Systematics of the Great Spangled Fritillary butterfly (*Speyeria cybele*)

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Systematics and Evolution

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Abstract

The taxonomic rank of species remains a fundamental unit in the study of biodiversity. However, speciation processes are diverse, making it challenging to delimit species. This difficulty is conflated by methodological issues including the use of too few characters, low sample sizes, and prior name changes unsupported by empirical data. To operationalize species taxonomy, numerous species concepts have been proposed to communicate morphological and evolutionary uniqueness, with some also considering biological processes.

The Great Spangled Fritillary butterfly, *Speyeria cybele,* provides a challenging case study in species delimitation as its species identity has remained uncertain since its original description. This North American butterfly is well known for its large size, swift flight, and sexually dimorphic wing size and colour pattern. The uncertainty about *S. cybele*'s species status is largely based on differences between populations east of the Rocky Mountains and those to the west of the Rocky Mountains. Some authors have treated *S. cybele* as a single variable species with transitions in colour pattern where populations contact. Others recognize western and eastern populations as two distinct species, with western populations being split off as *Speyeria leto,* which may be sympatric with *S. cybele* in some regions. However, prior taxonomic and phylogenetic studies of this butterfly have used too few characters or limited sampling of populations, and have not sufficiently quantified morphological differences.

My study used whole-genome single nucleotide polymorphisms (SNPs), mitochondrial DNA sequences, and wing colour-pattern and size data to assess the population structure of *S. cybele*. SNPs revealed four major genetic groups that admix to varying extents when populations are in

contact. Mitochondrial DNA showed two major haplogroups. The first was restricted to western North America and the second haplotype was geographically unconstrained. Wing morphometrics showed clear sexual dimorphism across North America, and other characters were usually clinal with no sharp boundaries between genetic groupings. By applying the genomic integrity species concept, which distills several other concepts in an effort to provide an objective calibration for species delimitation, I recommend recognition of *S. cybele* as a single species across North America.

Preface

This thesis is an original work by Leah G Jackson. No part of this thesis has been previously published.

"Apparently, the northern Montana Alberta front is a rich area to attack the *cybele-leto* problem. It will take a vast deal of exploring to determine how much of a fringe area will yield these direct intergradations"

- Arthur H. Moeck, Geographic Variability in *Speyeria*, 1975

Acknowledgements

This thesis was only possible because of the extensive sampling efforts and observations that were contributed to this study. Ed Gage and Randy Gage, thank you for helping to build the largest *S. cybele* specimen set ever collected for a research study, and for sharing your detailed observations of *Speyeria* behavior and habitat across the United States. Daniel Glaeske, thank you for specimens contributing to the prairie and boreal range of *S. cybele*, and for taking me into the field to collect specimens. Ted Pike, thank you for your specimens contributing to our understanding of the Rocky Mountain contact zones, showing me the tucked-away butterfly patches of the Porcupine Hills, and sharing your vast knowledge and evolutionary theories on butterflies. Many others have contributed specimens to this project; I am so proud to have synthesized all these efforts into a thesis and to build upon the previous work by Dr. Erin Campbell on *Speyeria*.

Thank you to my co-supervisors, Dr. Felix Sperling and Dr. Erin Campbell. Felix, I appreciate your support in pursuing a career in entomology since the second year of my undergraduate degree. You helped me focus all my enthusiastic project ideas into meaningful science. Erin, you are a bioinformatic genius, and I am so lucky to have learned from you! And I can't forget the legend himself, my committee member John Acorn, who has inspired me so much in science and is an enabler who keeps giving me excellent pets. To my colleagues associated with the Sperling Lab— Brevan Wagner, Brittany Wingert, Colin Chiu, Hannah Stormer, Janet Sperling, Lisa Lumley, Marcos Rodriguez, Victor Shegelski, Wei Han Lau, and Shawn Abraham— you taught me so much and were the best lab mates I could have asked for.

I have had amazing support from friends and family during my thesis. To my partner Kristian, thank you for pushing me when I was discouraged and celebrating every bit of progress I made. Most importantly, thank you to my big ol' tabby cat, Karim. Karim was on my lap helping me complete this thesis every moment he physically could. People who have ever glanced at my computer probably noticed all the cat hair decorating my keyboard, which Karim is always happy to provide.

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List of abbreviations

bp – base pair(s)

COI – cytochrome c oxidase I gene

CV – cross validation

ddRAD/ddRADseq – double-digest restriction site-associated DNA sequencing

DNA – deoxyribonucleic acid

FAMD – factor analysis of mixed data

F_{ST} – fixation index

FW – forewing

GBS – genotyping-by-sequencing

HW – hindwing

IBIS - Institut de biologie intérgrative et des systèmes

MBSU – Molecular Biology Service Unit

mtDNA – mitochondrial DNA

nMDS – non-metric multidimensional scaling

PCA – principal component analysis

PCR – polymerase chain reaction

RADseq – restriction site-associated DNA sequencing

SC – species concept

SNP – single nucleotide polymorphism

VFW – ventral forewing

VHW – ventral hindwing

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Chapter 1

General Introduction

1.1 Taxonomy and species concepts

Taxonomy allows humans to organize and characterize biodiversity, and to communicate biological differences across disciplines. However, it is challenging to define and universalize taxonomic ranks, and this is especially true for species. As a result, species concepts have attracted a great deal of study in the attempt to organize the enormity of biological diversity. This application of concepts should also capture the biological processes of speciation and diversification, but species delimitation is only as informative as the data used. With high-throughput sequencing and bioinformatic advances, it is increasingly feasible to generate more information to apply species concepts to cases with unresolved taxonomy. But patterns of variation within and between species may still be difficult to apply taxonomy to without reassessment, corroboration, integration of new technologies, and greater sampling efforts.

To reconcile taxonomic conflicts, integrative species delimitation compensates for biases in any one data type by using multiple sources of characters and analyses (Padial et al., 2010; Schlick-Steiner et al., 2010). Integrative taxonomy often combines high-throughput sequencing techniques such as analysis of whole genome single nucleotide polymorphisms (SNPs) with morphometric analysis and mitochondrial DNA sequencing (Chaplin et al., 2020; Campbell et al., 2022; Oury et al., 2023; Wingert et al., 2024). Single nucleotide polymorphism (SNP) genotyping techniques like restriction site-associated DNA sequencing (RADseq) and genotyping-by-sequencing (GBS) can be an effective tool for detecting recent divergences in organisms (Baird et al., 2008; Elshire et al., 2011; Andrews et al., 2016). When SNPs are used within an integrative delimitation framework they may help resolve the taxonomy of species, even when morphological complexities and uncharacterized ecological interactions make it challenging to detect patterns of diversification (e.g. Chaplin et al., 2020; Campbell et al., 2022; Oury et al., 2023; Wingert et al., 2024).

Many species concepts or definitions have overlapping themes and parameters, and are variably amenable to quantitative evaluation. Applications of most species concepts have relied on observable characters, including the pre-Darwinian typological species concept (e.g Linnaeus,

1753), morphological species concept (Cronquist, 1978), and the phenetic species concept (Ridley, 1993). Some species concepts have relied on additional inference, such as reproductive isolation for the biological species concept (Mayr, 1942), or monophyly and evolutionary uniqueness for the evolutionary species concept (Wiley, 1981), the phylogenetic species concept (Stace, 1989; Agapow et al., 2004), and the cohesion species concept (Templeton, 1989). Genetic information can be readily incorporated into applications of many species concepts. For example, both the biological species concept (Mayr, 1942) and the morphological species concept (Cronquist, 1978) can subsequently be tested via admixture in genetic markers. Such markers can give evidence of interbreeding or determine the distinctiveness of patterns first seen in morphology, without needing hybrid-offspring experiments, lab rearing, or access to parents of known ancestry (e.g. oak trees, Lagache et al., 2013; planktonic foraminifera, Quillévéré et al., 2013). The general lineage species concept integrates multiple criteria to delimit species in evolutionarily complex systems (de Queiroz, 2005). What unites these species concepts is diagnosability and consistently shared sets of characters, which is captured by the genomic integrity species concept (Sperling, 2003).

The genomic integrity species concept (Sperling, 2003) has two parts. The first defines species as populations that maintain their genomic integrity when they contact each other, even if they are exchanging some genes. The second deals with populations that are not in contact with their closest relatives but are more different from them than the difference between related sibling species pairs that are contacting each other without merging. Genomic integrity refers to a species genome remaining resistant to being broken up, as indicated by distinct genotypic combinations, even if they experience some gene flow between species or natural selection that shifts their genome composition. The second part of the genomic integrity species concept uses the degree of overall genetic or phenotypic difference between sympatric or parapatric species to calibrate the degree of difference between allopatric populations. If the degree of difference between allopatric populations is greater than that threshold, they should be considered separate species. That threshold may be relatively broad due to variation in overall similarity among sibling species that contact each other. The lower end of the range of overall similarity values within a genus or related genera serves as an operationally convenient value. The genotypic cluster species concept of Mallet (1995) is similar to that of Sperling (2003) but is less explicit

about the calibration of allopatric populations/species and is grounded more on species nominalism than on inferences about biological processes. While many species concepts exist, I will be relying primarily on the genomic integrity species concept (Sperling, 2003), and its criteria for species delimitation, since it encourages calibration of differences between related lineages and is integrated with other species concepts.

Butterflies can be good model organisms for exploring and testing the utility of integrative approaches to taxonomy given highly visible morphology, interest in them from naturalists, and because they are often used as indicator species (Fleishman and Murphy, 2009; Roe et al., 2009). In North America, *Speyeria* is a particularly interesting butterfly genus since it is composed of both rare and common species with extensive morphological variation (Campbell et al., 2020, 2022; Ren et al., 2020).

1.2 Overview of *Speyeria cybele*

1.2.1 Taxonomic history

Papilio cybele was described by Fabricius (1775; Figure 1.1) from "America", distinguishing it from *Papilio idalia* in "Northern America," and *Papilio aglaja* in "European violet habitat." Fabricius (1807) later placed these species into a new genus, *Argynnis*. After Fabricius, taxonomic activity can be grouped into three periods: the late 1800s, mid 1900s, and 2000s.

In the first period of taxonomic activity, three more species similar to *A. cybele* were described (Figure 1.1), including *A. leto* Behr 1862 from California, *A. carpenterii* Edwards 1876 from New Mexico, and *A. charlottii* Barnes 1897 from Colorado. *Argynnis carpenterii* was subsequently treated as a subspecies of *A. cybele* by Holland (1898, 1905), and *A. charlottii* was listed as a subspecies of *A. leto* by Barnes and McDunnough (1917; Figure 1.1).

In the mid 1900's, three more subspecies were described in *Argynnis cybele*: *A. c. krautwurmi* Holland 1931 from Michigan, *A. c. novascotiae* McDunnough 1935 from Nova Scotia, and *A. c. pseudocarpenteri* Chermock and Chermock 1940 from Manitoba. The North American species of *Argynnis* were then all assigned to a separate genus, *Speyeria*, by dos Passos and Grey (1945a). The status of *Speyeria leto* as a separate species from *S. cybele* was maintained until

subspecies *S. c. letona* dos Passos and Grey 1945 was described and dos Passos and Grey formally listed *S. leto* as a subspecies of *Speyeria cybele* (dos Passos and Grey, 1945b, 1947). At the same time, the subspecies *S. c. pugetensis* Chermock and Frechin 1947 was described from Washington. For the next 75 years, the genus name *Speyeria* replaced *Argynnis* in North America, and *S. cybele* was usually treated as a single species that included *S. c. leto* (Figure 1.1; cf. Howe, 1975).

The current taxonomic period began with the description of *S. cybele eileenae* Emmel, Emmel and Mattoon 1998 from California (Figure 1.1). Its genus was changed back to *Argynnis* by Zhang et al. (2020), who treated *Speyeria* as a subgenus on the basis of genetic distance comparisons with related genera. Zhang et al. (2022) then re-examined the species delimitation of *Argynnis cybele*, elevating *A. leto* as a species and describing the subspecies *A. cybele neomexicana* Grishin 2022 from New Mexico. These changes were accepted by Pelham (2023), who recognized four subspecies within *A. leto* (*A. l. leto, A. l. letona, A. l. pugetensis,* and *A. l. eileenae*) and seven in *A. cybele* (*A. c. cybele, A. c. pseudocarpenteri, A. c. krautwurmi, A. c. novascotiae, A. c. carpenterii, A. c. charlottii*, and *A. c. neomexicana*). Later that year, Hammond and McCorkle (2023) continued to treat *Speyeria* as a genus and defined two "subspecies groups" within one species, *S. cybele.* Within the *S. cybele leto* subspecies group, Hammond and McCorkle (2023) described the subspecies *S. c. caitlinae* Hammond and McCorkle 2023 from British Columbia, and *S. c. colorado* Hammond and McCorkle 2023 from Colorado. Like earlier taxonomic changes, these recent revisions relied on limited population sampling (Zhang et al., 2022), or were supported by relatively few morphological characters (Hammond and McCorkle, 2023).

In this thesis, I treat *S. c. leto* as a subspecies of *S. cybele*, reflecting my research results in Chapter 2. Following de Moya et al. (2017), I also use *Speyeria* as the genus name. This maintains consistency with recent work on *Speyeria* that uses the same data types (Campbell et al., 2020, 2022) and demonstrates greater weighting on recent historical usage among the criteria for recognizing a genus name (Vernygora et al., 2024).

1.2.2 Life history and conservation

Speyeria cybele ranges across much of Canada and the United States (Figure 1.2; Brock and Kaufmann, 2003; Dunford, 2009; James and Nunnallee, 2011), except for the southern tier of US states and northern Canada, and is considered common in the majority of regions it inhabits (Howe, 1975; Layberry et al., 1998; Brock and Kaufman, 2003). Occurrence data for *S. cybele* is denser in eastern North America (Figure 1.2), despite the greatest taxonomic diversity existing in western North America, which may reflect the density of human observers. Further, the range limits of *S. cybele* extend into Florida (Heppner, 2003) and northeastern Alberta (Riva et al., 2020; eButterfly, 2024). *Speyeria cybele* adults can be found nectaring on flowers in mesic meadows, open fields, and woodland edges, and are also common in habitats experiencing secondary succession, like roadsides (e.g. Allen, 1997; Fisher, 2005; James and Nunnallee, 2011; deMaynadier et al., 2023).

Speyeria cybele has a univoltine lifecycle, which begins in late summer or early fall with females ovipositing on or near violets (*Viola* sp.; James and Nunnallee, 2011). Various species of violets are reported as the larval host plant, and immatures overwinter in leaf litter near a violet as unfed first instar larvae (Allen, 1997; Douglas and Douglas, 2005; Dunford, 2009; James and Nunnallee, 2011). After emergence from winter diapause, larvae undergo six instars feeding on violets and then pupate near violets, with emergences starting in June and females remaining in the pupal stage one to three weeks longer than males (James and Nunnallee, 2011). However, many *S. cybele* life history details remain uncertain, as immatures are rarely found.

Field guides usually describe the adult flight of *S. cybele* as fast, active, and strong (e.g. Klots, 1951; Howe, 1975; Bird et al., 1995; Layberry et al., 1998; Poole, 2009; James and Nunnallee, 2011; Monroe and Wright, 2017). Males patrol open areas and forest edges, while females are more elusive early in the flight period and are more commonly encountered later in the season (Howe, 1975). After mating, adult females are believed to go into reproductive diapause, where females suspend reproductive behaviour for 3-5 weeks before beginning to oviposit and continuing until they die in late summer or early fall (Howe, 1975; Douglas and Douglas, 2005; James and Nunnallee, 2011).

Hybrid matings under lab conditions between *S. cybele* and other *Speyeria* species, including *S. aphrodite*, *S. callippe*, *S. diana*, *S. edwardsii*, *S. hydaspe*, *S. idalia*, *S. mormonia*, *S. nokomis*, and *S. zerene*, show no adverse Haldane effects and all hybrids appear to be fertile (Hammond et al., 2013; Hammond et al., 2020). The same ease of artificial hybridization applies to *S. cybele* populations from different parts of its range, such as the Pacific Northwest, Colorado and Michigan (Hammond et al., 2013; Hammond et al., 2020). However, mating behavior has not yet been tested between different geographic phenotypes of *S. cybele* when they contact each other under natural conditions.

The common and widespread occurrence of *Speyeria cybele* contrasts with some other *Speyeria* species that are declining in abundance and threatened by habitat loss, insecticides, and climate change (Howe, 1975; Allen, 1997; Layberry et al., 1998; Fisher, 2005; Schweitzer et al., 2011; James and Nunnallee, 2011; Breed et al., 2013; Monroe and Wright, 2017). Nonetheless, *S. cybele* may be declining in Kansas (Howe, 1975), and is a species of special concern in northeastern British Columbia (Guppy and Shepard, 2001; Heron, 2012) and Idaho (Idaho Fish and Game, 2024) due to low occurrences in those regions. Therefore, it is important to determine the population structure of *S. cybele*, to determine if there are unique populations of this butterfly that may need conservation monitoring and management.

1.2.3 Morphological variation

Wing size in adult *S. cybele* is smaller in males than females, and also broadly distinguishes *S. cybele* populations that occur east versus west of the Rocky Mountains, and at higher latitudes (Moeck, 1975; Dunford, 2009; Hammond and McCorkle, 2023). In the north, Acorn (1993) and Allard (2013) report size ranges of 51-68 mm in butterflies from Alberta and Manitoba. Toward the eastern end of its range, *S. cybele* is reported to have a maximum wingspan of about 100 mm (e.g. Douglas and Douglas, 2005; Acorn and Sheldon, 2016) compared to a maximum wingspan of about 80 mm in western populations (Howe, 1975; Emmel, 1998).

In addition to larger wingspans, wing colour and pattern have also been used to distinguish eastern and western populations (e.g. Howe, 1975; Dunford, 2009). Wing surfaces in eastern populations are characterized by orange dorsal ground colour and bold black patterning

(Edwards, 1872; Allen, 1997; Douglas and Douglas, 2005). Eastern females are paler dorsally than males, and sexual dimorphism is not as pronounced as in western populations (Dunford, 2009; Hammond et al., 2020). Western females are characterized by heavy black patterning with a pale yellow-white ground colour, contrasting with bright orange western *S. cybele* males with reduced black patterning (e.g. dos Passos and Grey, 1945b; Bird et al., 1995; Layberry et al., 1998; Glassberg, 2001; Dunford, 2009; Hammond et al., 2020). Western populations exhibit reduced silver markings on the ventral hindwing surfaces, and these silver spots are not considered to be sexually dimorphic (Layberry et al., 1998). Several authors have remarked on the presence of intergrades in northeastern Montana and southwestern Alberta (Eff, 1980; Bird et al., 1995; Glassberg, 2001; Zhang et al., 2022), however the extent to which wing variation between eastern and western populations is clinal or bimodal in contact zones is unresolved. The transition between western and eastern wing phenotypes is even more complex in New Mexico, Utah, and Colorado, where several subspecies have been described (Figure 1.2; Eff, 1980; Fisher, 2005).

1.2.4 Genetic variation

Prior genetic work has almost exclusively focused on broader phylogenetic relationships within *Speyeria*, and most of these studies used very few *S. cybele* specimens (Simonsen 2006; Dunford, 2007; de Moya et al., 2017; Campbell et al., 2017, 2020; Zhang et al., 2022). Of these broader phylogenetic relationship studies, Campbell et al. (2020) analyzed nuclear double-digest restriction-site associated (ddRAD) SNPs and mitochondrial DNA (mtDNA) of 10 *S. cybele* specimens, and showed sub-clustering of *S. cybele* into eastern or western groups. Campbell et al. (2020) recommended more sampling to assess the degree of admixture and introgression of *S. cybele* populations, especially in regions like southern Alberta where eastern and western populations may co-occur.

Zhang et al. (2022) presented expanded sampling using whole genome re-sequencing of 42 specimens, including holotypes of most taxa. Based on monophyly and principal component analysis (PCA) of genomic data, they suggested that *A. cybele and A. leto* be recognized as distinct species. However, two of the three specimens that they labelled as intergrades between these two taxa came from a potential contact zone in Montana and occupied a phylogenetically

intermediate position. In addition, the collection locality of the *A. c. carpenterii* lectotype from New Mexico was also called into question by Zhang et al. (2022), as it was genetically very similar to *A. cybele* specimens from eastern North America. However, the type locality for *A. c. carpenterii* was not resampled, and instead Zhang et al. (2022) described a new subspecies from a nearby county in New Mexico, based on two monophyletic specimens that appeared genetically similar to *A. c. charlottii*. While Zhang et al. (2022) provided genome-wide sequencing of each specimen, increased sampling from additional localities, in particular from potential contact zones, is clearly required to resolve the population structure, and by extension, the taxonomy of the species.

1.2.5 Evolution of *Speyeria cybele*

Glacial cycles in the Pleistocene have shaped modern butterfly population structure and facilitated rapid speciation of butterfly lineages (e.g. Marques et al., 2024; Maresova et al., 2021; de Moya et al., 2017). Glacial refugia in North America are proposed to be the sources of once-separated fauna that, during interglacial periods like the current Holocene, then disperse into deglaciated regions and reconnect with previously separated populations (Remington, 1968; Hewitt, 2000; Swenson and Howard, 2005; Maresova et al., 2021). Within contact zones, lineages either homogenize their genetic diversity if they can still interbreed, or they maintain their distinctiveness if the populations are too divergent to interbreed when they reconnect (Hewitt, 2000; Maresova et al., 2021).

According to de Moya et al. (2017), the common ancestor of all North American *Speyeria* probably diverged from Palearctic Argynnini about 6.1 mya, with a single dispersal event from Asia that was facilitated by a land connection across the Bering Strait 4.8-7.4 mya. Dispersal into North America began a period of rapid radiation and diversification of *Speyeria* within the last 5.2 mya that likely was facilitated by a pre-established diversity of violets (Marcussen et al., 2012; de Moya et al., 2017). *Speyeria cybele* diverged about 2.3 mya from its sister group in *Speyeria,* and divergences between eastern and western *S. cybele* date to about 1.0 mya, during the long glacial and interglaciation periods of the Pleistocene (de Moya et al., 2017). A more recent divergence time for the base of *Argynnis* (including *Speyeria*) is indicated by Chazot et al. (2019), who estimated 9.2 mya for the divergence of *Argynnis* from *Brenthis*, its sister clade,

while de Moya et al. (2017) estimated 11.1 mya for the same divergence. On the other hand, an order of magnitude older divergences for *Speyeria* and *S. cybele* are proposed by Hammond and McCorkle (2023), although the basis for their time calibration is unclear.

To date, discussion of *S. cybele* population structure has revolved around an "east versus west" narrative, where the Rocky Mountains putatively function as a major barrier to gene flow in Colorado, Wyoming, Montana, and Alberta (e.g. Holland, 1905; Moeck, 1975; Howe, 1975, Bird et al., 1995; Layberry et al., 1998; Kondla, 2004; Fisher, 2005; Hardesty and Groothuis, 2013; Campbell et al., 2020; Zhang et al., 2022; Hammond and McCorkle, 2023). There are two primary hypotheses concerning the delimitation of eastern and western lineages of *S. cybele* in the Rocky Mountains: either these lineages do not meaningfully interact when in contact, suggesting the presence of two species, or they interbreed frequently enough to suggest they comprise a single species. Zones of contact are thought to occur along the eastern slopes of the southern Alberta and northern Montana Rocky Mountains (Bird et al., 1995), and the southern Rocky Mountains of Colorado (Fisher, 2005). Another contact zone has been theorized to exist in California between northern-western and southern-western populations (Emmel, 1998). Moeck (1975) reported that the relationship between eastern and western *S. cybele* is clinal, with introgression producing intermediate wing-pattern phenotypes. Zhang et al. (2022) included all northwestern subspecies as part of the species *S. leto*, while the remaining subspecies were retained in *S. cybele*. In Colorado, the situation is less clear, with the subspecies *S. c. carpenterii*, *S. c. charlotti, and S. c. neomexicana* occurring west of the Rocky Mountains in New Mexico and Colorado, despite being more closely related to eastern *S. c. cybele* than to western populations (Fisher, 2005; Zhang et al., 2022). Different mountain ranges separated by arid Great Basin deserts in Idaho, Utah, and Wyoming tend to have different wing patterns (Gage pers. obs., 2024), perhaps a result of large gaps between suitable habitat that limits interaction between these populations. Since habitat is not continuous across western North America, *S. cybele* population structure is more complex than simply "east versus west." Whether eastern and western *S. cybele* populations interact and share genes where they contact each other needs to be investigated with a modern, focused attempt.

1.2.6 Current knowledge gaps

In order to taxonomically reflect evolutionary processes in complex populations, species delimitation requires ongoing reassessment, investigation, corroboration, and integration of new technologies and more geographic sampling. The taxonomy of *S. cybele* is unresolved, and investigation is required to assess *S. cybele* morphological and genetic patterns. Large gaps in suitable habitats in different parts of its range and differences among taxonomic hypotheses warrant investigation into whether *S. cybele* population structure could be more complex than simply "east versus west," and if the current split of *S. cybele* and *S. leto* represents biological processes of this butterfly. Species delimitation impacts current and future conservation monitoring and management of this butterfly, and research like this facilitates testing of evolutionary and taxonomic hypotheses with new technologies (e.g. RADseq and GBS), while corroborating with morphological data and natural history observations.

1.3 Thesis objectives

This thesis will address the following questions:

I. What is the population genetic structure of *S. cybele*, and do populations show admixture when in contact?
II. What are the relationships between the genetic structure and wing colour and pattern variation in *S. cybele*?
III. Is *S. cybele* composed of more than one species, and how should this be recognized taxonomically?

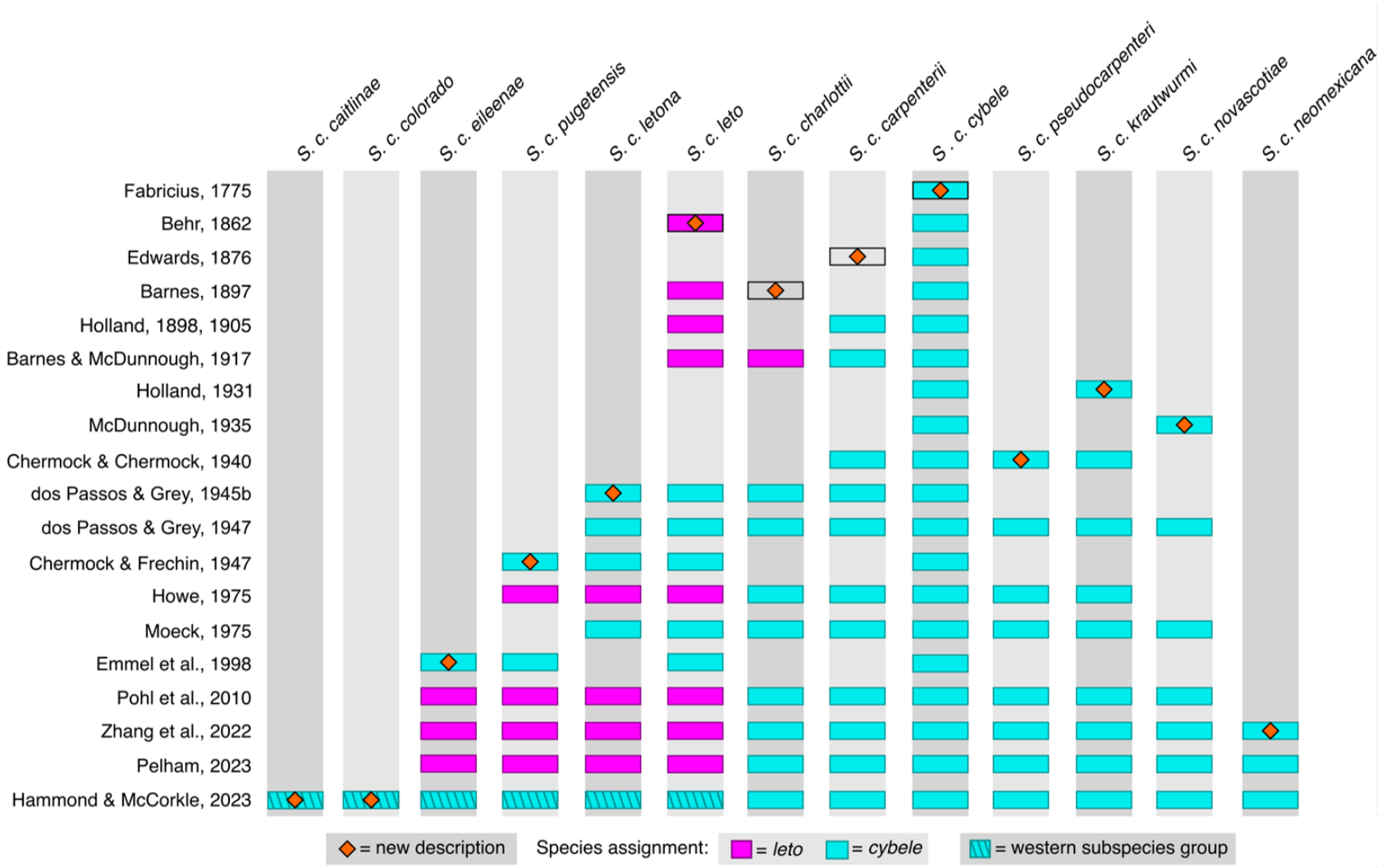

Figure 1.1. Taxonomic descriptions and treatments of *Speyeria cybele* and related taxa. Original descriptions as species have a black outline. Placements within *Speyeria cybele* or *Speyeria leto* are shown as turquoise or magenta, and only taxa included in each publication are shown.

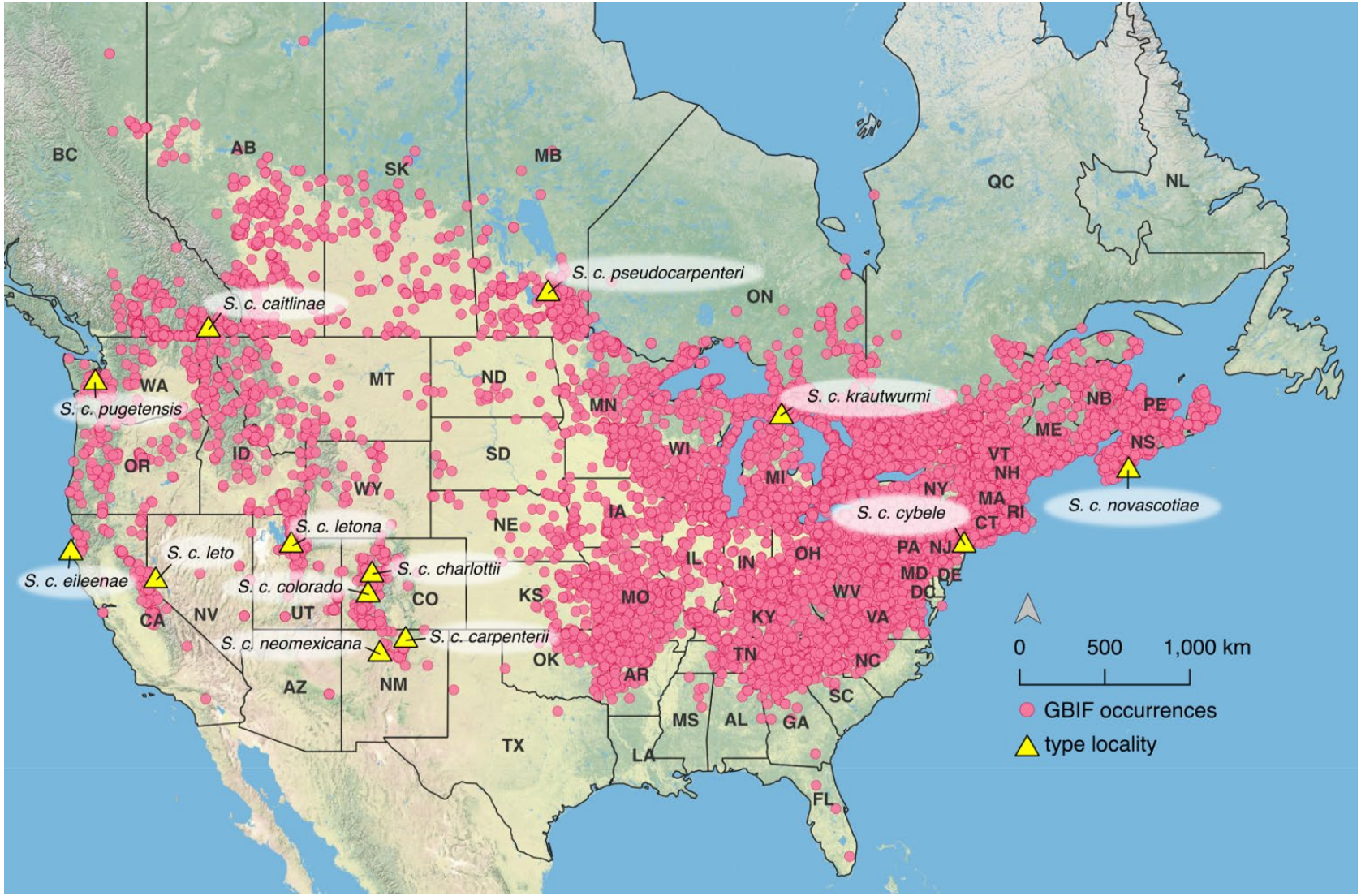

Figure 1.2. *Speyeria cybele* occurrences available on GBIF.org (accessed June 24th, 2024), and type localities of subspecies.

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Chapter 2

Genetic and morphological population structure of the Great Spangled Fritillary butterfly (*Speyeria cybele*)

2.1 Introduction

Species concepts and definitions have attracted a great deal of study. Applications of most species concepts are based on one of the following: observable characters (e.g. typological species concept, illustrated by Linnaeus 1753; morphological species concept, Cronquist, 1978; the phylogenetic species concept, Stace, 1989 and Agapow et al., 2004; phenetic species concept, Ridley, 1993), reproductive isolation (biological species concept, Mayr, 1942), evolutionary uniqueness (evolutionary species concept, Wiley, 1981; phylogenetic species concept, Stace, 1989 and Agapow et al., 2004; cohesion species concept, Templeton, 1989), or the use of several criteria (general lineage species concept, de Queiroz, 2005). What unites these species concepts is diagnosability and consistently shared sets of characters when different populations contact each other. The maintenance of a pattern of genetic clustering or cohesion in spite of some gene flow is emphasized in the genotypic cluster definition of Mallet (1995). This criterion can then be applied to calibrate a divergence threshold for recognizing allopatric populations as separate species within a larger group of related species. The combination of cohesion calibration of populations in contact (sympatry or parapatry) with ranking of allopatric populations is captured by the genomic integrity species concept (Sperling, 2003).

It is increasingly feasible to generate new information on organisms with unresolved taxonomy using high-throughput sequencing and bioinformatic advances. Techniques using analysis of genome-wide SNPs can be applied within an integrative delimitation framework as an addition to morphometric analysis and classical mtDNA sequencing (Chaplin et al., 2020; Campbell et al., 2022; Oury et al., 2023; Wingert et al., 2024). Reduced representation sequencing methods, like RADseq and GBS (Campbell et al., 2017), can be an effective tool for resolving recent divergences in the context of morphological complexity and gene introgression in semipermeable contact zones to gene introgression (Baird et al., 2008; Andrews et al., 2016). However, even with the integration of technological advances, taxonomic delimitations of species can still be obscured without ongoing reassessment, investigation, corroboration, and more geographic sampling.

The Great Spangled Fritillary*, Speyeria cybele* (Fabricius 1775), is a common butterfly across North America whose taxonomy has attracted debate since the late 1800s. Taxonomic discussion has generally focused on eastern versus western differences in wing variation and assumed that the Rocky Mountains are a major barrier to gene flow (e.g. Holland, 1905; Moeck, 1975; Howe, 1975; Layberry et al., 1998; Kondla, 2004; Fisher, 2005; Hardesty and Groothuis, 2013; Zhang et al., 2022; Hammond and McCorkle, 2023). Populations of eastern *S. cybele* versus western forms, often referred to as *S. leto* (Behr, 1862), are alternately viewed as two largely allopatric species, implying that they remain largely distinct in regions of sympatry along the Rocky Mountains (e.g. Howe 1975; Zhang et al., 2022), or as subspecies of one species, *S. cybele*, that intergrade when they are in contact (e.g. Moeck 1975; Fisher 2005). Except for Zhang et al. (2022), taxonomic changes for *S. cybele* have been based on single or a few characters, and most studies have relied on limited geographic sampling.

Speyeria cybele populations likely began diverging about 1.0 mya, amidst the glacial and interglacial periods of the Pleistocene (de Moya et al., 2017). This could have contributed to episodes of population isolation followed by secondary contact, likely influencing contemporary observations of diversity and taxonomic ambiguity (Alcaide et al., 2014). Major putative contact zones have been proposed in eastern slopes of the southern Alberta and northern Montana Rocky Mountains, and the southern Rocky Mountains (Moeck, 1975; Bird et al., 1995; Fisher, 2005). Moeck (1975) hypothesized that the relationship between eastern and western *S. cybele* may be clinal, with introgression producing intermediate wing-pattern phenotypes in other regions in addition to the Rocky Mountains. Additionally, *S. cybele* appears to exhibit more morphological diversity among populations along the southern Rocky Mountains, and isolated mountain ranges of Wyoming, possibly a result of lower levels of habitat connectivity (E. Gage pers. obs. 2024). Furthermore, previous genetic research has called for increased population level sampling (Campbell et al., 2020; Zhang et al., 2022), and wing morphology has yet to be quantified and analyzed on a broad geographic scale.

The evolutionary processes of speciation and diversification in *S. cybele* are currently challenging to capture taxonomically due to limited formal study. It is especially important to

determine whether this butterfly is composed of more than one species given current conservation concerns surrounding this and other *Speyeria* (Guppy and Shepard, 2001; Heron, 2012; Idaho Fish and Game, 2024). For example, *S. zerene* is listed as federally endangered in some regions of the United States and *Speyeria idalia* is listed as federally imperiled in the United States and is already extirpated from much of its range (Allen, 1997; Glassberg, 2001; Fisher, 2005; Monroe and Wright, 2017). *Speyeria diana* is listed as federally vulnerable in the United States and has been heavily impacted by habitat loss (Schweitzer et al., 2011; Monroe and Wright, 2017).

This study will address the following questions: (1) What is the population genetic structure of *S. cybele* across its range, and to what extent do populations interact when in contact?; (2) What are the relationships between the genetic structure and morphological variation in *S. cybele*?; and (3) Is *S. cybele* composed of more than one species, and how should this be recognized taxonomically? This research is facilitated by new technologies (e.g. RADseq and GBS), and corroborated with morphological data and natural history observations, to provide a foundation for ultimately testing broader evolutionary and taxonomic hypotheses.

2.2 Material and methods

2.2.1 Specimen collection and DNA extraction

Butterflies were collected with aerial nets in Canada and the United States across the range of *S. cybele*, with focus on sampling putative contact zones to characterize the interactions between populations in these regions (Appendix 1). Butterflies were identified as putative *S. cybele* using morphological field markings, consultation with the Bean Museum Collection at Brigham Young University, the personal collection of E. Gage, and comparison to pinned specimens in the E.H. Strickland Museum at the University of Alberta. A total of 340 *S. cybele* specimens were collected, potentially representing the current delimitation of 13 subspecies based on type specimen collection localities (Figure 1.2; Hammond and McCorkle, 2023; Pelham, 2023).

When possible, samples were frozen live at -20°C, or were preserved in 100% ethanol until frozen. All wings were clipped and stored in glassine envelopes for morphological analysis. DNA was extracted from leg and thoracic tissue using the Qiagen DNeasy Blood and Tissue kit,

with RNAse A treatment. Extracted DNA was ethanol precipitated and resuspended in Millipore water at a concentration of 20ng/µL then stored frozen at -20°C.

2.2.2 SNP sequencing and processing

Reduced-representation sequencing used 200 ng of extracted DNA per sample. Two library preparation methods were used, (1) double-digest RADseq (ddRAD; Peterson et al., 2012); and (2) two-enzyme GBS (Poland et al., 2012). Methodological details are in Appendix 1. Both library preparation methodologies used *MspI*/*PstI* restriction enzymes to digest genomic DNA. The ddRAD runs were produced at the Molecular Biology Services Unit (MBSU) at the University of Alberta, where 75bp, single-end sequencing was performed on an Illumina NextSeq 500. GBS library preparation was performed by the Institut de biologie intégrative et des systèmes (IBIS) at the Université Laval, and 100-bp, single-end sequencing was performed on an Illumina HiSeq 2000 at the McGill University-Genome Quebec Innovation Centre.

Data analysis was performed on the Cedar cluster hosted by the Digital Research Alliance of Canada. Raw reads generated from both sequencing methods were demultiplexed into individual files using the process_radtags in Stacks version 2.60 (Catchen et al., 2011; Catchen et al., 2013, Rochette et al., 2019). Reads that did not meet Illumina's chastity filters and had a Phred quality score below 30 were discarded. Fastp version 0.23.4 (Chen et al., 2018) was used to remove the 5' end *Pstl* site and sequences containing remnant Illumina adaptor sequences. Due to the difference in library preparation performed at MBSU and IBIS, after trimming the ddRAD sequences produced on the NextSeq were 62bp in length and the GBS sequences produced on the HiSeq were 87bp. In order to standardize length (recommended for *de novo* locus assembly in Stacks), all retained sequences were truncated to 62bp on the 3' end using Fastp. Sequence quality was then assessed using fastQC version 0.12.0 (Andrews, 2010) and multiQC version 1.20 (Ewels et al., 2016).

Locus construction was performed *de novo* using denovo_map.pl in Stacks version 2.60 (Catchen et al., 2011; Rochette et al., 2019), as there is currently not a sufficiently closely related reference genome for *S. cybele*. Following Paris et al. (2017) and Rochette et al. (2019), parameter testing for *M* and *n* was performed to optimize *de novo* SNP assemblies. The

parameter *M* is the number of mismatches allowed between sequence reads to form a locus, and *n* is the number of mismatches allowed between loci when aligning data for different individuals. Parameter testing was performed using a representative subset of sequences (n=175), with the dataset filtered for loci found in at least 80% of the specimens (r80). The parameter *M* was tested for values ranging from 0 to 6. Optimal *M* was determined by the plateau of assembled loci, polymorphic loci, and SNPs, and the peak of the log modulus transformation of new polymorphic loci. The parameter *n* was tested for values ranging from 0 to 6, using the optimal value for *M* that had been previously determined. Optimal *n* was determined by identifying the value at which the greatest number of assembled loci, polymorphic loci, and SNPs were recovered, while also taking into consideration the best practices suggested by Paris et al. (2017), namely that in most cases selecting a value for *n* that is equal to *M*-1, *M*, or *M*+1 is often sufficient. The *m* parameter, governing the minimum number of raw reads required to form a stack, was left at its default value of 3.

After parameter testing was complete, Stacks was re-run on the entire dataset (n=340) to assemble loci and perform initial locus filtering. To reduce genomic linkage in the dataset I output a single random SNP from each locus and missingness per locus at r80. VCFTOOLS version 3.0 (Danecek et al., 2011) was used to filter the dataset to a minimum genotype quality score of 30, a minimum minor allele frequency of 3%, and then the retained data was re-filtered to obtain a loci maximum missingness of 20%.

2.2.3 Cluster-based analysis of SNPs

Principal Component Analysis (PCA) was performed in RStudio version 2023.9.1.494 (Posit team, 2023) using the package adegenet (Jombart and Ahmed, 2011) on the SNP dataset of 340 specimens. The resulting PCAs were plotted with ggplot2 (Wickham, 2016). Clusters identified in the PCA were used to colour code groupings for analysis of individual SNP ancestries.

Individual SNP ancestries were estimated using a maximum likelihood model implemented by ADMIXTURE version 1.3.0 (Alexander et al., 2009). ADMIXTURE was performed for *K*= 1 to *K*=13 with ten replicates for each value of *K*. Ancestry proportions were tested up to *K*=13 to reflect the current subspecific taxonomy of *S. cybele* and results from the first two dimensions of

the PCA. Cross validation (CV) scores resulting from replicate runs for each tested value of *K* were plotted in Excel version 2308 (Microsoft Corporation, 2018) to identify the optimal value of *K* (Q-value), which should exhibit the lowest CV error. ADMIXTURE results were plotted as a bar graph using RStudio version 2023.9.1.494 (Posit team, 2023). Using the optimal *K*-value, each specimen's proportion of ancestry to each *K* group (Q value) were plotted as pie charts based on their collection location using QGIS version 3.28 (QGIS.org, June 24th, 2024).

Both PCA and ancestry-based analyses such as ADMIXTURE may be dominated by higher-level structuring (Elhaik, 2022). To evaluate hierarchical clustering in the dataset, SNP calling was repeated independently for each primary genetic grouping following the approach described in section 2.2.2, and then PCA and ADMIXTURE analyses were used to identify the presence of sub-clustering within these groups. Specimens with admixed ancestry in the initial ADMIXTURE analysis were assigned to sub-clusters based on the majority Q-value.

Pairwise F_{ST} (Weir and Cockerham 1984) was estimated using StAMPP version 1.6.3 (Pembleton et al., 2013) in RStudio, with 1000 bootstrap replicates. To assess the impact of admixture on estimates of genetic differentiation, clusters used in pairwise F_{ST} comparisons were conducted using two different Q-value thresholds: individuals were alternately grouped by a simple majority Q-value (Q-value >50%), and then also by omitting highly admixed specimens (those with Q-values less than 80% assigned to any single cluster).

2.2.4 Phylogenetic inference

Maximum likelihood phylogenetic analysis was used to identify population-level relationships within *S. cybele*. *Speyeria diana* and *S. aphrodite* were used as outgroups and to root this analysis (Appendix 1). SNPs were reassembled in Stacks and filtered the same way as for the previous datasets. Maximum likelihood inference was conducted in IQ-TREE 2.0.7 (Minh et al., 2020). First, model testing was run to infer the optimum substitution model based on the highest Bayesian Information Criterion (BIC) score (Kalyaanomoorthy et al., 2017). Then 1000 ultrafast bootstrap replicates (Minh et al., 2013) with the ASC+ flag was performed to generate the tree. Nodes that had lower than 50% bootstrap support were collapsed to produce a 50% majority-rule consensus tree. This tree was visualized in FIGTREE v1.4.4 (Rambaut, 2018) where specimens

were coloured by their SNP cluster identified by the majority Q-value obtained from ADMIXTURE.

2.2.5 mtDNA sequencing, alignment, and haplotyping

Following Hebert et al. (2003), Sanger sequencing was performed for the 5' barcoding region of mtDNA cytochrome c oxidase subunit I (COI) of n=98 specimens. Forward and reverse sequences for each specimen were assembled using *De novo Assemble* in Geneious version 2023.2.1 (www.geneious.com). Chromatograms of assembled sequences were manually checked for accuracy and trimmed to a final length of 678 bp. MAFFT v.6 (Katoh et al., 2019) was used for multiple sequence alignment with default settings. Alignment strategy was automatically set to MAFFT-L-INS-i. The aligned sequences were used to construct a minimum spanning network (Bandelt et al., 1999) in PopART (Leigh and Bryant, 2015).

2.2.6 Morphological character selection and scoring

In total, after consulting diverse field guides and visually comparing wings of all specimens (n=340), 16 morphological characters were selected for morphometric analysis (Figure 2.3). Dorsal forewing (FW) and dorsal hindwing (HW) ground colour was measured categorically by matching wing colour to 6 Behr paint colour cards (Figure 2.4A), with states 0-5 corresponding to colours Joyful Orange, Splendor Gold, Blazing Bonfire, Jackfruit, Honey Locust, and Spinning Silk. Paint colour cards were used since they were matte, allowing easy comparison to the matte ground colour, and RGB (red, green, and blue) values are available for replication in future experiments.

Forewing width was measured using a standard ruler. For butterflies, forewing size is typically measured from the base of the wing where it attaches to the thorax to the wing apex using specimens with attached wings (Hook et al., 2012). In this study, wings were clipped from the body and were not always clipped right to the base, so it was not possible to measure length consistently, and a proxy was needed for wing size. Accordingly, the forewings of 52 pinned *S. cybele* specimens located in the E. H. Strickland Museum were measured from apex to base (wing length), and then also measured maximum forewing width from vein A_{1+2}, through the junction of the subcostal vein and R_1, to the edge of the costal vein (wing width; Figure 2.3A).

Linear regression in Excel version 2308 (Microsoft Corporation, 2018) was performed on n=27 female and n=25 male pinned specimens to determine whether the wing width and wing length measurements were correlated, and if wing width could be used as a proxy for wing size; results indicated there was a linear correlation between forewing length and width (Appendix 10) for both sexes despite sexual dimorphism. Therefore, wing width was used as a proxy for wing size.

Thirteen discal silver spots on the ventral hindwing (Figure 2.3B) were measured using digital calipers across the axis with the greatest range in size variation (Figure 2.3C) and then standardized by dividing the spot measurement by wing width. The axis to measure each silver spot was determined by initial measurement of silver spots of many wings across different axes to determine the one with the most size variation.

2.2.7 Morphological analysis

Given sexual dimorphism in *S. cybele,* all morphological analyses considered males (n=255) and females (n=85) separately. Morphological analysis of 16 characters (Appendix 11) was conducted to determine whether recovered morphological patterns were consistent with observed genetic clusters. Standard deviation of each linear character measurement mean can be found in Table 2.2. The distributions of continuous character measurements were first assessed visually with histograms in Excel version 2308 (Appendix 13 and 14; Microsoft Corporation, 2018). While there were observable variations of continuous morphometric data corresponding to SNP genotype, there was extensive overlap between associated SNP clusters and the character measurements, so linear measurements of characters in combination with the wing colour states were used for further analysis.

In order to visualize covariance in morphological data and identify the contributions of each character, factor analysis of mixed data (FAMD) was performed using the FactoMineR package (Lê et al., 2008) in RStudio. Morphological character data included categorical (FW and HW colour) and continuous data (all other characters). To evaluate characters with disproportionate contributions to clustering results on the FAMD, character contributions were obtained using the package factoextra (Kassambara and Mundt, 2020). To compliment FAMD, nonmetric multidimensional scaling (nMDS) was performed to visualize the morphological distance

between specimens and identify the characters driving each morphological cluster without bias from unequal sample sizes, which is especially important since there were unequal sample sizes between SNP clusters. The FactoMineR package was used for the nMDS analysis, with Euclidean distance as a dissimilarity measure. The FAMD and nMDS plots were visualized using the ggplot2 (Wickham, 2016) and ggforce (Pedersen, 2024) packages in RStudio.

2.3 Results

2.3.1 SNP parameter testing and sequencing statistics

Stacks SNP parameter testing indicated that *M*=2 yielded the highest number of r80 assembled loci and polymorphic loci (Appendix 2A). The log modulus transformation showed *M*1/*M*2 yielded the highest number of new r80 polymorphic loci (Appendix 2B). Therefore, *M*=2 was selected. However, it should be noted that *M*=3 yielded the highest SNPs, since there were more mismatches allowed within each locus (Paris et al., 2017). Using *M*=2, *n*=4 yielded the greatest number of r80 assembled loci, polymorphic loci, and SNPs (Appendix 2C). However, Paris et al. (2017) recommends selecting an optimal *n* within *n*=*M*-1, *n*=*M*, and *n*=*M*+1. Therefore, *n*=3 (*n*=*M*+1) yielded the highest number of r80 assembled loci, polymorphic loci and SNPs within that range (Appendix 2C). Accordingly, *M*=2 and *n*=3 was used to assemble all SNP datasets.

2.3.2 Cluster-based analysis of SNPs

The n=340 SNP dataset had 430,479 total catalog loci prior to downstream filtering (Appendix 3). Filtering after assembly using VCFTOOLS retained 6069 SNPs and mean read depth per site ranged from 10.3 to 291.3 with an average of 57.3, and mean read depth per individual varied from 5.3 to 146.6 with an average of 59.9 (Appendix 3). The n=340 SNP dataset had a mean missingness of 0.11, and ranged from 0.012 to 0.72 for individuals and 0 to 0.2 for loci (Appendix 3).

Principal component analysis of the n=340 dataset SNPs showed four main clusters that broadly correspond to the eastern, northern, western, and southern regions of Canada and the United States (Fig. 2.1A; Appendix 4). There were many genetically intermediate specimens between clusters. Principal component axis 1 (34.2%) separated the western cluster from the other clusters. Principal component axis 2 (5.1%) separated the remaining primary genomic clusters

corresponding to southern, eastern, and northern portions of the *S. cybele* range. Principal component axis 3 (2.6%) further distinguished the northern cluster from all other clusters (Appendix 4).

ADMIXTURE analysis of the n=340 dataset SNPs gave an optimal *K* of 4 (Appendix 5). Like the PCA, these results recovered broadly eastern, northern, western, and southern clusters and further identified a large number of individuals with mixed ancestry (Q-values between 0.2-0.8) that generally corresponded to the intermediate specimens depicted in the PCA (Figure 2.1B). These admixed individuals were assigned to clusters on the ADMIXTURE plot and downstream analyses based on their majority Q-value (>50%). The *K* values 3, 5 and 6 were close to the CV error of the optimal *K* of 4 (Appendix 5). The ADMIXTURE plot of *K*=3 (Appendix 6) grouped ancestry into eastern, northern+western, and southern. The *K* values 5 and 6 further divided West into two groups (Appendix 6). This subdivision of West corresponded to the West subclusters seen in the SNP PCA (Figure 2.1 A). The one West group was more associated with California, Montana, and Wyoming, and the other was more associated with British Columbia, Idaho, Nevada, Oregon, Utah, and Washington. The ADMIXTURE plot of *K*=6 (Appendix 6) separated specimens admixed with northern and eastern ancestry into a separate cluster.

The geographic plot of individual Q-values against collection locations shows where SNP clusters contact one another (Figure 2.2). Specimens with an eastern majority Q-value ("East") were from Arkansas, Alabama, Indiana, Kentucky, Minnesota, Missouri, Nebraska, Nova Scotia, Oklahoma, Wisconsin, and Virginia. Specimens with a northern majority Q-value ("North") were from Alberta, Manitoba, Minnesota, Montana, Nebraska, Nova Scotia, Quebec, Ontario, Wisconsin, Wyoming, and Saskatchewan. Specimens with a western majority Q-value ("West") were from Alberta, British Columbia, California, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming. Specimens with a southern majority Q-value ("South") were from Colorado, New Mexico, Utah, and Wyoming. Admixed specimens (Q-values between 0.2-0.8) between North and East were from Nebraska, Nova Scotia, Ontario, Quebec, Virginia, and Wisconsin. Admixed specimens between North and West were from Alberta, Montana, and California. An admixed specimen between East and West was from Arkansas. An admixed specimen between South and West was from Wyoming.

When considering all specimens, pairwise population F_{ST} values (n=340; Table 2.1) were highest between the South and West SNP clusters (0.52), and lowest between North and East SNP clusters (0.11). Pairwise F_{ST} values for individuals exhibiting minimal admixture (n=291; Table 2.1) were higher in comparison but exhibited the same pattern. North and East SNP clusters were the most similar (0.14), and West and South SNP clusters were the most dissimilar (0.55).

The East subset (n= 61) had 12,733 SNPs, and 4927 SNPs after filtering. The East subset mean read depth per site ranged from 14.1 to 74.3 with an average of 43.4, and the mean read depth per individual varied from 7.8 to 409.4 with an average of 43.6. The East subset had a mean missingness of 0.09, and ranged from 0.04 to 0.37 for individuals, and 0 to 0.197 for loci (Appendix 3). The North subset (n=75) had 10 595 SNPs, and 4047 SNPs after filtering. The North subset mean read depth per site ranged from 10.5 to 369.0 with an average of 59.1, and the mean read depth per individual varied from 5.8 to 143.6 with an average of 61.0. Th North subset mean missingness of 0.12, and ranged from 0.02 to 0.64 for individuals, and 0 to 0.200 for loci (Appendix 3). The West subset (n=156) had 9211 SNPs, and 2801 SNPs after filtering. The West subset mean read depth per site ranged from 9.7 to 276.6 with an average of 58.3, and the mean read depth per individual varied from 16.0 to 131.9 with an average of 60.8. The West subset had a mean missingness of 0.11, and ranged from 0.01 to 0.67 for individuals, and 0 to 0.199 for loci (Appendix 3). The South subset (n=48) had 4797 SNPs, and 3229 SNPs after filtering. The South subset mean read depth per site ranged from 8.8 to 328.8 with an average of 58.7, and the mean read depth per individual varied from 16.4 to 166.8 with an average of 61.8. The South subset had a mean of 0.09, and ranged from 0.02 to 0.49 for individuals, and 0 to 0.188 for loci (Appendix 3).

Subcluster analysis for each of the four primary genomic groupings (Appendix 7 and 8) showed little to no genetic substructure within the East (K= 1), North (K= 1), and South (K= 2) SNP clusters. The West SNP cluster (Appendix 6 and 7C) showed the greatest amount of substructure (K=5) and largely corresponds with specimen collection localities. The first two PCA axes for each sub-cluster analysis (Appendix 8 A, B, C, and D) showed the South had greatest genetic variation, followed by the West, while the East had the lowest genetic variation.

2.3.3 Phylogenetic inference

The SNP assembly of *S. cybele* and the outgroup species *S. diana* and *S. aphrodite* (n=342) had 436,075 total loci and 1523 SNPs remained after filtering (Appendix 3). The phylogeny dataset had a mean read depth per site ranging from 11.4 to 166.3 with an average of 56.7, and the mean read depth per individual ranged from 5.0 to 140.8 with an average of 58.4 (Appendix 3). The phylogeny dataset had a mean missingness of 0.12, and ranged from 0.01 to 0.75 for individuals, and 0 to 0.199 for loci (Appendix 3).

The best SNP maximum likelihood tree model was TVM+F+R5 based on the highest BIC score. The 50% majority-rule maximum likelihood consensus tree (Appendix 9) produced four main groupings that corresponded to SNP clusters East, West, North and South. The East grouping showed low bootstrap support (<70%). At the base of the East group were North and East admixed individuals. The sister group to the East was the North grouping, which also had low bootstrap support, and the base had North and East admixed individuals. The West branched off from the North grouping through a stepwise "grade" of North individuals and individuals admixed between North and East, and North and West. This 'grade' from admixed specimens to the West grouping had ≥70% bootstrap support. The South grouping was well supported (≥95 bootstrap support) and branched off from the North grouping. Within each SNP grouping, most nodes were well supported.

2.3.4 mtDNA haplotyping

Minimum spanning network analysis of mtDNA indicated two major haplotype groupings, mtA and mtB (Figure 2.1C). Haplogroup mtA was associated with all SNP clusters and admixed individuals, with sub-haplotypes differing by 1 to 2 bp, and included some West SNP cluster specimens from Montana. Haplogroup mtB was at least 6bp different from mtA, and included only West SNP cluster specimens, which broke off into two subclusters that were 2bp apart, with group 1 only in Utah and Nevada specimens and group 2 in specimens from Idaho, British Columbia, Washington, and Montana. The sub-clustering within mtB did not correspond to the sub-clustering within the West SNPs.

2.3.5 Wing character distributions

Morphological character scoring and measurements (Appendix 10) show male wing width ranged from 16mm to 30mm (Figure 2.4A). East SNP cluster males had the largest mean wing width of 24.3mm with a standard deviation of 2.4mm (Table 2.2). South SNP cluster males had the smallest mean wing width of 19.0 mm with a standard deviation of 1.1mm. Female wing width ranged from 18mm to 31mm (Figure 2.4B). The East SNP cluster females had the largest mean wing width of 28.8mm with a standard deviation of 1.8mm. North SNP cluster females, and females admixed with North and West, had the smallest mean wing width of 20.5mm with a standard deviation of 1.3mm (Table 2.2).

Males from the East, North, and South SNP clusters showed FW and HW colours 0, 1, and 2 (Figure 2.4B). Males admixed as North/East SNP clusters also showed FW and HW colours 0, 1, and 2. West SNP cluster males showed FW colours 0, 1, and 2 but only HW colours 0 and 2. Males admixed as North/West showed FW and HW colours 1 and 2. A single admixed West/South male had FW colour 1 and HW colour 2. Females from the East cluster showed FW and HW colours 0, 1, and 2 (Figure 2.4B). North SNP cluster females showed FW colours 0, 1, 2, 3, and 5. West SNP cluster females, and females admixed with North and West, showed FW and HW colours 3 and 5. South SNP cluster females showed FW colours 4 and 5, and HW colours 3, 4, and 5. Females admixed as North/East SNP clusters showed FW and HW colours 1 and 2. The single female admixed with East and West showed FW and HW colour 2.

Among male specimens the ventral HW silver spots in the discal region were largest in East SNP and North SNP clusters, and smallest in the West SNP cluster (Table 2.2, Appendix 12). Spot B6 was largest in males admixed with North and West. Spot B10 was absent in West males and the single male admixed with West and South (DNA no. 13700). Spot B11 was largest in East SNP cluster males and was absent in South males and the single male admixed with West and South. Spot B12 was absent in West SNP cluster males, the single male admixed with West and South, and males admixed with North and West. Spot B13 was sometimes present in the North SNP cluster, North and West admixed, and North and East admixed males.

Among females the ventral HW silver spots in the discal region were the largest in the North SNP cluster, and smallest in the West cluster (Table 2.2, Appendix 13). Spot B11 was absent in West and South SNP cluster females, females admixed with North and West, and the single female admixed with East and West. Spot B12 was absent in West SNP cluster females, and the single female admixed with East and West. Spot B13 was absent in females admixed with North and East, North and West, and the single female admixed with East and West.

2.3.6 Wing character FAMD

The male-only FAMD analysis showed dimension 1 had a variance of 27.5% (Figure 2.5A), with silver spot B1 having the largest variance contribution (Appendix 15). Dimension 2 showed a 9.4% variance (Figure 2.5A), with wing width and FW colour having the largest variance contributions (Appendix 14). Dimension 3 showed a variance of 7.8%, and dimension 4 showed a variance of 7.4 % (Appendix 15), and FW and HW colour had the largest variance contributions to both dimensions (Appendix 14). Dimension 1 separated SNP clusters West from the North. Dimension 2 separated SNP clusters East from the South. Dimension 3 separated the South from the other SNP clusters. Dimension 4 separated two South males (DNA no. 10821 and 10263), an East and North admixed male (DNA no 11363), and a North male (DNA no. 10222) from the other SNP clusters.

The female-only FAMD analysis showed dimension 1 had a variance of 18.3% (Figure 2.5B), with FW and HW colour having the largest variance contributions (Appendix 16). Dimension 2 showed a 14.4% variance (Figure 2.5B), with largest variance contributions from wing width, FW colour, and HW colour (Appendix 16). Dimension 3 had a variance of 9.2 % and dimension 4 had a variance of 7.8%, with HW and FW colour having the largest variance contributions to both dimensions. Dimension 1 grouped females from the West SNP cluster and females admixed with North and West together away from the other females. Dimension 2 separated North SNP cluster females from the other groups. Dimension 2 grouped East SNP cluster females and the single East and West admixed female from the West, South, and females admixed with North and West. Dimension 3 (9.2%) and dimension 4 (7.8%; Appendix 17) grouped West SNP cluster females into FW and HW colours Jackfruit (colour 3), from California, Montana, New Mexico,

Oregon, and Utah, or Spinning Silk females (colour 5) from British Columbia, Montana, Oregon, Utah, and Wyoming.

2.3.7 Wing character nMDS

The male-only nMDS had a stress of 0.99, and the non-metric fit R2 was 0.99 (Figure 2.5C). The first-dimension distances were driven by FW and HW colour and wing width. Dimension 1 separated West SNP cluster males into two groups, a group of smaller and darker orange males collected from British Columbia, Montana, and Oregon, and a group of larger and orange males from throughout the West SNP cluster geographic range (Figure 2.2). Dimension 2 distances were driven by FW and HW colour, and silver spot B6, and separated males admixed with North and West from the other males.

The female-only nMDS had a stress of 0.10, and the non-metric fit R2 was 0.99 (Figure 2.5D). Wing width, FW and HW colour, and silver spot B9 drove dimension 1 distances. Dimension 1 grouped SNP clusters East, North, and North and East admixed females from the SNP clusters West, South, and North and West admixed females. Dimension 2 distances were driven by wing width and silver spots. Dimension 2 grouped North SNP cluster females from East SNP cluster females, and South SNP cluster females from West SNP cluster females.

2.4 Discussion

2.4.1. What is the population genetic structure of *S. cybele*, and do populations show admixture between clusters?

I recovered four primary genomic SNP clusters of *Speyeria cybele* in our sampled region: a population ranging broadly east of the Rocky Mountains in the United States (East), a population spanning much of central and eastern Canada (North), a population ranging broadly west of the Rocky Mountains in Canada and the United States (West), and another in the southern United States (South; Figure 2.1A and B). Each of these genomic populations exhibits admixture when in geographic contact, however the greatest degree of intergradation occurs between the North and West clusters east of the Rocky Mountains of Alberta and Montana, which was previously hypothesized by Moeck in 1975 (Figure 2.2). Our results also indicate substantial admixture

between North and East SNP clusters in a region between southern Canada and the northeastern United States (Figure 2.2).

Two specimens from Nova Scotia had some South genetic ancestry. While the amount of South ancestry was below the Q-value threshold to be considered admixed (0.2-0.8), these specimens may indicate lingering retained ancestral polymorphism in the northeastern portion of *S. cybele*'s range, or a remnant of prior secondary contact between these lineages during the Pleistocene (Nowell et al., 2011). Alternatively, these patterns may reflect our limited sampling of other genetic clusters, which was sparse in regions such as Kansas and the front range of Colorado, between the South and East SNP clusters, and Iowa, North Dakota, and South Dakota, which putatively connect the East and North SNP clusters. Another under-sampled region in our study is Sandoval Country, New Mexico, the type locality for *S. c. neomexicana*. These regions should be sampled in order to improve our characterization *S. cybele* population structure. In addition to increased geographic sampling, our results indicated relatively high polymorphism in the dataset. Therefore, future studies should conduct more fine-scale SNP assembly parameter testing for assessing population-level genomic divergence, especially with the addition of more specimens.

Speyeria cybele is a strong flier, and our results suggest that this species may be capable of long-distance dispersal. Some individuals show high admixture (~50%), but were collected over 500 km from their associated SNP clusters. The most notable specimens include a female from Arkansas admixed between West and East SNP clusters, and a male from California admixed between the West and North SNP clusters. Investigation into long-distance dispersal should focus on collecting *S. cybele* in sampling gaps to genetically identify putative long-distance dispersers, and where geographic boundaries of SNP clusters exist. Genetically identifying more potential *S. cybele* long-distance dispersers will help resolve whether this is an artefact of limited sampling of isolated subpopulations or a real biological phenomenon.

Speyeria cybele mtDNA shows two major haplogroups, mtA is associated with all four SNP clusters (Figure 2.1C), and mtB only corresponded to the West SNP cluster. Within mtB were two groups, which did not correspond to the two West SNP clusters when *K* was 5 and 6, as shown in Appendix 6. The lack of mtDNA variation could have resulted from under-sampling

geographic patterns of mtB, biogeography, or a cytoplasmic parasite like the bacterium *Wolbachia*. *Wolbachia* infections can reproductively isolate infected populations from non-infected populations and can result in less structure in the mtDNA as a result of selective sweeps (Kodandaramaiah et al., 2013). A similar *Wolbachia* infection event may have led to these two mtDNA haplotypes (Kodandaramaiah et al., 2013).

2.4.2 What are the relationships between genetic structure and wing variation in *S. cybele*?

Analysis of genetic clusters showed clinal transitions in zones of contact, while colour-pattern differences were more complicated than "east versus west." Morphological characters in females aligned more closely with genomic clusters compared to males, and males exhibited more subtle patterns of morphological variation. Each SNP cluster overlapped morphologically. The scored morphological characters did not correspond well to existing subspecific taxonomy. However, there may be other characters not sampled in this study that may be more consistent indicators of subspecies or genomic clusters. Future research should explore other morphological patterns or methods like geometric morphometrics that could better capture variation within *S. cybele.*

Genetically admixed specimens usually grouped morphologically with one of their putatively ancestral SNP clusters. For example, FAMD and nMDS results indicate that the Arkansas female admixed with East and West SNP clusters but had majority Q-value of East. This female was morphologically grouped with other East females. The FAMD and nMDS showed the California male admixed with North and West SNP clusters but had a majority Q-value of West. This male was morphologically grouped with other West males. However, FAMD and nMDS showed the Wyoming male admixed with West and South SNP clusters, with a majority Q-value of South, morphologically grouped with males admixed with North and West.

Given our findings, field guide descriptions should focus on characterizing regional morphology to capture variation across S. *cybele*'s range, even in less sexually dimorphic regions like that of the East SNP cluster. For example, wing width is bimodal in females and our results suggest that smaller specimens are more common in western portions of the species range while eastern specimens are usually larger. In contrast, males showed a normal distribution in wing width

across their geographic range suggesting less pronounced differences in size compared to females.

2.4.3 Biogeography of *S. cybele*

Genetic and morphological analysis indicate that *S. cybele* diversification is not as simple as "east versus west." Regions east of the Rocky Mountains showed extensive admixture between western and eastern *S. cybele* populations. Additionally, genomic SNPs indicate that *S. cybele* includes four genetic groups instead of two.

While all four genomic ancestries are found in Wyoming, each cluster appears to be primarily associated with a different mountain range or plateau. Genomic SNP results indicated little admixture between mountain ranges (although admixture was present within each mountain range). The Big Horn Mountains in northern Wyoming were associated with the West SNP cluster. In eastern Wyoming, the Black Hills Mountain range was associated with the admixture of North and East. Southward in northwestern Nebraska, a greater proportion of East SNPs were found in the Pine Ridge region. The Sierra Madre and Medicine Bow Mountain ranges in southern Wyoming were associated with the South SNP cluster. Mountain ranges in Wyoming could have acted as glacial refugia for *S. cybele* populations during the Pleistocene, and this could have been maintained post-glacially due to low occurrences of the putative hostplant *Viola* in low-elevation arid grassland habitat between these mountain ranges (Birks, 2019).

Contact zones facilitated by Pleistocene glacial cycles east of the Rocky Mountains of Alberta and Montana, and in the Midwestern and northeastern United States, have been proposed for other taxa (Swenson and Howard, 2005; Lyman and Edwards, 2022). The Alberta and Montana Rocky Mountain contact zone appears to be a transitional habitat between the prairies and mountains, and may have resulted from repeated glacial retractions and expansions during the Pleistocene (Graham et al., 2021). Contact zones along the east of the Rocky Mountains of Alberta and Montana have been established for woodpeckers (Natola et al., 2021), Canada jays (Graham et al., 2021), and Northern Flickers (Wiebe, 2000). In addition to avian examples, swallowtail butterflies (*Papilio*) show increased interspecific admixture east of the Rocky Mountains of Alberta (Dupuis and Sperling, 2016) The Midwestern and Northeastern United

States contact zone may have resulted from fauna and flora moving northward with the retraction of the Laurentide ice sheet (Dalton et al., 2022). Midwestern and Northeastern United States contact zones have also been observed in butterflies (*Speyeria atlantis-hesperis* complex, Campbell et al., 2022; *Limenitis arthemis*, Mullen et al., 2008; *Papilio glaucus* species group, Vernygora et al., 2022), fir trees (Cinget et al., 2015), aspen (Bagley et al., 2020), and mice (Garcia-Elfring et al., 2017).

Our results also suggest that populations of *S. cybele* located around the Rocky Mountains may have attributes of a ring species (Cain, 1954). A ring species consists of a series of populations encircling a large geographic barrier. Each adjacent population may exhibit gene flow and clinal morphological variation with one another, with putative genomic differences accumulating around the ring, until the two terminal populations are different enough that they exhibit little to no gene flow despite their geographic proximity (Irwin et al., 2001). Some North American examples of ring species are salamanders (Kuchta et al., 2009) and the western fence lizard (Bouzid et al., 2021). *Speyeria cybele* showed a ring-like structure around the northern Great Plains, with four SNP clusters that had clinal genetic variation except where West and the South SNP clusters appear to meet in Utah. However, at this point, it is unknown if the ring-like genetic structure of *S. cybele* is a biological process or an artefact of geographic sampling. Therefore, future studies should emphasize filling sampling gaps, to allow the ring species-like scenario to be fully investigated.

2.4.4 Is *S. cybele* composed of more than one species?

Speyeria leto as a separate species from *S. cybele* does not fulfill the requirements of the genomic integrity species concept (Sperling, 2003), since the recovered genomic clusters do not maintain integrity when in contact, and clusters that are not in geographic proximity are less or approximately equally different than populations that do contact and admix. For instance, pairwise F_{ST} comparisons with the western and northern populations suggested that these two populations exhibited the greatest amount of genomic differentiation from other populations (Table 2.1), yet these SNP clusters show extensive admixture east of the Rocky Mountains in Alberta and Montana (Figure 2.2). The southern population shows a lack of admixture which could be the result of landscape composition, or an artefact of sampling gaps between the South

and other SNP clusters. Despite the need for more sampling, the South SNP cluster still shows a small amount of admixture and possible ancestral polymorphism. Under the criteria of the genomic integrity species concept (Sperling, 2003), and until more sampling of the gap to the South population is conducted, I consider it preferable to remain taxonomically conservative and not elevate the South population as a species.

In addition to the genomic integrity species concept (Sperling, 2003), the criteria of other major species concepts do not support *S. leto* as a separate species from *S. cybele* due to extensive clinal genetic and morphological overlap, and evidence of interbreeding when populations are in contact (Mayr, 1942; Cronquist, 1978; Wiley, 1981; Templeton, 1989; Ridley, 1993; Mallet, 1995; de Queiroz, 2005). To best represent the biological processes of this butterfly, it is recommended that *S. leto* be sunk back into *S. cybele*.

There are thirteen *S. cybele* subspecies (Pelham, 2023; Hammond and McCorkle, 2023). However, the SNP data suggests that *S. cybele* is composed of only four primary genomic clusters, and morphometrics indicates that these clusters are clinal with transitions in zones of contact. Therefore, I suggest that the subspecies taxonomy should be re-evaluated to ensure that it represents the genetically distinct and relatively morphologically distinct populations. Based on these genetic and morphological results I recommend four subspecies based on the oldest or most unambiguous names of the four genetic subdivisions. The subspecies *S. c. cybele* would represent the East SNP cluster, encompassing Alabama, Arkansas, Indiana, Kentucky, Minnesota, Missouri, Nebraska, Oklahoma, Wisconsin, and Virginia. The subspecies *S. c. pseudocarpenteri* would represent the North SNP cluster, encompassing Alberta, Manitoba, Minnesota, Montana, Nova Scotia, Ontario, Quebec, Saskatchewan, and Wyoming. While *S. c. pseudocarpenteri* is not the oldest subspecies name in the northern region, specimens from near the type locality for the oldest name *S. c. krautwurmi* appear to be more similar to *S. c. cybele* based on our genetic and morphological results. The subspecies *S. c. leto* would represent the West SNP cluster, encompassing Alberta, British Columbia, California, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming. Based on its type locality, and until the possible mislabelling of its lectotype is more definitively resolved (Zhang et al., 2022), the subspecies *S. c. carpenterii* would represent the South SNP cluster, encompassing Colorado,

New Mexico, Utah, and Wyoming. All these proposed subspecies have evidence of transitions across the landscape, and evidence of occasional long-distance dispersal.

Speyeria cybele genetic patterns show parallels to the spruce beetle (*Dendroctonus rufipennis*), which also has a broad North American range (Maroja et al., 2007). Microsatellite data of *D. rufipennis* shows sympatric populations in northern North America, a contact zone in British Columbia, and another genetic group in the Rocky Mountains. In the contact zone, genetically divergent groups of *D. rufipennis* show admixing, while in the northern range, sympatric groups are more genetically similar and do not show admixing (Maroja et al., 2007). The lack of geneflow between sympatric groups of *D. rufipennis* is likely the result of the sympatric groups having different host tree preferences (Maroja et al., 2007). Both *S. cybele* and *D. rufipennis* highlight how genetic divergence does not necessarily reflect the potential for current gene flow between populations, and divergence may reflect the biogeographic history of these species. Therefore, calibration between distinct but mixing populations is needed to apply a taxonomic name change that reflects biological progresses.

2.4.5 Chapter conclusion

The use of multiple characters, increased sampling, and consideration of biological processes has allowed us to move closer to taxonomically reflecting the natural variation that occurs within *S. cybele sensu lato*. Genetic and morphological analyses suggest *S. cybele* represents a single species with four main genomic populations, and two mtDNA haplotypes. All SNP clusters showed admixture with each other, even when SNP clusters appear geographically separated across the continent. The wing morphology of the SNP clusters overlapped without sharp boundaries. However, this study illustrates that despite an eight-fold increase in sampling compared to Zhang et al. (2022), the most comprehensively sampled study of *S. cybele* to-date, ambiguities still exist and more sampling is needed.

Better sampling of *S. cybele* requires investigating geographic gaps, putative long-distance dispersers, associations with environmental factors, and the genome. There are important geographic sampling gaps in Kansas, the front range of Colorado, and eastern, western and southern portions of Wyoming. Increased geographic sampling could be used to investigate

whether *S. cybele* undergo long-distance dispersal. In addition to increased sampling, ecological niche modelling should be pursued to identify whether SNP clusters correlate to different niches and identify the genetic or environmental basis of phenotypic variation (Mikitová et al., 2021; Campbell et al., 2022). Population genomic investigation should also explore the sub-clustering shown in the West SNPs (Figure 2.1A, Appendix 6). Increased sampling and population genomic investigation would benefit greatly from a *Speyeria* high-quality whole-genome sequence to identify specific genes and genomic regions associated with the evolution of variation within *S. cybele* (Cicconardi et al., 2023).

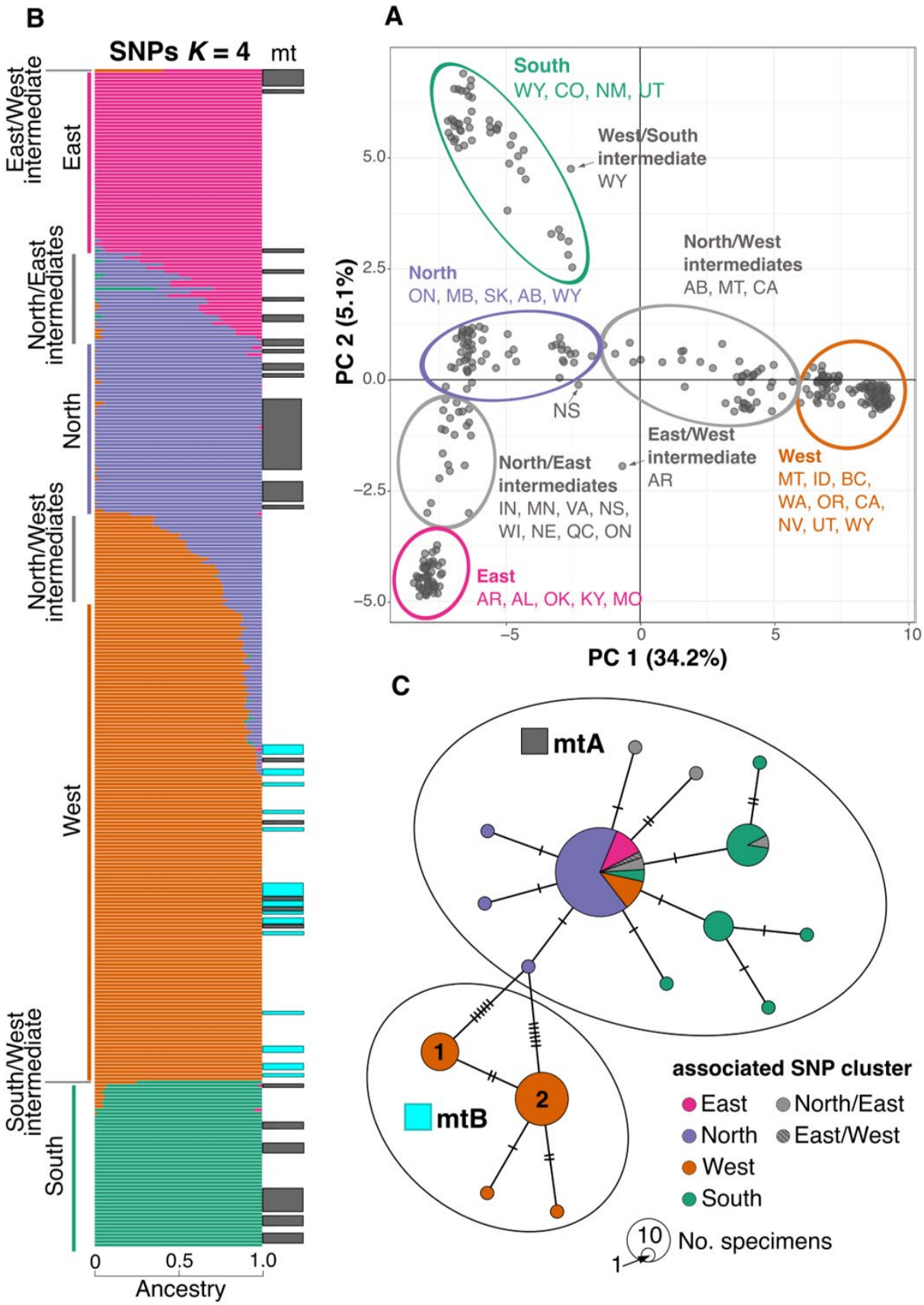
B
SNPs K = 4
mt
East/West intermediate
East
North/East intermediates
North
North/West intermediates
West
South/West intermediate
South
0
0.5
1.0
Ancestry
A
South
WY, CO, NM, UT
West/South intermediate
WY
North
ON, MB, SK, AB, WY
North/West intermediates
AB, MT, CA
NS
East/West intermediate
AR
North/East intermediates
IN, MN, VA, NS, WI, NE, QC, ON
West
MT, ID, BC, WA, OR, CA, NV, UT, WY
East
AR, AL, OK, KY, MO
PC 2 (5.1%)
PC 1 (34.2%)
5.0
2.5
0.0
−2.5
−5.0
−5
0
5
10
C
mtA
mtB
1
2
associated SNP cluster
East
North/East
North
East/West
West
South
10
1
No. specimens

Figure 2.1. Cluster-based analysis of 6069 SNPs (n=340 specimens) and mtDNA haplotypes (n=98 specimens). A). Principal component analysis ellipses approximate groups of convenience with exceptions, and do not represent statistical confidence intervals. The PCA clusters are coloured by ADMIXTURE population assignment shown in B. B). ADMIXTURE analysis of SNP data recovered *K*=4 as optimal. Labels on left correspond to SNP clusters, and intermediates were classified by Q values at 0.2 to 0.8 between clusters. Bars to the right of the ADMIXTURE plot show individual mtDNA haplotypes as shown in C. C). Minimum spanning haplotype network of mtDNA shows two major haplogroups; mtA is geographically widespread while mtB is only found in specimens of the West SNP cluster.

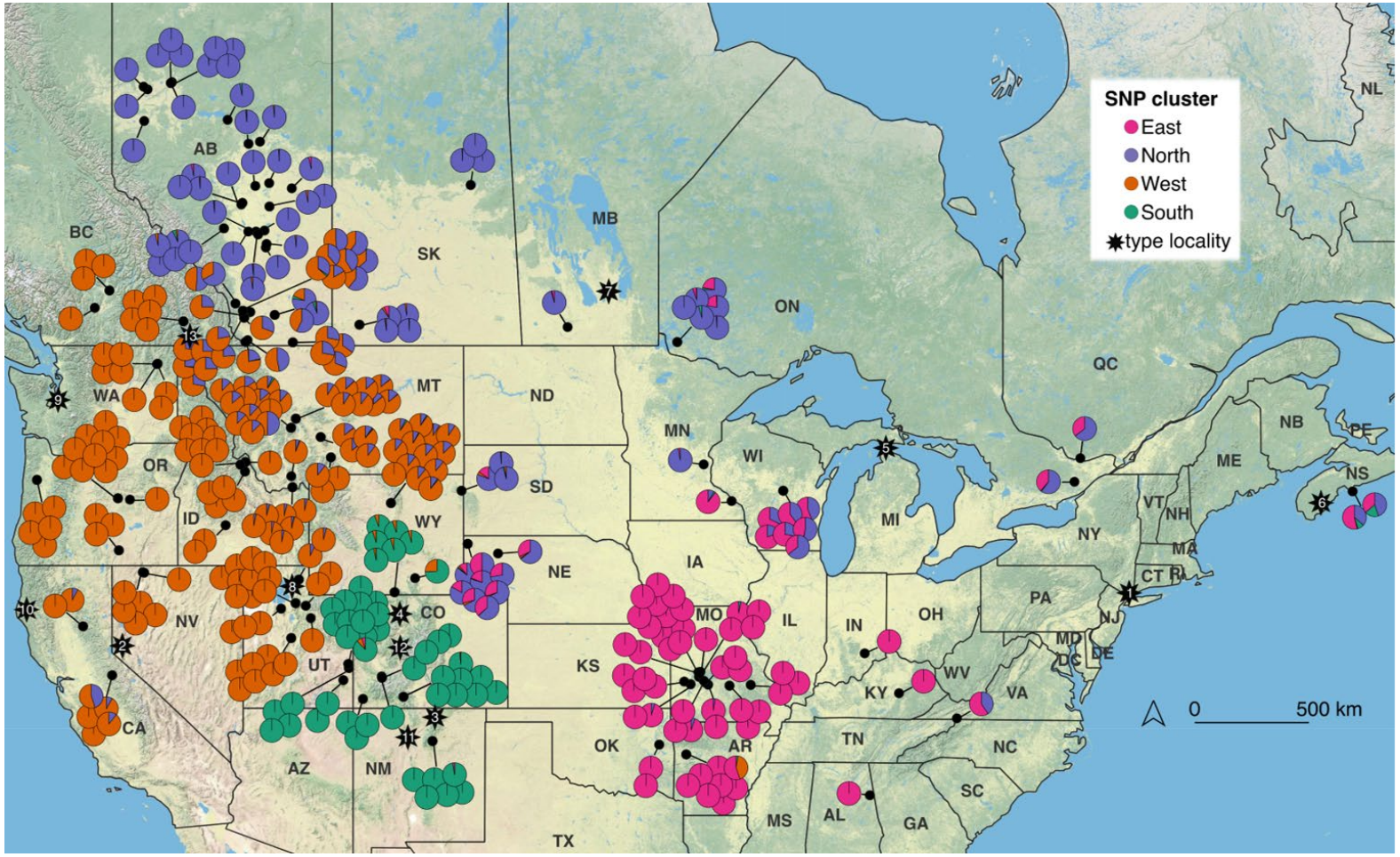

Figure 2.2 Geographic distribution of genomic clusters. Each pie chart represents a single specimen, and the colours indicate the ancestry proportions indicated in Fig. 2.1 B. Subspecies of the type localities are 1) *S. c. cybele*, 2) *S. c. leto*, 3) *S. c. carpenterii,* 4) *S. c. charlottii*, 5) *S. c. krautwurmi*, 6) *S. c. novascotiae*, 7) *S. c. pseudocarpenteri*, 8) *S. c. letona*, 9) *S. c. pugetensis*, 10) *S. c. eileenae*, 11) *S. c. neomexicana*, 12) *S. c. colorado*, and 13) *S. c. caitlinae*. Admixture between northern and western populations is frequent in the northern portion of the Rocky Mountains, while admixture between any population is less frequent in the Southern Rockies.

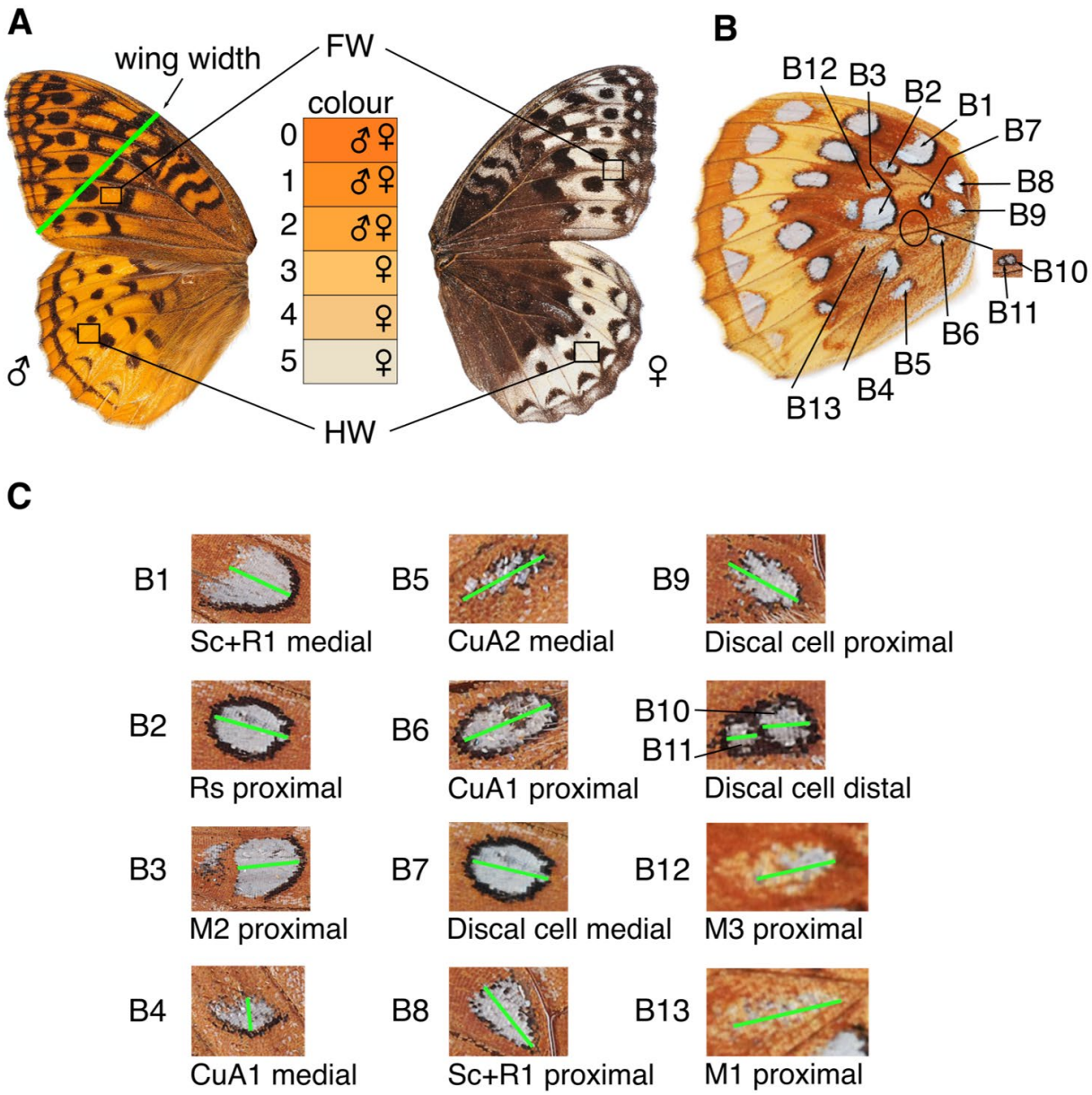

Figure 2.3. Wing morphology character scoring. A) Dorsal wing states: wing width as a measure of wing size, forewing (FW) ground colour, and hindwing (HW) ground colour. Ground colour was measured categorically as the best match to 0 to 5, corresponding to Behr Paint colours Joyful Orange, Splendor Gold, Blazing Bonfire, Jackfruit, Honey Locust, and Spinning Silk. B) Ventral hindwing discal spots were measured along the axes showing the greatest size variation between specimens, as shown by green lines in C).

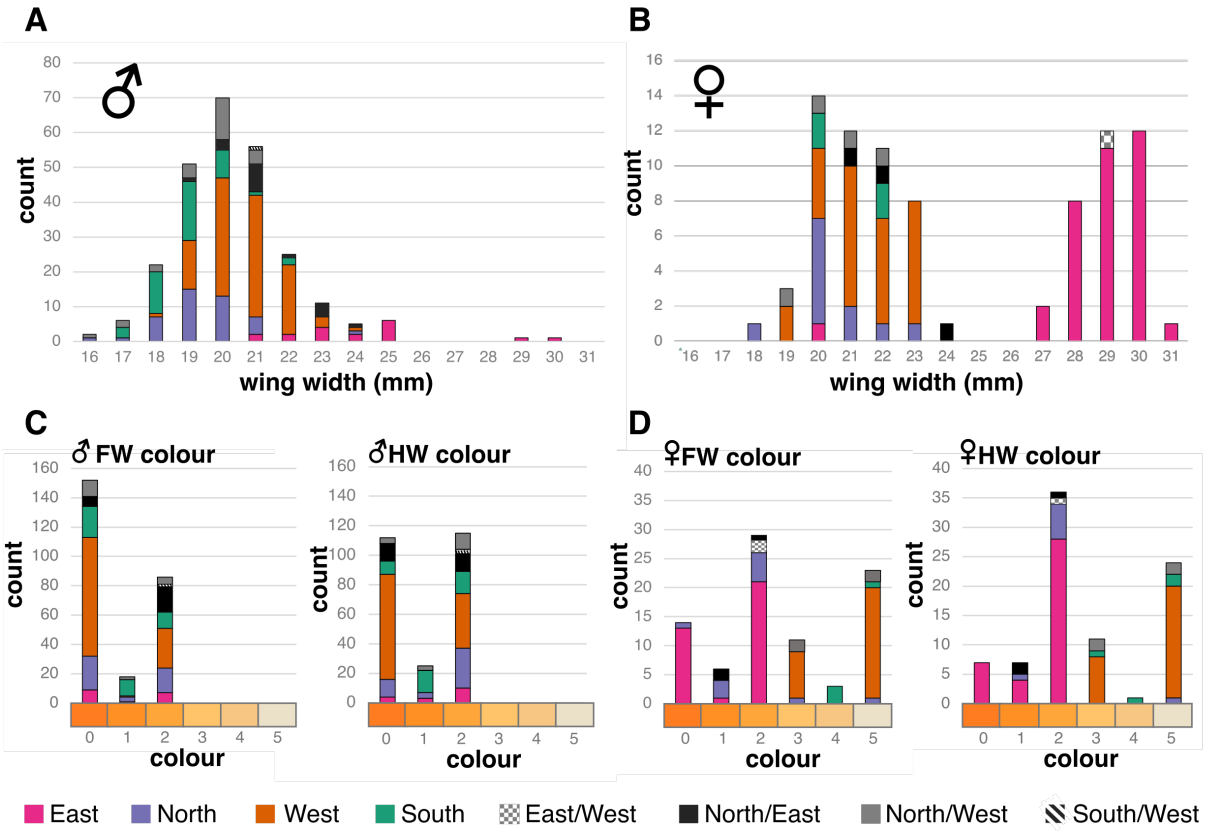

Figure 2.4. Histograms corresponding to associated SNP clusters of the **A** male (n=255) and **B** female (n=85) wing width, and **C** male and **D** female dorsal forewing (FW) and hindwing (HW) ground colour.

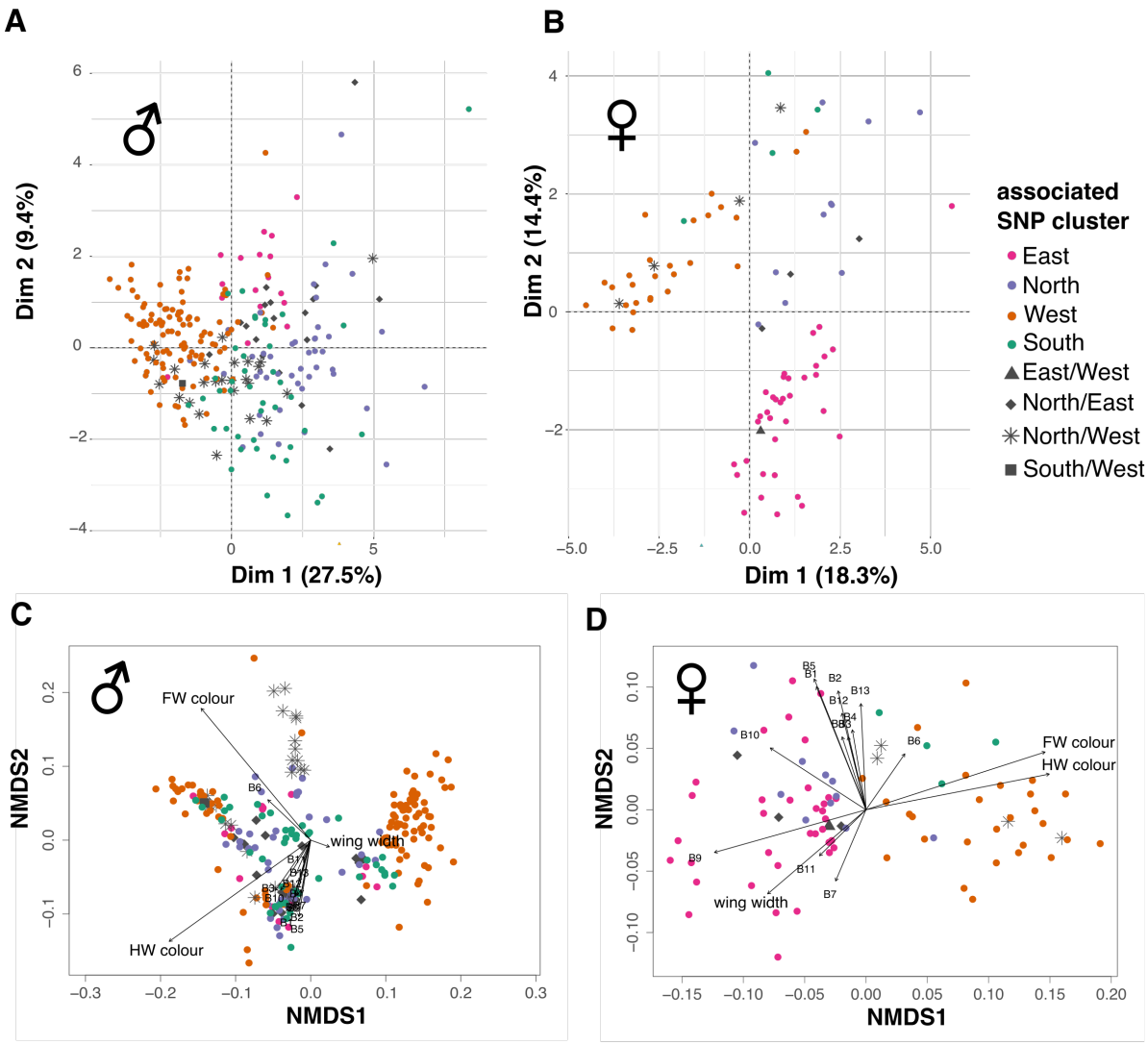

Figure 2.5. Wing morphological character analyses of male (n=255) and female (n=85) specimens. Separate **A** male and **B** female FAMD of wing morphological characters corresponding to associated SNP cluster. Non-metric multidimensional scaling results for **C** male specimens, and **D** female specimens.

Table 2.1. Pairwise F_{ST} comparisons for each combination of the four primary genomic clusters shown in Fig. 2.1. Values below the diagonal reflect calculations for all individuals (n=340), with admixed individuals assigned to clusters based on majority Q-value. Values above the diagonal reflect comparisons that only included individuals exhibiting minimal admixture (Q-values > 0.8, n=291).

		East	**North**	**West**	**South**
	n	53	55	136	47
East	75	-	0.14	0.54	0.24
North	61	0.11	-	0.50	0.16
West	156	0.50	0.42	-	0.55
South	48	0.22	0.14	0.52	-

Table 2.2. Linear wing character means for A) males (n=255) and B) females (n=85) for *K*=4 SNP clusters and their intermediates. Means and standard deviations of wing width are in millimetres (W) and are standardized sizes for silver spots (B1 to B13).

A)

♂	East	North/ East	North	North/ West	West	South	South/ West
n	17	18	43	25	108	43	1
W	24.3±2.39	21.4±1.33	19.4±1.31	19.4±1.33	20.7±1.09	19.0±1.13	21.0
B1	0.12±0.03	0.14±0.03	0.14±0.03	0.11±0.03	0.09±0.03	0.13±0.03	0.09
B2	0.06±0.03	0.05±0.03	0.04±0.03	0.01±0.02	0.01±0.02	0.03±0.04	0.08
B3	0.13±0.01	0.15±0.02	0.17±0.02	0.14±0.02	0.12±0.02	0.17±0.02	0.16
B4	0.06±0.02	0.08±0.02	0.08±0.02	0.07±0.02	0.06±0.02	0.08±0.02	0.05
B5	0.06±0.02	0.08±0.04	0.07±0.04	0.03±0.03	0.03±0.04	0.07±0.04	0.05
B6	0.10±0.01	0.11±0.02	0.12±0.02	0.13±0.02	0.10±0.03	0.10±0.03	0.10
B7	0.07±0.02	0.06±0.02	0.07±0.02	0.07±0.02	0.04±0.02	0.06±0.02	0.04
B8	0.06±0.02	0.08±0.02	0.09±0.03	0.06±0.03	0.04±0.02	0.06±0.02	0.02
B9	0.06±0.03	0.06±0.03	0.06±0.02	0.03±0.03	0.02±0.02	0.03±0.03	0.00
B10	0.04±0.04	0.06±0.04	0.06±0.04	0.03±0.03	0.00±0.01	0.02±0.03	0.00
B11	0.33±0.70	0.04±0.15	0.23±0.28	0.03±0.14	0.04±0.22	0.00	0.00
B12	0.03±0.04	0.02±0.03	0.03±0.05	0.00±0.02	0.00±0.02	0.01±0.05	0.00
B13	0.00±0.01	0.01±0.04	0.01±0.03	0.01±0.03	0.00±0.01	0.00±0.03	0.00

B)

♀	East	North/ East	North	North/ West	West	South	East/ West
n	35	3	11	4	27	4	1
W	28.8±2.81	22.3±2.53	20.5±1.29	20.5±1.29	21.4±1.25	21.0±1.15	29.0
B1	0.12±0.03	0.11±0.02	0.15±0.04	0.10±0.02	0.10±0.04	0.15±0.02	0.11
B2	0.05±0.03	0.03±0.01	0.05±0.05	0.03±0.04	0.04±0.04	0.08±0.01	0.06
B3	0.14±0.02	0.15±0.01	0.18±0.02	0.14±0.01	0.14±0.02	0.18±0.01	0.11
B4	0.07±0.02	0.07±0.01	0.08±0.03	0.09±0.04	0.06±0.02	0.09±0.01	0.05
B5	0.07±0.03	0.08±0.07	0.08±0.03	0.07±0.06	0.05±0.04	0.08±0.04	0.08
B6	0.09±0.03	0.12±0.01	0.12±0.03	0.10±0.02	0.11±0.03	0.11±0.01	0.09
B7	0.08±0.02	0.08±0.01	0.08±0.03	0.05±0.01	0.07±0.02	0.06±0.04	0.07
B8	0.06±0.01	0.07±0.02	0.11±0.04	0.08±0.04	0.06±0.01	0.07±0.01	0.06
B9	0.07±0.02	0.07±0.03	0.05±0.02	0.03±0.03	0.03±0.02	0.03±0.02	0.06
B10	0.06±0.03	0.09±0.01	0.07±0.04	0.02±0.02	0.01±0.02	0.02±0.04	0.04
B11	0.10±0.40	0.12±0.20	0.20±0.66	0.00	0.00	0.00	0.00
B12	0.02±0.03	0.03±0.03	0.01±0.02	0.01±0.02	0.00±0.02	0.04±0.04	0.00
B13	0.01±0.02	0.00	0.01±0.02	0.00	0.01±0.02	0.01±0.03	0.00

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Chapter 3
General Conclusions

3.1 Thesis overview

This thesis contributes to the study of integrative species taxonomy. Accurate species taxonomy is essential since it is often the basis for categorizing specimens for many conservation and ecological studies. Genetic data such as whole-genome SNPs and mtDNA was shown to reveal different dimensions to population structure, and when combined with inferences from morphometrics, can be used in an integrative taxonomic framework. Integrative taxonomy was shown to be especially useful for taxonomically ambiguous groups since it allowed evaluation on multiple levels of diversity within a species. This thesis illustrated how morphologically complex and geographically widespread species require multiple types of characters and a large number of specimens to accurately reflect their biodiversity using taxonomy. Ultimately, to taxonomically reflect the biology of an organism with a broad geographic range, many specimens, broad geographic sampling, and multiple data types and analyses are needed. Otherwise, taxonomic changes to species run the risk of over-splitting a group.

Taxonomic changes are often informed by the interpretation of results using species concepts. Species concepts are a conceptual framework for interpreting data and analyses, and vary in the extent to which they focus on phenotypic, genetic, and other biological characteristics like the propensity to inbreeding and ecological processes. Consequently, not all species concepts rely on biological processes and not all can be readily applied to allopatric populations, for example the genotypic cluster species concept (Mallet, 1995). However, the genomic integrity species concept (Sperling, 2003) does explicitly consider biological processes like geneflow and defines species boundaries by lack of genomic merging. The genomic integrity species concept (Sperling, 2003) compares the extent of genomic differences between allopatric populations to the extent of genomic differences of lineages that do contact each other. I have relied on this conceptual framework for interpreting the data and analyses in my thesis.

This thesis aimed to investigate the taxonomy of a broad-ranging North American butterfly. **Chapter 2** illustrates how using multiple characters, increased sampling, and consideration of biological processes allowed us to move closer to taxonomically reflecting the natural variation

that occurs within *S. cybele sensu lato*. Genetic analyses suggest *S. cybele* represents a single species with four main genomic populations, and two mtDNA haplotypes. All SNP clusters showed some admixture with each other, even when SNP clusters appear geographically separated across the continent. The wing morphology of the SNP clusters greatly overlapped, although this should be followed up with more in-depth morphometrics. This study illustrates that despite the eight-fold increase in sampling compared to Zhang et al. (2022), ambiguities still exist, and more sampling is needed of geographic gaps, putative long-distance dispersers, associations with environmental factors, and the genome.

3.2 Future research

Many of the observations and specimens that led to this project were provided by citizen scientists who wanted to know more about this charismatic butterfly. Future research should capitalize on the charismatic nature and public investment for conservation and education on this butterfly. While also adding to the progression of taxonomy and the public attention it receives, *Speyeria* could eventually serve as a model system for species evolution, similar to the use of *Heliconius* butterflies in tropical and subtropical regions (Cicconardi et al., 2023).

3.2.1 Genomics

To further explore genomics of *S. cybele*, I recommend analyses using a high-quality whole genome. While *de novo* reduced representation data are useful for identifying patterns, without a reference genome to assemble to, it is unknown where the SNPs exist in the genome (Fierst, 2015). If future work uses a *de novo* for assembly, more fine-scale parameter testing should be conducted. During parameter testing, M=2 yielded the highest number of r80 assembled loci and polymorphic loci and was used for assembly of all datasets. However, M=3 yielded the highest number of SNPs, and this should be investigated whether these additional SNPs are erroneous by comparing numbers of SNPs after filtering for minor allele frequency for each increment of M (Paris et al., 2017).

A whole genome assembly would allow for the identification of genomic architecture, chromosome interactions, and genomic functions (Fierst, 2015; Keeling et al., 2022), previously shown with fall migratory monarch butterflies (*D. plexippus*; Zhan et al., 2011; Talla et al.,

2020), and migratory hoverflies (*Episyrphus*; Doyle et al., 2022). In addition to a whole genome, transcriptome gene expression data could be used to identify genes associated with putative long-distance dispersal. Transcriptome gene expression has been previously used to identify genes associated with long-distance dispersal, including epidemic mountain pine beetle (*Dendroconus ponderosae*: Shegelski et al., 2021).

Mitonuclear discordance observed within *S. cybele* should be investigated by assessing mutation rate compared to other butterflies and the nuclear genome (e.g. Ney et al., 2018), and assessing the presence of putative *Wolbachia*-mediated genetic isolation (e.g. Kodandaramaiah et al., 2013). *Wolbachia* strains could be identified from raw SNP reads (Kim et al., 2016) or a PCR assay for *Wolbachia*-associated genes from the already extracted DNA (Kodandaramaiah et al., 2013).

3.2.2 Biogeography and ecology

In combination with a whole genome assembly, ecological niche modelling could quantify the niches and habitat associations of *S. cybele* genetic clusters and wing variation (Campbell at al., 2022). Ecological niche modelling of a Palearctic butterfly, *Argynnis paphia*, showed phenotypic plasticity is likely environmentally induced by elevation, bioclimatic variables, and bedrock (Mikitová et al., 2021). It would be worthwhile to perform similar ecological niche modelling to test for environmental phenotypic variation within *S. cybele* (Mikitová et al., 2021).

Speyeria cybele showed low genetic variation within the East and North SNP clusters. In contrast, the West SNP cluster showed genetic variation that was localized to collection locality (Appendix 7 and Appendix 8). The lack of sub-structuring in the East and North SNP clusters could be due to continuous habitat allowing for increased gene flow. Or, the lack of sub-structuring of the North SNP cluster could also be the result of populations experiencing range expansions leading to a founder affect (Hewitt 2000). Increased genetic sub-clustering of *S. cybele* could be the result of low gene flow between geographically separated populations, however this should be further investigated to see if this is a sampling artefact or a biological pattern. Increased genetic variation in western North America has also been observed in other fauna groups that radiated during the Quaternary glacial cycles (Hewitt 2000). Perhaps topology

generated by melting ice sheets lead to many habitat shifts in western *S. cybele* populations, which should be investigated with a phylogeographic approach (Hewitt 2000).

To further study biogeography and ecology of *S. cybele*, additional geographic sampling is needed. Regions in need of more sampling include in eastern Montana, eastern Colorado, and the United States Midwest. Sampling is also needed in the northeastern United States, the United States East Coast, and Canadian Maritime provinces.

3.2.3 Long-distance dispersal

Our results indicate that *S. cybele* is likely capable of long-distance dispersal, which has been established for other nymphalid butterflies. Within *Speyeria*, a stray *S. idalia* was found in in 2015 in southern Alberta (Anweiler, 2015), 400 km from its nearest other range record in Saskatchewan a century ago, and still farther from its current range where it may still be found in the Dakotas and Wyoming (NatureServe Explorer 2024). Long-distance dispersal is known for migratory nymphalid butterflies, such as painted lady (*Vanessa cardui*; Stefanescu et al., 2016) and the monarch butterfly (*Danaus plexippus*; Brower, 1995). Painted lady long-distance dispersal has been tested with stable isotopes on their wings (Stefanescu et al., 2016) and monarch butterfly long-distance dispersal has been tested using mark-and-recapture methods (review by Brower, 1995). However, these methods were used on butterflies with known migration routes and these methods may be difficult to test in occasional long-distance dispersers. Instead, initial investigation into long-distance dispersal should focus on collecting *S. cybele* in sampling gaps to genetically identify long-distance dispersers to help build more knowledge if this is an artefact of sampling or a real biological phenomenon.

3.2.4 Morphology

Morphological analysis of *S. cybele* wings show extensive overlap between SNP clusters, and increased morphological sampling should be pursued to reliably reflect genotype. While SNP clusters did show differences in size, colour, and silver spots, they were not enough to fully resolve the SNP clusters. Future research should quantify the dorsal wing-surface basal suffusion, which is often mentioned in field guides. When trying to measure dorsal basal

suffusion, it became apparent that its appearance is confounded by the size of the wing, density of the scales, contrast with the ground colour, and wing wear.

To understand the biological processes behind *S. cybele* wing variation, the basis of *S. cybele* morphological variation could be explored with high-quality whole genome data, and laboratory crossing of different populations, and testing of environmental variables (Kronforst and Papa, 2015). This future research is needed since sexually dimorphic ground colour has been previously hypothesized to driven by one or a few sex-specific alleles, that have become fixed at the extremes of *S. cybele's* range (Hammond et al., 2013, 2020). Sexually dimorphic females could also have similar physiology to white *Colias* females, who have different wing pigments in order to support greater fecundity (Hanly et al., 2023). Additionally, pale coloured West SNP cluster females could also be the result of selection for predation avoidance via Batesian mimicry of potentially distasteful butterflies like white admirals (*Limenitis arthemis*; Ritland, 1995). Alternatively, the degree of sexual dimorphism and other morphological variation may be the result of environmental variables (Montejo-Kovacevich et al., 2021).

Overall, I hope this thesis aids in future taxonomic changes that are rooted in objectivity, clarity, and biological processes. This project originally aimed to see if *S. cybele* was one species or two, one east and the other west. The question of whether there was a distinct western species from eastern *S. cybele* dates back to the 19th century. While *S. cybele* does appear to be a single species, the genetics are far more complicated, and showed four genomic grouping that admix with overlapping morphology. My greatest recommendations for future researchers working on *S. cybele* are to continue building a network of people willing to collect specimens and share their observations, to continue to uphold taxonomy to the scientific method, and to remember how beautiful this butterfly is when the work gets challenging.

"I sense that whatever character is studied, whether size, pattern, melanism or color shadings, the extremes would probably blend insensibly though the intermediates in geographically arranged series, if we had adequate material."

- Arthur H. Moeck, Geographic Variability in *Speyeria*, 1975

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Appendix

Biography

I grew up west of Calgary in the Alberta Foothills. My mom and dad are avid outdoorsmen who taught my sister and me the importance of wildlife and science from a young age. My parents let me follow my passion for nature, animals, and science by exploring the wilderness, raising and caring for various pets, and giving me things like natural history books and bug boxes. While growing up, I knew I wanted to be some sort of scientist, which led me to my undergraduate degree at the University of Alberta.

During summers in my undergraduate degree, I worked for Alberta Environment and Parks decontaminating and inspecting boats for aquatic invasive species (AIS). I inspected for AIS at a station in southeastern Alberta. At the station, I found a large variety of moths. In the semester following first summer inspecting for AIS, I asked my invertebrate zoology TA, Erin Campbell, to help me identify some of the moths I found. Erin then introduced me to Felix Sperling, who gave me the opportunity to volunteer in the E. H. Strickland Entomology Museum. The following summer while inspecting for AIS, I surveyed the moth biodiversity at the station and the nearby provincial park, Cypress Hills. This moth biodiversity project was my first project with Felix, and I continued doing undergraduate research projects with Felix until I finished my Bachelors of Science, with a Minor in Chemistry, in the fall semester of 2021.

I began this Masters project in the 2022 winter semester. The fall semester prior I worked on an undergraduate research project assessing the wing morphometrics of *S. cybele*. I enjoyed working on *S. cybele* under the supervision of Erin and Felix so much, that doing a Masters on this butterfly was a no-brainer. Now that I have come to the end of this Masters, I am excited to see where the next chapter will take me.

Appendix 1. Table of identification numbers, collection information, and sequencing performed. Specimens are listed in their order of appearance in the SNP admixture plot (Figure 2.1A). Specimens that were sequenced for mtDNA COI and included in the haplotype network of Figure 2.1C have an "*" in front of their DNA number.

DNA#	UASM	S	lat	lon	SNP Seq	Coll Date	Region	Locality	Collector(s)
*10837	397437	F	56.060	-118.390	July2016 NS	27-vii-2005	CA:AB	"Rd to Green Island"	DB
12339	381720	M	49.902	-112.611	Jan2020 NS	27-vii-2017	CA:AB	10 km N of Coaldale	T Pike
12340	381721	M	49.902	-112.611	Jan2020 NS	27-vii-2017	CA:AB	10 km N of Coaldale	T Pike
12338	381725	M	49.902	-112.611	Jan2020 NS	27-vii-2017	CA:AB	10 km N of Coaldale	T Pike
*10841	397441	F	53.476	-111.846	July2016 NS	14-vii-2000	CA:AB	Akasu Hill	C Schmidt
*9691	397079	M	54.710	-113.280	2013 HS	15-vii-2001	CA:AB	Athabasca	FAH Sperling
13611	431106	M	49.819	-113.998	Aug2023 NS	07-viii-2022	CA:AB	Beaver Cr Public Land Use Zone	LG Jackson
13612	431107	F	49.819	-113.998	Aug2023 NS	07-viii-2022	CA:AB	Beaver Cr Public Land Use Zone	LG Jackson
13610	431105	M	49.822	-113.984	Aug2023 NS	07-viii-2022	CA:AB	Beaver Cr Public Land Use Zone	LG Jackson
12348	381717	M	49.819	-113.998	Jan2020 NS	16-viii-2010	CA:AB	Beaver Cr	T Pike
12329	381783	M	49.819	-113.998	Jan2020 NS	27-vii-2012	CA:AB	Beaver Cr	T Pike
*10835	397435	M	55.280	-114.751	July2016 NS	9-viii-2015	CA:AB	Buck Mtn Rd near Pigeon Lk.	E Campbell
11374	397520	F	54.653	-113.797	Aug2017 NS	10-vii-2017	CA:AB	Cross Lk. Prov. Park	V Shegelski
*11376	397522	M	51.935	-112.981	Aug2017 NS	9-vii-2017	CA:AB	Dry Island Prov. Park	FAH Sperling
13617	431095	M	50.026	-114.055	Aug2023 NS	08-viii-2022	CA:AB	East Trout Cr Rd, Porcupine Hills Public Land Use Zone	LG Jackson
13615	431096	M	50.026	-114.055	Aug2023 NS	08-viii-2022	CA:AB	East Trout Cr Rd, Porcupine Hills Public Land Use Zone	LG Jackson
13613	431097	M	49.986	-113.719	Aug2023 NS	08-viii-2022	CA:AB	East Trout Cr Rd, Porcupine Hills Public Land Use Zone	LG Jackson
13619	431099	F	50.026	-114.055	Aug2023 NS	08-viii-2022	CA:AB	East Trout Cr Rd, Porcupine Hills Public Land Use Zone	LG Jackson
13614	431102	M	50.026	-114.055	Aug2023 NS	08-viii-2022	CA:AB	East Trout Cr Rd, Porcupine Hills Public Land Use Zone	LG Jackson
13616	431104	M	50.026	-114.055	Aug2023 NS	08-viii-2022	CA:AB	East Trout Cr Rd, Porcupine Hills Public Land Use Zone	LG Jackson
13618	431103	M	50.017	-114.054	Aug2023 NS	08-viii-2022	CA:AB	East Trout Cr Rd, Porcupine Hills Public Land Use Zone	LG Jackson
13620	431108	M	50.026	-114.055	Aug2023 NS	08-viii-2022	CA:AB	East Trout Cr Rd, Porcupine Hills Public Land Use Zone	LG Jackson
*9693	397081	M	53.530	-113.490	2013 HS	12-vii-2003	CA:AB	Edmonton	G Anweiler
*11375	397521	F	53.607	-112.862	Aug2017 NS	13-vii-2017	CA:AB	Elk Island Nat Park	B Sean
*9692	397080	F	56.130	-118.570	2013 HS	10-viii-2002	CA:AB	Fourth Cr, PRP 056	FAH Sperling
13603	431100	M	49.157	-113.842	Aug2023 NS	15-vii-2022	CA:AB	Hwy 6, nr Waterton Nat Park	LG Jackson
*10844	397444	M	53.071	-114.076	July2016 NS	26-vii-2015	CA:AB	Itaska Beach, Municipal Rd	FAH Sperling
13621	431085	F	52.971	-112.850	Aug2023 NS	01-viii-2022	CA:AB	Jubilee Park, Camrose	LG Jackson
13622	431092	M	52.971	-112.850	Aug2023 NS	01-viii-2022	CA:AB	Jubilee Park, Camrose	LG Jackson
*10845	397445	M	56.242	-117.285	July2016 NS	26-vi-2015	CA:AB	Kaufman Hill	JR Dupuis
*10221	397107	M	56.240	-117.290	2014 HS	31-vii-2014	CA:AB	Kaufman Hill	JR Dupuis
*10222	397108	M	56.240	-117.290	2014 HS	31-vii-2014	CA:AB	Kaufman Hill	JR Dupuis
*10326	397199	M	55.250	-118.540	2014 HS	6-vii-1998	CA:AB	Kleskun Hills	FAH Sperling
*9654	397044	F	52.280	-113.460	2013 HS	16-viii-2013	CA:AB	NW of Delburne RR 251	JR Dupuis
10847	397447	M	52.202	-113.362	July2016 NS	16-viii-2013	CA:AB	NW of Delburne, Rg Rd 243	JR Dupuis & BA Mori
*10840	397440	M	52.202	-113.362	July2016 NS	16-viii-2013	CA:AB	NW of Delburne, Rg Rd 243	JR Dupuis & BA Mori
*10226	397112	M	56.220	-117.330	2014 HS	1-viii-2014	CA:AB	Pat's Cr	JR Dupuis
*10229	397115	M	56.220	-117.330	2014 HS	1-viii-2014	CA:AB	Pat's Cr	JR Dupuis
*10842	397442	M	56.233	-117.297	July2016 NS	6-viii-2013	CA:AB	Pati Ck	JR Dupuis
*10843	397443	M	56.233	-117.297	July2016 NS	6-viii-2013	CA:AB	Pat’s Ck	JR Dupuis
*10846	397446	M	56.242	-117.285	July2016 NS	6-vii-2015	CA:AB	Peace River, Lower Kaufman Hill	JR Dupuis

DNA#	UASM	S	lat	lon	SNP Seq	Coll Date	Region	Locality	Collector(s)
*10331	397204	M	53.070	-114.080	2014 HS	4-viii-2012	CA:AB	Pigeon Lk, Itaska	FAH Sperling
*10836	397436	M	53.027	-114.127	July2016 NS	17-vii-2015	CA:AB	Pigeon Lk, Itaska Beach	FAH Sperling
*9613	397007	F	53.070	-114.080	2013 HS	7-viii-2010	CA:AB	Pigeon Lk, Itaska munic. Rd	FAH Sperling
12324	381724	M	49.986	-113.719	Jan2020 NS	18-vii-2017	CA:AB	Porcupine Hills, 10 km N of Hwy 532 on Trout Cr Rd	T Pike
12330	381773	M	49.819	-113.998	Jan2020 NS	27-vii-2012	CA:AB	Porcupine Hills, Beaver Cr	T Pike
12336	381727	M	49.819	-113.998	Jan2020 NS	04-viii-2012	CA:AB	Porcupine Hills, Beaver Cr Rec Area	T Pike
*10839	397439	M	52.309	-113.073	July2016 NS	16-viii-2013	CA:AB	Red Deer R @ Hwy 21	JR Dupuis & BA Mori
*9633	397025	F	52.280	-113.810	2013 HS	16-viii-2013	CA:AB	Red Deer River	JR Dupuis
*10807	397409	M	52.370	-114.920	July2016 NS	9-viii-2015	CA:AB	Rocky Mtn House	E Campbell
*10808	397410	M	52.370	-114.920	July2016 NS	9-viii-2015	CA:AB	Rocky Mtn House	E Campbell
*10809	397411	M	52.370	-114.920	July2016 NS	9-viii-2015	CA:AB	Rocky Mtn House	E Campbell
*10810	397412	M	52.370	-114.920	July2016 NS	9-viii-2015	CA:AB	Rocky Mtn House	E Campbell
*10811	397413	F	52.370	-114.920	July2016 NS	9-viii-2015	CA:AB	Rocky Mtn House	E Campbell
12337	381804	M	49.091	-111.786	Jan2020 NS	22-viii-2017	CA:AB	SW of Weir bridge	S Bishop
12341	381728	M	49.091	-111.786	Jan2020 NS	03-vii-2015	CA:AB	SW of Weir bridge	T Pike
12334	381805	M	49.091	-111.786	Jan2020 NS	03-vii-2015	CA:AB	SW of Weir bridge	T Pike
12335	381808	M	49.091	-111.786	Jan2020 NS	03-vii-2015	CA:AB	SW of Weir bridge	T Pike
*10834	397434	F	51.834	-113.013	July2016 NS	21-vii-2010	CA:AB	Tolman Bridge	JR Dupuis
12342	381678	M	49.897	-114.017	Jan2020 NS	08-vii-2015	CA:AB	W Sharples Cr Rd, Porcupine Hills	T Pike
12343	381647	F	49.103	-113.940	Jan2020 NS	4-viii-2012	CA:AB	Waterton Nat Park, Crandell Cpg	T Pike
12327	397694	M	50.234	-114.394	Jan2020 NS	22-vii-2017	CA:AB	Willow Cr PRA, Hwy 532, Porcupine Hills	S Bishop
12326	397693	M	50.234	-114.394	Jan2020 NS	21-vii-2017	CA:AB	Willow Cr PRA, Hwy 532, Porcupine Hills	E Campbell
*10831	397431	M	50.110	-120.790	July2016 NS	13-vii-2015	CA:BC	16 km E of Merritt, start of Kane Rd	FAH Sperling
*12236	397620	M	49.713	-116.759	Feb2019 NS	13-viii-2018	CA:BC	Kootenay Crawford Ck FSR, km 2-5	C Schmidt
*12237	397621	F	49.713	-116.759	Feb2019 NS	13-viii-2018	CA:BC	Kootenay Crawford Ck FSR, km 2-5	C Schmidt
*12238	397622	M	49.713	-116.759	Feb2019 NS	13-viii-2018	CA:BC	Kootenay Crawford Ck FSR, km 2-5	C Schmidt
*12239	397623	M	49.713	-116.759	Feb2019 NS	13-viii-2018	CA:BC	Kootenay Crawford Ck FSR, km 2-5	C Schmidt
*12235	397619	M	49.713	-116.759	Feb2019 NS	13-viii-2018	CA:BC	Kootenay Crawford Ck FSR, km 2-5	C Schmidt
*10779	397382	M	50.610	-120.120	July2016 NS	23-vii-2015	CA:BC	Pendelton Cr Rec Area	E Campbell
*10774	397377	F	50.610	-120.120	July2016 NS	23-vii-2015	CA:BC	Pendelton Cr Rec Area	J Lee
10782	397385	M	50.610	-120.120	July2016 NS	23-vii-2015	CA:BC	Pendelton Cr Rec Area	E Campbell
12323	397692	M	49.544	-99.297	Jan2020 NS	23-vii-2019	CA:MB	Glenboro	D Glaeske
L55	431071	F	44.460	-63.620	Aug2023 NS	10-viii-2022	CA:NS	Cootes Cove	TD Nelson
L54	431073	F	44.460	-63.620	Aug2023 NS	10-viii-2022	CA:NS	Cootes Cove	TD Nelson
12247	397624	M	44.769	-76.263	Feb2019 NS	14-vii-2018	CA:ON	Big Rideau L., Lally Rd, Lanark Co	C Schmidt & P Hall
*11367	397513	M	49.100	-94.315	Aug2017 NS	17-vii-2016	CA:ON	Morson	ZG MacDonald
*11363	397510	M	49.100	-94.315	Aug2017 NS	17-vii-2016	CA:ON	Morson	ZG MacDonald
*11369	397515	M	49.100	-94.315	Aug2017 NS	17-vii-2016	CA:ON	Morson	ZG MacDonald
*11362	397509	M	49.100	-94.315	Aug2017 NS	17-vii-2016	CA:ON	Morson	ZG MacDonald
11365	397511	M	49.100	-94.315	Aug2017 NS	17-vii-2016	CA:ON	Morson	ZG MacDonald
*11357	397504	M	49.100	-94.315	Aug2017 NS	9-vii-2016	CA:ON	Morson	ZG MacDonald

DNA#	UASM	S	lat	lon	SNP Seq	Coll Date	Region	Locality	Collector(s)
*10826	397428	M	45.524	-75.995	July2016 NS	5-vii-2015	CA:QC	Luskville	V Nazari
13604	431083	M	49.614	-108.759	Aug2023 NS	17-vii-2022	CA:SK	Pine Cr	LG Jackson
13601	431086	M	49.614	-108.759	Aug2023 NS	17-vii-2022	CA:SK	Pine Cr	D Glaeske
13602	431087	M	49.614	-108.759	Aug2023 NS	17-vii-2022	CA:SK	Pine Cr	LG Jackson
13605	431088	M	49.614	-108.759	Aug2023 NS	17-vii-2022	CA:SK	Pine Cr	LG Jackson
13606	431089	M	53.555	-103.701	Aug2023 NS	16-vii-2022	CA:SK	Tobyn Lake, Torch River Prov Park, Pruden's Pt Rd	D Glaeske
13608	431090	M	53.555	-103.701	Aug2023 NS	16-vii-2022	CA:SK	Tobyn Lake, Torch River Prov Park, Pruden's Pt Rd	D Glaeske
13609	431091	M	53.555	-103.701	Aug2023 NS	16-vii-2022	CA:SK	Tobyn Lake, Torch River Prov Park, Pruden's Pt Rd	D Glaeske
*10867	397467	M	38.591	-109.265	Aug2017 NS	13-vii-2016	US: UT	La Sal Mts, Castle Cr, Grand Co	E Gage
10861	397461	M	38.591	-109.265	Aug2017 NS	13-vii-2016	US: UT	La Sal Mts, Castle Cr and dolores triangle safari route, Grand Co	E Gage
*10849	397449	M	38.591	-109.265	July2016 NS	16-viii-2014	US: UT	La Sal Mts, Grand Co	E Gage
*10850	397450	M	38.591	-109.265	July2016 NS	16-viii-2014	US: UT	La Sal Mts, Grand Co	E Gage
*10851	397451	M	38.591	-109.265	July2016 NS	16-viii-2014	US: UT	La Sal Mts, Grand Co	E Gage
10852	397452	M	38.591	-109.265	July2016 NS	16-viii-2014	US: UT	La Sal Mts, Grand Co	E Gage
*10853	397453	M	38.591	-109.265	July2016 NS	16-viii-2014	US: UT	La Sal Mts, Grand Co	E Gage
10862	397462	M	38.591	-109.265	Aug2017 NS	13-vii-2016	US: UT	La Sal Mts, Grand Co	E Gage
10863	397463	M	38.591	-109.265	Aug2017 NS	13-vii-2016	US: UT	La Sal Mts, Grand Co	E Gage
*10864	397464	M	38.591	-109.265	Aug2017 NS	13-vii-2016	US: UT	La Sal Mts, Grand Co	E Gage
*10866	397466	M	38.591	-109.265	Aug2017 NS	13-vii-2016	US: UT	La Sal Mts, Grand Co	E Gage
*10848	397448	M	38.591	-109.265	July2016 NS	16-viii-2014	US: UT	La Sal Mts, Grand Co	E Gage
13749	431026	F	33.782	-85.561	Aug2023 NS	15-ix-2022	US:AL	Cleburne	E Gage & R Gage
*12249	397625	F	35.309	-93.907	Feb2019 NS	30-vi-2018	US:AR	Logan	E Gage & R Gage
12250	397626	F	35.309	-93.907	Feb2019 NS	30-vi-2018	US:AR	Logan	E Gage & R Gage
*12251	397627	F	35.309	-93.907	Feb2019 NS	30-vi-2018	US:AR	Logan	E Gage & R Gage
*12252	397628	F	35.309	-93.907	Feb2019 NS	30-vi-2018	US:AR	Logan	E Gage & R Gage
*12253	397629	F	35.309	-93.907	Feb2019 NS	30-vi-2018	US:AR	Logan	E Gage & R Gage
*12254	397630	F	35.309	-93.907	Feb2019 NS	30-vi-2018	US:AR	Logan	E Gage & R Gage
12264	397639	F	35.309	-93.907	Feb2019 NS	30-vi-2018	US:AR	Logan	E Gage & R Gage
*12265	397640	F	35.309	-93.907	Feb2019 NS	30-vi-2018	US:AR	Logan	E Gage & R Gage
13693	431101	M	38.191	-119.997	Aug2023 NS	16-viii-2022	US:CA	Pine Crest Cpg, Tuolumne Co	E Gage & R Gage
13685	431128	M	38.191	-119.997	Aug2023 NS	16-viii-2022	US:CA	Pine Crest Cpg, Tuolumne Co	E Gage & R Gage
13695	431141	M	38.191	-119.997	Aug2023 NS	16-viii-2022	US:CA	Pine Crest Cpg, Tuolumne Co	E Gage & R Gage
13684	431172	F	38.191	-119.997	Aug2023 NS	16-viii-2022	US:CA	Pine Crest Cpg, Tuolumne Co	E Gage & R Gage
13699	431173	M	38.191	-119.997	Aug2023 NS	16-viii-2022	US:CA	Pine Crest Cpg, Tuolumne Co	E Gage & R Gage
13694	431142	M	39.919	-121.320	Aug2023 NS	18-viii-2022	US:CA	Plumas Nat Frst, Butterfly valley tr, Pitcher plant marsh, Plumas Co	E Gage & R Gage
13704	431174	M	39.919	-121.320	Aug2023 NS	18-viii-2022	US:CA	Plumas Nat Frst, Butterfly valley tr, Pitcher plant marsh, Plumas Co	E Gage & R Gage
11861	397568	M	38.150	-107.750	Mar2018 NS	15-Aug-17	US:CO	Ridgeway, Ouray Co	E Gage
*10289	397174	F	38.100	-107.732	2014 HS	16-viii-2014	US:CO	Ridgeway, 3 miles S along Uncompahgre River, Ouray Co	E Gage & R Gage
*10288	397173	F	38.100	-107.732	2014 HS	16-viii-2014	US:CO	Ridgeway, 3 miles S along Uncompahgre River, Ouray Co	E Gage & R Gage
*10857	397457	M	38.100	-107.732	July2016 NS	16-viii-2014	US:CO	Ridgeway, 3 miles S along Uncompahgre River, Ouray Co	E Gage & R Gage
*10858	397458	F	38.100	-107.732	July2016 NS	16-viii-2014	US:CO	Ridgeway, 3 miles S along Uncompahgre River, Ouray Co	E Gage & R Gage

DNA#	UASM	S	lat	lon	SNP Seq	Coll Date	Region	Locality	Collector(s)
12315	397684	M	37.417	-106.759	Jan2020 NS	27-viii-2019	US:CO	San Juan Nat Frst, Elk Mtns, Frst Rd 667, Gunnison Co	R Gage
12318	397687	M	37.417	-106.759	Jan2020 NS	27-viii-2019	US:CO	San Juan Nat Frst, Elk Mtns, Frst Rd 667, Gunnison Co	R Gage
12317	397686	M	37.417	-106.759	Jan2020 NS	27-viii-2019	US:CO	San Juan Nat Frst, Elk Mtns, Frst Rd 667, Gunnison Co	R Gage
12321	397690	M	37.417	-106.759	Jan2020 NS	27-viii-2019	US:CO	San Juan Nat Frst, Elk Mtns, Frst Rd 667, Gunnison Co	R Gage
12314	397683	M	37.417	-106.759	Jan2020 NS	27-viii-2019	US:CO	San Juan Nat Frst, Elk Mtns, Frst Rd 667, Gunnison Co	R Gage
12316	397685	M	37.417	-106.759	Jan2020 NS	27-viii-2019	US:CO	San Juan Nat Frst, Elk Mtns, Frst Rd 667, Gunnison Co	R Gage
12319	397688	M	37.417	-106.759	Jan2020 NS	27-viii-2019	US:CO	San Juan Nat Frst, Elk Mtns, Frst Rd 667, Gunnison Co	R Gage
12320	397689	M	37.417	-106.759	Jan2020 NS	27-viii-2019	US:CO	San Juan Nat Frst, Elk Mtns, Frst Rd 667, Gunnison Co	R Gage
*10822	397424	M	37.340	-108.600	July2016 NS	5-viii-2015	US:CO	Taylor Cr, 0.5-2.4 mi N of SR 145, Montezuma Co	M Fisher
*10821	397423	M	37.340	-108.600	July2016 NS	5-viii-2015	US:CO	Taylor Cr, 0.5-2.4 mi N of SR 145, Montezuma Co	M Fisher
10823	397425	M	37.340	-108.600	July2016 NS	5-viii-2015	US:CO	Taylor Cr, 0.5-2.4 mi N of SR 145, Montezuma Co	M Fisher
12306	397675	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	1.4 mi from Hwy 93, Little Thompson Gulch. Wagonhammer Cr, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12298	397667	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12299	397668	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12300	397669	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12301	397670	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12302	397671	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12303	397672	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12304	397673	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12305	397674	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12307	397676	M	45.397	-113.941	Jan2020 NS	22-vii-2019	US:ID	Little Thompson Gulch, Salmon-Challis Nat Frst, Lemhi Co	E Gage & R Gage
12296	397665	M	45.100	-113.960	Jan2020 NS	22-vii-2019	US:ID	Perreau Cr Rd, 3.3 mi W of Hwy 93, Lemhi Co	E Gage & R Gage
12295	397664	M	45.100	-113.960	Jan2020 NS	22-vii-2019	US:ID	Perreau Cr Rd, 3.3 mi W of Hwy 93, Lemhi Co	E Gage & R Gage
12297	397666	M	45.100	-113.960	Jan2020 NS	22-vii-2019	US:ID	Perreau Cr Rd, 3.3 mi W of Hwy 93, Lemhi Co	E Gage & R Gage
12294	397663	M	45.100	-113.960	Jan2020 NS	22-vii-2019	US:ID	Perreau Cr Rd on Tormay Cr, 3.3 mi W of Hwy 93, Lemhi Co	E Gage & R Gage
*10236	397122	M	43.350	-114.840	2014 HS	16-vii-2014	US:ID	W of Fairfield, Cat Cr Rd	JR Dupuis
*10237	397123	M	43.350	-114.840	2014 HS	16-vii-2014	US:ID	W of Fairfield, Cat Cr Rd	JR Dupuis
10827	397429	M	38.968	-85.797	July2016 NS	22-vii-2015	US:IN	Muscatatuk Nat Wildlife Refuge, Monroe Co	V Nazari
13669	431029	M	37.548	-84.236	Aug2023 NS	28-viii-2022	US:KY	Madison Co SE of Berca	R Lardner
13681	431069	M	44.142	-91.846	Aug2023 NS	29-vii-2020	US:MN	Mound Prairie, Houston Co	D Glaeske
13787	431084	M	45.325	-93.113	Aug2023 NS	23-vii-2021	US:MN	Wyoming	D Glaeske
13673	431059	M	38.137	-93.325	Aug2023 NS	05-viii-2022	US:MO	BirdSong Conservation Area, St. Clair Co	R Lardner
13655	431047	F	37.869	-93.708	Aug2023 NS	11-vii-2022	US:MO	BirdSong Conservation Area, St. Clair Co	R Lardner
13657	431048	F	37.869	-93.708	Aug2023 NS	11-vii-2022	US:MO	BirdSong Conservation Area, St. Clair Co	R Lardner
13665	431054	F	37.869	-93.708	Aug2023 NS	11-vii-2022	US:MO	BirdSong Conservation Area, St. Clair Co	R Lardner
13671	431058	F	37.869	-93.708	Aug2023 NS	11-vii-2022	US:MO	BirdSong Conservation Area, St. Clair Co	R Lardner
13637	431037	M	37.910	-93.003	Aug2023 NS	12-vi-2022	US:MO	Branch, E. Branch @ RT 7	R Lardner
13638	431038	F	37.910	-93.003	Aug2023 NS	12-vi-2022	US:MO	Branch, E. Branch @ RT 7	R Lardner
13635	431035	F	38.137	-93.325	Aug2023 NS	09-vi-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13641	431041	M	38.137	-93.325	Aug2023 NS	09-vi-2022	US:MO	Granny's Acres Conservation Area	R Lardner

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13658	431049	F	38.137	-93.325	Aug2023 NS	05-viii-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13659	431050	F	38.137	-93.325	Aug2023 NS	09-vi-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13660	431051	F	38.137	-93.325	Aug2023 NS	05-viii-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13662	431052	F	38.137	-93.325	Aug2023 NS	07-ix-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13663	431053	F	38.137	-93.325	Aug2023 NS	05-viii-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13667	431056	F	38.137	-93.325	Aug2023 NS	05-viii-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13626	431062	M	38.137	-93.325	Aug2023 NS	09-vi-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13661	431063	F	38.137	-93.325	Aug2023 NS	05-viii-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13633	431068	M	38.137	-93.325	Aug2023 NS	05-viii-2022	US:MO	Granny's Acres Conservation Area	R Lardner
13625	431030	M	37.853	-92.910	Aug2023 NS	11-vi-2022	US:MO	Lead Mine Conservation Area Frst RD	R Lardner
13627	431031	M	37.853	-92.910	Aug2023 NS	11-vi-2022	US:MO	Lead Mine Conservation Area Frst RD	R Lardner
13645	431042	F	37.844	-90.972	Aug2023 NS	14-vi-2022	US:MO	Marktwain, Frst Palmer RD	R Lardner
13646	431043	F	37.844	-90.972	Aug2023 NS	14-vi-2022	US:MO	Marktwain, Frst Palmer RD	R Lardner
13648	431044	F	37.844	-90.972	Aug2023 NS	14-vi-2022	US:MO	Marktwain, Frst Palmer RD	R Lardner
13650	431045	F	37.989	-93.101	Aug2023 NS	12-vii-2022	US:MO	Mule Shoe Conservation Area, Hickory Co	R Lardner
13628	431065	F	37.989	-93.101	Aug2023 NS	12-vii-2022	US:MO	Mule Shoe Conservation Area, Hickory Co	R Lardner
13668	431066	F	37.989	-93.101	Aug2023 NS	12-vii-2022	US:MO	Mule Shoe Conservation Area, Hickory Co	R Lardner
13676	431061	M	37.965	-93.998	Aug2023 NS	07-viii-2022	US:MO	N of El Durado springs Hwy H x Hwy 0	JR Dupuis
13675	431067	M	37.965	-93.998	Aug2023 NS	07-viii-2022	US:MO	N of El Durado springs Hwy H x Hwy 0	JR Dupuis
13636	431036	M	37.817	-91.940	Aug2023 NS	14-vi-2022	US:MO	Route AA	R Lardner
13639	431039	F	37.817	-91.940	Aug2023 NS	14-vi-2022	US:MO	Route AA	R Lardner
13640	431040	F	37.817	-91.940	Aug2023 NS	14-vi-2022	US:MO	Route AA	R Lardner
13670	431057	F	38.134	-93.325	Aug2023 NS	06-viii-2022	US:MO	S. of Granny's Acres	R Lardner
13666	431055	F	38.313	-93.263	Aug2023 NS	10-vii-2022	US:MO	Warren, Dural Cr RD	R Lardner
13652	431046	F	38.314	-93.263	Aug2023 NS	10-07-2022	US:MO	Warsaw, Duran Cr RD	R Lardner
13651	431064	F	38.314	-93.263	Aug2023 NS	10-07-2022	US:MO	Warsaw, Duran Cr RD	R Lardner
13630	431032	M	38.246	-93.310	Aug2023 NS	10-vi-2022	US:MO	Warsaw, Dwyer RD	R Lardner
13631	431033	M	38.246	-93.310	Aug2023 NS	10-vi-2022	US:MO	Warsaw, Dwyer RD	R Lardner
13632	431034	M	38.246	-93.310	Aug2023 NS	10-vi-2022	US:MO	Warsaw, Dwyer RD	R Lardner
13674	431060	F	38.303	-93.355	Aug2023 NS	10-06-2022	US:MO	Warsaw. Sterett Cr, Village DR	R Lardner
13722	431098	F	46.484	-111.867	Aug2023 NS	21-viii-2022	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13721	431111	F	46.484	-111.867	Aug2023 NS	21-viii-2022	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13708	431113	F	46.484	-111.867	Aug2023 NS	21-viii-2022	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13733	431114	F	46.484	-111.867	Aug2023 NS	21-viii-2022	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13707	431119	F	46.484	-111.867	Aug2023 NS	21-viii-2022	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13715	431120	F	46.484	-111.867	Aug2023 NS	21-viii-2022	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13773	431121	M	46.484	-111.867	Aug2023 NS	21-viii-2022	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13719	431133	F	46.484	-111.867	Aug2023 NS	22-viii-2021	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13736	431138	M	46.484	-111.867	Aug2023 NS	22-viii-2021	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13728	431146	M	46.484	-111.867	Aug2023 NS	22-viii-2021	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13709	431155	F	46.484	-111.867	Aug2023 NS	22-viii-2021	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage

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13710	431156	M	46.484	-111.867	Aug2023 NS	22-viii-2021	US:MT	Elkhorn Wildlife Mgmt area, Helena. McClellan Cr, Jefferson Co	E Gage & R Gage
13687	431127	M	45.550	-110.056	Aug2023 NS	24-vii-2022	US:MT	Gallatin Nat Frst, Hyalite Canyon Rd, Gallatin Co	E Gage & R Gage
13683	431153	M	45.550	-110.056	Aug2023 NS	24-viii-2022	US:MT	Gallatin Nat Frst, Hyalite Canyon Rd, Gallatin Co	E Gage & R Gage
13692	431154	M	45.550	-110.056	Aug2023 NS	24-viii-2022	US:MT	Gallatin Nat Frst, Hyalite Canyon Rd, Gallatin Co	E Gage & R Gage
*12260	397636	M	44.948	-111.856	Feb2019 NS	30-vii-2018	US:MT	Monument Ridge, Madison Co	E Gage & R Gage
*12256	397632	M	44.593	-111.801	Feb2019 NS	30-vii-2018	US:MT	Red Rocks Nat Wildlife Refuge, Beaverhead Co	E Gage & R Gage
*12258	397634	M	44.593	-111.801	Feb2019 NS	30-vii-2018	US:MT	Red Rocks Nat Wildlife Refuge, Beaverhead Co	E Gage & R Gage
12259	397635	M	44.593	-111.801	Feb2019 NS	06-viii-2018	US:MT	Red Rocks Nat Wildlife Refuge, Beaverhead Co	E Gage & R Gage
12262	397637	F	44.593	-111.801	Feb2019 NS	30-vii-2018	US:MT	Red Rocks Nat Wildlife Refuge, Beaverhead Co	E Gage & R Gage
*12255	397631	M	44.593	-111.801	Feb2019 NS	30-vii-2018	US:MT	Red Rocks Nat Wildlife Refuge, Beaverhead Co	E Gage & R Gage
12257	397633	M	44.593	-111.801	Feb2019 NS	30-vii-2018	US:MT	Red Rocks Nat Wildlife Refuge, Beaverhead Co	E Gage & R Gage
*12263	397638	M	44.593	-111.801	Feb2019 NS	30-vii-2018	US:MT	Red Rocks Nat Wildlife Refuge, Beaverhead Co	E Gage & R Gage
13771	431123	M	46.194	-110.521	Aug2023 NS	23-viii-2022	US:MT	Smith Cr Rd Buillie Butte, Meagher Co	E Gage & R Gage
13769	431131	M	46.194	-110.521	Aug2023 NS	23-viii-2022	US:MT	Smith Cr Rd Buillie Butte, Meagher Co	E Gage & R Gage
13777	431135	M	46.194	-110.521	Aug2023 NS	23-viii-2022	US:MT	Smith Cr Rd Buillie Butte, Meagher Co	E Gage & R Gage
13779	431137	M	46.194	-110.521	Aug2023 NS	23-viii-2022	US:MT	Smith Cr Rd Buillie Butte, Meagher Co	E Gage & R Gage
13778	431117	M	44.078	-107.325	Aug2023 NS	14-vii-2022	US:MT	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13760	431109	M	46.769	-111.646	Aug2023 NS	23-viii-2022	US:MT	Trout Cr Cyn TH, Big Belt Mtns, Lewis & Clark Nat Frst, Lewis & Clark Co	E Gage & R Gage
13781	431110	M	46.769	-111.646	Aug2023 NS	23-viii-2022	US:MT	Trout Cr Cyn TH, Big Belt Mtns, Lewis & Clark Nat Frst, Lewis & Clark Co	E Gage & R Gage
13764	431112	F	46.769	-111.646	Aug2023 NS	23-viii-2022	US:MT	Trout Cr Cyn TH, Big Belt Mtns, Lewis & Clark Nat Frst, Lewis & Clark Co	E Gage & R Gage
13758	431115	F	46.769	-111.646	Aug2023 NS	23-viii-2022	US:MT	Trout Cr Cyn TH, Big Belt Mtns, Lewis & Clark Nat Frst, Lewis & Clark Co	E Gage & R Gage
13772	431116	F	46.769	-111.646	Aug2023 NS	23-viii-2022	US:MT	Trout Cr Cyn TH, Big Belt Mtns, Lewis & Clark Nat Frst, Lewis & Clark Co	E Gage & R Gage
13774	431118	F	46.769	-111.646	Aug2023 NS	23-viii-2022	US:MT	Trout Cr Cyn TH, Big Belt Mtns, Lewis & Clark Nat Frst, Lewis & Clark Co	E Gage & R Gage
13784	431122	F	46.769	-111.646	Aug2023 NS	23-viii-2022	US:MT	Trout Cr Cyn TH, Big Belt Mtns, Lewis & Clark Nat Frst, Lewis & Clark Co	E Gage & R Gage
13783	431132	M	46.769	-111.646	Aug2023 NS	23-viii-2022	US:MT	Trout Cr Cyn TH, Big Belt Mtns, Lewis & Clark Nat Frst, Lewis & Clark Co	E Gage & R Gage
13748	431075	F	42.409	-102.462	Aug2023 NS	16-vii-2022	US:NE	Smith Lk, State Wildlife Mgmt area, Sheridan Co	E Gage & R Gage
13747	431072	M	42.731	-103.842	Aug2023 NS	16-vii-2022	US:NE	Sowbelly Canyon, Sioux Co	E Gage & R Gage
13744	431074	M	42.731	-103.842	Aug2023 NS	16-vii-2022	US:NE	Sowbelly Canyon, Sioux Co	E Gage & R Gage
13745	431076	M	42.731	-103.842	Aug2023 NS	16-vii-2022	US:NE	Sowbelly Canyon, Sioux Co	E Gage & R Gage
13743	431077	M	42.731	-103.842	Aug2023 NS	16-vii-2022	US:NE	Sowbelly Canyon, Sioux Co	E Gage & R Gage
13742	431078	M	42.731	-103.842	Aug2023 NS	16-vii-2022	US:NE	Sowbelly Canyon, Sioux Co	E Gage & R Gage
13746	431079	M	42.731	-103.842	Aug2023 NS	16-vii-2022	US:NE	Sowbelly Canyon, Sioux Co	E Gage & R Gage
13751	431080	M	42.731	-103.842	Aug2023 NS	16-vii-2022	US:NE	Sowbelly Canyon, Sioux Co	E Gage & R Gage
13752	431082	M	42.731	-103.842	Aug2023 NS	16-vii-2022	US:NE	Sowbelly Canyon, Sioux Co	E Gage & R Gage
12312	397681	M	35.803	-105.437	Jan2020 NS	9-viii-2019	US:NM	Beulah, End of pond road, San Miguel Co	E Gage & R Gage
12313	397682	M	35.803	-105.437	Jan2020 NS	9-viii-2019	US:NM	Beulah, End of pond road, San Miguel Co	E Gage & R Gage
12308	397677	M	35.803	-105.437	Jan2020 NS	9-viii-2019	US:NM	Beulah, End of pond road, San Miguel Co	E Gage & R Gage
12309	397678	M	35.803	-105.437	Jan2020 NS	9-viii-2019	US:NM	Beulah, End of pond road, San Miguel Co	E Gage & R Gage
12310	397679	M	35.803	-105.437	Jan2020 NS	9-viii-2019	US:NM	Beulah, End of pond road, San Miguel Co	E Gage & R Gage
12311	397680	M	35.803	-105.437	Jan2020 NS	9-viii-2019	US:NM	Beulah, End of pond road, San Miguel Co	E Gage & R Gage
13753	431149	M	41.776	-118.604	Aug2023 NS	07-vii-2022	US:NV	Alta Cr, Humbolt Co	E Gage & R Gage

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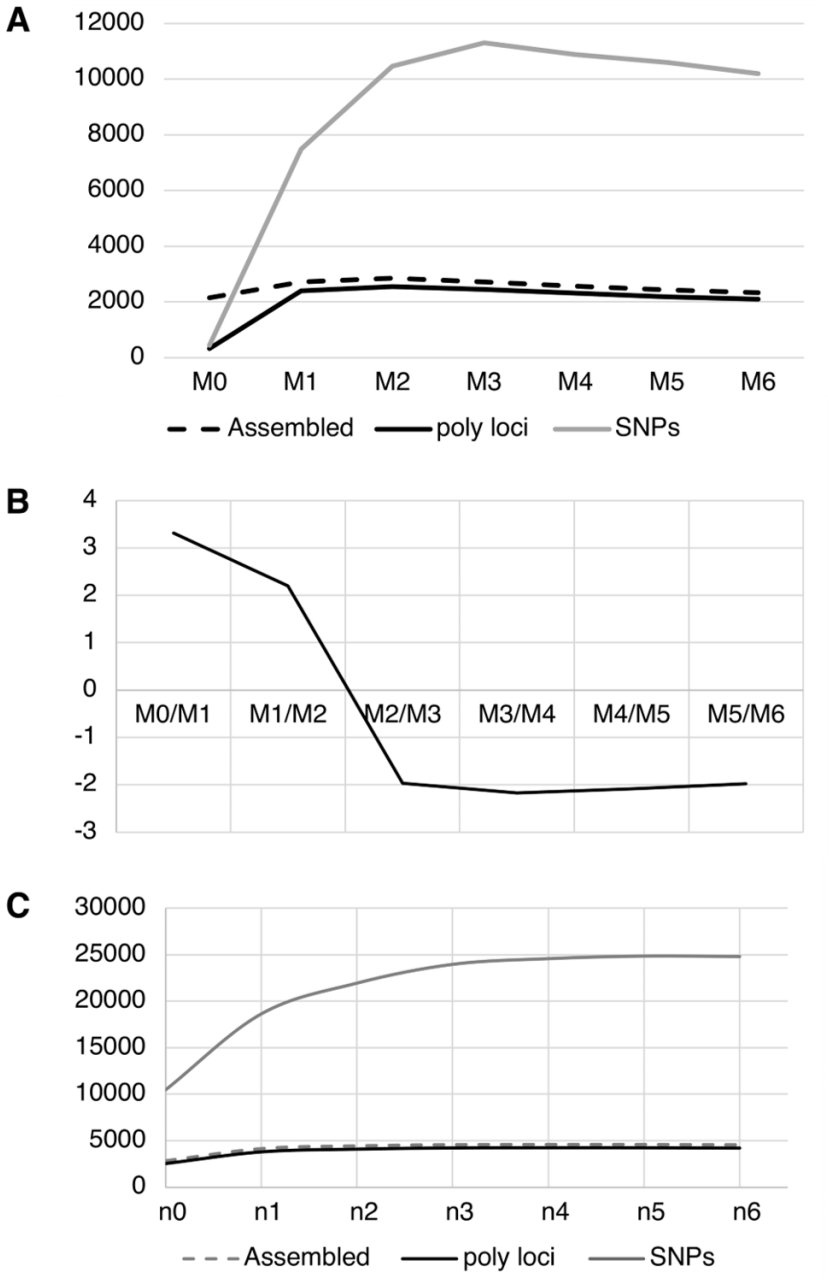
13759	431152	M	41.776	-118.604	Aug2023 NS	07-vii-2022	US:NV	Alta Cr, Humbolt Co	E Gage & R Gage
13756	431175	M	41.776	-118.604	Aug2023 NS	07-vii-2022	US:NV	Alta Cr, Humbolt Co	E Gage & R Gage
13757	431176	M	41.776	-118.604	Aug2023 NS	07-vii-2022	US:NV	Alta Cr, Humbolt Co	E Gage & R Gage
13775	431177	M	41.776	-118.604	Aug2023 NS	07-vii-2022	US:NV	Alta Cr, Humbolt Co	E Gage & R Gage
*10248	397134	F	41.760	-118.540	July2016 NS	30-vii-2014	US:NV	Alta Cr, Humbolt Co	E Gage
13762	431027	M	35.671	-95.135	Aug2023 NS	14-ix-2022	US:OK	Camp Gruber, Cookson Hills, Muskogee Co	E Gage & R Gage
13767	431028	F	35.671	-95.135	Aug2023 NS	14-ix-2022	US:OK	Camp Gruber, Cookson Hills, Muskogee Co	E Gage & R Gage
13705	431147	M	42.513	-119.691	Aug2023 NS	20-viii-2022	US:OR	Hart mountain hot springs Cpg, N along Rock creek, Lake Co	E Gage & R Gage
13690	431158	M	42.513	-119.691	Aug2023 NS	20-viii-2022	US:OR	Hart mountain hot springs Cpg, N along Rock creek, Lake Co	E Gage & R Gage
13703	431159	M	42.513	-119.691	Aug2023 NS	20-viii-2022	US:OR	Hart mountain hot springs Cpg, N along Rock creek, Lake Co	E Gage & R Gage
13686	431157	M	44.191	-119.189	Aug2023 NS	20-viii-2022	US:OR	Malheur Nat Frst, 9 mi W. of Hwy 395 on Hwy 163. Grant Co	E Gage & R Gage
13734	431148	F	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13727	431150	F	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13706	431160	F	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13723	431161	F	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13725	431162	M	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13729	431163	F	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13731	431164	M	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13732	431165	F	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13735	431166	M	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
13737	431167	F	44.234	-119.738	Aug2023 NS	20-viii-2022	US:OR	Ochoco Nat Frst, Rager Ranger Sta, USDA Agri center, Crook Co	E Gage & R Gage
12290	397659	M	44.827	-123.417	Jan2020 NS	27-vii-2019	US:OR	Stott Mt. Grant Cr, Josiah Wills Rd, Polk Co	E Gage & R Gage
12291	397660	M	44.827	-123.417	Jan2020 NS	27-vii-2019	US:OR	Stott Mt. Grant Cr, Josiah Wills Rd, Polk Co	E Gage & R Gage
12292	397661	M	44.827	-123.417	Jan2020 NS	27-vii-2019	US:OR	Stott Mt. Grant Cr, Josiah Wills Rd, Polk Co	E Gage & R Gage
12293	397662	M	44.827	-123.417	Jan2020 NS	27-vii-2019	US:OR	Stott Mt. Grant Cr, Josiah Wills Rd, Polk Co	E Gage & R Gage
*10304	397186	M	44.837	-123.413	2014 HS	21-viii-2014	US:OR	Stott Mtn, Grant Cr 0.8 mi down Josiah Wills Rd, Polk Co	E Gage
*11377	397523	M	38.017	-109.488	Aug2017 NS	14-vii-2017	US:UT	Abajo Mts., San Juan Co	E Gage & R Gage
11378	397524	M	38.017	-109.488	Aug2017 NS	14-vii-2017	US:UT	Abajo Mts., San Juan Co	E Gage & R Gage
*10854	397454	M	39.507	-111.859	July2016 NS	5-viii-2014	US:UT	Deep Canyon, 6.4 miles S of Levan, Juab Co	E Gage & R Gage
*10255	397141	M	39.507	-111.859	2014 HS	5-viii-2014	US:UT	Deep Canyon, 6.4 miles S of Levan, Juab Co	E Gage & R Gage
10256	397142	M	39.507	-111.859	2014 HS	5-viii-2014	US:UT	Deep Canyon, 6.4 miles S of Levan, Juab Co	E Gage & R Gage
10257	397143	M	39.507	-111.859	2014 HS	5-viii-2014	US:UT	Deep Canyon, 6.4 miles S of Levan, Juab Co	E Gage & R Gage
10258	397144	M	39.507	-111.859	2014 HS	5-viii-2014	US:UT	Deep Canyon, 6.4 miles S of Levan, Juab Co	E Gage & R Gage
*10855	397455	M	39.507	-111.859	July2016 NS	5-viii-2014	US:UT	Deep Canyon, 6.4 miles S of Levan, Juab Co	E Gage & R Gage
*10856	397456	M	39.507	-111.859	July2016 NS	5-viii-2014	US:UT	Deep Canyon, 6.4 miles S of Levan, Juab Co	E Gage & R Gage
10824	397426	M	40.204	-110.969	July2016 NS	28-vi-2015	US:UT	Deep Cr Canyon, Wasatch Co	E Gage & R Gage
10262	397147	F	38.450	-109.240	2014 HS	16-viii-2014	US:UT	La Sal Mts, Grand Co	E Gage
10263	397148	M	38.450	-109.240	Feb2016 NS	16-viii-2014	US:UT	La Sal Mts, Grand Co	E Gage
*10264	397149	M	38.450	-109.240	2014 HS	16-viii-2014	US:UT	La Sal Mts, Grand Co	E Gage
*10265	397150	M	38.450	-109.240	2014 HS	16-viii-2014	US:UT	La Sal Mts, Grand Co	E Gage
*10865	397465	M	38.591	-109.265	Aug2017 NS	13-vii-2016	US:UT	LaSal Mts, Castle creek, Grand Co	E Gage

DNA#	UASM	S	lat	lon	SNP Seq	Coll Date	Region	Locality	Collector(s)
13718	431144	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13711	431178	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13712	431179	F	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13713	431180	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13716	431181	F	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13717	431182	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13720	431183	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13724	431184	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13726	431185	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13730	431186	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
13714	431151	M	40.721	-111.658	Aug2023 NS	15-viii-2022	US:UT	Lambs canyon, Salt Lake Co	E Gage & R Gage
*10266	397151	M	40.530	-112.320	2014 HS	2-viii-2014	US:UT	Middle canyon, Tooele, Tooele Co	E Gage
*10267	397152	M	40.530	-112.320	2014 HS	2-viii-2014	US:UT	Middle canyon, Tooele, Tooele Co	E Gage
10832	397432	M	40.629	-111.197	July2016 NS	15-vii-2014	US:UT	Ponderosa group Cpg, Wasatch Nat Frst	E Campbell & J Lee
10833	397433	M	40.629	-111.197	July2016 NS	15-vii-2014	US:UT	Ponderosa group Cpg, Wasatch Nat Frst	E Campbell & J Lee
*10206	397095	M	41.530	-111.510	2014 HS	18-vii-2014	US:UT	Wasatch Nat Frst	E Campbell & J Lee
10212	397101	M	41.530	-111.510	July2016 NS	18-vii-2014	US:UT	Wasatch Nat Frst	E Campbell & J Lee
13680	431070	M	36.638	-81.607	Aug2023 NS	06-vi-2022	US:VA	W AA Wardlaw, Whitetop Mtn	A Roe
*10872	397472	M	48.453	-117.932	Aug2017 NS	29-vii-2016	US:WA	Jct. of Cole Cr and Hollar Cr on Hollar Cr road, Stevens Co	E Gage
*10869	397469	M	48.453	-117.932	Aug2017 NS	29-vii-2016	US:WA	Jct. of Cole Cr and Hollar Cr on Hollar Cr road, Stevens Co	E Gage
*10870	397470	M	48.453	-117.932	Aug2017 NS	29-vii-2016	US:WA	Jct. of Cole Cr and Hollar Cr on Hollar Cr road, Stevens Co	E Gage
*10871	397471	M	48.453	-117.932	Aug2017 NS	29-vii-2016	US:WA	Jct. of Cole Cr and Hollar Cr on Hollar Cr road, Stevens Co	E. Gage
10286	397171	M	48.440	-117.960	2014 HS	24-viii-2014	US:WA	Reidell Cr Rd, Stevens Co	E Gage & R Gage
*10287	397172	M	48.440	-117.960	2014 HS	24-viii-2014	US:WA	Reidell Cr Rd, Stevens Co	E Gage & R Gage
*10868	397468	M	48.453	-117.932	Aug2017 NS	29-vii-2016	US:WA	Reidell Cr Rd, Stevens Co	E Gage
*10817	397419	M	44.480	-89.500	July2016 NS	21-vi-2015	US:WI	Buena Vista Grassland, Portage Co	W Anderson
10818	397420	M	44.480	-89.500	July2016 NS	21-vi-2015	US:WI	Buena Vista Grassland, Portage Co	W Anderson
10814	397416	M	44.480	-89.500	July2016 NS	21-vi-2015	US:WI	Buena Vista Grassland, Portage Co	W Anderson
10819	397421	M	44.480	-89.500	July2016 NS	27-vi-2015	US:WI	Buena Vista Grassland, Portage Co	W Anderson
10815	397417	M	44.480	-89.500	July2016 NS	21-vi-2015	US:WI	Buena Vista Grassland, Portage Co	W Anderson
10820	397422	M	44.480	-89.500	July2016 NS	27-vi-2015	US:WI	Buena Vista Grassland, Portage Co	W Anderson
10816	397418	M	44.480	-89.500	July2016 NS	21-vi-2015	US:WI	Buena Vista Grassland, Portage Co	W Anderson
13738	431189	M	41.093	-107.162	Aug2023 NS	19-vii-2022	US:WY	Battleground Cpg, frst rd 807. Medicine Bow-Routt Nat Frst, Carbon Co	E Gage & R Gage
13739	431190	M	41.093	-107.162	Aug2023 NS	19-vii-2022	US:WY	Battleground Cpg, frst rd 807. Medicine Bow-Routt Nat Frst, Carbon Co	E Gage & R Gage
13741	431191	M	41.093	-107.162	Aug2023 NS	19-vii-2022	US:WY	Battleground Cpg, frst rd 807. Medicine Bow-Routt Nat Frst, Carbon Co	E Gage & R Gage
13750	431192	M	41.093	-107.162	Aug2023 NS	19-vii-2022	US:WY	Battleground Cpg, frst rd 807. Medicine Bow-Routt Nat Frst, Carbon Co	E Gage & R Gage
13763	431193	M	41.093	-107.162	Aug2023 NS	19-vii-2022	US:WY	Battleground Cpg, frst rd 807. Medicine Bow-Routt Nat Frst, Carbon Co	E Gage & R Gage
13740	431194	M	41.093	-107.162	Aug2023 NS	19-vii-2022	US:WY	Battleground Cpg, frst rd. 807 Medicine Bow-Routt Nat Frst, Carbon Co	E Gage & R Gage
13700	431188	M	41.576	-106.231	Aug2023 NS	18-vii-2022	US:WY	Medicine Bow Nat Frst, Rock Cr Trail No. 106, Carbon Co	E Gage & R Gage
13776	431093	M	44.481	-104.119	Aug2023 NS	15-vii-2022	US:WY	Dugout gulch botanical trail, Crook Co	E Gage & R Gage
13782	431094	M	44.481	-104.119	Aug2023 NS	15-vii-2022	US:WY	Dugout gulch botanical trail, Crook Co	E Gage & R Gage

DNA#	UASM	S	lat	lon	SNP Seq	Coll Date	Region	Locality	Collector(s)
13765	431081	M	44.481	-104.119	Aug2023 NS	15-vii-2022	US:WY	Sand Cr, Beulah, Crook Co	E Gage & R Gage
13754	431124	M	44.078	-107.325	Aug2023 NS	14-vii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13766	431125	M	44.078	-107.325	Aug2023 NS	14-vii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13689	431126	M	44.078	-107.325	Aug2023 NS	14-vii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13755	431129	M	44.078	-107.325	Aug2023 NS	14-vii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13770	431130	M	44.078	-107.325	Aug2023 NS	14-vii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13688	431134	M	44.078	-107.325	Aug2023 NS	26-viii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13780	431136	M	44.078	-107.325	Aug2023 NS	14-vii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13761	431139	M	44.078	-107.325	Aug2023 NS	14-vii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13697	431140	M	44.078	-107.325	Aug2023 NS	26-viii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13702	431143	M	44.078	-107.325	Aug2023 NS	26-viii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13691	431145	F	44.078	-107.325	Aug2023 NS	26-viii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
13696	431187	M	44.078	-107.325	Aug2023 NS	26-viii-2022	US:WY	Ten Sleep canyon, Leigh creek, Fish hatchery rd., Washakie Co	E Gage & R Gage
#10682	397305	M	35.860	-86.660	July2016 NS	2015	US:TN	Reared	D McCorkle via E Gage & R Gage
!10340	397211	M	46.840	-110.700	Feb2016 NS	23-vii-2014	US:MT	Little Belt Mtns, Hwy 89 mile 53	FAH & T Sperling, & S Ferguson

Symbols "!"S. aphrodite and "#" S. diana were outgroups used for the phylogenetic analysis. Ns refers to NS, and Hs refers to HS

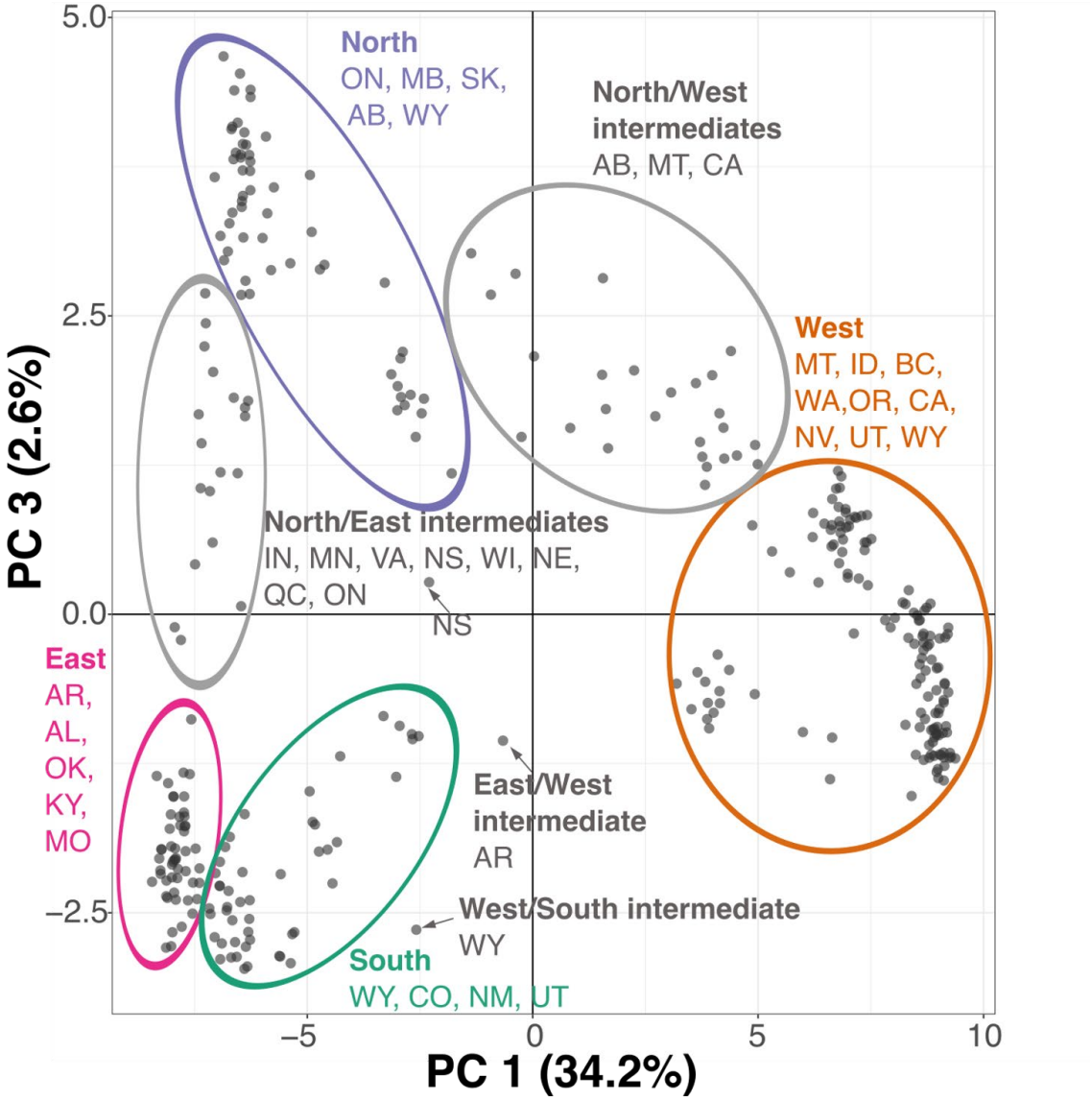
Appendix 2. Parameter testing results for Stacks *de novo* SNP assembly of n=175 specimens. A) All assembled r80 loci, polymorphic loci, and number of SNPs for *M*0 to *M*6, and B) the log modulus transformation (sign(x)*(log10(abs(x)+1)) of the total number of new r80 loci for each increasing increment of *M*, with *M*=2 recovering the highest number of new polymorphic loci. C) Metrics from testing number of assembled r80 loci, polymorphic loci, and number of SNPs for *n*0 to *n*6. Within *n=M, n=M-1*, and *n=M*+1 following Paris et al. (2017), *n*=3 (*n=M*+1) was optimal.



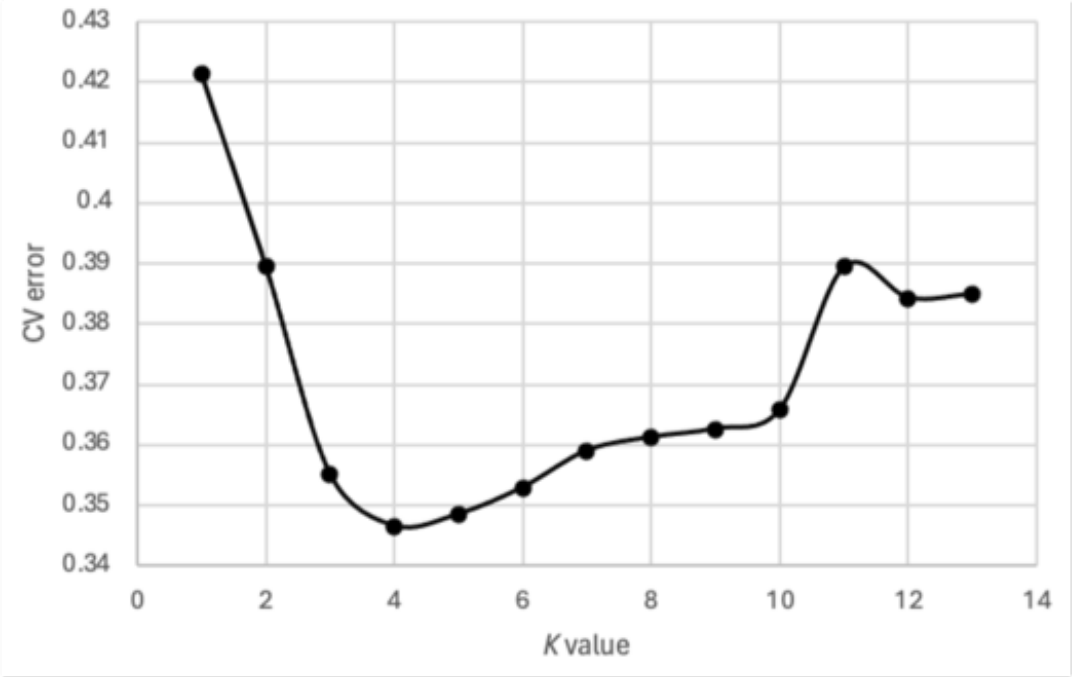
Appendix 3. Depth information for each assembled SNP dataset. Each dataset shows total catalog loci (total_cat), SNPs, mean individual depth (mean_i), individual minimum and maximum depth (i_min/i_max), mean locus depth (mean_l), locus minimum and maximum depth (l_min/l_max), mean missingness (miss_il), individual minimum and maximum missingness (min_i/max_i), and locus minimum and maximum missingness (min_l/max_l).

	n	total_cat	SNPs	mean_i	i_min/i_max	mean_l	l_max/l_min	miss_il	min_i/max_i	min_l/max_l
all	340	430479	6069	59.88	5.3/146.6	57.33	10.3/291.3	0.11	0.01/0.72	0/0.200
East	61	202987	4927	43.60	7.8/409.4	43.40	14.1/74.3	0.09	0.04/0.37	0/0.197
North	75	170605	4047	60.95	5.8/143.6	59.07	10.5/369.0	0.12	0.02/0.64	0/0.200
West	156	231668	2801	60.85	16.0/131.9	58.31	9.7/276.6	0.11	0.01/0.67	0/0.199
South	48	96780	3229	61.78	16.4/166.8	58.65	8.8/328.8	0.09	0.02/0.49	0/0.188
phylo	342	436075	1523	58.36	5.0/140.8	56.66	11.4/166.3	0.12	0.01/0.75	0/0.199

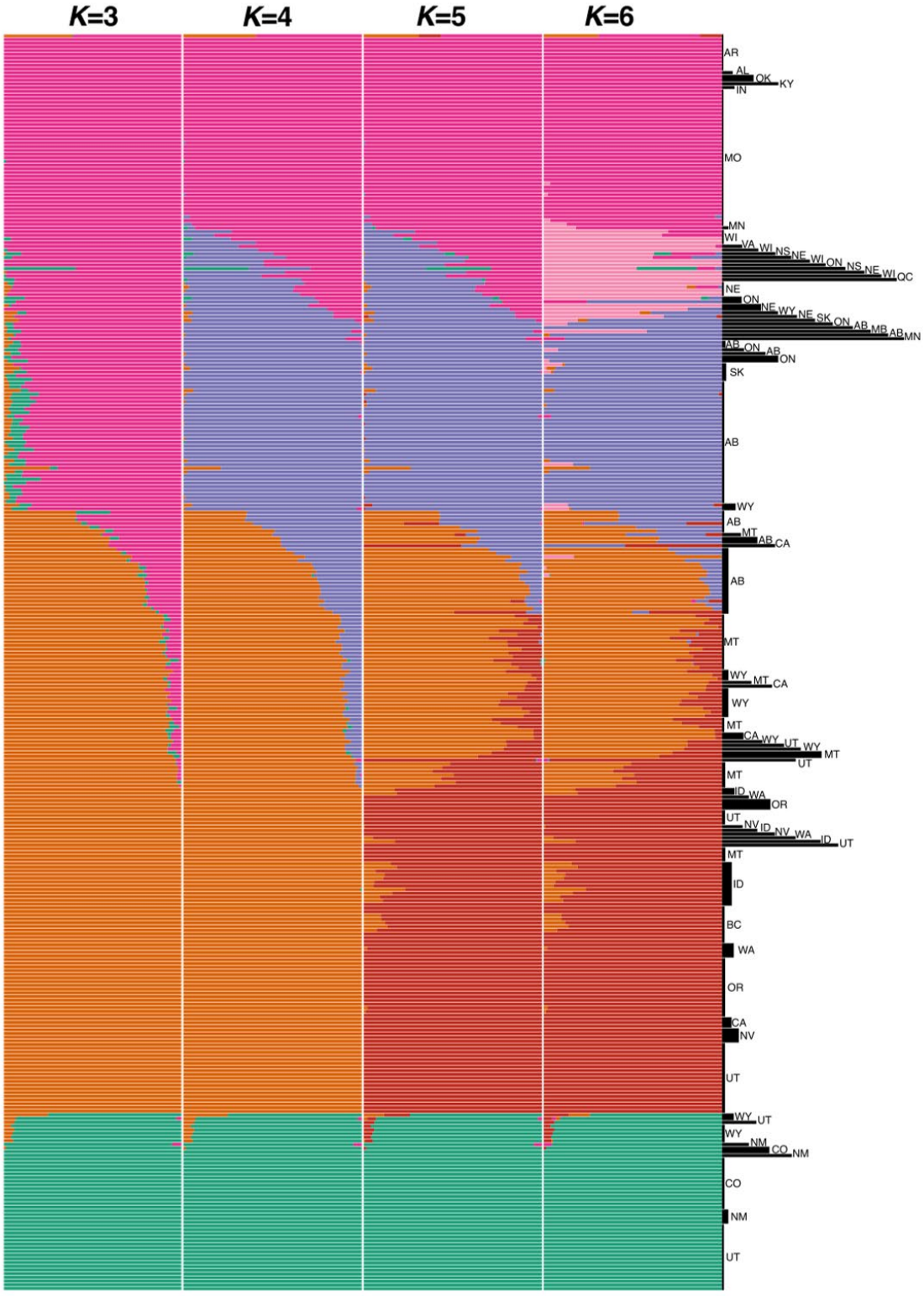
Appendix 4. Principal component analysis of n=340 SNP dataset, axis 1 and axis 3, showing approximate distribution of major clusters from Admixture analysis. Principal component analysis oval groups are approximate and are not confidence intervals, but are groups of convenience with exceptions.



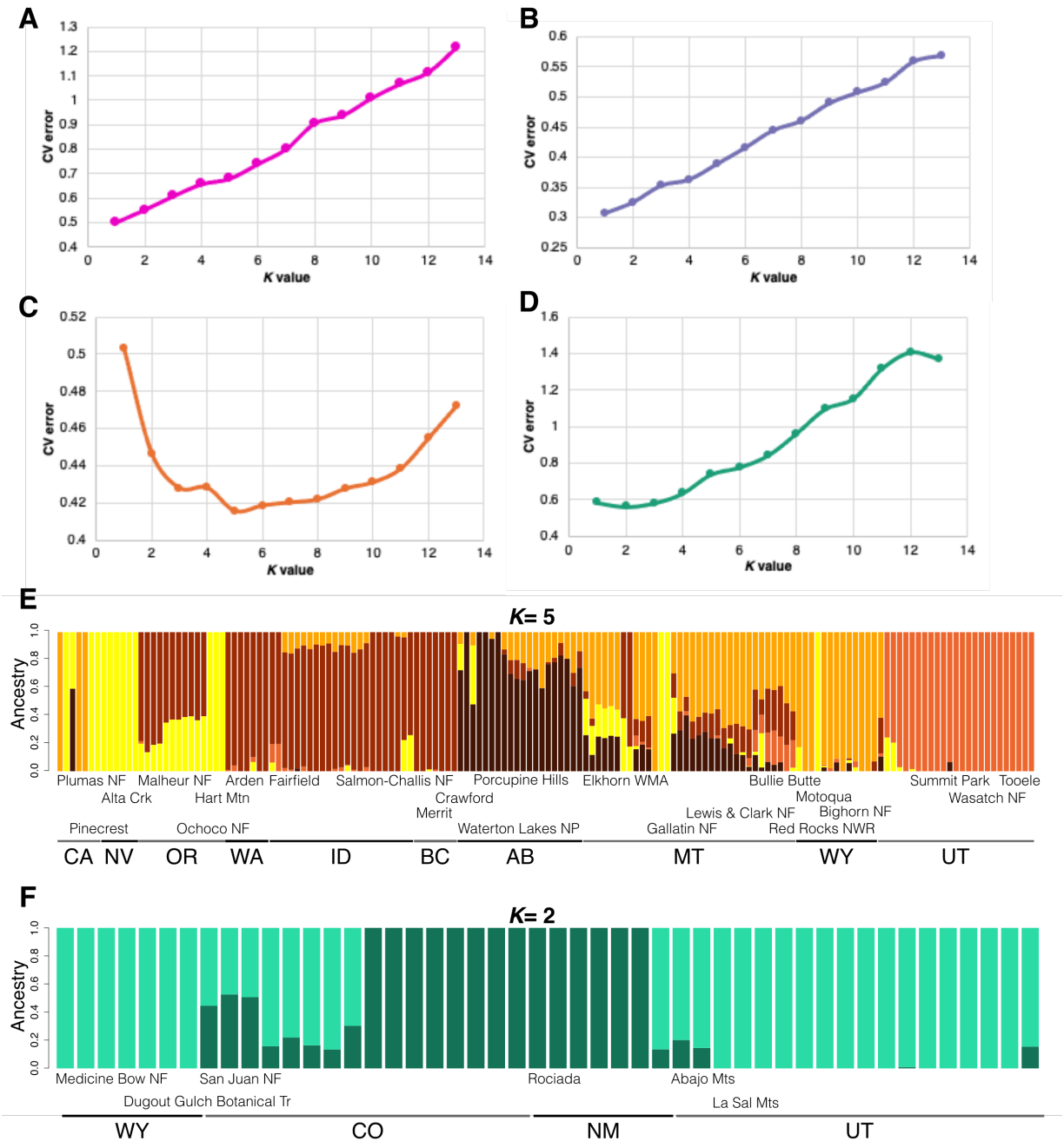
Appendix 5. Plot of ADMIXTURE CV error from *K* =1 to *K* =13. I chose *K*=4 to analyze the SNP data, as the value minimizes the CV error.



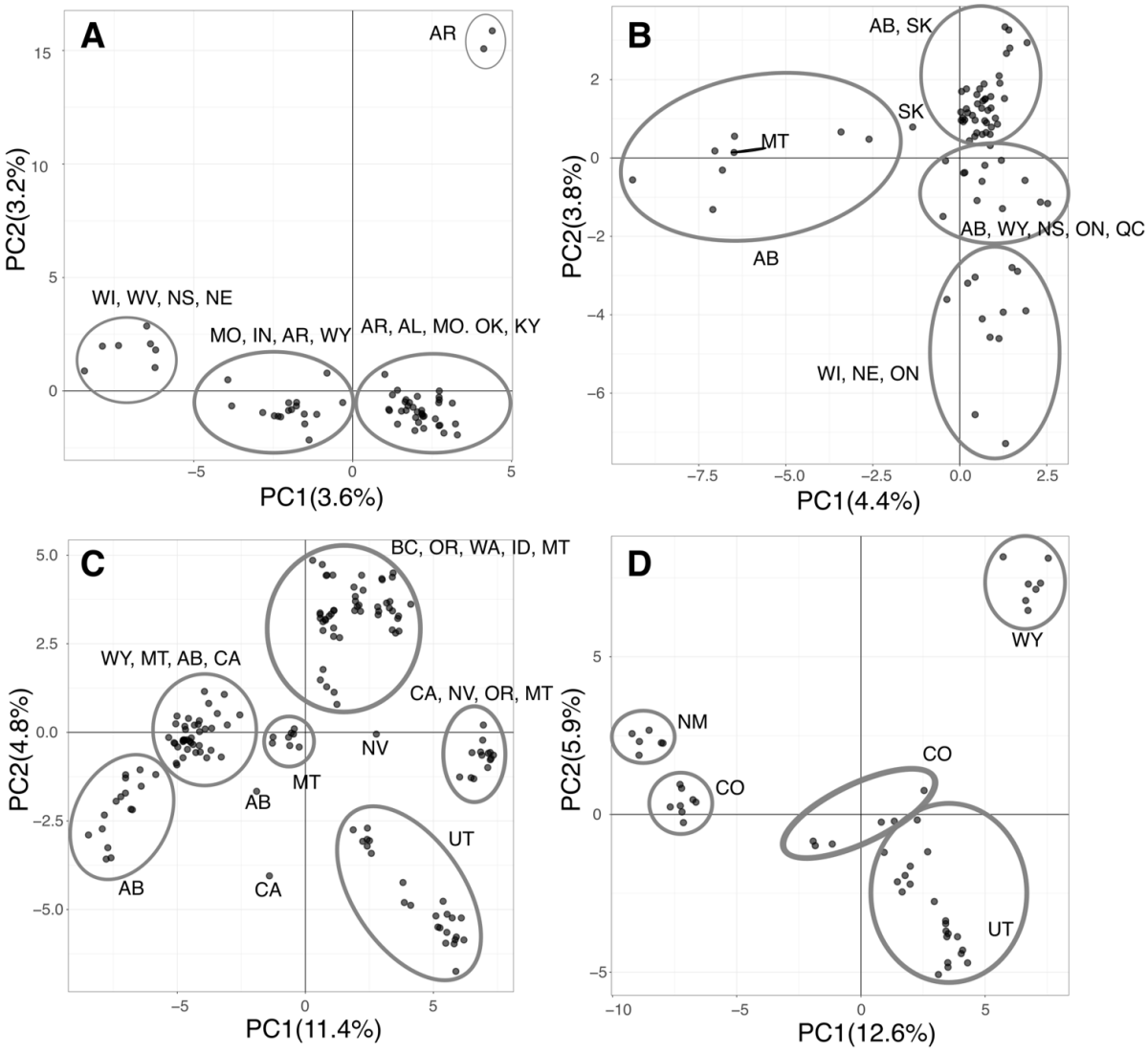
Appendix 6. ADMIXTURE *K*=3 to 6 plots of the n=340 data. The *K*-value 4 *K*=4 was to analyze the SNP data, as the value minimizes the CV error.



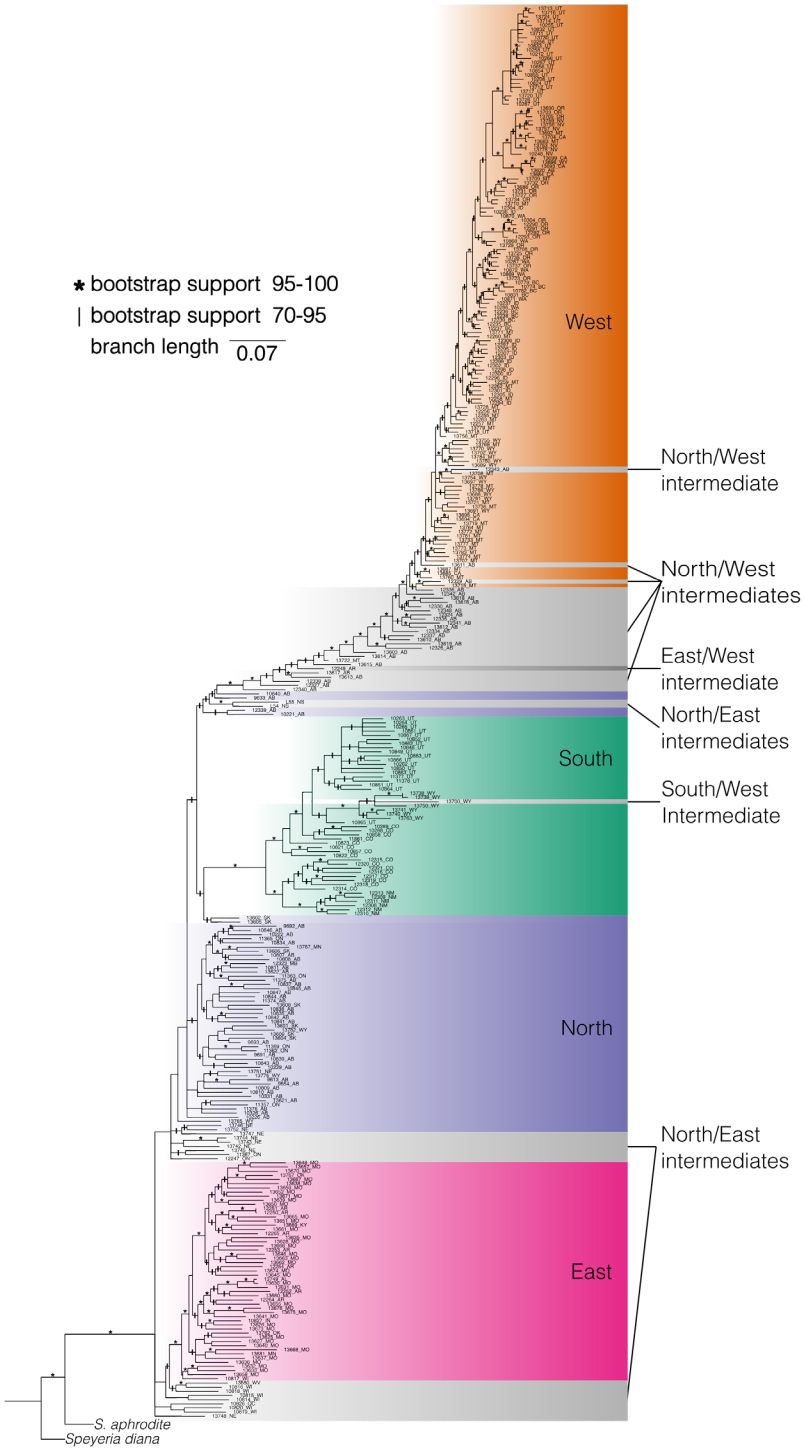
Appendix 7. Plot of ADMIXTURE CV error from *K* =1 to *K* =13 for each SNP cluster A) East (n=61) optimized at *K* =1, B) North (n=75) optimized at *K*=1, C) West (n=156) optimized at *K* =5, and D) South (n=48) optimized at *K* =2. The optimal *K*-value is the one with the minimum CV error. ADMIXTURE analysis of SNP sub-clusters E) West and D) South.



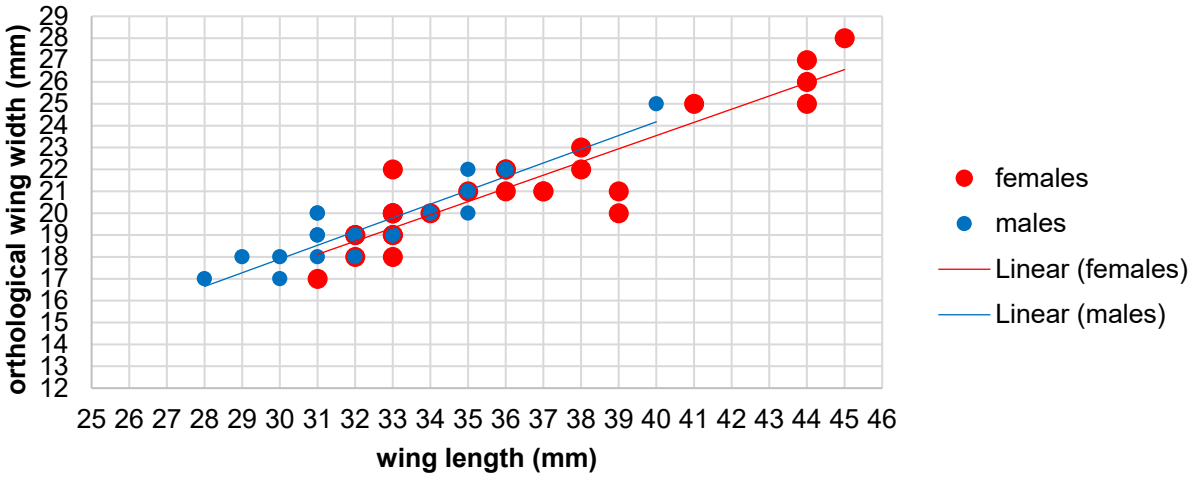
Appendix 8. Principal component analyses of each major SNP subcluster: A) East, B) North, C) West, and D) South.



Appendix 9. Maximum likelihood 50% majority-rule consensus tree of *S. cybele* (n=340) made from 1523 SNPs, with *S. aphrodite* and *S. diana* as outgroups. Specimens are coloured by associated SNP cluster, with admixed clusters indicated by a "/" in the cluster labels.



Appendix 10. Linear regression of wing width and wing length of pinned E. H. Strickland Museum specimens, n=27 females and n=25 males. Females had a Pearson' correlation (r) efficient of 0.915, and r= 0.913 for males. Linear regression shows wing width is a suitable proxy for wing length, however male and female data should be analyzed separately.



Appendix 11. Morphometric character scores and measurements. Wing width (W) and ventral hind wing discal silver spots B1 to B13 are raw measurements in mm.

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
9613	AB	N	F	20	2	2	3.56	0.45	3.72	2.16	1.75	3.16	1.15	1.91	0.85	2.22	0	0.38	0
9633	AB	N	F	20	5	5	1.5	0	4.53	1.53	1.08	3.08	2.32	3.11	1.04	2.03	0	0	0
9654	AB	N	F	20	1	3	3.31	1.19	3.58	1.42	1.88	2.88	2.09	3.16	0.92	0.55	0	0	0
9691	AB	N	M	19	0	0	2.89	0	3.35	1.6	1.77	2.26	0.51	1.61	0	1.31	0.58	0	0
9692	AB	N	F	20	2	2	4.06	0	4.01	2.3	1.14	3.01	1.43	1.46	0.85	2.19	0	0	0
9693	AB	N	M	19	2	0	3.06	1.34	2.53	1.33	1.54	1.76	0.95	1.95	1.1	2.35	0	2.67	0
10206	UT	W	M	21	0	0	2	0.97	2.09	0.99	2	1.47	0	1.63	0	0	0	0	0
10212	UT	W	M	20	0	0	1.19	0	2.3	1.03	0.89	1.81	0.51	1.1	0.37	0	0	0	0
10221	AB	N	M	19	2	0	3.58	1.04	3.22	1.67	0.51	2.19	1.33	1.38	0.91	1.89	0	1.46	0
10222	AB	N	M	20	1	2	3.76	0.47	4.06	1.9	2.55	3.06	1.07	1.94	2.52	1.71	2.18	2.43	0
10226	AB	N	M	19	0	2	3.54	0	2.83	1.64	1.34	2.58	1.48	1.53	0.89	1.57	0	2.61	0
10229	AB	N	M	19	2	2	3.28	0	3.93	1.51	0.91	2.04	1.7	1.62	1.04	1.44	0	0	0
10236	ID	W	M	21	2	0	2.71	0	2.26	0.48	0.61	2.21	0.91	1.49	0.44	0	0	0	0
10237	ID	W	M	21	0	0	2.59	0	2.8	1.14	0	3.04	0.89	1.02	0.43	1.4	0	0	0
10248	NV	W	F	23	3	3	2.17	1.55	2.56	1.6	1.37	3.29	1.22	1.03	1.21	0	0	0	0
10255	UT	W	M	19	0	0	1.15	0	2.63	0.71	0	1.82	0.69	0.78	0	0	0	0	0
10256	UT	W	M	19	0	0	2.22	0	2.41	0.98	0	2.12	0	0	0	0	0	0	0
10257	UT	W	M	20	2	0	2.48	1.3	2.67	1.14	1.6	2.68	1.49	0.74	0	0	0	0	0
10258	UT	W	M	23	0	0	1.44	0.87	1.99	0.77	0	1.42	0.6	1.17	0	0	0	0	0
10262	NM	S	F	20	3	4	3.33	1.45	3.64	1.68	1.83	1.9	1.74	1.79	1.03	0	0	1.63	1.12
10263	NM	S	M	19	1	2	3.34	2.92	3.93	2.06	3.7	1.9	1.38	1.34	0.6	1.51	0	5.24	3.93
10264	UT	S	M	20	1	1	2.94	0	3.12	1.32	1.06	1.03	1.36	1.12	0.92	0	0	0	0
10265	UT	S	M	19	1	1	2.91	0	2.92	1.44	1.15	1.69	0.58	1.02	0.72	1.5	0	0	0
10266	UT	W	M	22	2	1	2.84	1.8	2.6	1.37	1.57	2.05	0	0.99	0	0	0	0	0
10267	UT	W	M	22	0	0	2.44	1.05	2.54	1.15	0.9	0.84	1.4	1.08	0.6	0	0	0	0
10286	WA	W	M	20	2	2	2.81	0	2.08	1.32	0	2.26	0.56	0.74	0	0	0	0	0
10287	WA	W	M	20	0	0	2.83	0	3.35	1.75	0	2.74	1.2	1.37	0.7	0	0	0	0
10288	WY	S	F	22	5	4	3.91	2.03	4.1	1.93	1.52	2.33	0.35	1.51	0.72	1.86	0	0	0
10289	WY	S	F	20	5	5	2.43	1.45	3.48	1.45	0	2.12	1.01	1.23	0.57	0	0	1.31	0

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
10304	OR	W	M	21	0	0	2.69	0.74	2.21	1.15	0.62	3.11	0.73	0.98	0.77	0	0	0	0
10326	AB	N	M	20	2	0	2.71	0	2.26	0.48	0.61	2.21	0.91	1.49	0.44	0	0	0	0
10331	AB	N	M	19	1	2	2.33	0	3.24	1.31	0.76	2	0.92	1.42	1.44	0	0	0	0
10774	BC	W	F	23	5	5	1.57	0	2.24	0.99	2.2	2.21	1.21	1.07	0	0	0	0	0
10779	BC	W	M	21	0	0	2.18	1.3	3.3	1.29	0.77	2.8	1.57	1.46	0.72	0	0	0	0
10782	BC	W	M	21	0	0	2.5	0	3.86	1.3	1.45	1.42	0.96	0.69	0.49	0	0	0	0
10807	AB	N	M	20	0	2	2.29	0	3.42	1.18	0.9	3	1.85	0.81	0.44	2.06	0.96	0	0
10808	AB	N	M	20	0	2	2.39	1.02	3.35	1.49	2.24	2.74	1.47	1.7	0.55	1.68	0	0	0.62
10809	AB	N	M	20	0	1	2.74	0	3.48	1.35	1.72	2.14	1.22	1.67	1	1.64	0	0	0
10810	AB	N	M	21	0	2	2.5	0.72	3.42	1.75	1.26	3	1.36	1.79	0.48	0.84	0	0	0
10811	AB	N	F	20	2	1	2.7	1.93	3.1	2.11	1.35	2.23	1.39	1.99	1.32	0.69	0	1.1	0
10814	WI	N/E	M	23	0	0	4.31	2.11	3.57	2.39	2.83	1.57	1.24	1.97	2.3	0	0	1.73	4.19
10815	WI	N/E	M	23	0	0	3.06	0.64	4.04	1.52	1.63	1.99	0.54	1.66	1.98	2.03	0.35	0	0
10816	WI	N/E	M	22	0	0	3.5	1.4	3.15	1.73	0	2.37	1.27	2.02	1.4	1.87	0	0	0
10817	WI	N/E	M	21	0	0	3.23	0.3	3.08	1.64	1.49	2.38	0.6	1.28	1.64	2.12	0.63	0	0
10818	WI	N/E	M	24	2	0	3.51	0.64	3.51	1.21	0.5	2.63	1.41	1.71	0.7	2.33	0	0	0
10819	WI	N/E	M	23	2	0	3.9	0.59	3.87	2.06	3.11	2.13	1.08	2.66	1.6	0.97	0	1.17	0
10820	WI	N/E	M	23	2	0	3.15	0.39	3.15	1.65	1.14	2.23	1.99	2.2	1.06	0	0	0	0
10821	CO	S	M	18	1	2	2.79	0	3.25	1.81	1.33	2.61	0.65	1.76	0.97	1.25	1.87	1.9	0
10822	CO	S	M	17	2	0	1.72	0	2.64	0.94	0	2.46	1.4	0.95	0.51	0	0	0	0
10823	CO	S	M	19	1	0	2.5	0.6	3.1	1.29	0.76	2.14	0.64	1.36	0.59	1.35	0	0	0
10824	UT	W	M	21	0	0	2.81	0	2.35	1.18	0.28	2.86	1	0.89	0.38	0	0	0	0
10826	QC	N/E	M	20	2	0	3.2	1.24	2.24	2.38	2.28	2.6	0.57	1.53	1.14	1.98	0	0	0
10827	IN	E	M	23	2	0	4.03	1.52	3.33	1.18	1.14	2.59	1.1	1.08	2.4	0	0	0	0
10831	BC	W	M	22	2	0	2.18	1.3	3.3	1.29	0.77	2.8	1.57	1.46	0.72	0	0	0	0
10832	UT	W	M	21	0	0	2.46	0	2.63	1.35	1.25	1.77	1.09	0.72	0.39	0	0	0	0
10833	UT	W	M	20	0	0	2.45	0	2.05	2.47	0	2.39	0.58	0.91	0.31	0	0	0	0
10834	AB	N	F	21	1	1	3.83	2.42	4.05	2.24	2.64	2.34	1.41	3.11	0.91	2.08	1.54	0	0
10835	AB	N	M	16	1	2	3.4	1.46	2.81	1.76	0	2.13	1.83	2.02	1.24	1.75	0	0	0

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
10836	AB	N	M	20	0	0	3.34	1.34	4.05	1.96	1.37	2.48	1.53	1.65	0.97	0	0	0	1.88
10837	AB	N	F	20	1	1	3.64	1.89	3.76	1.78	2.37	2.12	2.09	2.33	1.4	0	0	0	0
10839	AB	N	M	20	1	2	2.26	0	3.37	1.26	0	1.51	1.96	1.65	0	0.89	0	0	0
10840	AB	N	M	20	2	0	2.29	1.54	3.2	1.92	2.55	1.77	0.89	1.51	1.38	0.66	0	2.37	3.49
10841	AB	N	F	21	2	0	3	1.74	3.78	0.61	2.04	3.02	0.83	2.9	1.17	2.2	0.89	0	2.06
10842	AB	N	M	17	2	2	1.46	0	2.99	1.38	1.17	2.35	1.74	1.08	0.89	1.37	0	0	0
10843	AB	N	M	18	2	2	1.83	0	3.71	1.2	0.93	2.13	0.92	1.65	0.95	2.26	0	2.41	0
10844	AB	N	M	19	0	0	3.18	1.43	2.86	1.29	1.16	2.55	0.85	2.37	1.44	2.21	1.17	0	0
10845	AB	N	M	19	2	0	2.51	0.83	3.62	1.42	0.94	1.99	1.17	1.36	0.78	1.57	0	0	0
10846	AB	N	M	20	2	0	3.21	0	4.16	1.89	1.29	1.83	0.27	2.15	1.49	1.65	0	0	0
10847	AB	N	M	18	2	0	2.3	0.8	3.06	1.33	1.57	2.14	2.29	1.53	1.04	0.77	0	0	0
10848	UT	S	M	18	2	0	2	0.68	3.34	1.2	1.54	1.91	1.08	0.92	0	1.1	0	0	0
10849	UT	S	M	17	1	1	1.81	0	2.77	1.27	0.96	1.83	1.18	0.7	0.48	1.25	0	0	0
10850	UT	S	M	19	1	1	3.07	0	3.71	1.5	1.73	2.69	1.62	1.95	0.9	0	0	0	0
10851	UT	S	M	18	1	2	2.9	1.47	3.62	1.4	1.53	2.37	1	0.56	0.86	0.61	0	0	0
10852	UT	S	M	18	2	0	1.67	0	2.66	1.31	1.71	1.63	1.24	1.13	0	1.01	0	0	0
10853	UT	S	M	19	2	0	2.36	2.61	3.2	1.88	1.82	1.38	1.25	1.64	0	0	0	0	0
10854	UT	W	M	21	0	0	1.54	0	2.45	0.63	0	1.64	0	0.81	0	0	0	0	0
10855	UT	W	M	21	0	0	1.19	0	2.95	1.18	0	2.11	0.61	1.14	0.41	0	0	0	0
10856	UT	W	M	19	0	0	2.84	0	2.33	1.03	0.83	2.1	0.75	0.85	0	0	0	0	0
10857	CO	S	M	19	2	0	2.44	0	3.12	1.67	1.07	1.43	1.26	0.44	0	0	0	0	0
10858	CO	S	F	22	4	4	3.18	1.76	3.61	2.32	2.03	2.51	2.01	1.57	0	0	0	0	0
10861	UT	S	M	20	2	2	3.47	2.48	3.55	1.86	1.75	1.7	1.59	1.91	2.01	0.91	0	0	0
10862	UT	S	M	20	2	0	3.33	0	3.18	1.71	1.89	1.59	1.81	1.71	0	1.31	0	0	0
10863	UT	S	M	19	2	0	3.14	0.6	3.46	0.78	0.55	1.51	0.89	1.44	0.69	0.71	0	0	0
10864	UT	S	M	18	2	0	3.26	0	3.23	1.64	1.48	1.98	1.27	1.06	0	0	0	0	0
10865	UT	S	M	18	2	0	2.98	0	3.08	1.17	1.23	2.64	1.21	1.64	1.24	0	0	0	0
10866	UT	S	M	18	2	0	2.85	0	2.64	1.01	1.37	1.79	1.15	0.81	0	0	0	0	0
10867	UT	S	M	19	2	0	2.28	1.42	3.04	2.07	2.47	1.65	1.44	1.42	1.03	0.61	0	3.61	0

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
10868	WA	W	M	22	2	2	3.11	0	2.29	1.06	1.59	2.76	0.57	0.95	0.39	0	0	0	0
10869	WA	W	M	21	2	2	3.12	1.72	2.32	1.22	1.6	2.17	1.39	2.76	1.08	0	0	0	0
10870	WA	W	M	21	2	2	1.44	0	2.05	1.43	1.95	2.35	1	0.85	0.45	0	0	0	0
10871	WA	W	M	21	2	2	1.87	0.3	2.88	1.69	1.15	1.7	0.94	1.25	1	0	0	0	0
10872	WA	W	M	22	0	0	4.03	0	3.19	1.18	2.56	2.18	1.04	1.11	0	0	0	0	0
11357	ON	N	M	21	2	0	1.95	0	3.53	1.32	0	2.19	1.59	1.94	0.95	0	0	0	0
11362	ON	N	M	21	2	0	2.13	0.77	3.2	1.29	1.06	2.45	2.19	1.88	0.81	1.6	0	0	0
11363	ON	N/E	M	21	1	2	3.56	1.65	3.46	2.15	2.3	2.44	0.65	1.65	1.01	1.93	1.63	1.98	1.41
11365	ON	N	M	20	2	1	3.62	1.06	3.35	1.91	1.4	2.12	1.06	1.5	1.5	0	0	2.24	0
11367	ON	N/E	M	20	2	1	3.6	1.56	3.32	1.42	1.81	2.21	1.15	1.46	0	1.94	0	0	0
11369	ON	N	M	24	2	1	3.97	1.59	3.61	1.91	2.31	2.3	1.73	2.2	0.9	2.24	0	1.27	0
11374	AB	N	F	23	1	2	1.98	0	3.64	1.61	2.43	1.57	1.83	1.09	0.56	1.84	0	0	0
11375	AB	N	F	22	2	2	3.83	0.87	3.96	1.01	1.15	2.17	0.89	1.86	0.65	2.36	0	0	0
11376	AB	N	M	19	0	0	2.65	0	3.2	1.39	1.51	2.24	1	1.27	1.31	1.73	0	0	0
11377	UT	S	M	22	1	2	3.31	1.08	3.5	1.9	0	1.68	0.67	2.37	0.81	1.59	0	0	0
11378	UT	S	M	22	1	1	3.25	0	4.14	1.98	1.58	2.53	0.33	1.64	0.84	1.41	0	0	0
11861	CO	S	M	19	1	1	2.03	0.78	2.73	1.32	0.58	1.44	1.27	1.11	0	0.38	0	0	0
12235	BC	W	M	21	0	0	1.43	0	2.35	1.1	0	2.64	1.25	0.7	0	0	0	0	0
12236	BC	W	M	21	2	2	1.63	0	2.19	1.54	0	2.29	1.04	1.04	0	0	0	0	0
12237	BC	W	F	22	5	5	2.49	0.59	3.41	0.69	0	2.6	1.83	1.18	0	0	0	0	0
12238	BC	W	M	20	0	2	1.5	0.71	2.51	1.6	2.17	3.37	1.45	0.55	0	0	0	0	0
12239	BC	W	M	22	0	0	2.16	0.66	2.77	1.36	0	2.29	1.15	0.58	0	0	0	0	0
12247	ON	N/E	M	21	2	2	2.89	1.61	3.21	1.88	2.74	2.28	0.63	2.16	0.96	1.14	0	1.55	0
12249	AR	W/E	F	29	2	2	3.18	1.8	3.33	1.42	2.21	2.48	2.11	1.72	1.75	1.25	0.4	0	0
12250	AR	E	F	30	0	0	3.18	5.36	4.67	1.98	2.37	3.25	2.82	1.87	1.63	2.13	1.96	1.43	0
12251	AR	E	F	29	0	0	2.77	0.67	3.66	1.72	1.52	1.32	2.36	1.72	2.07	0.89	0	0	0
12252	AR	E	F	28	0	0	3.55	1.56	3.09	1.42	2.3	1.04	2.02	2.04	2.34	0	0	0	0
12253	AR	E	F	30	0	0	3.88	2.25	4.8	3.83	2.62	1.75	3.06	1.77	1.22	2.94	0	0	0
12254	AR	E	F	29	0	0	2.85	0.69	4.11	1.6	2.07	2.27	2.01	1.82	2.37	1.09	2.2	0	0

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
12255	MT	W	M	20	0	0	1.89	0	2.49	1.13	0.67	1.36	0.47	0.83	0	0	0	0	0
12256	MT	W	M	20	0	0	1.36	0	2.21	1.54	0	0.87	1.28	0.53	0	0.54	0	0	0
12257	MT	W	M	20	0	0	1.55	0	2.64	1.37	0	0.67	0.63	1.15	0	0	0	0	0
12258	MT	W	M	21	0	0	1.9	0	2.2	1.86	0	1.45	0.91	0.81	0.54	0	0	0	0
12259	MT	W	M	21	0	0	1.5	0	2.61	1.52	0	1.03	0.58	1.38	0.43	0	0	0	0
12260	MT	W	M	20	0	0	2.52	0	2.81	2.11	0	1.23	1.72	0.93	0.41	0	0	0	0
12262	MT	W	F	22	3	3	1.65	0	3.39	1.11	0	1.26	1.57	1.16	0.43	0	0	0	0
12263	MT	W	M	20	0	0	1.31	0	2.45	1.35	1.5	1.28	0.78	1.1	0.42	0	0	0	0
12264	AR	E	F	28	0	0	4.21	0.9	4.25	1.92	2.22	1.2	1.92	1.07	2.67	2.29	0	0	0
12265	AR	E	F	30	0	0	4.03	1.17	4.49	1.54	1.87	1.97	2.71	1.9	3.35	2.07	0	0	0
12290	OR	W	M	22	0	0	1.76	0	1.91	0.9	0	0.39	1.05	1.06	0	0	0	0	0
12291	OR	W	M	22	0	0	1.35	0	2.96	1.45	0	1.33	1.18	0.63	0	0	0	0	0
12292	OR	W	M	20	0	0	1.54	0	2.03	1.35	0	1.18	0.79	0.86	0	0	0	0	0
12293	OR	W	M	22	0	0	1.63	0	2.17	1.09	0	1.32	0.62	1.05	0	0	0	0	0
12294	ID	W	M	19	0	0	1.64	0	2.41	1.07	0.82	1.24	1.22	0.63	0.51	0	0	0	0
12295	ID	W	M	20	0	0	1.11	0	1.91	0.71	0	2.69	1	0.84	0	0	0	0	0
12296	ID	W	M	21	0	0	1.56	0	2.2	1.03	1.01	0	2.37	0.73	1.11	1.17	0	0	0
12297	ID	W	M	20	0	0	1.39	0	1.79	0.99	1.34	2.84	1.47	1.31	0.38	0	0	0.67	0
12298	ID	W	M	21	0	0	1.31	0	2.48	0.93	0	1.54	0.79	1.06	0	0	0	0	0
12299	ID	W	M	20	0	0	2.07	0	1.95	1.3	0.8	2.01	1.08	0.54	0	0	0	0	0
12300	ID	W	M	21	0	0	1.44	0	2.33	0.86	0.45	2.21	0.55	0.54	0	0	0	0	0
12301	ID	W	M	21	0	0	1.91	0	2.68	1.01	1.61	1.8	1.14	1.32	0.47	0	0	0	0
12302	ID	W	M	20	0	0	1.62	0	2.54	0.89	2.05	1.2	0.82	1.24	0	0	0	0	0
12303	ID	W	M	21	0	0	1.09	0	2.17	0.99	0	1.78	0.96	0.45	0	0	0	0	0
12304	ID	W	M	21	0	0	1.92	0	2.6	1.13	0.96	1.13	0.63	1.16	0.51	0.96	0	0	0
12305	ID	W	M	22	0	0	1.42	0	1.98	0.87	0	1.37	0	0.88	0	0	0	0	0
12306	ID	W	M	19	0	0	1.5	0	1.84	0.99	0	1.06	0	0.56	0.85	0	0	0	0
12307	ID	W	M	20	0	0	1.15	0	1.71	1.09	1.45	1.37	0.48	0.61	1.12	0	0	0	0
12308	CO	S	M	20	0	0	2.29	1.5	3.76	1.58	1.54	1.59	2	1.51	0.66	0	0	0	0

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
12309	CO	S	M	18	0	0	1.91	1.36	3.09	1.15	1.53	1.21	1.42	1.26	1.01	0.91	0	0	0
12310	NM	S	M	18	0	0	2.15	0	2.89	1.02	1.47	1.24	1.37	0.99	1.15	0.49	0	0	0
12311	NM	S	M	19	0	0	2.93	1.73	3.47	1.75	1.43	2.26	1.4	1.17	0	0	0	0	0
12312	NM	S	M	20	0	0	1.92	0.92	2.94	1.77	2.01	1.13	1	1.58	1.07	0	0	0	0
12313	NM	S	M	19	0	0	2.32	0.87	2.85	1.75	1.85	1.53	0.83	1.32	0.72	0.7	0	0	0
12314	CO	S	M	20	0	0	1.95	0.6	3.75	1.53	0.45	1.83	2.07	0.85	0.9	0.51	0.47	0	0
12315	CO	S	M	20	0	0	2.5	1.58	3.72	1.31	0.98	1.78	1.57	1.14	1.26	0	0	0	0
12316	CO	S	M	18	1	1	2.04	0.75	3.12	1.42	1.28	1.62	1.15	1.6	0.79	0	0	0	0
12317	CO	S	M	18	1	1	1.75	0	3.79	1.35	0.65	2.1	1.69	1.69	0.87	0	0	0	0
12318	CO	S	M	21	0	0	2.25	1.16	3.48	1.22	2.04	0.99	1.82	1.06	0.62	0	0	0	0
12319	CO	S	M	19	0	1	2.33	0.88	4.01	1.35	2.24	1.32	1.6	1.6	1.06	0	0	0	0
12320	CO	S	M	19	1	1	1.84	0.89	3.05	1.09	1.7	2.11	1.31	0.87	0.56	0	0	0	0
12321	CO	S	M	19	1	1	3.55	0	3.31	1.41	2.3	3.35	1.57	1.36	1.27	0	0	0	0
12323	MB	N	M	18	2	2	3.63	0.61	3.07	1.7	1.71	2.21	1.22	3.17	1.36	1.73	0	0	0
12324	AB	N/W	M	18	0	2	1.87	0	2.58	1.39	0	2.04	1.43	0.78	0	0	0	0	0
12326	AB	N/W	M	20	2	2	1.4	0	2.41	1.39	0	2.15	1.56	0.69	0	0	0	0	0
12327	AB	N/W	M	20	2	2	3.04	0	2.67	1.76	2.24	1.44	1.93	1.13	0	0	0	0	0
12329	AB	N/W	M	20	0	2	1.25	0	2.65	0.73	0	2.46	1.15	0.66	1.26	0	0	0	0
12330	AB	N/W	M	20	0	2	1.61	0	2.39	1.04	0	2.87	0.6	0.62	0	0	0	0	0
12334	AB	N/W	M	19	0	2	1.72	0	2.62	1.37	0.99	2.16	1.38	1.07	0.72	0	0	0	0
12335	AB	N/W	M	19	0	2	2.4	0	2.41	1.3	0.65	2.38	1.55	1.52	0.69	0.44	0	0	0
12336	AB	N/W	M	21	0	2	2.27	0	3.01	1.53	1.27	2.05	1.36	0.87	0	0.32	0	0	0
12337	AB	N/W	M	20	0	2	1.67	0.58	2.67	1.34	1.6	2.79	1.26	1.26	0.5	1.16	0	0	0
12338	AB	N/W	M	20	0	2	2.21	0.3	2.7	1.06	0.42	2.55	1.58	1.34	0.54	0.94	0	0	0
12339	AB	N	M	18	0	2	2.62	0.78	3.14	1.13	1.74	2.61	1.04	1.51	0.83	1.06	0	0.68	0
12340	AB	N/W	M	20	0	2	2.12	1.42	3.51	1.41	1.13	2.91	1.47	1.38	0.94	0	0	0	0
12341	AB	N/W	M	19	0	2	2.54	0	2.78	1.87	0.65	2.29	1.25	1.48	0.83	0.48	0	0	0.83
12342	AB	N/W	M	20	0	2	1.56	0	2.2	0.88	0	1.74	0.68	1.1	0	0	0	0	0
12343	AB	N/W	F	21	5	5	2.3	0	2.71	1	0	1.74	1.06	1.21	0	0.62	0	0	0

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
12348	AB	N/W	M	20	0	2	1.81	0	3.02	1.18	0	2.75	1.19	1.12	0	0	0	0	0
13601	SK	N	M	18	2	0	2.82	1.22	3.21	1.74	0.68	2.53	1.46	1.19	0.79	1.31	0	0	0
13602	SK	N	M	19	2	0	2.94	1.07	3.22	1.97	1.5	2.3	1.47	2.52	1.18	0.68	0.68	0	0
13603	AB	N/W	M	21	2	0	2.23	0	3.55	1.79	0.94	2.6	1.19	1.32	0	0.84	0	0	0
13604	SK	N	M	19	2	2	3.04	2.22	3.15	1.29	1.61	2.51	1.56	1.25	1.83	0	0	0	0
13605	SK	N	M	19	2	0	1.96	0.2	2.77	1.34	1.4	1.77	1.45	1.36	0.72	0	1.61	0	0
13606	SK	N	M	18	2	0	2.31	0	3.71	1.66	0.9	2.07	1.39	1.16	1.64	1.1	0	2.19	0
13608	SK	N	M	18	2	0	2.89	0.46	3.05	1.5	2.08	2.55	1.32	2.13	1.1	1.72	0	0.61	0
13609	SK	N	M	20	2	0	2.5	0.56	3.2	1.85	2.29	2.75	2.14	3.09	1.35	0	0	0	0
13610	AB	N/W	M	20	2	0	2.01	0	2.65	1.26	0	2.6	1.18	0.69	0.56	0.26	0.72	0	0
13611	AB	N/W	M	17	2	0	2.49	0	2.26	1.49	0	2.36	1.71	0.88	0.78	0.58	0	0	0
13612	AB	N/W	F	19	3	3	1.5	1.52	2.37	2.4	1.85	1.85	0.81	1.36	1.16	0.84	0	0	0
13613	AB	N/W	M	20	2	0	3.4	0	3.65	1.89	0	2.33	1.06	2.09	1.04	1.65	0	0	0
13614	AB	N/W	M	19	2	2	1.8	0	2.4	1.11	0	2.61	1.01	0.84	1.21	0	0	0	1.83
13615	AB	N/W	M	17	2	0	2.04	0.45	2.72	0.78	0.55	2.55	1.69	2.3	0.8	0	0	0	0
13616	AB	N/W	M	16	2	2	1.14	0	2.67	0.71	0.62	2.53	0.98	0.7	0.81	0	0	0	0
13617	AB	N/W	M	18	2	2	2.63	0.64	2.74	1.64	1.31	2.27	1.67	2.11	1.57	1.57	0	2.22	2.32
13618	AB	N/W	M	20	2	0	2.78	0.64	2.53	1.91	1.92	2.92	1.16	1.35	0	0	0	0	0
13619	AB	N/W	F	20	3	3	2.48	1.09	2.95	2.3	2.61	2.6	1.35	2.69	0.82	0	0	0.68	0
13620	AB	N/W	M	21	2	2	2.4	0	3.13	0.99	1.32	2.28	1.1	2.73	0.77	0.8	0	0	0
13621	AB	N	F	18	2	2	2.08	0	3.05	1.43	1.26	2.21	1.48	1.68	1.25	0.85	0	0	0
13622	AB	N	M	19	2	0	1.69	1.79	3.1	1.28	2.04	2.86	1.61	1.1	1.23	1.77	0	0	0
13625	MO	E	M	25	0	2	2.5	1.26	3.18	1.54	2.06	2.99	2	1.07	1.61	2.26	0	1.67	0
13626	MO	E	M	23	2	0	3.57	1.63	3.31	2.29	0.95	2.56	2.09	0.8	1.59	1.78	0	0	0
13627	MO	E	M	25	0	0	3.13	1.39	3.68	1.39	1.59	2.53	3.23	1.64	1.28	2.02	0	1.16	0
13628	MO	E	F	28	2	2	3.76	2.08	3.84	2.32	0.9	3.16	1.95	1.87	1.58	3.09	0	2.67	0
13630	MO	E	M	22	0	0	3.17	2.47	2.71	1.52	1.03	2.03	1.5	1.94	1.95	0	0	0.89	0
13631	MO	E	M	23	1	2	3.02	2.19	3.66	1.97	1.76	1.98	0.93	1.41	2.02	0	0	0	0
13632	MO	E	M	24	2	0	2.95	1.39	3.23	1.83	1.56	2.59	1.65	1.93	1.1	1.58	0	0.82	0

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
13633	MO	E	M	23	2	0	2.58	0.56	2.9	1.05	1.21	2.75	1.01	1.37	1.91	0.85	0	0.67	1.15
13635	MO	E	F	20	2	2	4.17	1.25	4.05	2.19	3.36	2.52	2.79	1.84	2.44	2.82	0	0	1.92
13636	MO	E	M	24	2	0	3.43	2.56	3.27	1.62	1.25	2.24	1.28	1.29	2.86	1.22	0	2.67	0
13637	MO	E	M	25	2	0	3.53	1.54	3.44	1.55	1.98	2.13	1.52	2.14	1.39	0	0	2.12	0
13638	MO	E	F	29	2	2	3.21	1.68	3.36	1.9	1.31	3.5	3.15	1.92	1.96	1.82	0	0.92	0
13639	MO	E	F	28	2	0	3.07	0	3.72	1.84	0.96	1.59	2.76	1.76	2.23	2.22	0	0	0
13640	MO	E	F	29	2	0	3.74	1.57	4.12	1.67	2.76	2.38	1.33	1.71	1.1	2.19	0	0	0
13641	MO	E	M	29	2	2	3.03	1.48	3.49	1.33	1.08	2.59	1.66	1.71	1.46	2.24	0	0	0
13645	MO	E	F	28	2	2	4.15	2.49	4.38	2.07	2.58	2.04	0.9	1.6	1.29	2.11	0	3.15	0
13646	MO	E	F	29	2	0	2.19	1.11	4.19	1.42	0	3.15	2.67	1.56	2.19	0.51	1.42	0	0
13648	MO	E	F	27	2	2	3.5	0.7	2.91	1.25	2.09	2.94	3.08	1.35	1.43	1.8	0	0	0
13650	MO	E	F	29	2	2	3.17	0.78	4.27	2.03	2.37	3.2	3.32	2.12	1.48	1.13	0.69	0	0
13651	MO	E	F	30	2	2	3	1.33	4.2	2.24	1.08	3.31	2.58	1.51	1.94	2.31	0	0	0
13652	MO	E	F	30	2	2	4.06	1.36	3.89	1.88	2.78	1.86	1.99	1.63	1.32	1.45	0	0.58	2.79
13655	MO	E	F	31	2	2	3.24	0.77	4.71	2.17	2.49	3.09	2.14	1.93	1.75	2.04	0	0.91	0
13657	MO	E	F	28	2	0	2.09	0.69	4.28	1.06	0	2.9	2.21	1.62	1.98	2.27	0	0	0
13658	MO	E	F	30	1	2	3.58	0.82	3.76	2.09	3.18	2.27	2.15	1.58	1.79	1.24	0	1.12	0
13659	MO	E	F	29	2	2	5.2	2.4	3.8	2	3.14	3.07	1.9	1.96	2.01	2.09	0	0	2.11
13660	MO	E	F	28	2	2	2.92	0.89	3.67	1.79	1.52	2.56	1.61	1.35	2.08	1.39	0	0.71	0
13661	MO	E	F	30	2	2	2.14	1.43	3.59	1.75	0.86	3.21	2.45	1.85	3.01	1.62	1.84	0.61	0
13662	MO	E	F	29	2	2	3.66	1.96	3.32	1.98	2.32	2.76	1.53	1.78	1.43	1.07	0	0.48	0
13663	MO	E	F	27	2	2	3.48	1.06	4.09	1.46	1.25	2.12	1.38	1.98	2.32	1.52	0	0	0
13665	MO	E	F	29	2	2	3.61	1.99	4.22	1.83	3.3	2.79	2.57	1.93	1.9	1.69	0	1.49	0
13666	MO	E	F	30	2	2	3.27	0.63	3.54	1.72	2.88	3.76	2.91	1.87	2.7	1.58	0	0.62	0
13667	MO	E	F	29	2	2	4.1	1.02	3.52	1.51	2.36	3.59	1.34	1.82	1.97	1.58	0	0.5	0
13668	MO	E	F	30	2	2	4	2.47	5.03	2.1	3	3.5	2.41	2.25	1.49	1.36	0	0.75	0
13669	KY	E	M	21	2	2	1.6	0	2.76	1.25	0	1.81	0.88	0.76	0	0	0	0	0
13670	MO	E	F	30	2	2	3.84	1.78	3.98	2.1	2.81	3.69	2.59	2.05	2.63	0.39	0	1.43	0
13671	MO	E	F	30	2	0	3.16	1.03	3.83	1.43	1.16	3.56	2.11	1.46	2.05	1.63	0	0.47	0

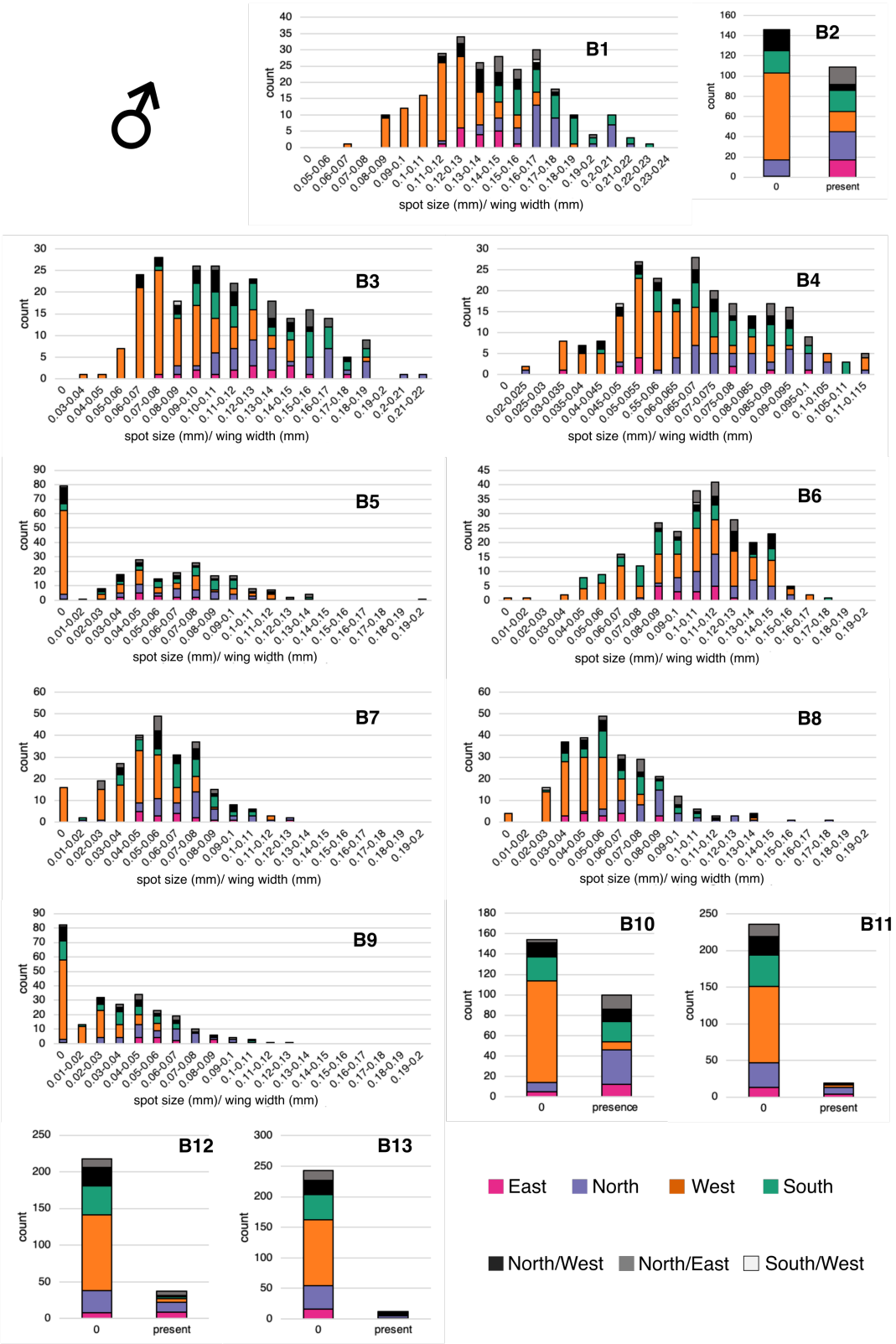
DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
13673	MO	E	M	25	1	2	2.88	1.41	3.61	1.66	1.22	2.25	1.18	1.6	1.2	0.65	0	1.64	0
13674	MO	E	F	30	2	0	3.61	1.03	3.92	1.83	2.1	3.71	2.25	1.7	2.77	0.99	0	0.52	0
13675	MO	E	M	25	2	2	2.24	1.02	2.94	1.66	1.37	2.39	1.54	1.23	1.29	1.02	0	0	0
13676	MO	E	M	25	1	2	3.35	0.95	3.07	1.29	0.97	3.19	1.41	1.17	1.13	1.6	0	0.57	0
13680	WV	N/E	M	21	2	0	1.94	0	2.6	1.2	0.71	2.66	1.63	1.24	1.1	0	0	0	0
13681	MN	E	M	21	0	0	2.73	1.38	3.04	1.67	2.19	2.51	1.77	1.41	1.92	0.5	0	0	0
13683	MT	W	M	20	2	2	1.7	0.82	2.31	1.05	0.64	2.89	0.96	1.02	0.3	0.68	0	0	0
13684	CA	W	F	21	3	3	1.3	0	2	0.98	0	2.33	1.6	1.23	0.75	0	0	0	0
13685	CA	W	M	22	0	0	1.55	0	2.46	1.22	0.52	2.49	1.3	0.62	0	0	0	0	0
13686	OR	W	M	21	0	0	1.98	0	2.26	2.24	0.99	2.71	1.4	0.95	0.53	0.24	0	1.77	0
13687	MT	W	M	18	2	0	1.27	0	2.13	0.57	0	1.27	0.92	0.54	0	0	0	0	0
13688	WY	W	M	20	2	2	1.44	0	3.07	1.17	0	3.04	1.15	0.64	0	0	0	0	0
13689	UT	W	M	19	2	2	1.33	0	2.39	0.78	0	2.28	0.45	0.76	0	0	0	0	0
13690	OR	W	M	23	0	0	1.56	0	1.86	1.09	0	2.42	1.41	1.26	0	0	0	0	0
13691	WY	W	F	21	5	5	1.4	0	2.43	0.97	0	2.46	0.79	1.08	0	0	0	0	0
13692	MT	W	M	21	0	0	1.61	0	2.26	1.06	0.69	1.82	0.64	1.16	0.61	0	0	0	0
13693	CA	N/W	M	21	2	0	1.35	0	1.8	0.84	0	2.94	0.72	0.75	0.52	0.4	0	0	0
13694	CA	W	M	21	0	0	1.56	0	2.2	1.55	1.1	2.35	0.79	1.19	0.32	0	0	0	0
13695	CA	W	M	21	2	2	1.42	0	2.06	1.31	0	2.52	1.49	0.55	0	0	0	0	0
13696	WY	W	M	20	2	2	1.26	0	1.84	0.6	0	1.33	0.69	0.69	0	0	0	0	0
13697	WY	W	M	20	2	2	2.01	1.7	2.33	1.44	0	2.31	0	0.56	0.9	0	0	0	0
13699	CA	W	M	20	0	0	1.51	0	2.45	1.26	0	2.46	1.47	0.8	0.9	0	0	0	0
13700	WY	S/W	M	21	2	2	1.86	0	3.4	0.98	0.96	2.15	0.91	0.51	0	0	0	0	0
13702	WY	W	M	21	2	2	1.36	0	2.28	1.1	1.49	1.96	0	1.17	0	0	0	0	0
13703	OR	W	M	22	0	0	1.49	0	1.93	1.18	0	2.16	1.29	1.23	0.76	0	0	0	0
13704	CA	W	M	24	2	2	1.67	0	2.16	1.69	0	2.42	0	0.65	0	0	0	0	0
13705	OR	W	M	22	0	0	1	0	1.53	0.72	0	1.65	0	0.86	0.34	0	0	0	0
13706	OR	W	F	23	5	5	2.45	1.42	2.63	1.23	1.98	2.8	1.48	1.16	0.96	0	0	0	0
13707	MT	W	F	21	5	5	1.85	0	2.87	1.37	0	2.44	1.67	1.24	0.49	1.04	0.69	0	0

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
13708	MT	W	F	20	5	5	2.23	1.7	3.08	1.45	1.68	2.81	1.2	1.11	0.99	0	0	0	2.24
13709	MT	W	F	23	5	5	4.86	3.48	3.88	2.31	2.9	2.7	1.76	1.53	0.82	0	0	1.81	0
13710	MT	W	M	22	0	0	2.23	0	3.05	1.57	1.55	3.19	1.21	1.11	0.68	0	0	0	0
13711	UT	W	M	21	0	0	1.29	0	2.29	1.05	0	1.71	1.28	0.84	0	0	0	0	0
13712	UT	W	F	23	5	5	1.3	1.51	2.73	1.52	1.98	2.46	1.08	0.99	0	0	0	0	0
13713	UT	W	M	20	2	0	1.68	0	2.42	1.41	0	1.75	0.74	0.97	0.89	0	0	0	0
13714	UT	W	M	22	2	0	1.87	0.73	2.76	1.44	2.93	0.97	0.52	0	0	0	0	0	0
13715	MT	W	F	20	5	5	2.45	1.33	2.63	1.09	1.1	1.26	0.85	1.67	0.65	0	0	0	0
13716	UT	W	F	22	5	5	1.52	0.89	2.71	1.56	1.2	1.05	1.03	0.8	0.57	0	0	0	0
13717	UT	W	M	20	0	0	1.92	0	2.32	1.06	0	1.74	0.69	0.69	0.97	0	0	1.52	0
13718	UT	W	M	19	2	2	2.02	0	2.92	0.87	0	2.47	0	1.17	0	0	0	0	0
13719	MT	W	F	21	5	5	1.81	0.51	3.36	0.99	1.59	3.38	1.12	1.41	0	0	0	0	0
13720	UT	W	M	20	2	0	1.81	0.51	3.36	0.99	1.59	3.38	1.12	1.41	0	0	0	0	0
13721	MT	W	F	22	3	3	2.25	1.86	3.36	1.4	1.21	0.93	2.94	1.63	1.29	0	0	0	0
13722	MT	N/W	F	22	5	5	2.06	0	3.42	1.59	1.11	2.27	1.11	1.36	0.71	0	0	0	0
13723	OR	W	F	23	5	5	2.22	0.75	3.28	1.18	0	2.51	1.7	1.08	0.66	0	0	0	0
13724	UT	W	M	22	2	2	1.97	0	2.16	1.21	0	1.96	1.02	1.16	0.31	0	0	0	0
13725	OR	W	M	21	0	0	1.89	1.71	2.05	1.44	2.41	2.76	1.09	0.92	1.15	0	0	2.94	1.73
13726	UT	W	M	21	2	2	1.09	0	2.3	0.65	0	2.29	0.82	0.9	0	0	0	0	0
13727	OR	W	F	22	3	3	3.03	1.35	3.6	1.21	1.95	2.6	1.5	1.34	0.93	0.59	0	0	0
13728	MT	W	M	19	2	2	1.33	0	2.2	1.09	0	2.63	0.93	0.68	0.43	0	0	0	0
13729	OR	W	F	21	5	5	2.89	0	3.33	2.88	1.88	1.68	2.81	1.27	0.69	0	0	0	0
13730	UT	W	M	22	2	2	1.62	0	2.44	1.96	0.85	3.11	0.76	0.69	0.64	0	0	0	0
13731	OR	W	M	22	0	0	2.15	0.63	2.52	1.92	2.07	3.23	2.6	1.34	0.51	0	0	0	0
13732	OR	W	F	21	3	3	3.77	1.74	3.48	1.89	2.95	2.85	1.71	2.1	1.27	0.52	0	0	0
13733	MT	W	F	23	5	5	2.49	0.97	3.26	1.21	0.67	3.14	1.77	1.17	0.76	0	0	0	0
13734	OR	W	F	22	3	3	1.35	0.53	2.85	1.44	1	2.31	1.71	1.29	0.91	0	0	0	0
13735	OR	W	M	20	0	0	2.19	0.27	2.29	2.25	1.95	2.87	1.04	1.07	1.45	0	0	2.18	0
13736	MT	W	M	20	2	2	1.25	0	2.38	1.31	0	1.88	0	1.19	0	0	0	0	0

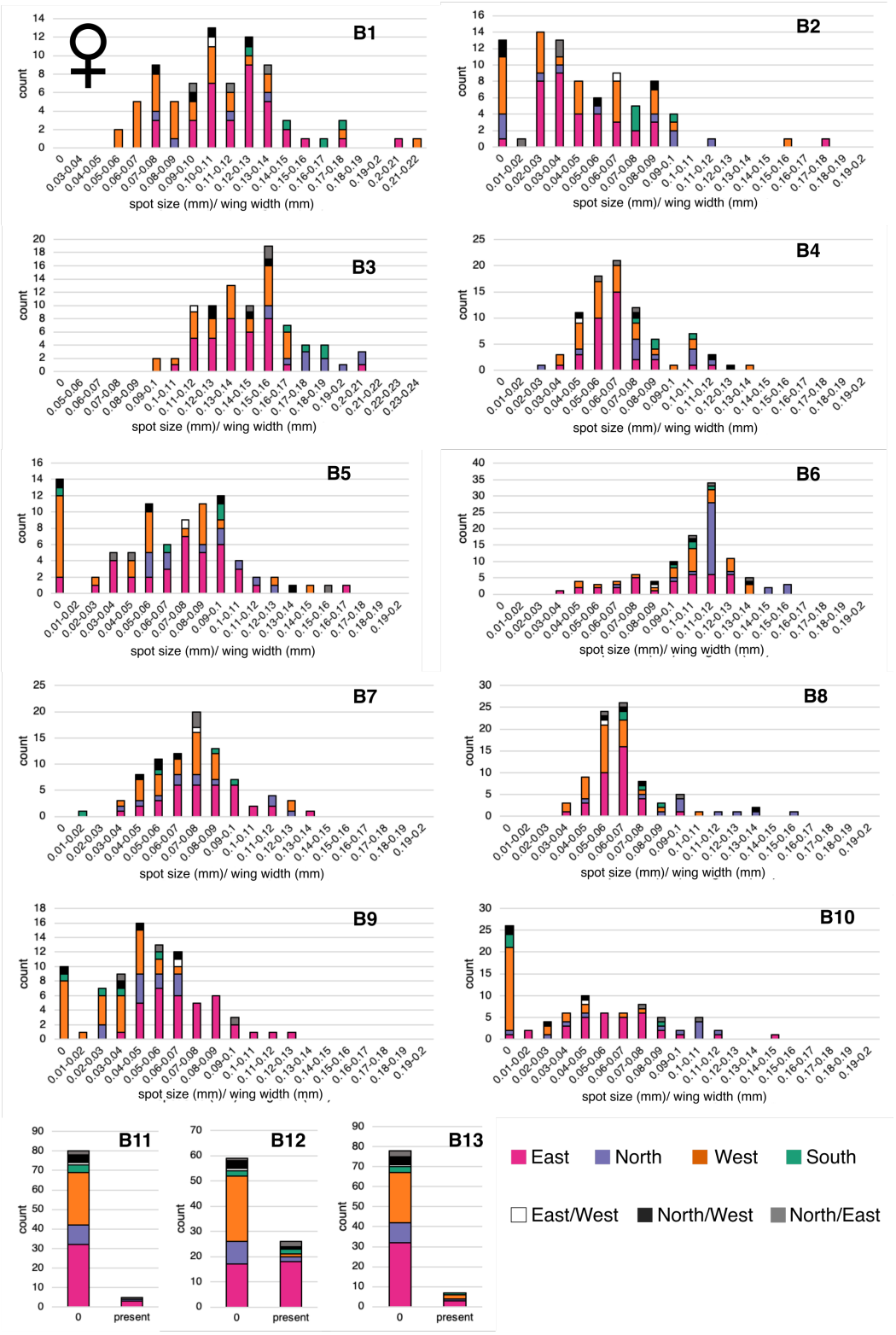
DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
13737	OR	W	F	21	3	3	1.85	1.97	2.92	1.55	1.18	2.81	1.77	1.23	0.87	0	0	0	0
13738	WY	S	M	19	2	2	2.95	0	3.96	1.35	0.79	2.05	0.78	1	0	0	0	0	0
13739	UT	S	M	20	2	2	2.01	0	3.13	1.9	1.09	1.71	0.91	1.13	0	1.02	0	0	0
13740	WY	S	M	17	2	2	2.28	0	3.83	1.4	0	1.76	0.94	0.53	0	0	0	0	0
13741	WY	S	M	18	2	2	1.37	0	3.51	1.46	1.21	1.89	1.18	0.55	0.33	0	0	0	0
13742	NE	N/E	M	21	2	0	2.41	1.04	3.05	1.9	0.84	2.32	1.6	1.34	0.69	1.14	0	0	0
13743	NE	N/E	M	21	2	2	2.18	0.52	3.27	1.39	1.69	2.33	1.22	0.98	1.65	1.77	0	0	0
13744	NE	N/E	M	19	1	2	2.2	1.16	2.29	1.71	1.2	1.56	0.99	1.08	1.3	1.4	0	0	0
13745	NE	N/E	M	21	1	1	3.15	0.87	4.04	2.05	2.3	2.59	1.63	1.5	0.81	1.31	0	0	0
13746	NE	N/E	M	21	2	0	2.01	1.22	2.93	1.41	1.88	2.7	1.25	1.99	0.92	0.47	0	1.08	0
13747	NE	N/E	M	20	2	2	2.6	0.59	2.87	1.53	1.3	2.09	1.77	1.82	1.86	1.69	0	1.21	0
13748	NE	N/E	F	24	2	2	2.2	0.45	3.38	1.87	1.14	3.12	1.7	2.22	0.92	2.44	0	0	0
13749	AL	E	F	28	2	2	3.79	0.86	4.3	1.58	2.11	3.36	2.56	1.82	1.57	1.27	0.74	0	0
13750	WY	S	M	19	2	2	1.63	0	3.35	1.3	0	1.13	1.06	0.74	0.75	0	0	0	0
13751	NE	N	M	21	2	0	2.22	0.75	2.94	1	0.75	2.33	1.19	1.56	1.64	0	0	0	0
13752	NE	N	M	21	2	0	2.54	0	2.97	1.59	0.89	2.63	1.6	1.13	1.38	1.59	0	0	0
13753	NV	W	M	23	0	0	3.05	1.46	3.29	1.74	0	2.36	1.11	1.42	0.67	0.61	0	0	0
13754	WY	W	M	21	0	0	1.63	0	1.71	0.75	0	1.2	0.78	0.51	0.73	0	0	0	0
13755	WY	W	M	20	0	0	1.67	0	2.33	1.02	0	2.39	1.02	0.67	0	0	0	0	0
13756	NV	W	M	21	0	0	2.2	0	3.44	1.3	0.98	2.58	1.2	1.19	0	0	0	0	0
13757	NV	W	M	21	0	0	2.75	1.57	2.97	2.1	1.3	3.22	0.87	1.61	1.02	0	0	0	0
13758	MT	W	F	20	5	5	1.11	0	2.72	0.86	0	2.42	0.99	1.37	0	0.68	0	0	0
13759	NV	W	M	22	0	0	1.87	0	2.63	1.31	0	2.8	0	1.17	0.77	0.58	0.92	0	0
13760	MT	W	M	20	0	2	0.68	0	1.99	0.7	0	2.02	0	0.68	0.72	0	0	0	0
13761	WY	W	M	19	0	0	1.57	0	2.11	1.4	0	2.16	0.92	1.48	0	0	0	0	0
13762	OK	E	M	30	2	1	2.9	1.6	3.62	0.95	1.32	3.08	2.23	1.1	1.39	2.87	0	0	0
13763	WY	S	M	19	2	2	2.35	0	2.84	1.02	0	1.54	0.57	1.01	0	0	0	0	0
13764	MT	W	F	21	5	5	1.56	0.94	2.68	2.16	0	2.45	1.89	1	0	1.36	0	0	0
13765	WY	N	M	20	2	2	2.72	1.51	3.33	1.88	1.71	2.16	1.46	1.7	1.21	1.68	0.75	1.83	1.47

DNA	locality	Genotype	S	W	HW	FW	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
13766	WY	W	M	19	0	0	1.12	0	2.43	1.22	0	2.29	0.65	0.94	0	0	0	0	0
13767	OK	E	F	29	1	1	3.41	1.36	3.96	2.36	1.75	2.27	2.16	1.8	2.44	1.92	0	1.21	0
13769	MT	W	M	21	2	0	1.6	0	2.76	1.25	0	1.81	0.88	0.76	0	0	0	0	0
13770	WY	W	M	19	2	0	1.29	0	2.35	1.24	2.11	0.84	0.98	0	0	0	0	0	0
13771	MT	W	M	20	2	2	1.45	0	2.63	0.77	0	1.95	1.13	0	0	0	0	0	0
13772	MT	W	F	19	5	5	1.37	0.79	1.92	1.1	0	2.29	1.28	1.02	0	0.77	0	0	0
13773	MT	W	M	19	2	2	2.22	0	2.35	1.13	0	2.44	0	0.72	0	0	0	0	0
13774	MT	W	F	19	5	5	1.34	0.45	2.61	0.71	0	2.57	1.38	0.71	0.81	0.73	0	0	0
13775	NV	W	M	22	0	0	2.17	1.55	2.56	1.6	1.37	3.29	1.22	1.03	1.21	0	0	0	0
13776	WY	N	M	19	0	0	2.45	0.97	2.6	1.57	2.02	1.73	0.95	1.3	0.71	1.19	0	0	0
13777	MT	W	M	19	2	0	1.42	0	1.77	1.08	0	1.93	0.78	0.77	0	0	0	0	0
13778	WY	W	M	20	2	0	1.27	0	2.42	1.06	0	2.45	0.91	0.64	0.27	0	0	0	0
13779	MT	W	M	19	2	2	1.76	0	2.64	1.29	1	2.6	0.78	1.13	0	0	0	0	0
13780	WY	W	M	20	2	0	1.91	0	3.29	1.69	1.46	2.47	0.76	1.07	0	0	0	0	0
13781	MT	W	M	20	2	2	1.6	0	2.55	1.51	0	2.06	0.92	1.01	0.6	0	0	0	0
13782	WY	N	M	19	2	2	2.02	1.47	2.61	1.99	2.15	2.27	1.47	0.96	0.83	1.45	0	2.57	0
13783	MT	W	M	20	0	0	2.09	0.88	2.42	1.29	0.85	2.04	0	0.86	0	0	0	0	0
13784	MT	W	F	20	5	5	1.73	0.58	3.29	1.64	0.94	2.41	1.65	1.27	0.44	1.4	0	0	1.26
13787	WI	N	M	20	0	2	3.34	2.05	4.2	2.54	3.48	2.25	1.63	2.46	1.65	0.93	0	0	0
L54	NS	N/E	F	21	1	1	2.74	0.76	3.2	1.41	3.24	2.44	1.67	1.44	2.09	1.64	0	1.42	0
L55	NS	N/E	F	22	1	1	2.51	0.84	3.41	1.29	0.8	2.26	1.75	1.27	1.31	1.88	0	0.73	0

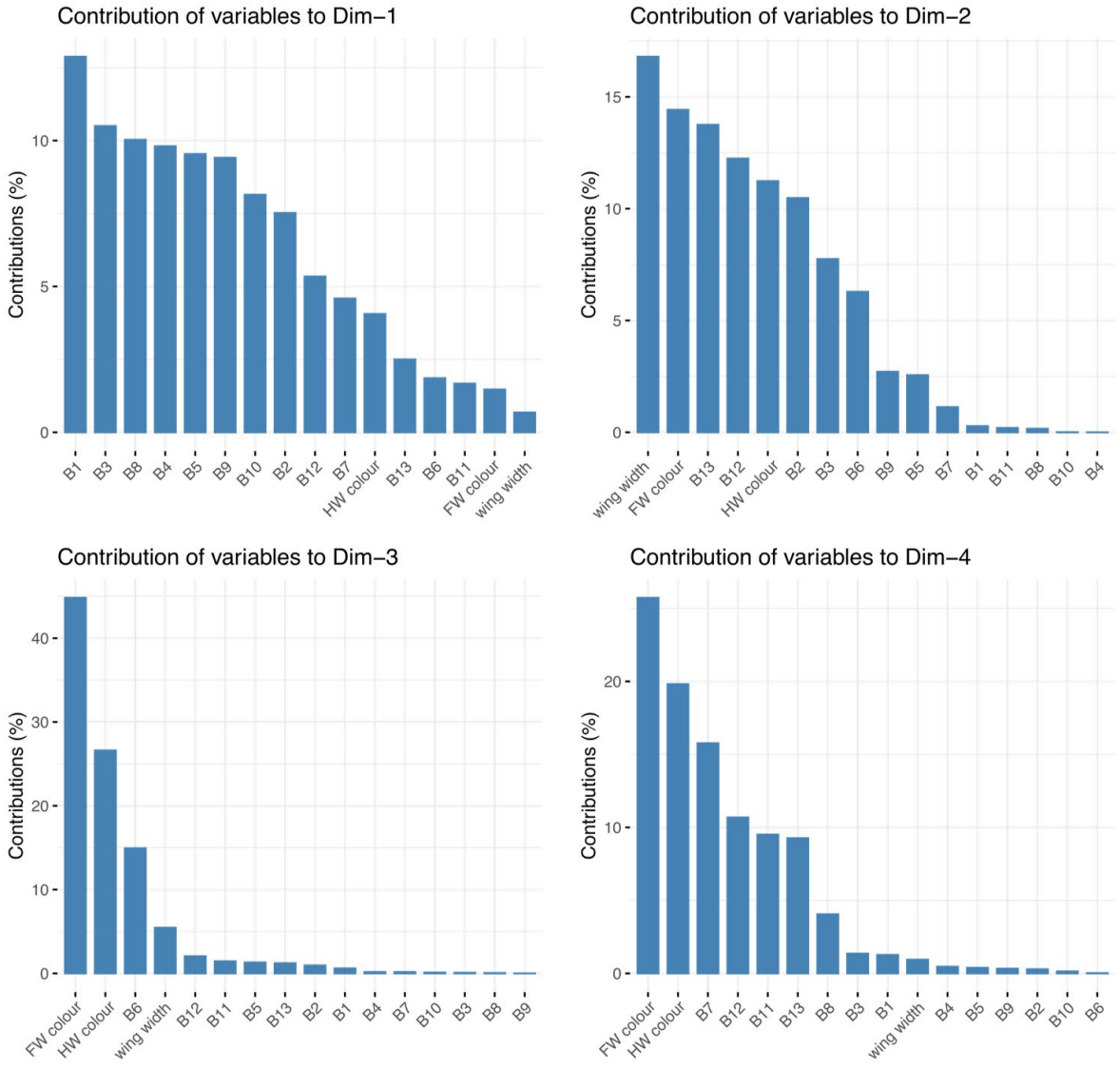
Appendix 12. Male (n=255) character histograms used for interpretation of morphometric analysis.



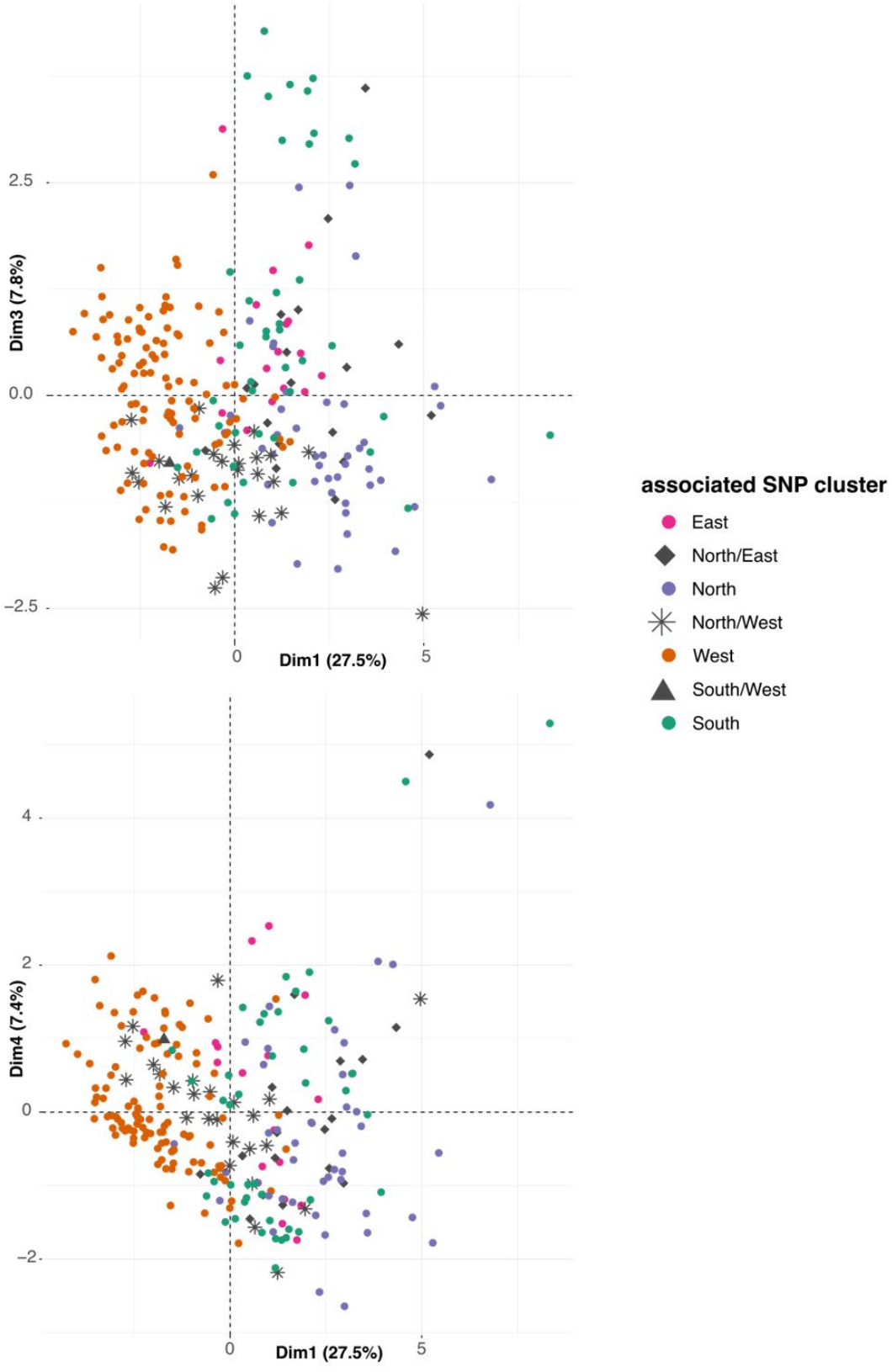
Appendix 13. Female (n=85) character histograms used for interpretation of morphometric analysis.



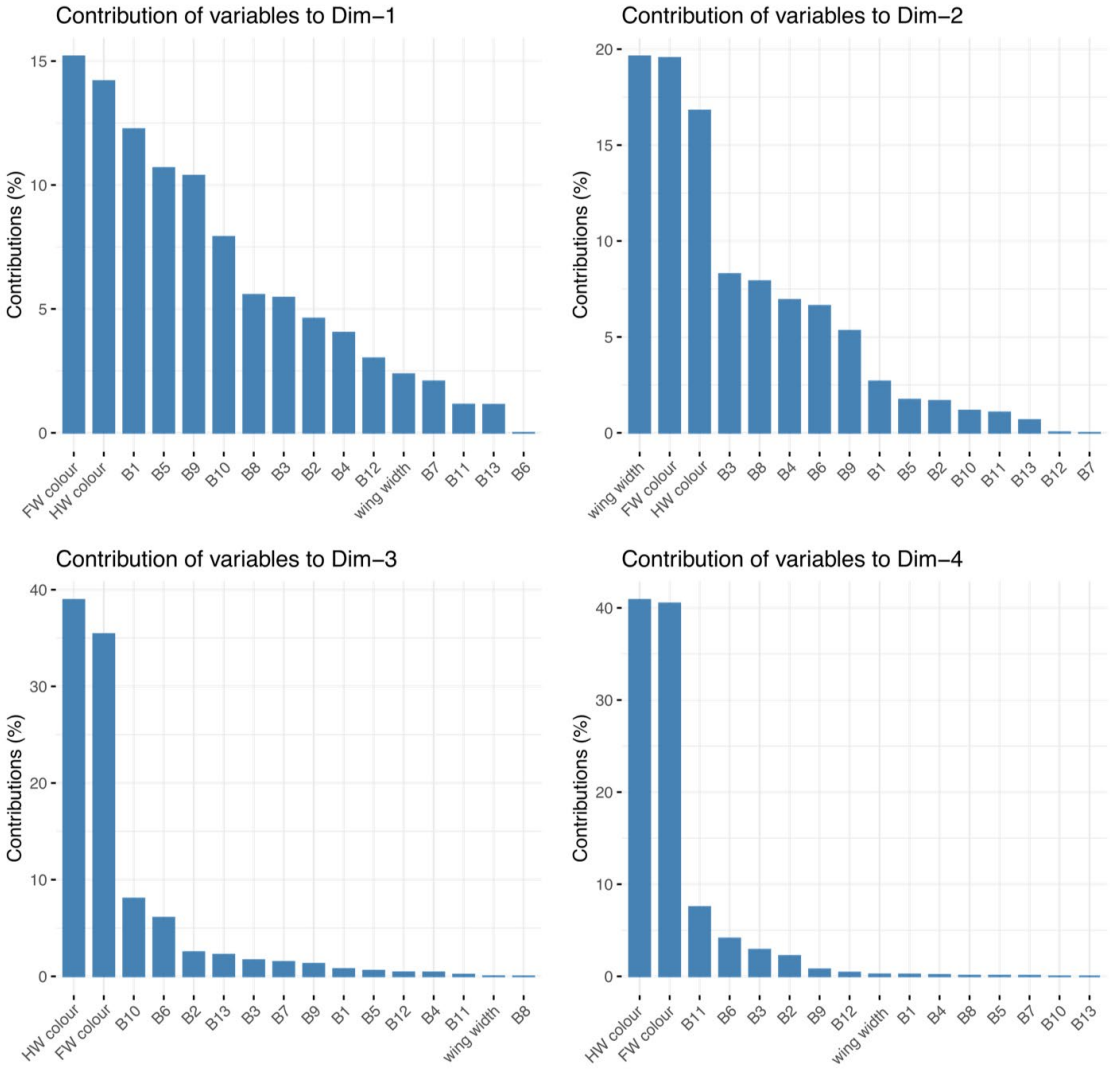
Appendix 14. Male (n=255) FAMD character contributions for dimension 1 to dimension 4.



Appendix 15. Male FAMD dimension 1 and 3, and dimension 1 and 4.



Appendix 16. Female (n=85) FAMD character contributions of dimension 1 to dimension 4.



Appendix 17. Female dimension 1 and 3, and dimension 1 and 4.