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The impact of long-term artificial removal experiment and natural local extinctions on the genetics of an alpine butterfly

Keon Young Park, *The University of Western Ontario*

Supervisor: Keyghobadi, Nusha, *The University of Western Ontario*

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Abstract

In the current age of widespread anthropogenic effects across the globe, organisms are vulnerable to habitat fragmentation and localized decline in population more than ever before. Therefore, insight into metapopulation-level dynamics and the genetic basis of inter-patch dispersal is key to understanding the regional persistence of a species in the face of potential localized extinction or population bottleneck. In this study I used samples from a previously conducted localized long-term population removal experiment to investigate the effects of an extended population reductions on the genetic structure of a local population, and to explore the genetic basis of dispersal and recolonization using both the removal experiment and past natural local extinctions within a well-studied metapopulation system of an alpine butterfly species (*Parnassius smintheus)*. Overall, while my results did not find evidence for the genetic basis of dispersal in this species within the markers assayed, genetic metrics across the experiment empirically demonstrated this system's resilience against localized population reductions, as both genetic diversity and influx of dispersers into the focal patches were maintained throughout the experiment despite the yearly removals of the local populations for eight consecutive years.

Keywords

Metapopulation, Dispersal, Extinction-recolonization dynamics, Source-sink dynamics, Genetic rescue, Genetic diversity, Local extinction, Local population bottleneck, Metapopulation persistence, Single nucleotide polymorphism, Candidate gene, Phosphoglucose isomerase, *Parnassius smintheus*

Summary for Lay Audience

In the current age of widespread human land-use change across the globe, more and more organisms are left existing in a fragmented landscape, where they are vulnerable to localized decline or even extinctions more than ever before. In these fragmented landscapes, dispersal of individuals among habitat patches play an important role in the recovery and persistence of local populations, especially in the face of dramatic population reductions. Additionally, dispersal is a trait that can be affected by the genetics of an individual, and therefore, disturbances that cause local population reductions can lead to the increased representation of dispersers within a patch, when the patch becomes recolonized by individuals from other habitat patches. In my project, I investigated the genetic characteristics of an alpine butterfly species, the Rocky Mountain apollo (*Parnassius smintheus*) within a regional population network located in the Canadian Rockies. On top of natural local extinctions that have occurred in the past, in two of the patches, all observed butterflies were annually captured and artificially removed from year 2001 to 2008 as part of a population dynamics experiment. I tracked the impact of this long-term removal on the genetic characteristics of these patches throughout the experiment, assessed the origin of the dispersers coming into them, and explored a genetic basis of dispersal and recolonization in this system using recolonization events caused by both the experimental removal and natural extinctions. Genetic metrics across the experiment demonstrated this system's resilience against local population reductions even over a prolonged period, as both available genetic diversity and influx of dispersers into the decimated patches were maintained throughout the experiment, despite the continuous removal over eight years.

Co-Authorship Statement

Chapters 2 and 3 will be published with Nusha Keyghobadi, Stephen F. Matter, and Jens Roland as co-authors. Dr. Matter and Dr. Roland conducted the long-term artificial removal experiment which served as the primary basis of my thesis, as well as providing data from the mark-recapture population survey conducted annually within the study system. Additionally, Dr. Matter provided the dried tissue samples from individuals captured as part of the artificial removal experiment, necessary for my genetic research.

All chapters will be published with Dr. Keyghobadi as co-author. Through the entirety of my thesis, Dr. Keyghobadi has supervised and contributed to developing the research question, designing the conceptual and analytical methods of the study, and writing of the manuscript.

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Chapter 1

1 General Introduction

With widespread land-use change and habitat encroachment due to human activity in the modern era, natural landscapes across the globe are continuously being deteriorated. As a result, habitat fragmentation - the division of an organism's preferred habitat by discontinuities in the landscape, is becoming increasingly common, threatening many species across the globe (Fahrig, 2003; Crooks et al., 2017). Habitat fragmentation can have many negative effects, such as the decrease in total area of suitable habitat available to support populations (Fletcher et al., 2018), as well as degradation of habitat quality in remaining patches whose boundaries are exposed to highly contrasting environments and land covers (i.e., edge effects; Laurence et al., 2007). Additionally, habitat fragmentation can isolate local populations from one another, thereby limiting movement of individuals and gene flow among them (Schlaepfer et al., 2018). Limited movement and gene flow, in combination with the decreased population size, can lead to both increased local demographic stochasticity (Oubourg, 1993) and loss of genetic diversity due to higher vulnerability to genetic drift (Templeton et al., 1990). Consequently, populations occurring in fragmented landscapes can experience increased risk of local extinctions or population bottlenecks, which in turn increase the risk of regional extirpation or global extinction, especially for endemic species whose range is limited (Crooks et al., 2017).

Widespread anthropogenic habitat fragmentation has led to the growing importance of the ecological paradigm of the metapopulation, especially in conservation

biology, population genetics, and evolution (Akcakaya et al., 2007). The metapopulation concept was first formalized and coined by Levins (1969) and describes a regional population of a single species that occupies a network of multiple, spatially distinct habitat patches, each harbouring a semi-independent local population. While each local population is vulnerable to extinction from stochastic events at any point in time, extinctions in different habitat patches are not synchronous and movement of individuals from other habitat patches within the network allows for recolonization of the resulting empty patches (Molofsky & Ferdy, 2005). Therefore, while individual habitat patches within a metapopulation may temporarily be unoccupied, the overall system persists through this continuous and dynamic balance between local extinction and recolonization (Hanski,1998).

Additionally, local population dynamics are affected by variation among the habitat patches that form the metapopulation system, as different patches often vary in size, connectivity, resource availability, and other environmental conditions that affect the quality of the habitat patch (Fernández et al., 2016). This variation results in different carrying capacity and growth rate among populations in different patches, which can lead to the uneven interchange of individuals among the local populations, with some patches potentially dependent on the influx of immigrants for persistence (i.e., “sink” populations), while other patches may support large local populations that consistently provide emigrants to other patches within the system (i.e., “source” populations) (Dias 1996; Puliam, 1998). Within metapopulations, both extinction-recolonization dynamics and source-sink dynamics are driven by dispersal, the movement and settlement of individuals from their natal patch to another (Bohrer et al., 2005). Dispersal serves as the

main mechanism for colonization, gene flow and population rescue within a metapopulation (Tesson & Edelaar, 2013), and for this reason understanding the causes and consequences of dispersal is central in our understanding of the ecology and evolution of metapopulations (Wang et al., 2015; Saastamoinen et al.,2018)

While the metapopulation concept has been applied to and studied across many different taxa (Opdam, 1991; Elmhagen & Angerbjörn, 2001), butterflies are particularly well represented in the metapopulation literature (Ovaskainen & Saastamoinen, 2018). Many butterfly species naturally exist in obvious patchy distributions associated with their larval host or nectaring plants (Hanski & Thomas, 1994). This, in addition to the ease of observing and studying butterflies, at least in the adult stage, as well as the availability of extensive occupancy, distribution, and natural history data has made this group particularly well-suited for empirical study of metapopulations. For example, metapopulations of the Bay checkerspot butterfly in California and the Glanville fritillary butterfly in the Åland Islands of Finland represent some of the best-studied metapopulation systems in the world and serve as model systems for the understanding the ecology and evolution of spatially structured populations in general (Harrison et al., 1998; Hanski & Meyke, 2005).

In this thesis, I explore aspects of local extinction and recolonization, population bottlenecks, and dispersal in a metapopulation of the Rocky Mountain apollo butterfly, *Parnassius smintheus*. *Parnassius smintheus* is an alpine butterfly that inhabits high-altitude meadow patches in the Rocky Mountains of North America (Roland et al., 2000). These patches harbour the butterfly's larval host plant, *Sedum lanceolatum*, a perennial succulent plant that also serve as source of nectar for adults (Roslin et al, 2008), and are

naturally fragmented by forests. *Parnassius smintheus* is univoltine, having one generation per year. After over-wintering as first instar pharate larvae inside the eggs, the larvae feed on *Sedum*, completing five instars until pupating (Sperling & Kondla, 1991). In southwestern Alberta, where my study occurred, the adults emerge in July and August, flying, mating, and ovipositing until as late as early September. Notably, this relatively brief adult flight period is the only window of time in which individuals can disperse from one habitat patch to another, as larvae are not known to disperse from their natal location (Matter et al., 2004). Between-patch dispersal is generally uncommon and occurs mostly over short distances in this species, with approximately 10% of adults dispersing between patches over an average distance of 130m per flight season, with the maximum observed travelling distance of an individual being approximately 2 km (Roland et al., 2000).

My research focused specifically on a metapopulation of *P. smitheus* on Jumpingpound Ridge, in the Rocky Mountains of Kananaskis Country, Alberta, Canada (50° 57'N, 114° 54'W) (Matter & Roland, 2007). This metapopulation system consists of 23 alpine meadow habitat patches that are 0.2 ha to 22.7 ha in size and <2000m above sea level, separated by intervening coniferous forests (Roland et al., 2000). This metapopulation has been the subject of long-term research and monitoring, with each local population surveyed by mark-recapture in most years since 1995 (Matter et al., 2014). As part of the yearly mark-recapture surveys, small pieces of wing tissue have been collected from a sample of individuals captured each year and stored in 95% ethanol for genetic analyses (Keyghobadi et al., 1999; Keyghobadi et al., 2005). Several studies have used these tissue samples to genotype and describe the genetic diversity within and

among the local populations on Jumpingpound Ridge over time, highlighting the importance of patch connectivity in shaping the genetic structure of the metapopulation and maintaining allelic diversity within local populations (Keyghobadi et al., 2005; Jangjoo et al., 2016). In addition, a long-term experiment was conducted on Jumpingpound Ridge from 2001 to 2008, in which all observed adults in two of the habitat patches were removed each year; the purpose of this experiment was to induce local extinctions in those patches and test the predicted effects of these extinctions on the dynamics of neighbouring populations (Matter & Roland, 2009; Matter & Roland, 2010). Although the local populations in the two experimental patches were never driven completely extinct, the yearly removal of adults did result in long-term population bottlenecks (Matter & Roland, 2009).

Parnassius smintheus, while currently not endangered, is potentially highly vulnerable to climate change in two distinct ways. First, increased fragmentation of its habitat is occurring as forest cover surrounding alpine meadows is encroaching into the meadows due to rising tree line from warmer temperatures in the mountains (Roland & Matter, 2007). Not only does this reduce the total amount of available habitat, but it also increases isolation of patches from each other, as the intervening forest matrix in this system is known to significantly reduce between-patch movement of butterflies compared to open meadow environments (Roland et al., 2000: Ross et al., 2005; Keyghobadi et al., 2005). Additionally, this species is particularly vulnerable to variation in temperature and snowfall during their over-wintering period as pharate larvae, drastically reducing the survivorship of the larvae and population growth when extreme temperatures, either warm or cold, occur during early winter (Roland & Matter, 2016, Roland et al., 2021). As

weather conditions globally grow more stochastic and unpredictable under climate change, organisms such as *P. smintheus* that are sensitive to extreme weather conditions are increasingly threatened (Nadeau et al., 2017). Research into the metapopulation dynamics, consequences of severe local declines in population size, and dispersal and connectivity of this species may provide valuable insight into its conservation status in the face of these challenges posed by climate change.

In my thesis, I explore the metapopulation-level processes of extinction-recolonization, source-sink dynamics, and dispersal in *P. smintheus.* I do this by tracking the population genetic response to two types of demographic events that have occurred in the past in the Jumpingpound metapopulation: natural local extinctions and long-term population bottlenecks caused by the experimental removals described above. My two main objectives are to quantify the genetic effects of the long-term, induced bottlenecks on the experimental populations and to characterize the genetic basis of dispersal and recolonization in *P. smintheus.* In my first data chapter, I track multiple genetic metrics of the local populations subjected to long-term removal of the adult population across eight generations and identify the source populations of immigrants. In my second chapter, I search for genetic markers putatively associated with dispersal and recolonization, focusing on the candidate gene, Phosphoglucose isomerase (Wheat & Hill, 2014); I do this using both local natural extinction events and the experimentally induced population bottlenecks, under the hypothesis that alleles conferring better dispersal ability or propensity should increase in frequency following recolonization and immigration into empty or nearly empty patches, respectively.

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Chapter 2

2 The impact of long-term artificial removal experiment on the genetics of an alpine butterfly

2.1 Introduction

In the current age of increasing anthropogenic effects and stochastic environmental conditions across the globe, organisms are vulnerable to habitat fragmentation and localized decline in population more than ever before (Thomas et al., 2004; Dirzo et al., 2014). With this, the importance and applicability of the metapopulation concept continues to grow, as an increasing number of organisms exist within a progressively fragmented landscape. First defined by Levins (1969), a metapopulation is a network of populations existing in distinct, spatially separated habitat patches in which movement of individuals from one patch to another (dispersal), serves as the key mechanism of interaction. The persistence of a metapopulation is dependent on the dynamic balance between declines and extinctions of local populations, and subsequent rescue and recolonization by dispersers from another population within the network. With this balance, while local patches may go temporarily extinct, the overall system remains (Hanski, 1998).

Habitat patches that harbour local populations within a metapopulation network are often of variable sizes and quality due to such factors as resources available and environmental conditions present, leading to uneven carrying capacity and reproductive output among individual patches (Puliam, 1988; Dias, 1996). Such disparity can give rise to source-sink dynamics among interacting patches within the network. High-quality patches within a metapopulation not only are able to sustain their local populations but

may also act as continual sources of dispersers into lower-quality habitat patches. Conversely, in low-quality patches where the death rate regularly exceeds the birth rate, the persistence of a local population will depend on influx of immigrants, therefore acting as a population sink within the network (Puliam, 1988). Source-sink dynamics among patches directly affect the direction and rates of dispersal and recolonization within a population network (Dias, 1996; Chen et al, 2014).

Despite the overlap between the important processes involved in extinction-recolonization and source-sink dynamics in the persistence of a metapopulation, the bodies of literature for these fields are largely separated, with most research exploring population dynamics within the framework of only one of the above fields. Literature on metapopulation dynamics mainly explores various factors that affect the likelihood of local extinctions or inter-patch dispersal, as well as describing the demographic or genetic structure of local populations in relation to the extinction-recolonization cycles (Wang & Altermatt, 2019; Ovaskainen & Saastamoinin, 2018). Arguably the most well-explored study system in this field of literature is the Glanville fritillary butterfly (*Melitaea cinxia*) metapopulation in the Åland Islands, where extensive research on metapopulation dynamics has been conducted, exploring genetic, environmental, and spatial factors that affect extinction, dispersal, and recolonization (Hanski,1998; Saccheri et al., 1998). In particular, local extinction risk for this species has been shown to increase with decreasing population size, density of neighbouring populations, and habitat size, as well as increased presence of cattle grazing (Hanski et al., 1995). In addition, Saccheri et al. (1998) have empirically supported the association of lower

genetic diversity and higher inbreeding with increased local extinction risk within the same system.

Source-sink dynamics within a population network have been explored across a wide range of taxa, with much of the literature describing the patterns of density-dependent population dynamics and identifying the sources or sinks within each system based on various approaches of estimating immigration/emigration, such as demographic modelling (Paquet et al., 2020), population assignment of migrants (Rennala & Mountain, 1997), or parentage analysis (Peery et al., 2008). Thomas et al. (1997) described the shifts in source-sink dynamics of a butterfly (*Euphydryas editha*) population network based on population density data in response to a summer frost which eliminated all individuals within a single habitat type (clear-cut) that normally served as the source population within the system. That study concluded that source populations, which have higher average population density under normal circumstances, can become sinks when faced with sudden catastrophic disturbances such as those caused by climatic effects.

In either body of literature, long-term field research regarding the effects of bottlenecks in local populations, in the context of an interconnected network of populations, is scarce due to difficulties in detecting such events with observational data, and the need for persistent monitoring and sampling of populations over a prolonged period. In those few studies that address such long-term system dynamics, the researchers were limited to addressing the effects of catastrophic events that affected many local populations across the system intermittently (Lamy et al., 2012, Jangjoo et al., 2016) or described the population dynamics of such events purely based on observational density

and mark-recapture data, without further exploring the genetic composition of the local populations (Thomas et al., 1997). Here, I examine the genetic effects of experimentally induced, localized, long-term population bottlenecks within a metapopulation network of an alpine butterfly, *Parnassius smintheus* Doubleday, 1847, using genotypic data. I also describe the consequent dispersal dynamics between the bottlenecked sink populations and potential, surrounding source populations.

Parnassius smintheus is an Apollo butterfly species that inhabits primarily high-altitude alpine meadow habitats throughout the Eastern Rocky Mountains in Canada and the United States. The larvae's main host plant is a species of perennial succulent, *Sedum lanceolatum*, although the larvae are known to be able to feed on other sedum species as well, such as *Sedum rosaceae* (Roslin et al., 2008). The species is univoltine, completing a single generation each year. The larvae pupate after completing five instars, from which the adults emerge around July to August to mate and oviposit (Sperling & Kondla, 1991). This annual flight season is the only window of time in which individuals can disperse from their natal patch to another, as the larvae are not known to be able to disperse between patches. Dispersal is generally limited to neighbouring habitat patches; average recapture distances of marked adults within a flight season are only about 130 m for both males and females, and less than 10% of individuals are typically recaptured outside of the habitat patch in which they were originally captured and marked (Roland et al., 2000).

The metapopulation I studied occurs on Jumpingpound Ridge in the Rocky Mountains of Kananaskis Country, Alberta. The metapopulation consists of 23 high altitude (> 2000 m) meadow habitat patches ranging in size from 0.2 ha to 22.7 ha,

separated by intervening forest (Roland et al., 2000). This system has been the subject of long-term population monitoring and genetic sample collection since 1995, with mark-recapture population assays performed during the annual flight season to monitor population size and movement. Simultaneously with the mark-recapture, genetic samples (wing clips) have been collected from marked individuals for genetic analyses exploring the effects of land cover, connectivity, and population dynamics on genetic diversity and differentiation (Keyghobadi et al., 1999, Caplins et al., 2014).

Starting in 2001, the south-eastern portion of the network (**Figure 2.1**) was subject to an eight-year long experiment in which all adults observed in two adjacent patches (P and Q, **Figure 2.1**) were captured and removed each year, to examine effects of simulated local extinctions on the population dynamics of the surrounding patches (Matter & Roland, 2010). Specifically, the aim of the removals was to test the hypothesis that the extinction of these patches would reduce immigration into surrounding patches, leading to negative effects on their population growth and persistence, proportional to their distance from P and Q. While the removals aimed to induce local extinction within these patches, the populations in P and Q did not go completely extinct (Matter and Roland 2010). However, the reproductive output of these patches was reduced by more than 75% by the removals, leading to severe demographic bottlenecks (Matter & Roland, 2010). The population bottlenecks induced in patches P and Q in turn did not result in higher likelihood of extinctions in surrounding patches but did lead to reduced immigration into the surrounding patches and increased synchrony in population growth among the local populations (Matter & Roland, 2009); although recent re-analysis with additional data from years following the removals suggests that the artificial bottleneck

induced in P and Q may not have been the cause for the increase in synchrony among the surrounding populations (Matter et al., 2022).

The populations in patches P and Q persisted, despite the yearly removal of all observed adults over seven generations, either because a portion of the population each year failed to be observed and was left behind, or because they were rescued by immigration from other populations in the network, or some combination of these two factors. Here, using samples of the adults removed from patches P and Q during each year of the removal experiment, I track the changes in genetic diversity and composition of those populations through the course of the experiment to test these competing hypotheses. In an isolated population experiencing a sustained or recurring bottleneck, such as that induced in patches P and Q, we would expect the local population to decline significantly in genetic diversity (Lande, 1988). However, within an interconnected population network, I hypothesized that continuous immigration from surrounding patches would counter this effect (Jangjoo et al., 2016). Therefore, to the extent that the populations in P and Q persisted due to rescue via immigration, I predicted that they would maintain consistent levels of genetic diversity despite the continuous removal of individuals.

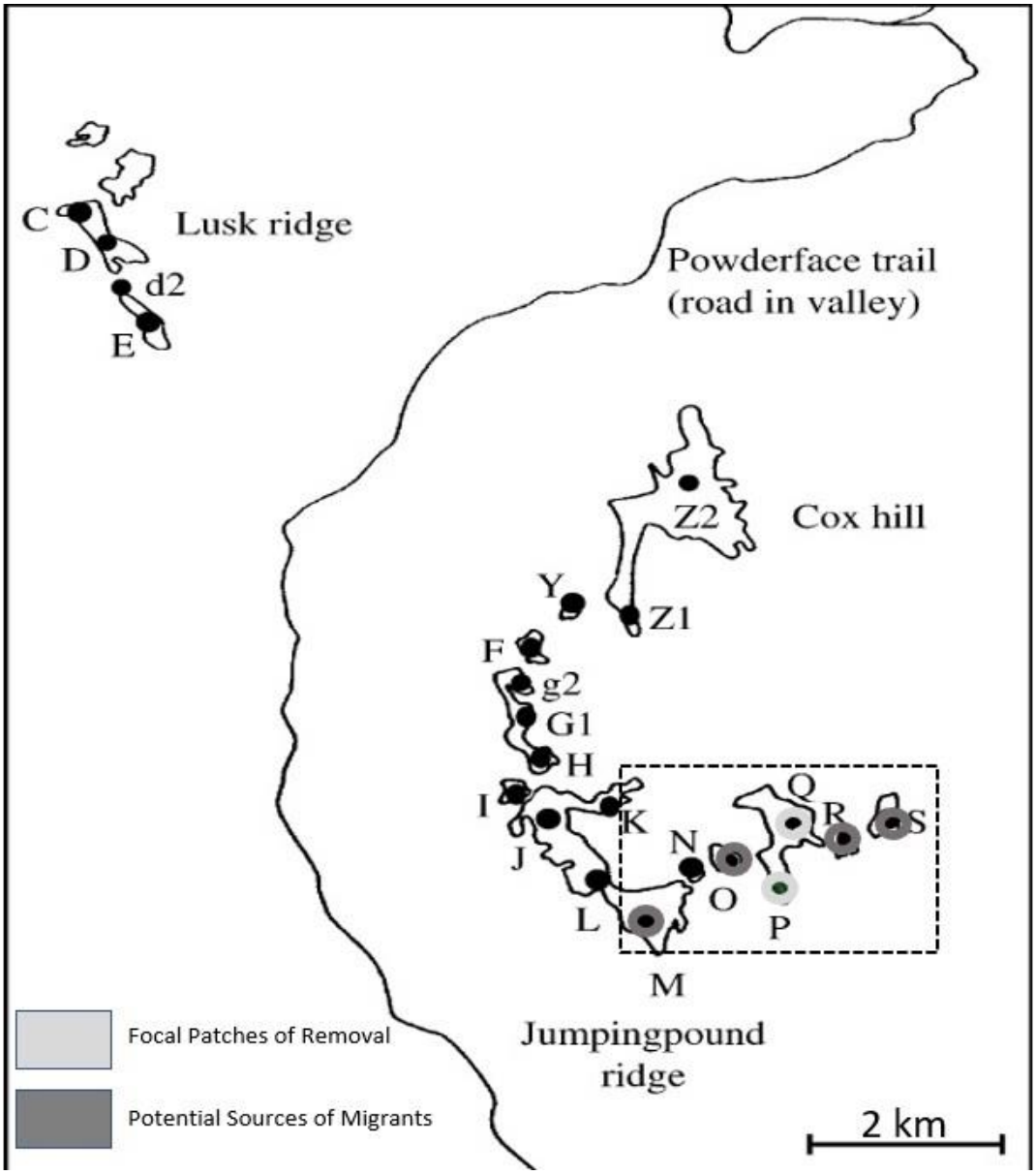

Figure 2.1: Map of the Jumpingpound Ridge study system located in Kananaskis Country, Alberta (modified from Keyghobadi et al., 2005), showing outlines of meadow habitat patches. Each habitat patch is labelled with a black dot and unique letter label. Dotted lines outline the section of the population network studied here, with patches P and Q being the target of artificial removals and other patches in the dotted square being within potential dispersal distance (2 km) of *Parnassius smintheus*.

2.2 Methods

2.2.1 Sample Collection

The removal experiment on patches P and Q was conducted from 2001 to 2007 to investigate the effects of severe and continuous population reduction in patches P and Q on the population dynamics in patches that are connected to them (Matter & Roland, 2009; Matter & Roland, 2010). Patches P and Q were surveyed every one to three days during the flight season each year, and all observed butterflies were captured by hand netting and removed from the site. In total, 4,830 butterflies were removed from P and Q over the eight-year period (Matter & Roland, 2010). The removed individuals were labelled with the date and location of capture, and then stored, dried, and pinned in a collection at the University of Cincinnati.

I sampled from this collection of pinned specimens from patches P and Q, removing one leg per individual across each year of the removal experiment. For years in which the number of captured individuals in a patch was lower than 30, I sampled all available individuals. Otherwise, I sampled between 30-60 individuals per patch per year. The samples from 2001 represent the basal or initial state of the populations, as they would not yet have responded to the experimental manipulation. Additionally, leg tissues from 42 frozen whole butterflies, removed during the 2008 flight season were also used; these represent the final state of the populations after the experimental removals in combination with dried samples from 2008. In total, I sampled a total of 470 individuals from patches P and Q across eight years (**Table 2.1**).

2.2.2 DNA Extraction and Genotyping

I extracted DNA from sampled tissues using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD). The tissues were first broken down by homogenization in a microcentrifuge tube, before being lysed and incubated at 56 ° C for 24 hours. The extracted DNA was eluted with 200µl of purified water.

I used the purified DNA to genotype each individual at two previously developed single nucleotide polymorphism (SNP) panels, encompassing a total of 197 SNPs. The first panel (Panel A) contained 32 SNPs located within the phosphoglucose isomerase locus, *Pgi*, which has been hypothesized to affect dispersal ability or propensity in several arthropods (Orsini et al., 2009; Wheat & Hill, 2014). This panel was developed by Jangjoo (2018) to assay potential selective variation between dispersers and non-dispersers. The second, larger panel (Panel B) contained 165 physically unlinked SNP loci (35 putatively functional SNPs and 130 putatively neutral or non-functional SNPs) developed by Chaulk and Lucas based on previous RADseq analysis (Lucas, 2022). The potential function of each locus was determined by mapping to a transcriptome (Jangjoo, 2018), with those loci with 90% match being considered putatively functional. This panel was developed for the purposes of assessing genetic variation in this species, with approximately random distribution of assayed SNPs across the genome. The SNP genotypes were determined on the Agena iPlex Gold MASSarray platform (Sequenom, San Diego, CA), which can perform robust genotyping assays using samples of low DNA concentrations. Differentially weighted nucleotide primers are attached to the target SNPs within amplified DNA samples, and SNP variants are then identified through mass spectrometry.

2.2.3 Data Analysis – Annual measures of population metrics

I performed most analyses in the R statistical platform (R development core team, 2017), with the sample from each year in each of patch P and Q being used as an individual unit of analysis. I estimated yearly allelic richness in each patch, rarefied to 14 individuals, using the package 'PopGenReport' (Adamack & Gruber, 2014), followed by Wilcoxon tests for significant differences in mean allelic richness between consecutive years in a given patch (e.g., 2001 versus 2002, 2002 versus 2003, etc.) and between the first and final year of the experiment (i.e., 2001 versus 2008). I performed tests for Hardy-Weinberg equilibrium (HWE) for each patch in each year using the package 'pegas' (Paradis, 2010), with the number of Monte-Carlo replicates set to 1000. I estimated pair-wise F_{ST} between samples from consecutive years in each patch, as well as between the first and final years of the experiment, and expected heterozygosity in each patch each year using the package 'hierfstat' (Goudet, 2005), with number of bootstraps set to 10000. I compared expected heterozygosity between consecutive years, as well as between the first and last year of the experiment using the package 'adegenet' (Jombart, 2008), from which a Monte-Carlo comparison test with the number of simulations set to 999 iterations, were conducted to test for significant difference. I performed exact tests for linkage disequilibrium in each patch each year using 'Genepop' (Rousset, 2008) with dememorization set to 10000. I then fit each of the following metrics with a separate linear mixed model in the 'nlme' package (Pinheiro et al., 2017) with year as the predictor (i.e., reflecting time since the start of the removal experiment), and with patch identity (P or Q) as a random factor: mean allelic richness, expected heterozygosity, proportion of SNPs out of HWE, and year-to-year F_{ST}.

2.2.4 Population assignment of dispersers

After the first year of the removal experiment, a majority of individuals captured and removed from patches P and Q are expected to be first or second-generation immigrants from other patches. I used an approach implemented in Geneclass 2.0 (Piry et al., 2004) to assign individuals removed from patches P and Q to potential patches of origin from a set of neighbouring, potential source populations. I determined these assignments only for the years 2005 and 2008, years in which allele frequency data from neighbouring populations were available (Lucas, 2022). I used the Rennala & Mountain (1997) method of migrant assignment available in Geneclass 2.0, which ranks the most likely source population, by probability, for each individual being assigned. This approach is applicable when all potential sources of migrants within the system are accounted for in the reference population, without the possibility of other external sources. Based on *a priori* knowledge that *P. smintheus* only travels 130 m on average, to a maximum of 2 km, within one flight season (Roland et al., 2000), I determined that five neighbouring patches (M, N, O, R, S) present within the 2 km maximum dispersal distance from P and Q were the possible source populations of the annual migrants (**Figure 2.1**). This assumption is further substantiated by previous estimates of patch connectivity, which indicate that only patches M, O, R, and S are sufficiently connected to patches P and Q to be influenced by any changes in the population sizes of the latter (Caplins et al., 2014). Allele frequencies for Panel B SNPs (165 loci) for the reference/source populations in 2005 and 2008 were obtained from Lucas (2022) from a total of 107 genotyped individuals. Patch N is a small patch that harbours a very small local population), and no samples were collected or available from that patch in those

years. Considering that N is the smallest in both patch and population size among the considered source populations with consistently low population estimates in the single digits each season (Matter et al., 2014), the omission of N would have minimal effects on my assignment results.

2.3 Results

2.3.1 Changes in population genetic metrics across the removal experiment

Of the 197 SNPs assayed, eight could not be successfully genotyped in more than 35% of the individuals genotyped and were therefore removed from the dataset. Of the 470 individuals genotyped, six individuals were successfully genotyped at less than 80% of all SNPs and were also removed from the dataset. After the removal of those SNPs and individuals, the total genotype failure rate across all remaining 464 individuals and 189 SNP loci used for further analyses was 5.2%.

Populations in both patches P and Q showed a slightly increasing trend in mean allelic richness over the years, with minor oscillations throughout the experiment (**Figure 2.2 A**). The mean allelic richness in a given year ranged from 1.712 to 1.792 (SE ± 0.005) for patch P and 1.708 to 1.791(SE ± 0.009) for patch Q. Wilcoxon tests for significant differences between consecutive years within a patch indicated that no consecutive years were significantly different in mean allelic richness for patch P, while only years 2 and 3 were significantly different ($p = 0.009$) for patch Q. Wilcoxon tests between year 1 and 8 indicated no significant difference in mean allelic richness through the course of the entire experiment for P ($p = 0.239$), but near significance for Q ($p = 0.055$). Year was a significant predictor ($p = 0.016$) of mean allelic richness overall, with a gradual increase in allelic richness over the duration of the experiment (**Figure 2.2 A**).

The expected heterozygosity in both patches declined following the initial year of the removal, followed by recovery to pre-removal levels over the following three years, eventually stabilizing in the final three years of the experiment (**Figure 2.2 B**). The expected heterozygosity in a given year during the experiment ranged from 0.210 to 0.240 for patch P, and 0.202 to 0.238 for Patch Q. In patch P, the expected heterozygosity differed significantly between years 1 and 2 ($p = 0.033$) and years 4 and 5 ($p = 0.001$). In patch Q, the only consecutive pair of years between which expected heterozygosity was significantly different were years 4 and 5 ($p = 0.001$). Between years 1 and 8, the first and final years of the study, only patch Q showed a significant difference in expected heterozygosity ($p = 0.006$) and was higher in the final year. Year was a significant predictor of expected heterozygosity overall ($p = 0.005$).

The proportion of SNPs out of HWE in each patch fluctuated throughout the experiment, with an overall increasing trend towards the end of the experiment, particularly in patch Q (**Table 2.1 & Figure 2.2 C**). Of note, patch Q had approximately 12. 3% of total loci significantly out of HWE during year 8, which was by far the largest proportion of loci in disequilibrium observed, with the next closest being year 6 from patch P with 8.02%. Year was a significant predictor of the number of loci in disequilibrium ($p = 0.002$).

Year-to-year, pair-wise F_{ST} estimates in both patches were low to moderate, and ranged between 0.002 to 0.013 (mean = 0.006, SE = 0.001) in patch P, and between 0.002 to 0.008 (mean = 0.005, SE = 0.0008) in patch Q **(Figure 2.2 D).** None of the consecutive pair-wise F_{ST} values were significantly different from zero (95% confidence interval bracketed zero) other than for the comparison of years 5 and 6 in patch P. Additionally,

pair-wise F_{ST} estimates between years 1 and 8, the first and final years of the study, were low and not significantly different from zero in either patch (P: F_{ST} = -0.002; Q: F_{ST} = -0.004). There were multiple, comparatively larger fluctuations in year-to-year pair-wise F_{ST} in patch P, while year-to-year F_{ST} values in Q stayed stable from the beginning to end of the experiment (**Figure 2.2 D)**. Overall, year-to-year pair-wise F_{ST} did not change significantly over the course of the experiment ($p = 0.770$; **Figure 2.2 D**).

Table 2.1: Yearly sample size and population metrics for sites P and Q throughout the removal experiment. MAR is mean allelic richness, H_e is expected heterozygosity, and 'Loci out of HWE' is the proportion of loci significantly out of Hardy-Weinberg equilibrium.

Patch	Year of Collection	Sample Size	MAR	H_e	loci out of HWE
P	2001	24	1.755	0.236	0.027
P	2002	21	1.745	0.210	0.011
P	2003	28	1.779	0.213	0.037
P	2004	29	1.770	0.215	0.059
P	2005	27	1.767	0.243	0.011
P	2006	30	1.772	0.240	0.080
P	2007	29	1.792	0.237	0.054
P	2008	41	1.777	0.236	0.064
Q	2001	21	1.709	0.219	0.021
Q	2002	30	1.770	0.202	0.037
Q	2003	14	1.780	0.218	0.005
Q	2004	31	1.791	0.218	0.043
Q	2005	28	1.778	0.240	0.043
Q	2006	26	1.757	0.233	0.043
Q	2007	30	1.788	0.237	0.064
Q	2008	61	1.781	0.238	0.123

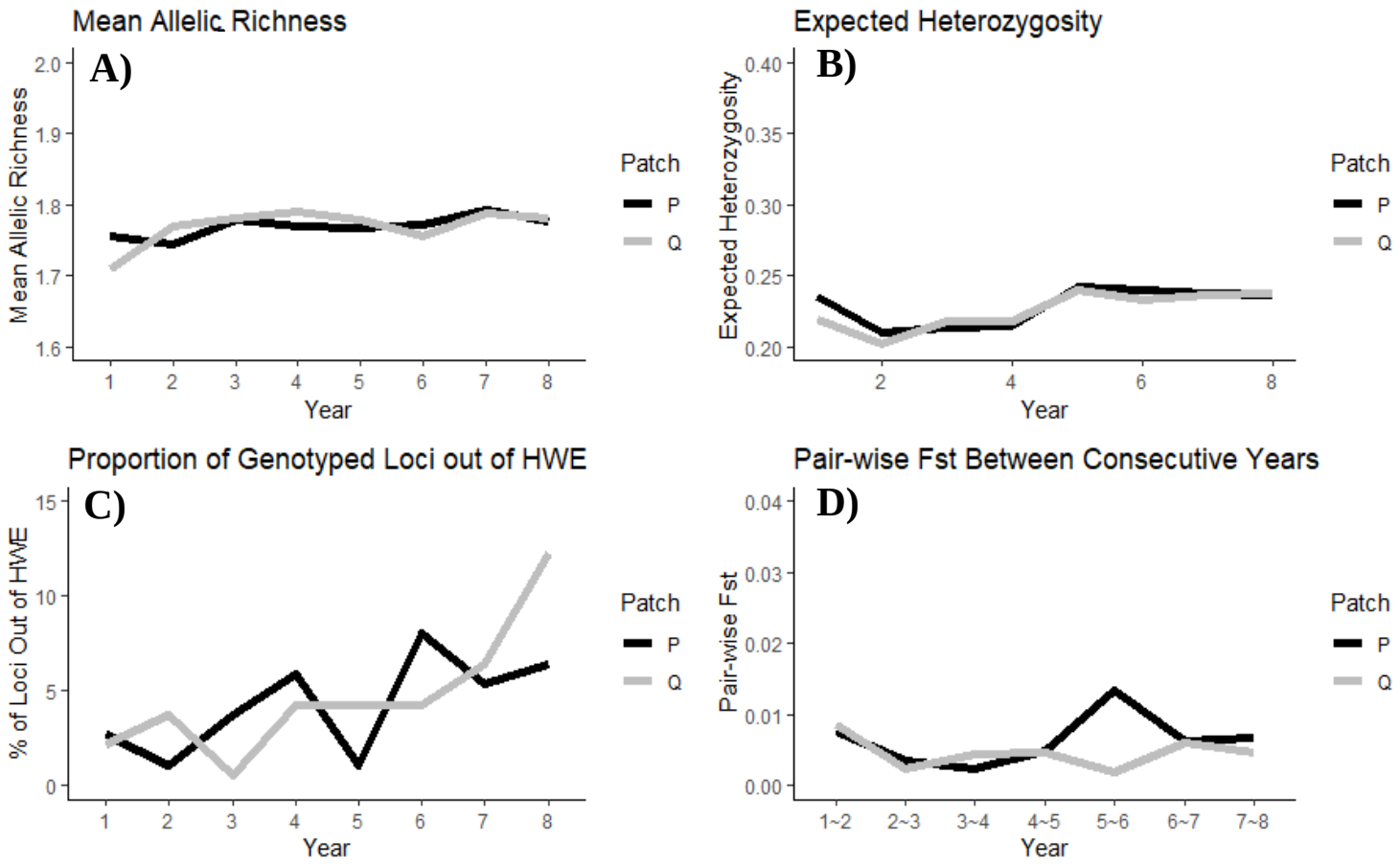

Figure 2.2: Yearly population genetic metrics within local populations in patches P and Q, through the course of an eight-year experiment in which all adults observed in each patch, each year were removed starting in 2001: A) mean allelic richness (MAR), B) Proportion of SNPs not in Hardy-Weinberg equilibrium (HWE), C) Temporal pair-wise F_{ST} between samples collected in consecutive years , and D) expected heterozygosity (H_e). MAR and H_e changed significantly over time, increasing overall through the experiment ($p = 0.016$, $p = 0.005$, respectively). Proportion of loci out of HWE also increased over time ($p = 0.002$). Year-to-year pair-wise F_{ST} did not change significantly over time ($p = 0.77$), and only pair-wise F_{ST} between years 5 and 6 in patch P was significantly greater than zero.

2.3.2 Population assignment of migrants

The probability of assignment to the highest-ranked potential source population, averaged among all individuals collected from P and Q in a given year (2005 or 2008), was 84.19% (se ± 1.23%), compared to 13.30% (se ± 1.07%) for the second most-likely source population **(Table 2.2)**. In 2005, patch P had relatively even proportional contribution of inferred immigrants from patches M (33%), R (30%), and S (37%), with no individuals being assigned to patch O (out of 27 individuals sampled from P in 2005). However, the relative contribution of inferred immigrants to patch P from patch M was more than halved in 2008 (9%), while patch O's contribution increased dramatically to 20%. Contributions from patches R and S to both P and Q remained relatively constant between 2005 and 2008 (**Figure 2.3**)

Table 2.2: The mean percentage probability of assignment of individuals sampled in patches P and Q in 2005 and 2008 (years 5 and 8 of the study), to four potential neighbouring source patches by Geneclass 2.0. For each individual, potential sources (M, O, R, S) were ranked based on the likelihood of the assigned individual originating from each patch. The mean probability of assignment across all individuals, for the first and second most likely patches of origin, are reported here. The probabilities of assignment sum to 100 across the four potential source patches tested, for each individual assigned.

Year of removal	Patch	Assigned Individuals	Mean probability for 1st assignment (%) ± SE	Mean probability for 2nd assignment (%) ± SE
2005	P	27	84.318 ± 2.795	13.354 ± 2.481
2005	Q	28	86.923 ± 2.911	12.043 ± 2.138
2008	P	44	85.229 ± 2.203	11.932 ± 1.825
2008	Q	61	82.118 ± 2.138	14.851 ± 1.824

The largest contributor of inferred immigrants to patch Q in 2005 was patch M, with more than half (57%) of individuals captured in Q being assigned there. Patch S, followed by R, were the next largest contributors to inferred immigration into patch Q, with about a third of the samples from Q in 2005 being assigned to one of these two potential sources (21% for S, 11% for R). Patch O once again had minimal contribution in 2005, with only one individual captured in patch Q being assigned to O, out of 28 total individuals. Interestingly, patch Q also saw a large decrease in inferred immigration from M, and an increase in immigration from O, from 2005 to 2008. The proportion of individuals captured in Q that assigned to patch M declined more than 40 percentage points from 2005 to 2008, while those assigned to patch O increased by 10 percentage points. Patch R became by far the largest contributor of inferred immigrants to Q in 2008, with 46% of 61 sampled individuals being assigned there. The inferred dispersal from patch S to Q also increased from 2005 to 2008, accounting for assignment of 30% of the individuals sampled in patch Q in the latter year (**Figure 2.3**).

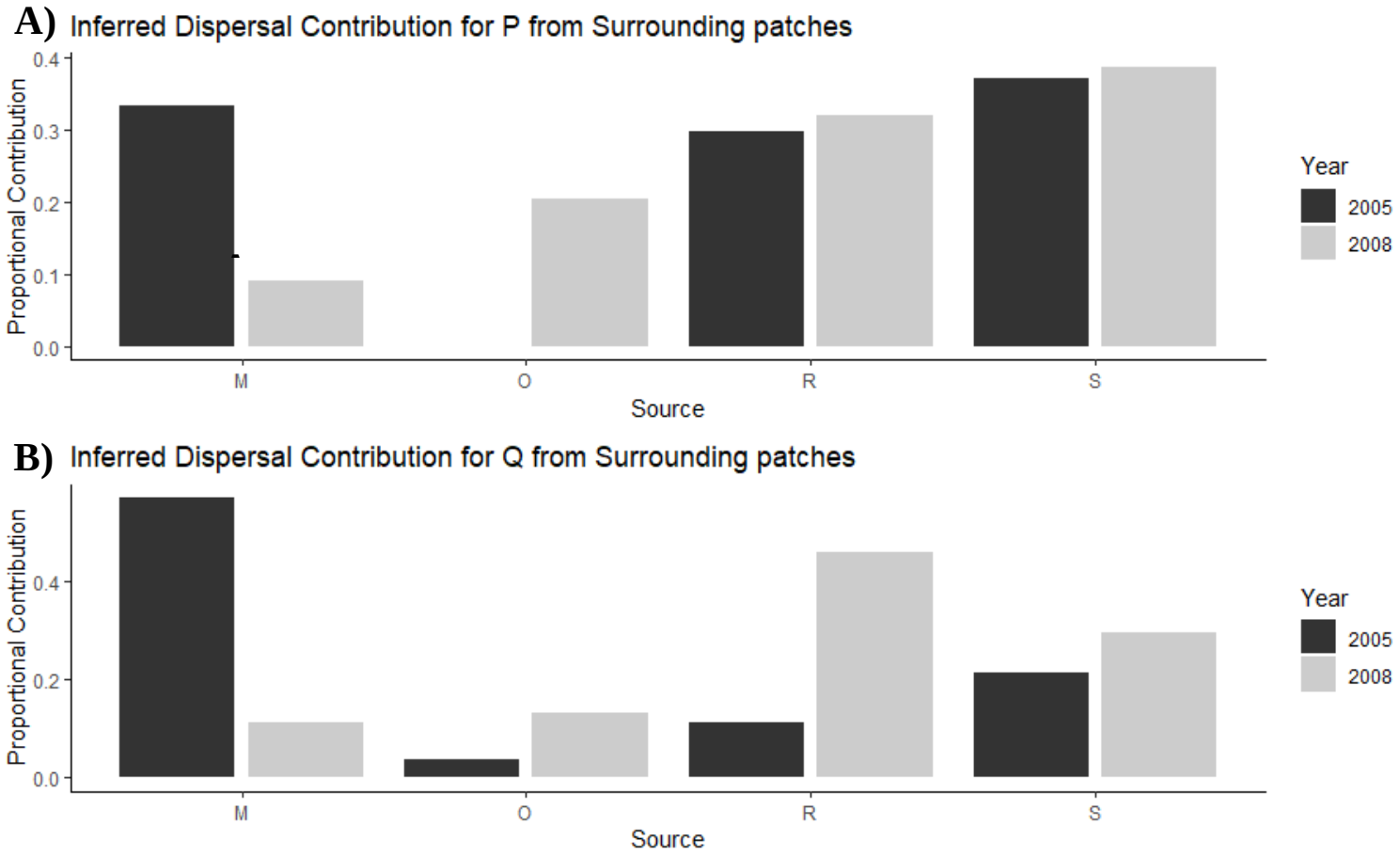

Figure 2.3: Proportion of samples collected in **A)** patch P, and **B)** patch Q, in the 2005 (dark bars) and 2008 (light bars) flight seasons, assigned to each of four potential source populations (in neighbouring patches M, O, R, and S) located within 2 km. Proportional inferred contribution of dispersers was calculated by dividing the number of individuals assigned to each of the potential source patches by the total number of individuals sampled fin each focal patch (P or Q) in each year.

2.4 Discussion

2.4.1 Population genetic metrics throughout the removal experiment

Despite the persistent removal of individuals from each population over eight consecutive years, the genetic metrics measured in P and Q highlighted the resilience of the population network to such localized reductions in population. Any isolated population experiencing such a high level of mortality and reduced reproductive output (Matter & Roland, 2010) consistently over several generations, would be expected to show a significant decline in genetic diversity (Smith et al., 1991). However, genetic diversity metrics for both P and Q did not decline, and in fact showed a moderate but significant increasing trend over time, for both mean allelic richness and expected heterozygosity (**Figure 2.2**). Only mean allelic richness in patch Q showed an initial decline directly after the start of the removal experiment (years 1 to 2), but subsequently increased, albeit with some fluctuations in the later years after the level of allelic diversity had stabilized around years 4 to 5 (**Figure 2.2**). The maintenance and slight increase in genetic diversity in patches P and Q is most readily explained by the continual replenishment of those populations by dispersers from surrounding populations, resulting in both demographic and genetic rescue (Jangjoo et al., 2016). The increase in the proportion of loci out of Hardy-Weinberg equilibrium in populations sampled from P and Q over time is also consistent with immigration from multiple sources, as the consistent influx of dispersers from a pool of partially differentiated source populations would be expected to lead to deviations from Hardy-Weinberg equilibrium. Based on temporal pair-wise F_{ST}, the populations did not differentiate significantly from year to year, or across the entire duration of the experiment in response to the removals except between

years 5 and 6 in patch P, and even in this case, the magnitude of differentiation was quite small (F_{ST} = 0.013). While the increasing proportion of loci out of Hardy-Weinberg equilibrium suggest a constant influx and increased representation of dispersers from several source populations each year into patches P and Q, the small and largely non-significant temporal F_{ST} estimates indicate a relatively stable pool of dispersers over time and continuous inter-mixing of the dispersers, leading to low levels of differentiation between samples from consecutive years throughout the experiment.

The maintenance of genetic diversity over time, as well as the inference of yearly dispersal into P and Q by measures of temporal genetic differentiation and Hardy-Weinberg equilibrium, suggest that dispersal from nearby patches served as the main mechanism contributing to the persistence of populations in patches P and Q in the face of the experimentally induced local population bottlenecks. Furthermore, the moderate increase in genetic diversity I observed suggests that the removals may actually have led to elevated rates of immigration into patches P and Q relative to natural levels experienced before the experiment. Therefore, while the removal of individuals from these patches reduced movement into surrounding populations (Matter & Roland, 2010), my results indicate that there may conversely have been increased movement into these patches, such that they acted as demographic sinks.

2.4.2 Population assignment of dispersers

I observed a large decrease in the relative contribution of in dispersers from patch M to patches P and Q between 2005 and 2008. Patch M has the largest area and population size among the potential source patches (Matter et al., 2014), and the number of inferred dispersers that it provided into P and Q declined by approximately 23 and 45

percentage points, respectively. This was compensated by increased relative contribution from other surrounding patches, particularly from patch O into patch P (~10 percentage point increase) and from patch R into patch Q (~35 percentage point increase). Despite the severe decline in patch M's inferred disperser contributions from 2005 to 2008, population decline in M itself was not detected between 2005 and 2008 based on mark-recapture population estimates (Matter et al., 2014; Jangjoo et al., 2016). In fact, in 2008 the largest population size estimate for M over the last two decades was obtained, with the estimated local population size more than triple that estimated in 2005 (Matter et al., 2014). *Parnassius smintheus* is more likely to emigrate from patches that contain smaller populations (Roland et al., 2000), and adult males prefer to immigrate into more densely populated patches (Matter et al., 2004). The species therefore displays inverse density-dependent dispersal behaviour that would favour remaining in large, dense populations and may explain the observed decline in immigration from patch M in the year with the highest population density in that patch.

It is well-established that genetic diversity of a local population can recover quickly from an acute decline in population size as a result of immigration from other, occupied patches (Bossuyt, 2007). Previous work on this system found that after a metapopulation-wide bottleneck resulting from poor overwintering conditions, patch connectivity, which facilitates immigration, was associated with faster recovery of allelic diversity (Jangjoo et al., 2016). It has also been shown that gene flow after such metapopulation-wide bottlenecks rapidly decreases genetic differentiation among populations and re-establishes patterns of isolation-by-distance, reversing the effects of

the high levels of genetic drift that are experienced during the bottlenecks (Jangjoo et al., 2020; McEachern et al., 2010).

Here, I have shown that such genetic rescue can occur even under continuous, long-term, and repeated decimation of the local population, and even when many of the potential source patches of the dispersers (e.g., N, O, R, S) are smaller in both population size and area than the focal patch (P and Q; Matter & Roland, 2010). However, it should be noted that neighbouring, local populations of *P. smintheus* on Jumpingpound Ridge are not highly genetically differentiated from each other (Keyghobadi et al. 1999; Jangjoo et al. 2020), meaning that a smaller number of unique alleles are likely to be completely lost in local bottleneck or extinction events. This in turn can allow for a quicker recovery of genetic diversity with migration, and the maintenance of alleles across the entire metapopulation. Therefore, genetic resilience of a metapopulation system in response to an extended localized population bottleneck requires further investigation, potentially through a similar removal experiment using systems in which the local populations are more genetically differentiated. Additionally, while local populations may be rescued both demographically and genetically through immigration, extensive intermixing and homogenization of interacting populations may lead to synchronization of population dynamics and increased vulnerability of the entire network to regional disturbances (Hanski & Woiwood, 1993; Goldwyn & Hastings, 2008). Nonetheless, my study does lend further support to the hypothesis that dispersal among populations in interconnected networks plays a critical role in conferring resilience to extreme local demographic declines and bottlenecks (Caplins et al. 2014; Jangjoo et al. 2016), once again

highlighting the importance of dispersal in the persistence of metapopulations (Wang et al., 2015).

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Chapter 3

3 Characterising a genetic basis of dispersal and recolonization in an alpine butterfly metapopulation

3.1.1 Introduction

Dispersal, defined as the movement and settlement of individuals from their natal habitat patch to another patch, shapes many aspects of a population, from geographic distribution to available variation in the gene pool (Tesson & Edelaar, 2013). While also associated with movement of organisms, dispersal is distinct from seasonal migration; dispersal pertains to movement of an individual away from its source, usually within a local area and occurring once, while seasonal migration is the cyclic and regional mass movement of individuals between locations (Duarte & Mali, 2019). Therefore, dispersal plays an especially important role in networks of spatially distinct habitat patches and populations, where between-patch movement of individuals acts as the main mechanism of interaction between local populations. Population networks, known as metapopulations, are dependent on the dynamic balance between continuous local extinctions and subsequent recolonization by inter-patch dispersers, such that while a local population may temporarily go extinct, the overall population network remains (Levins 1967; Hanski, 1998). Furthermore, in the event of a large population decline in a local patch, dispersal from other source patches within the network contributes to the recovery of population numbers as well as genetic diversity (i.e., demographic and genetic rescue, respectively) (Frankham, 2015). Despite the importance of dispersal as a fundamental ecological process, and while there have been many works exploring the physiological or macroecological aspects of dispersal, the genetics of dispersal has been

comparatively underexplored (Saastamoinen et al., 2018). Consequently, the genetic basis of dispersal is not well-understood in many systems.

Dispersal behavior is a complex phenotype affected by genetic and environmental factors, including both the internal (i.e., physiological state) and external environments (Clobert et al., 2009). Additionally, dispersal requires not only the capability to move over or through the landscape (physiological or morphological traits that benefit movement), but also the propensity or motivation to leave one habitat patch for another (i.e., the behavioural likelihood of an individual to disperse, regardless of its movement ability). Therefore, empirically quantifying dispersal is difficult especially within a natural setting, where dispersal history of individuals can be difficult to assay and where high dispersal ability and propensity do not necessarily coincide (Steyn et al., 2016). As a result, variation in dispersal ability among individuals has often been quantified through proxy measurements, for example morphological traits associated with movement such as wing shape. or size and strength of wing musculature, or by measuring locomotive performance such as flight duration and velocity under controlled lab conditions (Roff et al., 1997; Zera & Brisson, 2012).

Uncovering the genetic basis of variation in dispersal is further complicated by the fact that this phenotype is likely under polygenic control in most organisms (Roff, 1986; Mackay et al., 2009, Saastamoinen et al., 2018). However, despite the support for polygenic control of this trait, the majority of research on the genetic basis of dispersal has relied on characterizing variation at a limited set of loci selected based on an *a priori* hypothesis (i.e., candidate loci), in relation to proxy measures of dispersal-related morphology or performance described above (Wheat & Hill, 2014; Saastamoinen et al,

2018). One of the first candidate genes to be associated with dispersal behaviour was the foraging gene discovered in *Drosophila melanogaster* (Pereira & Sokolowski, 1993). Variation in this gene affects movement behaviour and dispersal distance in both *D. melanogaster* larvae and adults, where individuals with the "rover" allele display increased dispersal distance and food-searching behaviour compared to individuals homozygous for the "sitter" allele (Edelsparre et al., 2014). However, of the potential candidate genes shown to affect dispersal ability or propensity in arthropods, arguably the most widely researched is the phosphoglucose isomerase gene *(Pgi)*, which encodes its namesake enzyme (Hanski, 2011; Wheat & Hill, 2014; Niitepõld & Saastamoinen, 2017). The enzyme phosphoglucose isomerase catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate during the second step of glycolysis, playing a critical role in the cellular generation of ATP (Eanes, 2011). While it is still not fully understood how the observed variation at this gene specifically affects the molecular biochemistry of animals, variation at the gene has nonetheless been shown to affect a number of metabolism- and dispersal-related phenotypes such as fecundity, thermal tolerance, dispersal distance, and dispersal performance across multiple arthropod species (Saastamoinen et al., 2009; Wheat et al, 2010; Niitepõld et al, 2009). Examples include the willow leaf beetle (*Chrysomela aeneicollis)*, in which variation at the *Pgi* locus is associated with differential survival and running speed in response to heat stress (Rank et al., 2007), as well as the *Colias* butterflies and the European map butterfly (*Araschnia levana*), in which variation in *Pgi* affects flight performance and distance within a lab setting (Watt, 1977; Mitikka & Hanski, 2010).

To date, there have only been two systems in which the genetics of dispersal has been explored within a metapopulation. The first was an artificial mesocosm network, in which researchers tested the effect of various patch configurations (patch quality, size, and spatial placements) on dispersal propensity and gene expression of spider mites, concluding that heterogeneity in available habitats negatively impacted dispersal rate, as well as finding 152 and 182 differentially expressed genes among the patch configuration treatments (De Roissart et al., 2015; De Roissart et al., 2016). The other such system is a natural metapopulation of the Glanville fritillary (*Melitaea cinxia*), a widespread European butterfly species, located in the Åland Islands of Finland (Hanski & Meyke, 2005). The *Pgi* gene as a candidate locus for dispersal ability has arguably been most thoroughly explored in this species, in which specific single nucleotide polymorphism (SNP) variations and genotypes within *Pgi* have been associated with many fitness and dispersal related traits including flight metabolic rate, dispersal likelihood, larval survival rate, female clutch size, and body temperature during flight (Orsini et al., 2009; Saastamoinen et al., 2009: Kallioniemi & Hanski, 2011). Furthermore, when analyzed at a metapopulation level, the frequency of an allele within *Pgi* (*Pgi-f*) was significantly higher within isolated, or newly colonized habitat patches while another allele (*Pgi-d*) was more common in well-connected, already established populations. This suggests that recolonization of empty habitat patches, particularly isolated ones, selects for the *Pgi-f* allele, supporting the hypothesis that this allele enhances dispersal ability in this species (Haag et al., 2005). Here, I explore the potential role of variation in *Pgi* in determining dispersal ability and recolonization success in another natural metapopulation system, that of the Rocky Mountain Apollo butterfly, *Parnassius smintheus*. Specifically, I

examine whether local recolonization and immigration select for specific *Pgi* alleles in a metapopulation of *P. smintheus,* on Jumpingpound Ridge in the Canadian Rockies.

Recent research in the Jumpingpound Ridge system found that *P. smintheus* individuals that were recaptured in a different patch from that in which they were originally marked (i.e., dispersers) had higher prevalence of the minor (less common) allele at two SNPs within *Pgi* (PGI 1018 and 1241) compared to non-dispersers (i.e., individuals only ever recaptured in the same patch in which they were originally marked) (Jangjoo, 2018). Furthermore, within the dispersers, those individuals that were heterozygotes or minor allele homozygotes at PGI 1018 had significantly higher mean dispersal distance compared to those that were major allele homozygotes. As a continuation to this research, I investigate whether recolonization and immigration events within the Jumpingpound metapopulation select for these same potentially dispersal-related SNPs in *Pgi.* I examine recolonization and immigration in response to two types of local, catastrophic population declines: natural extinctions and artificially induced population bottenecks, respectively. Natural extinctions were events in which local habitat patches were found with zero observed individuals in a yearly population survey of the system, while artificial population bottlenecks were events in which researchers removed all observed butterflies from two habitat patches for eight consecutive years as part of an experimental population dynamics study (Matter & Roland, 2010; Matter et al., 2014).

I hypothesized that variation in *Pgi* contributes to variation in dispersal ability among individuals of *P. smintheus*. Furthermore, I hypothesized that genetic variations within *Pgi* associated with increased dispersal ability or propensity would be selected for

during recolonization and immigration events. Consequently, I predicted an increase in frequency of dispersal-related SNPs (minor alleles of PGI 1018 and 1241) within local populations directly after natural recolonizations. I also predicted that these dispersal-related SNPs would increase in frequency as a response to the artificial population bottlenecks induced by the yearly, repeated removal of butterflies from two local populations, as these removals appear to have led to the continuous influx of dispersers into the patches each year (Chapter 2). I expected to see the most pronounced increase in frequency of dispersal-related SNPs between the first and second year of the removals, as the individuals removed in the first year of the experiment represented the resident populations that had not yet been exposed to any selective pressure for dispersal. In contrast, most individuals collected in subsequent years would have represented dispersers that immigrated to the experimental patches the same year, leading to a more consistent representation of dispersal-related genotypes among the samples collected in years 2 to 8 of the study.

3.2 Methods

3.2.1 Study system & organism

Parnassius smintheus is a univoltine butterfly species that inhabits primarily high-altitude alpine meadow habitats throughout the Eastern Rocky Mountains in Canada and the United States (Sperling & Kondla, 1991). The larvae's main host plant is a species of perennial succulent, *Sedum lanceolatum*, although the larvae are known to be able to feed on other sedum species as well, such as *Sedum rosaceae* (Roslin et al, 2008). The larvae pupate after completing five instars, from which the adults emerge around July to August to mate and oviposit during the annual flight season.

The metapopulation studied here occurs on Jumpingpound Ridge in the Rocky Mountains of Kananaskis Country, Alberta. The metapopulation consists of 23 high altitude (> 2000 m) meadow habitat patches ranging in size from 0.2 ha to 22.7 ha (Roland et al., 2000). This system has been the subject of long-term population monitoring and genetic sample collection since 1995, with mark-recapture population assays performed during the yearly summer flight season to monitor population size and movement on a patch-by-patch basis (Matter et al., 2014).

Starting in 2001, the south-eastern portion of the network was subject to an eight-year long experiment in which all adults observed in two adjacent patches were captured and removed each year, to examine effects of simulated local extinctions on the population dynamics of the surrounding patches (**Figure 3.1**) (Matter & Roland, 2010). For a more thorough description of the study organism and system, as well as long-term studies conducted within the study metapopulation, please refer to section 2.1 (pg. 17-18).

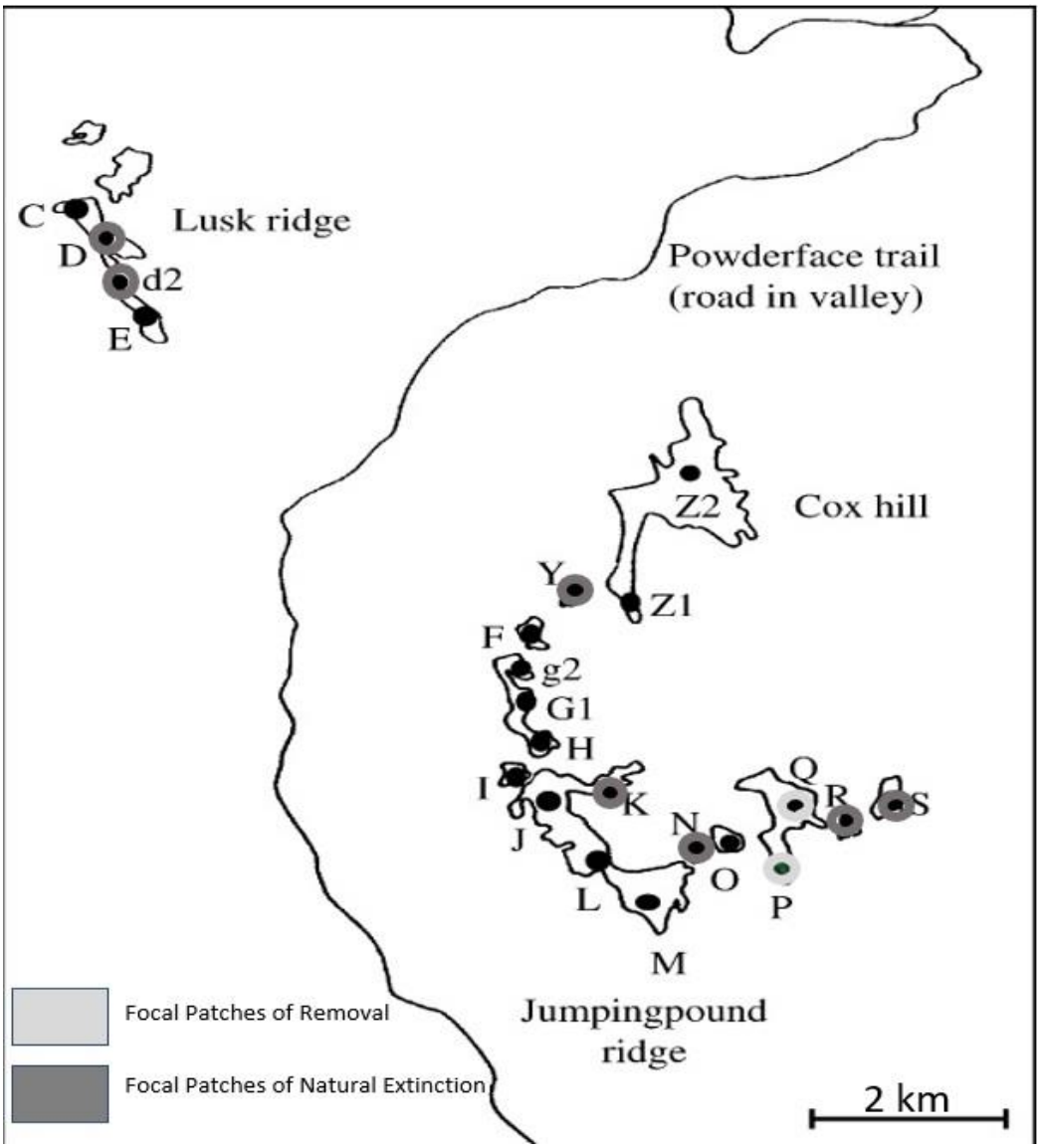

Figure 3.1: Map of the Jumpingpound Ridge study system located in Kananaskis Country, Alberta. Individual habitat patches are indicated with black dots and letter labels. Patches containing populations that have experienced natural extinctions or experimental removals are circled, with light grey circles representing patches that experienced long-term artificial removal of adults from 2001 to 2007, and dark grey circles representing patches that have experienced natural extinctions. Of those patches that experienced natural extinctions, patches D, N, R, S, and Y had archived tissue samples available for genotyping. (Modified from Keyghobadi et al., 2005).

3.2.2 Sample collection and identification of natural extinctions

Mark-recapture surveys were performed on the Jumpingpound Ridge metapopulation each year by catching adult butterflies using hand-nets and marking the hind wings of each newly captured individual with a unique three letter code using permanent marker (Roland et al., 2000). Along with the butterfly's unique ID, the spatial location, date, and time of each capture or recapture event was recorded. A small clip (~0.2 cm^2) of wing tissue was collected from newly captured individuals and placed in 95-100% ethanol for genetic research (Keyghobadi et al., 1999). The mark-recapture surveys took place throughout the adult flight season, during which time each habitat patch was visited approximately every 3 to 5 days.

Naturally occurring local extinction events were identified when no adults were observed in a patch during an entire, given flight season. A recolonization was identified once individuals were observed in a patch that had been deemed extinct in a previous year. In total, there were 22 separate local extinction events and 19 subsequent recolonizations across seven different patches from 1995 to 2020; three patches (N, R, and Y) were locally extinct in 2019, but had not yet been recolonized by 2020. Using all available wing clips that had been sampled in the year immediately before each recorded extinction, and in the year of each recorded recolonization, I genotyped a total of 33 pre-extinction and 16 post-extinction samples. Population sizes, and hence numbers of sampled individuals, are generally very low immediately before extinctions and immediately after recolonizations. As a result, I had very small sample sizes from individual patches, and had to pool the pre- and post-extinction samples across all available patches. Two individual samples were considered both pre- and post-extinction

due to a patch experiencing back-to-back extinction, recolonization, then another extinction event. These two individuals were genotyped but were excluded from further analyses (**Table 3.1**).

Table 3.1: Number of samples available from populations experiencing natural local extinction events, immediately before ('Pre') the recorded extinction and immediately after ('Post') recolonization. Two individuals from patch N (designated with asterisk) were collected during 2008, which falls between two separate extinction events in 2007 and 2009-2014. These individuals were excluded from further analyses. Therefore, out of 47 total available samples, 45 were analyzed: 31 samples representing pre-extinction populations and 14 representing post-extinction populations.

Patch	Year of extinction	# of samples Pre	# of samples Post
D	2011	6	2
N*	2007	0	2*
N*	2009 - 2014	2*	7
N	2018 - ongoing	4	0
R	2019	10	0
S	2011	6	0
Y	2010 - 2014	1	5
Y	2019 - ongoing	4	0

*Samples count as both pre and post extinction populations due to falling between two separate extinction events.

Separate from the above natural extinction events, a long-term experimental bottleneck occurred from 2001 to 2007 in two patches (P, Q), as a result of an experiment to investigate the effects of continuous removal of adults on the populations dynamics in surrounding, connected patches (Matter & Roland, 2009; Matter & Roland, 2010). The removed individuals were labelled with the date and location of capture, then stored, dried, and pinned in a collection at the University of Cincinnati. I sampled from these pinned butterfly specimens removed from patches P and Q, taking one leg as genetic samples for each sampled individual. For years in which the number of captured individuals in a patch was lower than 30, I sampled all available individuals. Otherwise, I sampled between 30-60 individuals per patch per year. In addition to the dried samples, tissues from 42 frozen whole butterflies removed during the 2008 flight season, representing the final state of the populations after the experimental removals, were also used. In total, I sampled a total of 470 individuals from patches P and Q across eight years (**Table 3.2**).

Table 3.2: Number of individuals genotyped from patches P and Q, for each year of the removal experiment. Total sample size for P = 229 and total sample size for Q = 241.

Patch	Year of Collection	Sample Size
P	2001	24
P	2002	21
P	2003	28
P	2004	29
P	2005	27
P	2006	30
P	2007	29
P	2008	41
Q	2001	21
Q	2002	30
Q	2003	14
Q	2004	31
Q	2005	28
Q	2006	26
Q	2007	30
Q	2008	61

3.2.3 DNA Extraction and Genotyping

DNA was extracted from sampled tissues using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD). The tissues were first broken down by homogenization in a microcentrifuge tube, before being lysed and incubated at 56 ° C for 24 hours. The extracted DNA was eluted with 200µl of purified water. Each sample was genotyped at two previously developed single nucleotide polymorphism (SNP) panels, encompassing a total of 197 SNPs. The first panel (Panel A) contained 32 SNPs located within the candidate locus *Pgi,* developed by Jangjoo (2018) to assay potential selective variation between dispersers and non-dispersers. The second, larger panel (Panel B) contained 165 physically unlinked SNP loci (35 putatively functional SNPs and 130 putatively neutral or non-functional SNPs, based on mapping to an RNAseq library representing gene expression in adult thoracic tissue during flight) developed by Chaulk and Lucas based on previous RADseq analysis (Lucas, 2022). Assessing SNP variation outside of *Pgi,* both expressed and non-expressed, provides a measure of change in allele frequencies for putatively non-selected and non-dispersal related SNPs, as a point of comparison for any observed changes in *Pgi* SNP frequency; furthermore, by assaying these additional SNPs, I could search for other potential genetic loci associated with dispersal and recolonization. For more details regarding the genotyping methods, please refer to section 2.2.2 (pg. 21).

3.2.4 Units of analyses

For natural extinctions, all genotypes were grouped into two samples: pre-extinction and post-extinction, the latter representing the first generation of recolonizers. For the removal-experiments, individual genotypes were separated by location (P or Q) and year of collection (2001, 2002, …., 2008), and the variation among years was analyzed separately for each of the two habitat patches.

3.2.5 Detecting loci associated with recolonization or immigration

The first approach I used to identify SNPs potentially associated with recolonization or immigration was the outlier approach used to detect loci putatively experiencing selection, as implemented in the software BAYESCAN 2.1 (Foll & Gaggiotti, 2008). Outliers are genetic markers that show significantly different levels of genetic differentiation among populations assayed, compared to the expected values assuming no selection (Lewontin & Krakauer, 1973). The significance threshold values used for determining SNPs under selection were q-value < 0.05 and Bayes factor greater than +1.5 or less than -1.5. Q-value represents the false discovery rate associated with detecting an outlier, while Bayes factor represents the strength of evidence for selection based on Bayesian statistics. A positive Bayes factor greater than the +1.5 threshold indicates substantial evidence for diversifying selection occurring at a particular locus, while a negative Bayes factor less than -1.5 indicates evidence for purifying selection (Foll & Gaggiotti, 2008). For the natural extinctions, I tested for outliers between the pre-extinction (n = 31) and post-extinction (n = 12) samples, to identify loci potentially experiencing selection as a result of the recolonization process. For the removal

experiment, I tested for selection across different years, separately for each focal patch, P and Q.

The second approach I used to identify SNPs potentially associated with recolonization or immigration was a series of multinomial logit models, implemented in R (R development core team, 2017) using the package mclogit (Elff, 2016), in which the genotype frequencies at each individual SNP locus were compared between pre- and post-extinction samples, (for natural extinctions) or among years (for experimental removals). For natural extinctions, I expected that any alleles associated with dispersal or recolonization should increase in frequency in the post-extinction samples, and for the experimental removals I expected that alleles associated with dispersal or immigration would increase in frequency over time. To do this, the SNP genotypes at each locus were represented in a trinomial form: the minor (less common) allele homozygote genotype was represented as “0”, heterozygote genotype was represented as “1”, and the major allele homozygote was represented as “2”, for each assayed SNP. The observations of each of the three possible genotypes were then modeled as a function of either pre- versus post- extinction status (natural extinction dataset) or year (experimental removal dataset), separately for each SNP. Significance values were adjusted for multiple tests using the Benjamini-Hochberg correction (Bejamini & Hochberg, 1995).

All assayed SNPs that were monomorphic or lacked the minor allele homozygote in the relevant dataset (i.e., natural extinction or experimental removal dataset) were removed from the multinomial modelling analysis. In total, 110 SNPs were kept for the natural extinction dataset, out of 197 total SNPs genotyped; of the removed SNPs, 26 were monomorphic and 61 SNPs lacked the minor homozygote from the sampled

populations. For the experimental removals from patch P, 154 of 197 total SNPs were kept for the multinomial modelling; of the removed SNPs, 15 were monomorphic and 28 SNPs lacked the minor homozygote among the samples. For experimental removals from patch Q, 156 of 197 total SNPs were kept for the multinomial modelling; of the removed SNPs, 16 were monomorphic and 25 SNPs lacked the minor homozygote among the samples from that patch.

3.3 Results

Bayescan analysis for detecting outlier SNPs under selection yielded no significant results from either the natural extinctions or the removal experiment. None of the assayed SNPs, based on the q-value threshold of ≤ 0.05 and Bayes factor threshold of $\geq|1.5|$ showed evidence of being selected for as a result of natural recolonizations, or over time as a result of the experimental removals. All Bayes factor values from outlier tests at each SNP were between -0.047 and -0.045 BF for the natural extinctions. For the removal experiment, BF values for P were between -0.040 and -0.12, while they were between 0.040 and -0.11 for Q.

Multinomial logit modelling of genotype frequencies at individual loci yielded similar results, with no SNPs detected to have significantly changed in genotype frequency, either after recolonization (i.e., comparing pre- versus post-extinction samples) or across years of the experimental removals, after correction for multiple testing. For natural extinctions, even before the correction for multiple tests, no SNPs showed significant differences in genotype frequency in the pre- versus post-extinction samples. For the analyses of experimental removals, eight SNPs from patch Q and one

SNP from patch P showed significant differences in genotype frequencies across years, prior to the correction for multiple tests. Two of these (PGI_1512, p = 0.033; PGI_626, p = 0.017) were SNPs within *Pgi,* but were different from previously identified SNPs (PGI 1018 and 1241) associated with dispersers by Jangjoo (2018). However, after correction for multiple tests, none of the above SNPs remained statistically significant (**Table 3.3**).

Table 3.3: SNP loci that changed significantly in genotype frequency across years of the removal experiment, as determined by multinomial regression and before a correction for multiple tests was applied. Original model P-values ('P-value') were adjusted for multiple testing using the Benjamini – Hochberg statistical correction ('P-adjusted'); after correction, none of the SNPs displayed significant change in genotype frequencies over time (all $P > 0.05$).

Patch Analyzed	SNP	P-value	P-adjusted
P	PS_18450	0.039	0.998
Q	PGI_1512[a]	0.033	0.792
Q	PGI_626[a]	0.017	0.792
Q	PS_107844	0.034	0.792
Q	PS_141537[b]	0.002	0.318
Q	PS_175642	0.022	0.792
Q	PS_41732	0.030	0.792
Q	PS_60289	0.039	0.809
Q	PS_69474[b]	0.019	0.792

[a] located within the *Pgi* locus

[b] mapped to transcriptome expressed in adult thoracic tissue during flight

3.4 Discussion

No SNPs within or outside *Pgi* changed significantly in allele frequencies following recolonizations or in response to experimentally induced bottlenecks in local populations of *P. smintheus*. I was unable to detect significant changes in frequency even for SNPs that have previously been associated with dispersers within the system (i.e., PGI 1018 and 1241), using either method of detecting potential SNPs associated with dispersal and recolonization. Nine loci changed in frequency through the course of the removal experiment, as determined from the multinomial modelling, and two of these were within *Pgi* (**Table 3.3);** however, none of these were statistically significant after correction for multiple testing. Hence, I did not find conclusive evidence that *Pgi* underlies differential dispersal ability or propensity in this species.

While *Pgi* has been associated with a variety of ecologically important traits including dispersal across a wide variety of arthropods (Wheat & Hill, 2014), it is also true that *Pgi* is far from a universal gene underlying adaptative traits. A number of studies investigating *Pgi* as a candidate locus for adaptive traits have reported negative results. For example, researchers hypothesized that the broad thermal tolerance of the invasive grass grub beetle, *Costelytra zealandica,* was conferred by variation in *Pgi*, and that this was the main reason this species was so widespread across New Zealand. However, it was found that variation in *Pgi* did not influence thermal tolerance or growth rate in this species (Lefort et al., 2014). It has also been reported that there is close to no variation within *Pgi* among bumblebees, both within and across five different species, indicating that *Pgi* sequence variation cannot underlie adaptations in this group (Ellis et al., 2013). While *Pgi* does show considerable sequence variation in *P. smintheus,* and some alleles

have been found to differ in frequency between dispersing and non-dispersing individuals (Jangjoo, 2018), my results suggest that any effect of *Pgi* on dispersal is not strong enough to have been detectable in naturally occurring recolonization events, or among immigrants moving into experimentally induced 'sink' populations. This is not necessarily surprising, considering that dispersal is a complex trait and likely to be influenced by multiple genes as well as environmental conditions (Saastamoinen et al. 2018). At the same time, my study featured a number of caveats and limitations that could have limited my power to detect any associations between *Pgi* variation and either recolonization or immigration; these include the high connectivity of the patches used in the experimental removals, and the low sample sizes associated with natural extinctions.

To date, the genetic basis of dispersal has only been investigated in one other natural, metapopulation system, that of the Glanville fritillary butterfly in the Åland Islands, where it was found that specific alleles within the *Pgi* locus were more prevalent within local populations that either had low connectivity with other neighbouring populations, or were newly established (Haag et al., 2005). In the case of the removal experiment on Jumpingpound Ridge, both focal patches from which individuals were removed were geographically well-connected with other neighbouring patches, such as M, O, R, and S, which served as the sources of immigrants (Chapter 2; **Figure 3.1**). Due to the high connectivity of these patches, which would facilitate the dispersal of individuals into them, it is possible that individuals of lower dispersal ability or propensity could have moved to P and Q without meaningful risk of dispersal mortality (i.e., any selection associated with dispersal might have been very weak). Further corroborating this hypothesis, previous studies have demonstrated that local populations

of the Jumpoingpound Ridge metapopulation with higher connectivity recover their allelic diversity faster after sudden population size crashes (i.e., bottlenecks), and that the genetic signatures of population bottlenecks within the local populations are rapidly lost due to genetic rescue by dispersers (Caplins et al. 2014; Jangjoo et al., 2016: Jangjoo, et al., 2020). While these previous studies excluded patches P and Q and their directly connected patches (M, O, R, S), due to potential confounding effects caused by long-term removal experiment, my results suggest that a similar, rapid genetic recovery occurred within P and Q even under a repeated, continuous population bottleneck caused by the removals (Chapter 2). The high rates of immigration into patches P and Q in response to the removals, facilitated by their high connectivity, could make it difficult to detect signatures of SNPs associated with dispersal and recolonization from the removal experiments. Future studies that repeat the experimental removals in more isolated patches (e.g., patches Y or S, **Figure 3.1**) might better separate strong versus weak dispersers and have more power to identify genes underlying dispersal success.

For the natural extinctions, which primarily did occur in patches with low connectivity, the numbers of available samples representing pre- and post-extinction populations were necessarily low, due to small population sizes both before and after local extinction events. This led not only to low statistical power in my analyses, but also a low amount of genetic variation being captured within the sample. For example, I observed that many more SNPs were monomorphic or lacked the minor homozygotes in the natural extinction dataset compared to the experimental removal dataset (61 SNPs lacking minor homozygotes and 26 monomorphic loci in the natural extinction dataset, compared to 15 and 16 monomorphic loci and 28 and 25 missing the minor homozygote

for P and Q, respectively), suggesting that a lower degree of genetic variation was captured in the sample from the former. For future research on the genetic basis of dispersal and recolonization in *P. smintheus*, it would be beneficial to assay the sampled individuals at a genome-wide level, with many more loci than were present in the SNP panels I used (i.e., thousands of loci versus a few hundred). This could increase the statistical power of the analyses, without necessarily having to increase the number of sampled individuals, as sampling more individuals before and after natural extinctions may not be realistically possible given the naturally small sizes of pre- and post-extinction populations. Additionally, while using a candidate locus in exploring the genetic basis of dispersal represents a strong hypothesis-driven approach and can reduce research costs, it can lead researchers to overlook other potential genes that influence the trait of interest, including those that either have additive or epistatic effects with the candidate genes in question (Saastamoinen et al., 2018).

Another caveat to note in using local extinction events to characterize the genetic basis of dispersal and recolonization is that it may be necessary to address dispersal and recolonization separately in some systems. While recolonization requires successful dispersal, these two processes are not synonymous, and can each be affected by distinct factors selecting for different traits (Kappes et al., 2014). While a local extinction event gives dispersers the opportunity to take advantage of a vacant patch, the recolonization of that patch also involves successful settlement, survival, and reproduction of those dispersers once they arrive at their destination. Therefore, the signatures of any genes associated with initial dispersal to the empty patch may be lost or obscured by this

additional set of complex processes and associated traits necessary for successful recolonization.

Although I did not find any SNPs associated with dispersal and recolonization in *P. smintheus*, using natural extinction events or experimental removals nonetheless has the potential to bypass many of the logistical problems associated with studying the genetic basis of dispersal in the field, such as accurately detecting dispersal history, or directly sampling individuals that have truly dispersed between habitat patches. At the same time, these approaches also do not suffer from some of the very simplifying assumptions of using proxy measures of dispersal based on morphological traits or locomotive performance measured in captivity. I believe these approaches could be expanded upon to further explore the genetics of dispersal in *P. smintheus*, particularly by sampling many more loci from across the genome and conducting experimental removals on the most isolated populations and may also be valuable in exploring the genetic basis of dispersal in other metapopulation systems.

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Chapter 4

4 General Discussion and Conclusion

As the number of species that exist within fragmented landscapes continues to grow globally, it is becoming crucial to understand metapopulation processes to predict the persistence and evolution of populations, and to inform management and conservation (Fahrig, 2003). My thesis contributes insight into the genetics of metapopulations, and metapopulation-level processes. I empirically demonstrate the potential genetic resilience of metapopulations against severe localized population declines, even when such decline occurs over an extended period. Importantly, my work corroborates and highlights the importance of dispersal, and consequently connectivity, among local populations in conferring this resilience (Keyghobadi et al., 2005; Jangjoo et al., 2016). Additionally, my research tests an underexplored approach to studying the genetic basis of dispersal in the wild, providing a framework for future studies that could employ a similar study design.

I explore key processes affecting the dynamics metapopulations specifically recolonization, dispersal, and source-sink dynamics, by quantifying the population genetic response to severe, local population declines and extinctions. In my first chapter, I tracked the genetic consequences of long-term population bottlenecks induced by artificial removal of adults on genetic diversity. I also investigated the sources of the immigrants that rescued the allelic diversity in the experimentally manipulated patches. This metapopulation system displayed strong resilience against local, long-term population bottlenecks, with genetic metrics indicating the continuous influx of migrants

from neighbouring patches, as well as the preservation and recovery of genetic diversity within the local populations despite heavy removal of adults for eight consecutive years. My results complement the previous results from this removal experiment (Matter & Roland, 2010), where the extinction risk of patches neighbouring P and Q did not increase as a result of the removals. This is an especially surprising and potentially encouraging result for this metapopulation, considering the relatively low dispersal rate detected (~10%) within the species in previous studies (Roland et al., 2000), as well as the fact that P and Q are centrally located patches in the patch network and contain some of the largest local populations in the metapopulation (Matter et al., 2004; Matter et al., 2014). The relative migrant contributions of potential source patches into patches P and Q did not show consistent patterns between the two years (2005,2008) examined, despite variability in patch size among potential sources and variability in population sizes between the years; this suggests that sink populations within this metapopulation are not reliant on any single source patch for the influx of dispersers, further contributing to resilience of the system as a whole (Keyghobadi et al., 2005; Jangjoo et al., 2016).

My results indicate that in the event of future local extinctions or bottlenecks, local populations may be minimally affected, even by long-term bottlenecks, as long as there are neighbouring, occupied patches that can provide recolonizers and immigrants. However, my results also highlight two key potential risks faced by the metapopulation, both of which will be exacerbated by climate change. First are system-wide disturbances that simultaneously affect all populations, such as unfavourable temperatures caused by extreme weather events, which are becoming increasingly frequent across the globe (Mann et al., 2017). Notably, *P. smintheus* is known to be particularly vulnerable to

extreme temperatures during its overwintering period as pharate larvae (Roland & Matter 2016; Roland & Matter, 2021). Second is the reduction in the overall connectivity among local populations. Several studies have empirically supported that reduction in connectivity among the local populations can lead to the degradation of the overall metapopulation system and its persistence (Secor et al., 2009; Carroll et al., 2020). Warmer temperatures in recent years have led to the rise in the alpine tree line in the Rockies, leading to the encroachment of surrounding conifer forests into the meadow habitats of *P. smintheus* (Ross et al, 2005; Roland & Matter, 2007). It has previously been found that forest matrices deter dispersal in this species, significantly limiting movement and gene flow between patches (Ross et al., 2005; Keyghobadi et al., 2005). While it does seem that the Jumpingpound Ridge system is not in immediate danger, continuous monitoring of the populations as well as connectivity amongst patches into the future is paramount.

In my second chapter, I explored the genetic basis of dispersal in *P. smintheus* by using the predicted increased representation of dispersers in patches following dramatic local population declines, specifically as a result of both natural extinctions and the experimentally induced population bottlenecks. My results provided no evidence of selection associated with dispersal within the candidate locus (*Pgi*) or in other SNP markers that I genotyped, despite two SNPs within *Pgi* being associated with dispersers in a previous study on this system (Jangjoo, 2018). This could be due to variation in *Pgi* having only very subtle or complex effects on *P. smintheus* dispersal, or to limitations in my statistical methodology and sample availability, especially for the natural extinctions. Nonetheless, using the recovery from dramatic population decline events to investigate

the genetic basis of dispersal is an unexplored approach that has the potential to bypass many of the limitations present in other studies of dispersal and dispersal genetics, such as determining accurate dispersal history of individuals, and without having to use proxy metrics of dispersal ability such as morphological traits or in-lab performance to do so (Saastamoinen et al.,2018). Therefore, there was still value in testing this underutilized approach in my study, and my work suggests potential improvements in study design for similar field experiments or observational studies involving local extinctions or bottlenecks.

I hope that my work inspires and informs further research using genetics to understand metapopulation dynamics and dispersal in *P. smintheus*, as both factors drive the overall persistence of these butterflies on Jumpingpound Ridge and likely throughout their range (Roland et al., 2000; Matter & Roland, 2010). Furthermore, the Jumpingpound Ridge metapopulation system can serve as a model for other organisms facing similar threats such as increasing habitat fragmentation and increasing population size variability, caused by human land use and global climate change. Further research into the topics I explored may produce important insights into key processes determining metapopulation dynamics and metapopulation persistence, and thereby inform the management and conservation of a broad range of taxa inhabiting increasingly fragmented landscapes.

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Curriculum Vitae

Name:	Keon Young Park
Post-secondary Education and Degrees:	University of Western Ontario London, Ontario, Canada 2014-2018 B.Sc. Hon. Spec. Biology
Honours and Awards:	Dean's Honour list 2017, 2018 Alberta Conservation Association Grant in Biodiversity 2019 - 2022
Related Work Experience:	Animal care and lab volunteer Advanced Facility for Avian Research, University of Western Ontario 2015 – 2018 Teaching Assistant University of Western Ontario 2018-2022
Conference Presentation:	Synthesizing genotype-environment association studies to unravel the main drivers of local adaptation IALE-North America 2021