colony.

Lastly, we confirmed that brown rats arrived from a western European source. Local history and other anecdotal evidence hypothesized that rats arrived with European settlers and whalers. In addition to origin, we also found support for only a single invasion into the archipelago, so continued introduction may not be an issue in this system. This information will be important for planning future management of invasive rats in Haida Gwaii and helps explain how invasive species interact with a novel environment.

4.2 Management implications

We were able to make several recommendations for invasive rat management in Haida Gwaii. The most powerful may be the identification of suitable targets for eradication. The Kunga-Titul Islands population are strong candidates for eradication as there was negligible connectivity detected between these islands and nearby sources such as Tanu and Lyell Islands. Consequently, there is minimal risk of re-invasion following eradication. Ellen Island may be

another suitable candidate for eradication, though there may be a risk of re-invasion from Kunghit Island, and a clearer understanding of the dynamics between these two populations, including factors affecting gene flow, is needed. Kunghit Island also appears to be sufficiently isolated from other rat populations (with the exception of Ellen Island) and could serve as another eradication target, though the relatively large target area may require a multi-stage effort such as was used on South Georgia Island (Piertney *et al.*, 2016). Similarly, a Lyell Island eradication could be beneficial, as it acts as a source population to many proximate islands, and historically it hosted a significant seabird colony, though care would be needed to ensure re-invasion does not occur from one of these islands.

We were also able to make some suggestions for defining eradication units. When two or more populations are exchanging migrants at a significant rate, these populations present a significant risk of re-invasion following an eradication (Robertson & Gemmell, 2004). In this event, connected populations must be eradicated simultaneously to ensure the complete removal of the invasive species. In this study, we noted that Faraday and Murchison Islands consistently clustered as a single population across analyses and even across species (Figure 3.7; Figure 3.9; Figure 3.10; Figure 3.12). As such, future eradications on these islands should occur concurrently, since re-invasion from one to the other is highly probable. We also noted that the brown rat populations on Tanu and Richardson Islands shared significant coancestry and should similarly be grouped into a single eradication unit (Figure 3.10). Interestingly, there appeared to be two discrete black rat populations on Lyell Island which were moderately differentiated (Figure 3.4: Figure 3.12). It may be possible to approach an eradication on this island as a two-stage process with minimal risk of re-invasion, though it is important to note that there appear to be few barriers to gene flow for brown rats on Lyell Island which could complicate eradication

planning. We also noted that the source of the failed eradication on the Bischof Islands was a re-colonization by rats from Lyell Island (Figure 3.15). As a result, increasing the eradication target area to include nearby Lyell Island populations is necessary for a successful Bischof Islands eradication. Increased biosecurity and/or regular trapping on the Bischof Islands may also be required. These same measures would also help to successfully eradicate rats on Faraday and Murchison Islands, as these islands were also colonized from Lyell Island. Lyell potentially is acting as a source to all neighbouring island populations, so removal of this population is likely needed to permanently remove rats from this region of the archipelago.

We also evaluated if there was a continued biosecurity threat of rats being transported to Haida Gwaii from mainland populations. We inferred that brown rats most likely originated from a western European population and may have even been from a single introduction (Figure 2.7). Our results suggest that continued introduction is unlikely, and efforts should be focused on eradication and within-archipelago biosecurity.

4.3 Limitations and future studies

We recognize that this study, while comprehensive, has some limitations. For Chapter 2, we aimed to identify the origin of brown rats in Haida Gwaii using a global SNP database. Using traditional population genetic methods, we were unable to confidently identify the brown rat origin likely because of genetic drift. The process of genetic drift describes the tendency for allele frequencies within a population to change randomly from generation to generation (Masel, 2011; Wright, 1931). The effect of genetic drift is particularly strong when populations are small, especially in the absence of gene flow among populations. The initial invasion of Haida Gwaii was presumptively an introduction of a small number of individuals, and the number of introductions was limited. These factors created an opportunity for genetic drift to drastically

influence allele frequencies, resulting in a population so novel that it appears to be only distantly genetically related. We attempted to compensate for genetic drift by modelling various demographic scenarios. Using coalescent modelling, we can incorporate stochastic changes in allele frequencies (such as those encountered through genetic drift, or even novel mutations) to better elucidate the series of events (*e.g.*, introductions, changes in population size, *etc.*) that led to the current populations. Using this approach, we were able to identify more confidently a western European origin for brown rats in Haida Gwaii, though model improvement is required. Another approach to avoiding the challenges of genetic drift would be to examine museum samples to better characterize the genetic composition of the historical Haida Gwaii population, which could improve both clustering- and coalescent-based estimates.

There were also some limitations in Chapter 3. While we were able to reconstruct patterns of connectivity among populations and make some inferences on environmental factors shaping these patterns, these analyses did not explicitly evaluate the effect of these factors on dispersal and gene flow. One approach for improving this aspect could be to use resistance-surface modelling such as that implemented by the software Circuitscape (Shah & McRae, 2008). This method uses aspects of circuit theory to explain genetic differentiation in terms of the difficulty, or "resistance", of an individual traversing a particular habitat and has successfully been used to describe patterns of gene flow in other systems. (McRae, 2006; McRae & Beier, 2007). By incorporating this information into management strategies, more effective eradications can be organized that offer the lowest risk of eradication failure (Piertney *et al.*, 2016).

Further research could include the development of a genetic toolkit to provide fast and reliable assessment of origin for novel invasions. Eradication success is much more probable and cost-effective if an invasion can be stopped early in the process (Cristescu, 2015; Moore *et al.*,

2011). However, knowledge of the invasion origin can play a critical role in assessing future biosecurity threats (Robertson & Gemmell, 2004). While the methods used in this study are optimal for large numbers of samples, they are inefficient for analyzing the relatively few individuals you would expect to sample in an early-stage invasion. One approach particularly well suited for such a scenario is genotyping-in-thousands by sequencing (GT-seq) (Campbell *et al.*, 2015). This sequencing technique allows for cost-effective sequencing of ~500 SNPs for any number of individuals using custom amplicons and massively-parallel multiplex polymerase chain reactions. Development of such a SNP panel for use in Haida Gwaii would allow for quick and inexpensive assessment of all new invasions to plan more effective future eradications.

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APPENDICES

Appendix A: Chapter 2 Supplementary Materials

Table A.1. Reference populations, sample size (*n*), and subpopulations of globally sampled brown rats *(R. norvegicus*) from Puckett *et al.* (2016; 2018) used in these analyses.

Population (abbreviation)	*n*	Subpopulations
Aleutian Islands (ALN)	13	Adak Island, USA; Greater Sitkan Island, USA; Attu Island, USA; Sedanka Island, USA
China (CHI)	33	Bangun Perkasa ship (USA); Harbin, China; Narati, China; Xiao Mu Xing, China; Nagasaki, Japan; Sakhalinskaya Oblast, Russia; Talon Village, Russia
South-East Asia (SEA)	30	Pursat, Cambodia; Sihanouk, Cambodia; Tarlac, Philippines; Banpong Village, Thailand; Songkla, Thailand; Udon Thani, Thailand; Hanoi, Vietnam
Northern Europe (NER)	92	Häme, Finland; Loimaa, Finland; Turku, Finland; De Mortel, Netherlands; Doetinchem, Netherlands; Oeffelt, Netherlands; Bergen, Norway; Göteborg, Sweden; Lövsta, Sweden; Malmö, Sweden; Skellefteå, Sweden; Stockholm, Sweden
Western Europe (WER)	38	Gramatneusiedl, Austria; Le Puy-en-Velay, France; Lyon, France; Marseille, France; Ahlen, Germany; Königshain, Germany; Magdeburg, Germany; Möggingen, Germany; Sassenberg, Germany; Liverpool, Great Britain; Nottingham, Great Britain; Budapest, Hungary;
Western North America (WNA)	29	Albuquerque, USA; Bay Area, USA; Sitka, USA; Sonoma Valley, USA
Eastern North America (ENA)	46	Baltimore, USA; New Orleans, USA; New York City, USA
South America (SAM)	25	Buenos Aires, Argentina; Pergamino, Argentina; St. Andrew Parish, Barbados; Salvador, Brazil; Antihuala, Chile; El Oliveto, Chile; Las Naices, Chile; Guatemala City, Guatemala; Curundú, Panama; San Francisco, Panama; Praslin, St. Lucia
San Diego (SND)	9	San Diego county, USA
Vancouver (VAN)	15	Vancouver, Canada

Table A.2. Comparison of genetic diversity metrics across the full (32k) and subset (12k) single nucleotide polymorphism (SNP) datasets. The full dataset was compiled by Puckett *et al.* (2016; 2018) from globally sourced brown rats. The subset of SNPs were those that were retained for *n*=299 brown rat samples collected from Haida Gwaii, BC after quality filtering. Diversity metrics were calculated using Genodive (Meirmans & Tienderen, 2004).

Dataset	N_{SNP}	N_A	N_E	H_o	H_s	H_t	G_{is}
12k	12 433	2.000	1.459	0.244	0.308	0.355	0.208
32k	31 878	1.996	1.371	0.205	0.260	0.301	0.213

N_{SNP} = number of SNPs; N_A = mean number of alleles per locus; N_E = mean number of effective alleles per locus; H_o = mean observed heterozygosity; H_s = mean heterozygosity within populations; H_t = total heterozygosity; G_{is} = inbreeding coefficient

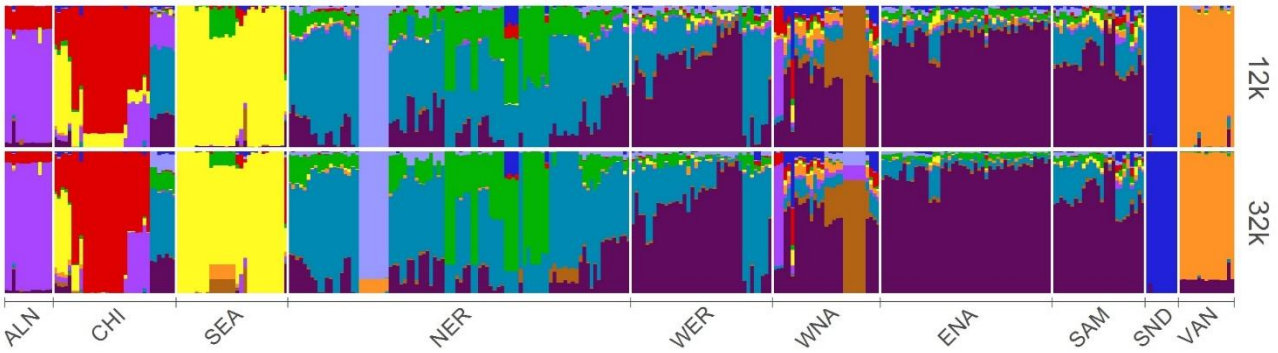

Figure A.1. Comparison of ADMIXTURE results for *k*=10 reference populations using the full (32k; Puckett *et al.* 2016, 2018, *n*=31 878 single nucleotide polymorphism (SNP) nuclear markers) and subset (12k; *n*=12 433 SNPs) datasets. Ten iterations were performed for each dataset and summarized using CLUMPP. Plot was constructed using the R-package *pophelper v2.2.6* (Francis, 2017). See Table I for abbreviation definitions.

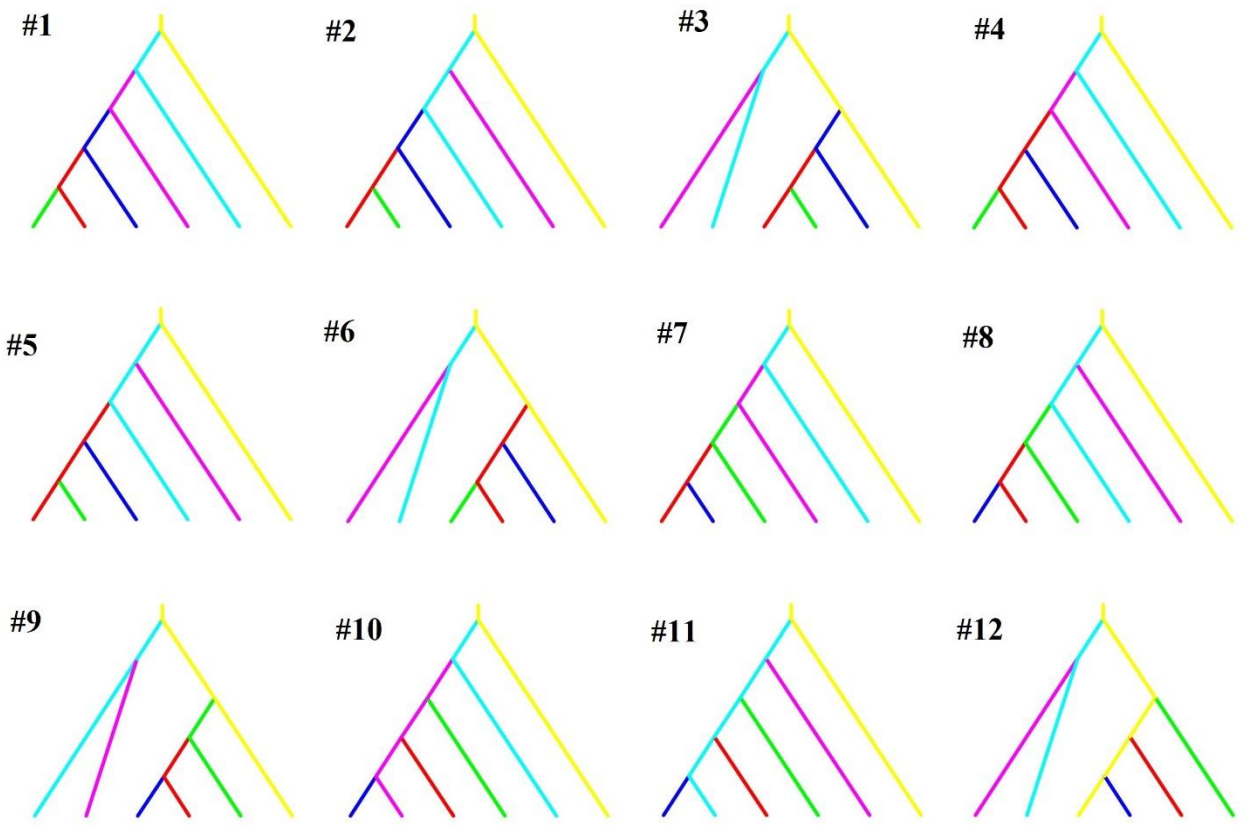

Figure A.2. Topologies for twelve invasion scenarios compared to infer global origin of brown rats (*Rattus norvegicus*) in Haida Gwaii, BC. Coalescent modelling was used to infer origin using DIYABC v2.1.0 (Cornuet *et al.*, 2014). Each colour represents a unique population are defined as follows: (green) Kunghit, Haida Gwaii; (red) Lyell, Haida Gwaii; (blue) Tlell Haida Gwaii; (pink) Vancouver; (cyan) western North America; and (yellow) western Europe.

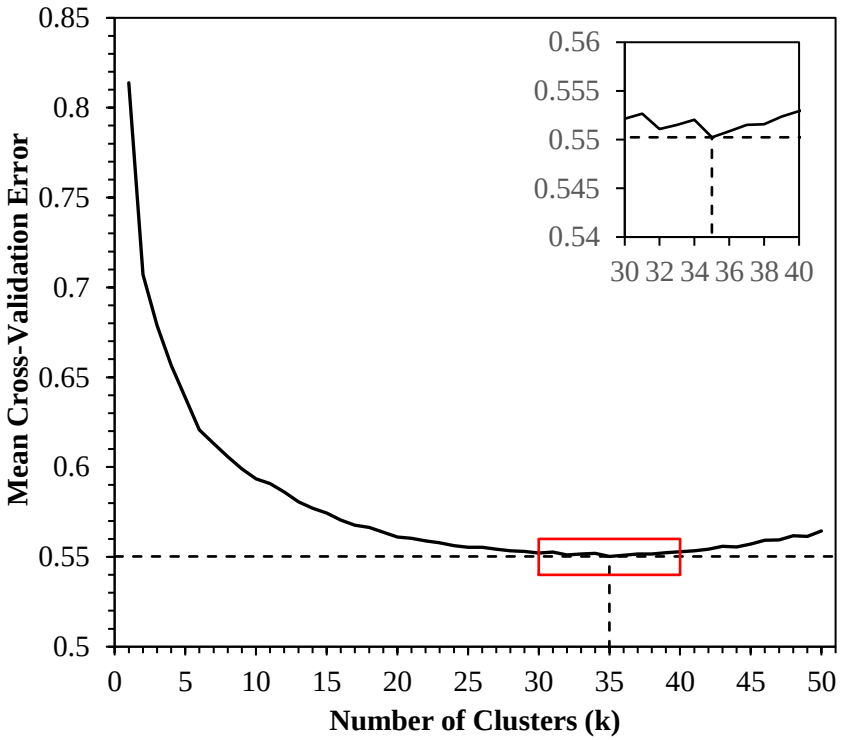

Figure A.3. Mean cross-validation error estimated across *k*=1-50 for an unsupervised Bayesian clustering analysis, implemented by the software ADMIXTURE (Alexander *et al.*, 2009), for a globally sampled brown rat dataset (*n*=480). Genotypic data for *n*=12 433 single nucleotide polymorphism nuclear markers were used as inputs. The cross-validation was calculated 10-fold (*i.e.* --cv=10 flag), and error estimates were averaged over *n*=10 iterations for each value of *k*. An optimal *k*=35 was identified. The inset shows a zoomed-in look of the red square from the main plot.

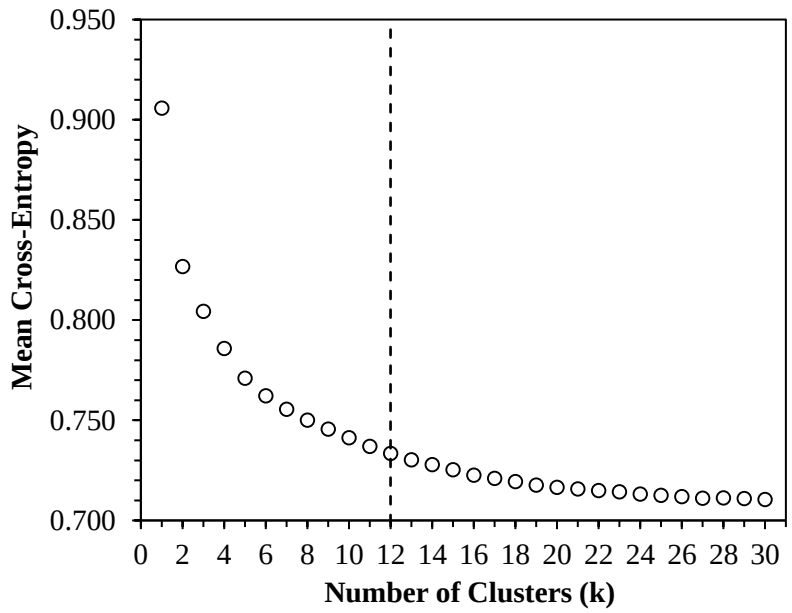

Figure A.4. Mean cross-entropy estimates for *k*=1-30 for a Bayesian clustering analysis implemented by the R-package *LEA* (Frichot & François, 2015) for a globally sampled brown rat dataset (*n*=480). Genotypic data for *n*=12 433 single nucleotide polymorphism nuclear markers were used as inputs. Cross entropy estimates were averaged over *n*=20 iterations for each value of *k*. The location of "elbow" (indicated by the dashed line) identified an optimal *k*=12.

Appendix B: Chapter 3 Supplementary Materials

Table B.1. Sequencing results for *n* = 7 ddRAD libraries. Each library was constructed with *n* = 96 individuals of black and brown rats (*Rattus rattus*, *R. norvegicus*, respectively). Libraries were sequenced on the Illumina Hi-Seq 2500 PE125 platform. (*) One rat from Hotspring island was sequenced over a series of sequencing spikes on the Illumina Mi-Seq PE250 platform.

Library	Number of reads	Number of bases	Average quality
1	217,226,502	54,306,625,500	34.5
2	236,254,061	59,063,515,250	35
3	257,468,458	64,367,114,500	34
4	256,098,451	64,024,612,750	34
5	255,569,370	63,892,342,500	34
6	254,221,630	63,552,907,500	34
7	224,663,074	56,165,768,500	34
Hotspring*	622,865	311,432,500	34

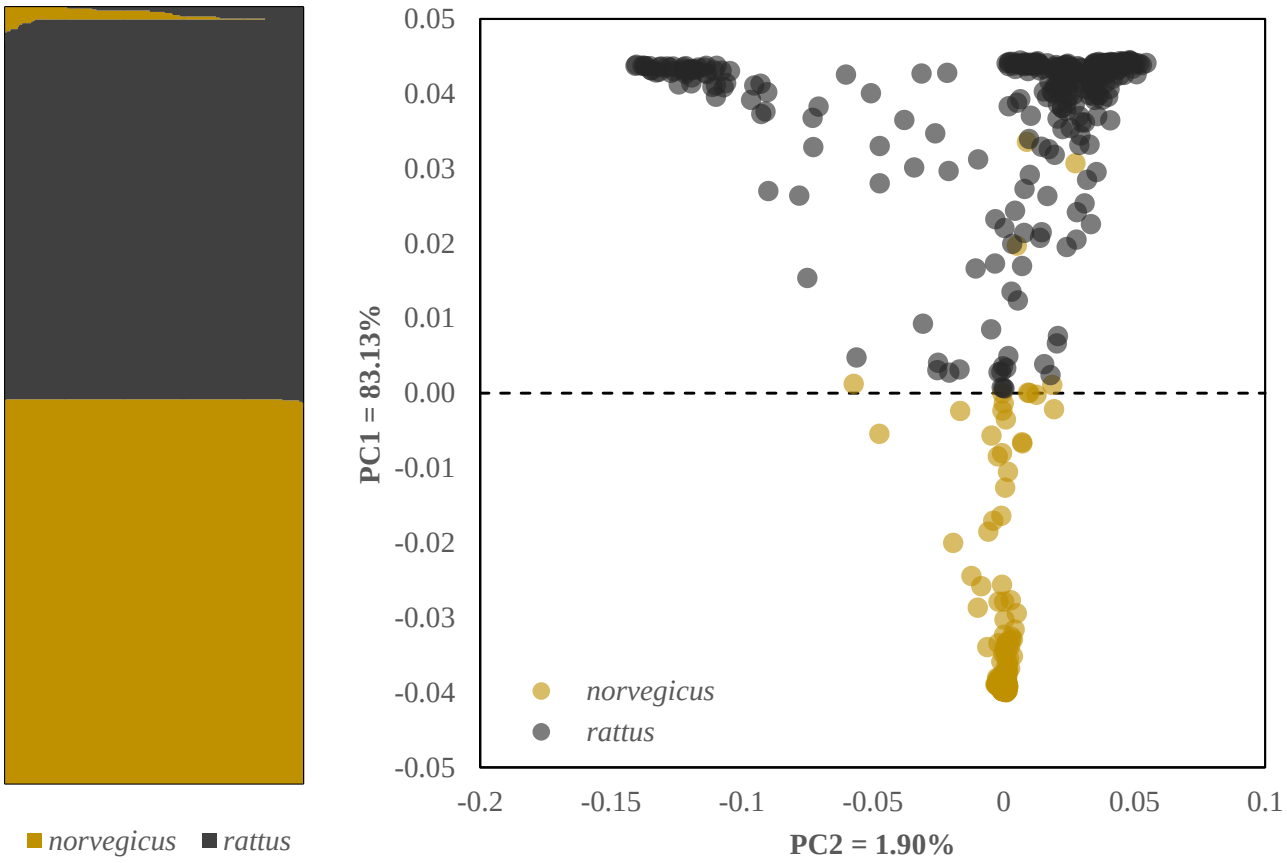

Figure B.1. ADMIXTURE plot (left) and principle component analysis (right) of *n* = 673 brown and black rats collected in Haida Gwaii, BC to genetically confirm species identity. Principle component circles are coloured based on ADMIXTURE results.

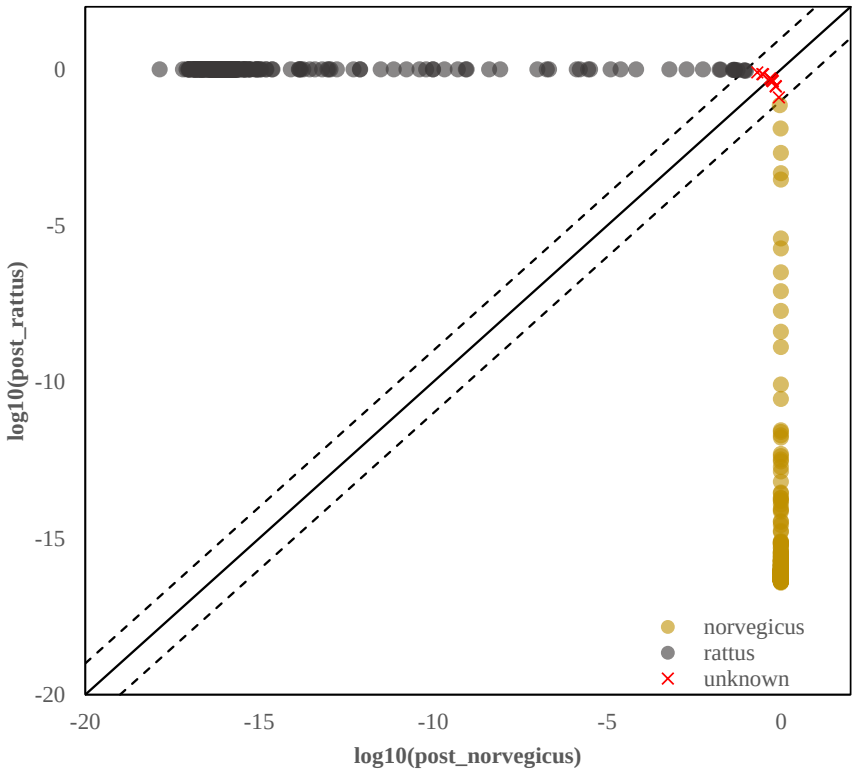

Figure B.2. Posterior probabilities (log_{10} transformed) for n = 673 brown and black rats collected in Haida Gwaii, BC for species identification. The diagonal separates assignment to species, and the dashed lines indicate 90% assignment to one species.

Table B.2. Sensitivity analysis summary statistics for the brown rat dataset (total n = 325).

r	min_maf	N	N_{SNP}	Mean Depth	Mean Miss. (%)
0.70	0.01	275	73019	12.00	10.58
	0.02	280	55435	12.91	10.51
	0.03	283	49030	13.56	10.21
	0.04	287	43135	14.37	9.89
	0.05	292	37091	15.10	9.94
0.80*	0.01	289	47843	14.81	5.97
	0.02	293	36521	15.87	5.91
	0.03	296	32915	16.47	5.76
	0.04	297	29607	17.32	5.58
	0.05*	300	25585	18.16	5.64
0.90	0.01	302	23626	21.38	2.16
	0.02	306	18967	21.96	2.18
	0.03	306	17734	22.39	2.13
	0.04	306	16656	22.92	2.09
	0.05	308	14569	23.77	2.14
0.95	0.01	313	12138	26.34	0.87
	0.02	313	9628	26.70	0.85
	0.03	313	9077	26.93	0.85
	0.04	313	8638	27.15	0.85
	0.05	313	7421	28.02	0.90

r = proportion of genotyped individuals to call a SNP
min_maf = minimum minor allele frequency
N = number of individuals with ≥6x mean depth of coverage
N_{SNP} = number of SNPs
Mean Depth = mean depth of coverage
Mean Miss. = mean missingness per individual
(*) denotes optimal parameter selection

Table B.3. Sensitivity analysis summary statistics for the black rat dataset (total n = 329).

r	min_maf	N	N_{SNP}	Mean Depth	Mean Miss. (%)
0.70	0.01	199	105037	12.83	7.08
	0.02	217	81467	13.83	6.98
	0.03	232	58889	15.07	7.21
	0.04	237	42949	15.51	7.50
	0.05	238	33666	15.63	7.71
0.80*	0.01	241	55493	16.43	4.23
	0.02	242	47579	17.35	4.12
	0.03	247	36583	18.40	4.14
	0.04	251	25755	18.93	4.24
	0.05*	254	19317	19.06	4.41
0.90	0.01	266	18762	22.72	2.05
	0.02	267	16898	22.92	2.14
	0.03	267	13559	23.23	2.12
	0.04	269	8687	23.34	2.01
	0.05	271	5542	23.54	1.97
0.95	0.01	290	1199	27.57	0.69
	0.02	290	941	27.70	0.76
	0.03	290	767	27.90	0.80
	0.04	290	544	27.75	0.79
	0.05	290	330	27.95	0.71

r = proportion of genotyped individuals to call a SNP
min_maf = minimum minor allele frequency
N = number of individuals with ≥6x mean depth of coverage
N_{SNP} = number of SNPs
Mean Depth = mean depth of coverage
Mean Miss. = mean missingness per individual
(*) denotes optimal parameter selection

Table B.4. Results of SNP filtering for the brown rat dataset (*n* =297). (*) The STACKS filtering step removed SNPs not genotyped in <80% of individuals, loci with minimum minor allele frequencies <5%, and loci with a maximum heterozygosity >0.5.

Filter	SNPs removed	SNPs remaining
None	-	35 728 551
STACKS*	35 697 534	31 017
Non-autosomal	580	30 437
Outliers ($q < 0.2$)	2 474	27 963
HWE ($p < 0.05$)	277	27 686

Table B.5. Results of SNP filtering for the black rat dataset (*n* =251). (*) The STACKS filtering step removed SNPs not genotyped in <80% of individuals, loci with minimum minor allele frequencies <5%, loci with a maximum heterozygosity >0.5, and allowing for only a single SNP per RADtag.

Filter	SNPs removed	SNPs remaining
None	-	20 803 160
STACKS*	20 763 799	39 361
Non-autosomal	716	38 645
Outliers ($q < 0.2$)	9 134	29 511
HWE ($p < 0.05$)	693	28 818

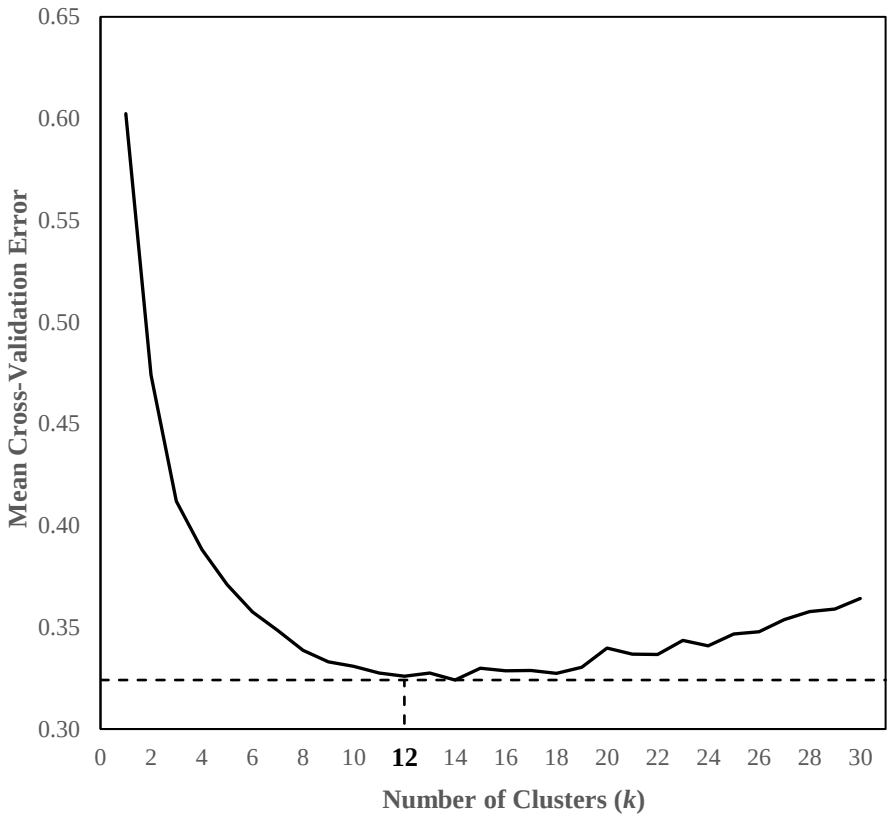

Figure B.3. Mean cross-validation error estimated across *k*=1-30 for an unsupervised Bayesian clustering analysis, implemented by the software ADMIXTURE (Alexander *et al.*, 2009), for brown rats (*Rattus norvegicus*; *n* = 325) sampled across Haida Gwaii, BC. The cross-validation was calculated 10-fold (*i.e.* --cv=10 flag), and error estimates were averaged over *n*=10 iterations for each value of *k*. An optimal *k*=12 was identified.

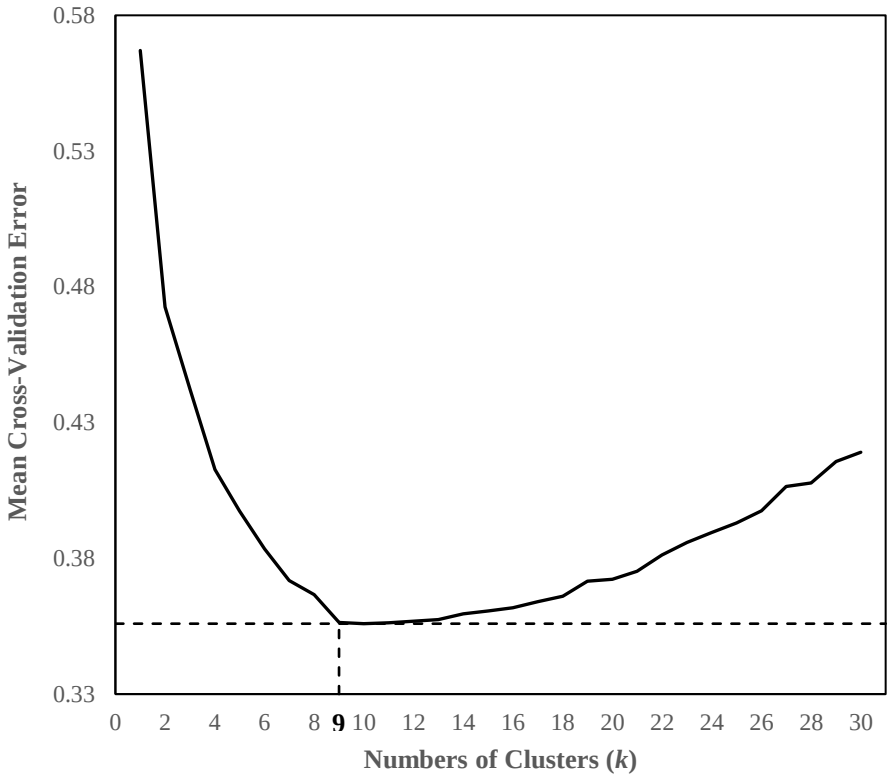

Figure B.4. Mean cross-validation error estimated across *k*=1-30 for an unsupervised Bayesian clustering analysis, implemented by the software ADMIXTURE (Alexander *et al.*, 2009), for brown rats (*Rattus rattus*; *n* = 251) sampled across Haida Gwaii, BC. The cross-validation was calculated 10-fold (*i.e.* --cv=10 flag), and error estimates were averaged over *n*=10 iterations for each value of *k*. An optimal *k*=9 was identified.

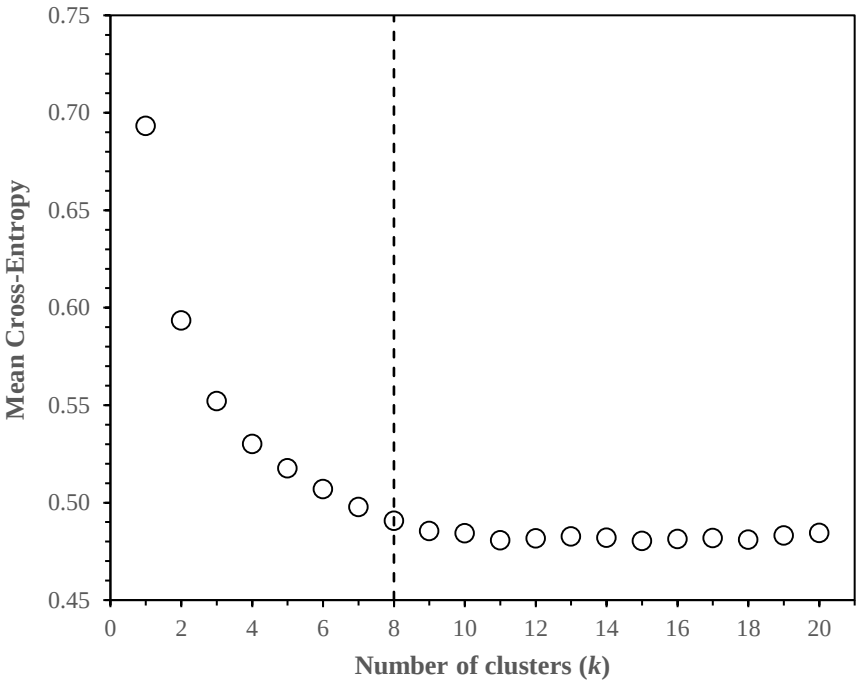

Figure B.5. Mean cross-entropy estimates for k=1-20 from a Bayesian clustering analysis implemented by the R-package *LEA* (Frichot & François, 2015) for n=325 brown rats (*Rattus norvegicus*) collected in Haida Gwaii, BC. Cross entropy estimates were averaged over n=10 iterations for each value of k. The location of "elbow" (indicated by the dashed line) identified an optimal k=8.

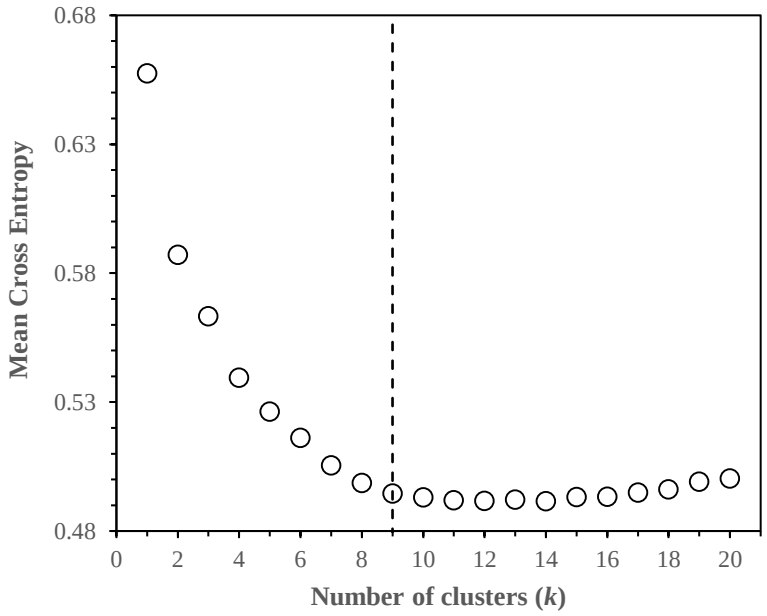

Figure B.6. Mean cross-entropy estimates for *k*=1-20 from a Bayesian clustering analysis implemented by the R-package *LEA* (Frichot & François, 2015) for *n*=251 black rats (*Rattus rattus*) collected in Haida Gwaii, BC. Cross entropy estimates were averaged over *n*=10 iterations for each value of *k*. The location of "elbow" (indicated by the dashed line) identified an optimal *k*=9.