# EVIDENCE OF INBREEDING & DIVERGENCE IN THE WESTERN PRAIRIE FRINGED ORCHID (*PLATANTHERA PRAECLARA*)

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Sarah Anne Tennefos

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# North Dakota State University
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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

SUPERVISORY COMMITTEE:

Steven Travers
Chair

Gary Clambey

Kenneth Lepper

Craig Stockwell

Approved:

04/09/2018
Date

Craig Stockwell
Department Chair

## ABSTRACT

The conversion of grasslands to agriculture land has made the tall grass prairie one of the world's most endangered ecosystems and has played a key role in the decline of one particular species of rare endangered orchid, *Platanthera praeclara* (western prairie fringed orchid, WPFO). Loss of genetic diversity, accumulation of mutations, and inbreeding all increase the risk of extinction in endangered species.

Through the use of microsatellite markers to characterize allele frequencies in six populations of WPFO, evidence of inbreeding was common and highest at the most extreme northern, southern and eastern populations. Thus, suggesting that in addition to the current conservation objectives, interventions to reduce levels of inbreeding should be an additional conservation objective. The populations that warrant the greatest effort in recovery are the populations located at the edges of the range, where plants are the most likely to experience an extinction vortex.

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# CHAPTER 1. OVERVIEW OF *PLATANTHERA PRAECLARA*

## 1.1. Introduction to the problems associated with rarity

Conservation Biology is concerned with the mediation of endangered and threatened species. It is estimated that 20% of plant species are endangered (Rabinowitz *et al*., 1986). Conservation of plant biodiversity is dependent on both understanding the causes of rarity and consequences. The hypothesized causes of rarity in plants vary across a wide range of explanations. For example, there are particular life history characteristics, such as niche specialization, population isolation, or specific mutualistic relationships with other organisms, that are associated with plant rarity and can increase a plant species' risk of extinction (Rabinowitz *et al*., 1986). Human activity has impacted plants through conversion of habitat, loss of pollinators, collecting for anthropogenic uses, and introduction of competitors, pathogens, and pests. For most species, rarity results from some combination of anthropogenic and natural evolutionary factors rather than a single cause.

There are two consequences of rarity that can have important impacts on the population biology of rare plants: 1) increased distances between individuals and populations; and 2) reduced numbers of potential mates (Rabinowitz *et al*., 1986). Large geographic distances between plants can decrease the probability that pollinators can transfer pollen between distantly spaced plants. Large distances between populations can also lead to reduced gene flow due to reduced seed dispersal between populations. Over time restrictions in gene flow among populations can lead to genetic divergence and speciation amongst types (Charlesworth and Charlesworth, 1987). The decreased number of genes migrating in and out of a population can, over time, lead to population divergence and the evolution of new ecotypes or even new species (Gilpin and Soule, 1986).

A second consequence of rarity, is that populations can have low densities of plants and thus few potential mates for sexual reproduction. As a consequence, the number of alleles of different genes can often be reduced as well as the overall genetic diversity of a population. In small populations it is often the case that nearby individuals, those individuals with which a plant is likely to mate, are likely to be close relatives. Thus, there is an increase in the likelihood of inbreeding (biparental inbreeding). In addition to this biparental inbreeding, selfing may often be more likely in small populations because pollinators may be more likely to remain within a plant when foraging and thus cause geitenogamous pollination (transfer of pollen between flowers on a single plant) and selfing. Inbreeding can then lead to decreased heterozygosity at the population level and increased expression of deleterious traits which ultimately can lead to reduced fitness (Charlesworth and Willes, 2009).

Rarity restricts the number of available mates; this increases the probability of interbreeding among closely related individuals, leading to inbreeding depression and a reduction in genetic and genotypic variability. Lower genetic variation then leads to a subsequent inability to adapt to an ever-changing environment. The genetic health of rare plants is of great concern to conservation biologists because the reduced number of alleles in small populations with low genetic diversity can potentially lead to an extinction vortex. An extinction vortex describes the biotic and abiotic processes that declining populations undergo as they are being pushed towards extinction (Gilpin and Soule, 1986). When a population becomes too small, it increases the likelihood of close relatives mating with each other. If the mating relatives carry the same recessive deleterious alleles, their offspring can inherit two copies and suffer the consequences of the deleterious phenotype.

The western prairie fringed orchid is an ideal species to study the effects of rarity on genetic health and population dynamics because it is a rare species with a highly specialized pollinator system. There are 37 species of *Platanthera* orchids native to North America. *Platanthera praeclara*, commonly known as the western prairie fringed orchid (WPFO), is a threatened and federally protected orchid in North America. Over the past century, there has been a decline in the number of plants due to loss of habitat, collection pressure, and poaching (Sharma *et al*., 2003; Ross, 2012). North America's native prairies have seen as much as a 99.9% decline in available habitat space since European settlement (Samson and Knopf, 1996). The WPFO is only found in North American tallgrass prairie ecosystems, generally occurring in small, isolated populations due to habitat destruction from human activity, usually conversion of prairie lands to agricultural plots.

**1.2. Why is *P. praeclara* rare – The history of the prairie region**

In the past, many species have shifted their distribution patterns to track changing climates (Lawler *et al*., 2015). If a species is unable to accommodate to the changing climatic conditions, it can struggle to thrive, ultimately increasing its rarity. The current climate change may not be as dramatic as the changes North Dakota has already undergone. However, studying ancient cases of global climate change may give scientists a better approach to predict the future of the tallgrass prairie. Carbon is one of the primary drivers of global climate change. Atmospheric carbon in the form of carbon dioxide amplifies the earth's natural greenhouse effect. Carbon dioxide is capable of absorbing and holding heat from the sun. Atmospheric carbon dioxide concentrations are rising mainly because of the burning of fossil fuels for energy. Fossil fuels like coal and oil contain carbon that plants pulled out of the atmosphere through photosynthesis many millions of years ago; humans have returned that carbon to the atmosphere

in just a few hundred years since the industrial revolution. Over the last 50 years, increased temperatures have allowed plants to have a longer glowing season. This has increased net primary production, which has over time decreased overall soil carbon by 4% from global grasslands (Parton *et al*., 1995).

The current pollution event involves changes to the carbon cycle and atmospheric $CO_2$ levels. Native prairie plants typically have deep and extensive root systems that, in addition to helping them survive dry conditions, also serve as important natural carbon sinks. In grassland ecosystems most of the carbon is stored in the nutrient rich soils (Parton *et al*., 1995). The potential for tallgrass prairie carbon storage rates vary between 0.30 and 1.7 metric tons per acre per year (Garcia-Alvarez, 2011). The carbon turnover rate in a grassland ecosystem is long-lived lasting anywhere from 100-10,000 years (Parton *et al*., 1995). So, the effects on global carbon cycles won't be felt until well into the future. The WPFO utilizes the C3, or Calvin-Benson metabolic pathway, which has low efficiency in hot, dry conditions (Arditti *et al*., 1982). This makes the orchids more vulnerable to changing climatic conditions.

The erratic climate in upper midwestern region oscillates between periodic flooding followed by years of severe drought. The availability of increased moisture results in higher than average number of orchid flowers during a growing season. This is then followed by years of severe droughts when the orchids seemingly disappear from the landscape (Sieg and Wolken, 1999). There is a positive correlation between flower densities and soil moisture levels (Sieg and Wolken, 1999).

The WPFO prefers slightly moist, alkaline, sandy soil with little to no shade (Sieg and Wolken, 1999). Identifying the optimum soil conditions for this federally protected orchid will provide researchers a better understanding of how to conserve the natural prairies of the

Midwest. Providing the WPFO with its ideal habitat will increase the likelihood of a mature plant entering its reproductive stage and potentially passing on its genes to the next generation.

### 1.3. What factors contribute to the interconnectedness and isolation of *P. praeclara* individuals – Distribution and ecology

The orchid populations are found in both the United States and Canada. As of 1996 it occurred in 175 tall grass prairie sites in 8 ecoregions that are in 41 counties across 6 states and at least one population in Manitoba (Figure 1.1; USFWS, 1996). The largest concentrations of WPFO are in the northern ecoregions of Minnesota, North Dakota, and Manitoba. These are the populations that have been closely monitored and studied, while the smaller populations have been generally ignored (Sharma, 2002). Distances between adjacent populations in a previous study ranged from 1 to 175km (Ross *et al*., 2013). The current known range of the WPFO extends through Kansas, Missouri, Iowa, Nebraska, Minnesota, North Dakota, and northward into Manitoba. Though WPFO existed in South Dakota and Oklahoma in the past is it believed that these states no longer contain the WPFO. There has been a dramatic reduction in the populations in Iowa, Kansas, Missouri, and eastern Nebraska (Alexander, 2006). It has been suggested that northward shifts in the range of the orchid reflect influences of climate change on the plant (Vitt, personal communication). Little is known about how far seeds are dispersed in this species or how far pollinators move pollinia between populations.

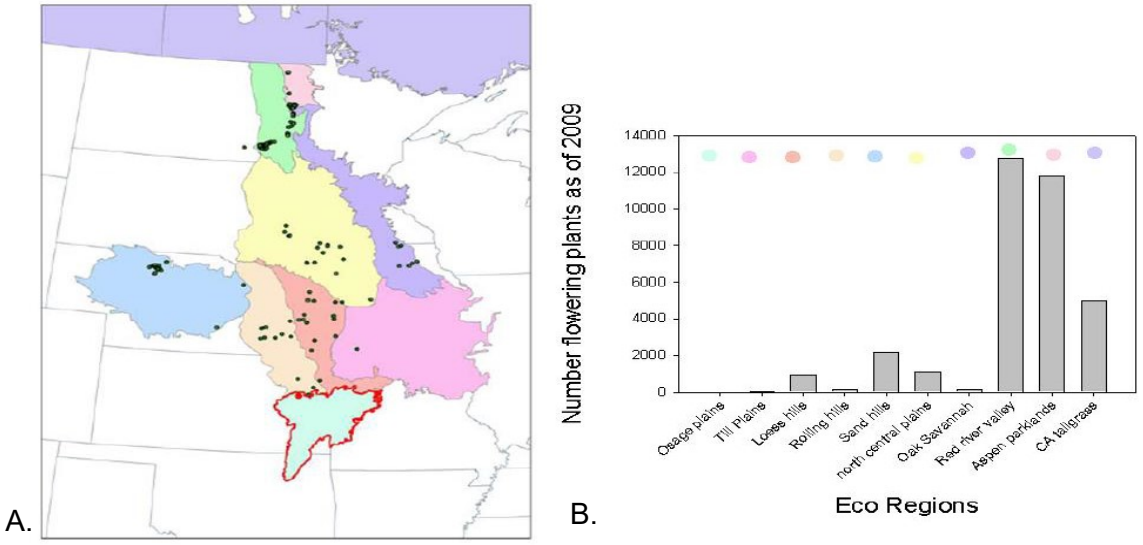


**Figure 1.1.** Historical range map of *Platanthera praeclara* showing ecoregions in different colors. Black dots are extant populations as of 2012. The red contour includes an ecoregion that once had WPFO but no longer does (Phil Delphey, personal communication). B. Counts of the number of flowering plants by ecoregions in 2009 (US Fish and Wildlife Service).

Several biological threats have been identified for WPFO including herbivory (by deer, cattle, and insects), herbicide use in agriculture lands, and reduced pollinator activity (Sharma *et al*., 2003). The survival to maturity decreased by around 60% when the orchids grew in actively grazed pastures, compared to a decrease of 23% in non-grazed pastures (Alexander, 2006). The use of herbicides in orchid habitats can have devastating effects on the WPFO, such as deformed flowers and a decreased number of flowering plants (Alexander, 2006). Any conservation strategy that aims to maintain or reestablish populations needs to provide land owners with quick and accurate techniques to maximize suitable habitat for the WPFO (Wolken *et al*., 2001).

The WPFO is considered to be a sister species of the eastern prairie fringed orchid (EPFO), *Platanthera leucophylla* (Sheviak and Bowles, 1986). The main range of the two species are geographically separated by the Mississippi River, *P. praeclara* only to the west and

*P. leucophylla* to the east and isolated populations to the west (Alexander, 2006). The two species coming into contact and hybridizing only in a limited portion of their distributions in Iowa (Wallace, 2003).

### 1.4. Morphological and physiological characteristics of *P. praeclara* that might influence pollination and recruitment of new plants

The WPFO is a perennial herb native to North American tallgrass prairie. The life history of the WPFO includes two life stages, vegetative growth and flowering growth. During the first life stage, plants are vegetative. If ideal conditions are not met the WPFO can remain vegetative indefinitely, become dormant, or a reproductive plant can return to a vegetative state. The vegetative-state average 24cm tall, usually only having one or two leaves throughout the entire growing season. The WPFO is capable of reproducing asexually during this stage by forming new primary tuber and perennating buds which can develop into new plants the following growing season (Sieg and Wolken, 1999). A plant can develop an inflorescence early in the growing season, around mid-April in southern ecoregions and late-May in northern ecoregions. The blooms, however, open between mid-June and late-July. Each inflorescence can have 10-24 creamy-white flowers on a raceme (Sharma *et al*., 2003). Multiple flowers can open and mature simultaneously at any given time. Access to the nectar is only possible for insect pollinators with extremely long proboscis and large heads-hawkmoths (Fox, 2008). The WPFO has the longest nectar spur of any northern temperate *Platanthera* species. The spurs range from 30-50 mm in length (Sharma *et al*., 2003). Eight hawkmoth species have been identified as pollinators of WPFO (Fox *et al*., 2013).

Successful pollination leads to development of capsules containing thousands of dust-like seeds. Nonetheless new seedlings are rarely observed (Sharma *et al*., 2003). WPFO seeds lack

any true endosperm or nutrition source for the germinating seedling. Survival of new individuals depends fungal associations with mycorrhizae. Mycorrhizal fungi are necessary for seedlings to germinate because the association between fungus and plant stimulates gluconeogenesis, mobilizes reserves and provides nutritional support until the seedling is able to photosynthesize (Sharma *et al*., 2003). Most mycorrhizal fungi are believed to be specific to both host species and region. Sharma and colleagues (2003) extracted 2 types of fungi from the WPFO; *Ceratorhiza* in plants from Minnesota and *Epulorhiza* in plants from Missouri.

For many plant species, mycorrhizae are a required component for adequate growth and reproduction. The Orchidaceae family is particularly reliant on these mycorrhizal associates. Mycorrhizae are the combined system of a fungus that infiltrates the root system supplying a mutually beneficial relationship. The fungus supplies the plant with nutrients and water and in return it is supplied with sugars the plant produces via photosynthesis once the young plant is established. A lack of available mycorrhizal fungi in the soil bank may be an additional factor contributing to the decline of this species, though it has yet to be formally studied.

**1.5. Population genetics and genetic rescue**

When discussing genetic rescue as applied to conservation biology, there are seven major issues that must be addressed: The likelihood of an inbreeding depression; the accumulation or loss of deleterious mutations within a population; a loss of genetic variation in small populations; genetic adaptation that may take place in captive populations and the possible implications on reintroduction success; future outbreeding depressions; fragmentation of populations and reduction in gene migration; and taxonomic uncertainties (Frankham, 2005).

Given the population characteristics and relative rarity of WPFO plants, they are at risk from low levels of genetic health. However, only a handful of studies have addressed direct

measures of genetic diversity and inbreeding in this genus and most are based on older molecular techniques with little ability to assess genetic diversity at a fine scale.

Allozymes are variant forms of an enzyme that are coded by different alleles at the same locus. Microsatellite marker studies have been used to uncover the subtle population genetic patterns not evident from using allozymes (Slatkin, 1995). Microsatellites or single sequence repeats (SSRs) are often highly polymorphic and offer the hope of greater understanding of population structure (Slatkin, 1995). Population geneticists look for different alleles, different genotypes between populations, and diverging populations. Genetic variation is what gives a plant the ability to adapt to changing environments, it's their genetic toolbox. The loss of genetic variation through the restriction of gene flow among populations can ultimately cause genetic drift. When observed in smaller populations, genetic drift can accelerate the decline of rare plants (Sharma *et al*., 2003). Thus, there is a real need to better understand broad population genetic patterns across a range of geographic scales in the WPFO in order to better understand the ability of this species to avoid extinction vortices.

In his M.S. thesis, Andrew Ross (2012), used microsatellite markers to observe the genetic variability of one of the largest single population of WPFO; it is located in the Sheyenne National Grassland (Ransom county, ND). He also analyzed nearby populations in western Minnesota. His results showed a lower observed heterozygosity than expected in most populations; all populations had at least some level of heterozygosity. He also found evidence for inbreeding (inbreeding coefficient ranged from 0.12 to 0.52). There was also little evidence of genetic divergence among the populations he studied, this indicated significant gene flow among WPFO populations on a relatively small geographical scale.

On a larger geographical scale, habitat fragmentation of functioning prairie ecosystems continues to be the greatest threat for many prairie plants. The long-term survival and mediation of the WPFO depends on local and national restoration efforts (Sharma *et al*., 2003). As a result of habitat fragmentation and destruction WPFO was listed as a threatened species in the U.S. in 1989 under the Endangered Species Act of 1973 and an endangered species in Canada in 2003 under the Species at Risk Act of 2002. The United States Fish and Wildlife Service released a detailed 5-year recovery plant in 1996, which outlined the actions believed to be required to recover and protect the WPFO. The introduction of cost effective genetic research tools has increased the understanding of the WPFO and should be included in the revised management plans.

This study assesses the genetic health of populations of the WPFO across a broad geographic distribution and assessed the consequences of rarity in WPFO in order to measure the genetic health and divergence of populations separated by hundreds of kilometers. Genetic assays were conducted to answer the following research questions:

1. What is the extent of genetic diversity within WPFO populations ranging from southern Manitoba to Iowa?

2. Which of the populations are in Hardy-Weinberg equilibrium?

3. Which of the populations has the evidence of inbreeding?

4. How much genetic divergence is there among WPFO populations?

5. What are the implications to the threatened species recovery play when genetic health of the populations is included?

# CHAPTER 2. AN ANALYSIS OF THE POPULATION GENETICS OF THE WESTERN PRAIRIE FRINGED ORCHID

## 2.1. Sample collection

The goals of the study were to assess intra-population levels of genetic diversity and heterozygosity as well as among-population patterns of gene flow and genetic divergence. The populations sampled met the following criteria: 1) ranging in size from small (< 20 individuals flowering) to large (> 1000); 2) the populations are located along an extensive latitudinal gradient; and 3) multiple eco-regions and habitats are represented by the chosen populations (Figure 2.1).

Previous studies have concentrated on a single population, in the Sheyenne National Grassland (Ransom county, ND; Ross, 2012). This study compares multiple sites across multiple ecoregions. The six sample sites Manitoba, Minnesota (Lake Bronson, Blue Mound, Rose Dell, and Lake Louise), and Iowa represent a variety of environments.

The Manitoba Tall Grass Prairie Preserve is located in southeastern Manitoba near Winnipeg. It is one of the last remaining stands of tallgrass prairie in Manitoba and is part of the Tallgrass Aspen Parkland conservation area in Manitoba and Minnesota. The area is characterized by a mosaic of habitat types, including tallgrass prairie, aspen woodland, sedge meadow wetlands, riparian woodland, and oak savanna.

The northernmost population in Minnesota is located in the Lake Bronson Scientific and Natural Area (SNA) in Kittson County. The typical Tallgrass Aspen Parkland habitat is a mosaic of wet prairie and wet meadow dominated by various sedges, both dotted with bog birch (*Betula glandulosa*), shrubby cinquefoil (*Dasiphora fruticosa*), and various willows.

Southwestern Minnesota sites occupy a unique landscape setting for the species on pockets of fine silt overlying or between outcrops of Sioux Quartzite. Plants at Blue Mounds State Park were collected from four patches of mesic prairie, not all of which have been historically counted from year to year.

The Rose Dell population is the largest unprotected population of *P. praeclara* in the United States. It also occurs on mesic prairie overlying bedrock approximately 18 km northwest of Blue Mound State Park. This privately-owned site has most recently been managed for prairie seed and/or for hay.

The easternmost population of *P. praeclara* sampled in this study, Lake Louise, is located Mower county in Minnesota. This small population is in diverse, high-quality wet-mesic prairie of an abandoned railroad right-of-way. Between 1998 and 2015 there were six years in which no plants were observed flowering.

The most southern population sampled, Dinesen, was in an Iowa state preserve in Shelby County, Iowa. The 20-acre remnant of tallgrass prairie has loess-topped ridges that are typical of this region of the plains in western Iowa.

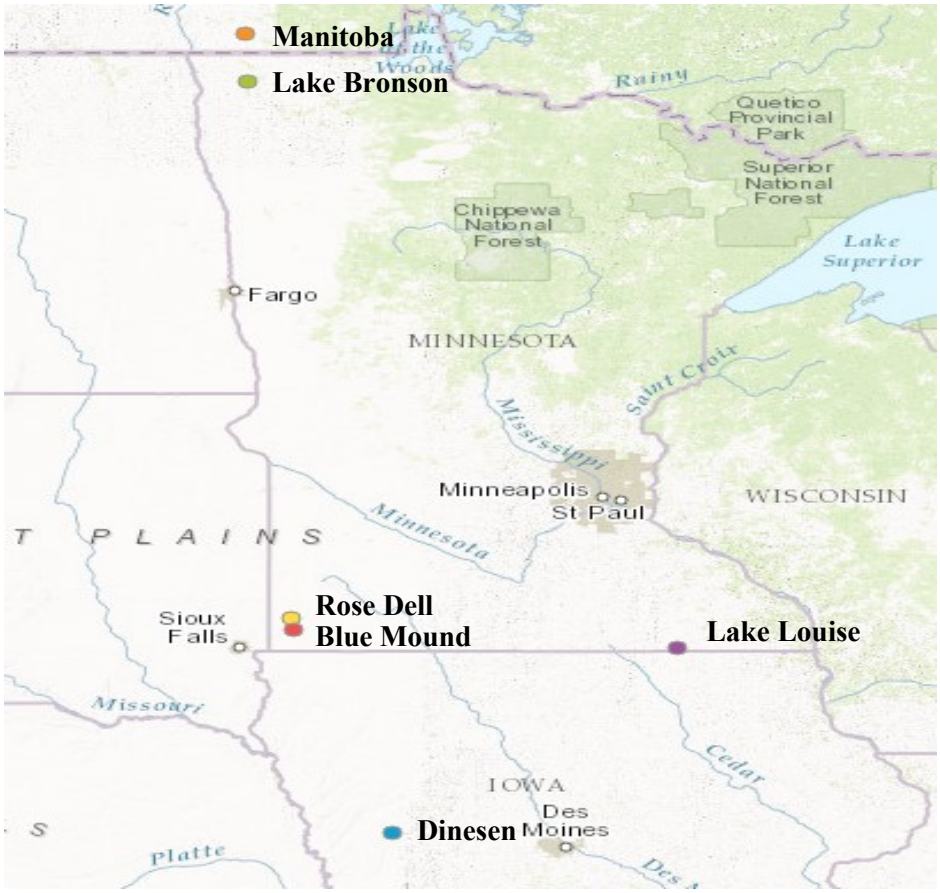


**Figure 2.1.** Map of populations sampled. The six populations are indicated by different colored circles.

**Table 2.1.** Geographic distances in kilometers are above the diagonal. Pairwise $F_{st}$ are below the diagonal.

| | Manitoba | Lake Bronson | Rose Dell | Blue Mound | Lake Louise | Dinesen |
|---|---|---|---|---|---|---|
| **Manitoba** | ---- | 47.91 | 595.71 | 608.15 | 703.38 | 835.38 |
| **Lake Bronson** | 0.059 | ---- | 547.80 | 560.29 | 660.27 | 787.63 |
| **Rose Dell** | 0.062 | 0.053 | ---- | 27.60 | 300.14 | 246.42 |
| **Blue Mound** | 0.121 | 0.075 | 0.058 | ---- | 323.46 | 243.32 |
| **Lake Louise** | 0.163 | 0.097 | 0.164 | 0.232 | ---- | 300.96 |
| **Dinesen** | 0.102 | 0.119 | 0.087 | 0.104 | 0.240 | ---- |

Leaf material was collected from flowering plants in six distinct populations (Manitoba, Lake Bronson, Rose Dell, Blue Mound, Lake Louise, and Dinesen) between 2011 and 2015 (Table 2.2). The distance between the northern-most population, Manitoba, and the southern-most, Dinesen, Iowa, was 845 km along a north south axis. The easternmost population, Lake Louise, was 314 km west of the others (Figure 2.1).

The sampling protocol was consistent between sites. Thirty-five flowering plants per population were randomly selected and labelled; populations with less than thirty individuals were completely sampled. Sampling consisted of removing approximately one square centimeter of green leaf tissue from a flowering plant, placing the tissue in a plastic microcentrifuge tube with a cap and storing it on dry ice in a cooler for less than 24 hours. At the end of a day of sampling, the cooler with the samples was transported by car to North Dakota State University where the samples were placed in zip-lock bags and stored in an ultracold freezer (-80 F).

**Table 2.2.** Characteristics of populations sampled. Populations are in order of decreasing latitude. Only the Rose Dell population was sampled in 2013 and 2015.

| Population | County, state | Number of flowering plants per year: (Mean) | (Min) | (Max) | Latitude | Longitude | Year sampled for genetic markers | # plants sampled for genetic markers |
|---|---|---|---|---|---|---|---|---|
| **Manitoba** | Manitoba, CA | 145[a] | 1 | 4758 | 49.16 | -96.67 | 2011 | 30 |
| **Lake Bronson** | Kittson, MN | 40[b] | 5 | 200 | 48.73 | -96.63 | 2013 | 29 |
| **Rose Dell** | Rock, MN | 478[d] | 0 | 1947 | 43.81 | -96.22 | 2015 | 33 |
| | | | | | | | 2013 | 10 |
| **Blue Mound** | Rock, MN | 67[e] | 0 | 436 | 43.69 | -96.19 | 2013 | 17 |
| **Lake Louise** | Mower, MN | 6[f] | 0 | 22 | 43.52 | -92.52 | 2013 | 13 |
| **Dinesen** | Shelby, IA | 37[g] | 1 | 126 | 41.71 | -95.25 | 2014 | 34 |

**a:** based on 23 years of MN DNR census data. **b:** based on 32 years of TNC census data **c:** based on 10 years MNDNR census data. **d:** based on 29 years MNDNR census data. **e:** based on 19 years MN DNR census data over, courtesy of Derek Anderson. **f:** based on census data over 14 years, courtesy of Lori Beiderman.

**2.2. DNA extraction**

Genomic DNA was extracted from each of the samples according to the standard protocols of the Quiagen, DNeasy Kit (Qiagen, Valencia, CA). Each sample was ground with liquid nitrogen in its microcentrifuge tube using a pellet pestle for approximately 30 seconds. The final volume of extracted DNA solution was approximately 100ul.

Microsatellite markers were used to evaluate the genetic patterns of the WPFO samples. As a genetic marker, microsatellites are particularly useful in conservation genetic studies examining among-population patterns due to their high rate of polymorphism (Allendorph and Luikart, 2007) and have been used successfully in orchid studies (e.g. Swarts and Dixon, 2009).

A DNA template from each plant was used in PCR reactions to amplify microsatellite regions at eight previously identified loci (Ross *et al*., 2013). In order to fluorescently label the PCR products, a three primer "CAG tag" protocol was used (Oetting, 1995). One forward and one reverse primer were combined with a third oligonucleotide containing a sequence complementary to a binding site on the forward primer and the 5' end is labeled with a fluorescent tag. We used a total of four tags (VIC, PET, NED, and FAM). A PCR reaction master mix was prepared for each sample consisting of 8.8µl of DD $H_20$, 4µl 5X buffer, 1.2µl $MgCl_2$, 0.8µl dNTP, 1µl of CAG-sequence primer at 0.5µM, 1µl of non CAG-sequence primer at 5µM, 1µl of fluorescently-labeled primer at 5µM, 0.2 µl of "Hotstart GoTaq" polymerase (VWR International) per reaction. Two microliters of template DNA was added to each reaction for a final volume of 20µl. The microsatellite amplification was performed on an Eppendorph AG 22331 thermocycler using the following touchdown profile: initial denaturization of 94° C for 2 min, 16 cycles of (1) a denaturization step at 94° C for 30 seconds, (2) an annealing step starting at 65° C for 30 seconds in the first cycle and decreasing 0.5° C each subsequent cycle, and (3) an

elongation step of 72° C for 30 seconds. The initial 16 cycles were followed by 20 cycles of a denaturization step (1) of 94° C for 30 seconds, (2) an annealing step at 57° C for 30 seconds, an elongation step (3) of 72°C for 30 seconds, with a final 4° C hold.

Successful amplification was check for in each reaction with agarose gel electrophoresis. Four µl of PCR product from each reaction was mixed with 2µl of 5x loading dye and loaded in individual wells in a 2% agarose gel. The gel was run at 100 volts for 2 hours, and then stained in an approximately 5 mg/mL ethidium bromide solution and photographed under UV light in a Fluorochem FC2 analyzer (Alpha Innotech).

Fragment length analysis and initial allele calls were conducted by the Plant Microbe Genomics Facility at Ohio State University (http://pmgf.osu.edu). PCR products were shipped on dry ice to the facility where they were analyzed using an Applied Biosystems 3730 analyzer.

**2.3. Statistical analysis**

Linkage disequilibrium and deviations from Hardy-Weinberg expectations was tested for using the program GENEPOP. The Excel-based add in GenAlEx was used to calculate mean numbers of private alleles, expected and observed heterozygosity and inbreeding coefficients (Peakall and Smouse, 2006). We also tested the hypothesis of isolation by distance by conducting a Mantel test on inter-population distances and allele frequencies using GenAlEx. Genetic structure among populations was visualized with the program Structure which uses a Bayesian approach to identifying statistical clusters. The proportion of variance in allele frequencies due to population was identity by conducting an AMOVA in the program GenAlEx. The DiveRsity package (Keenen *et al*., 2013) in R was used to test for among population structure and calculate D-Jost (Nei and Chesser 1993, Jost 2008). Pairwise $R_{st}$ values comparing all possible pairs of populations were calculated with the DiveRsity package. For this a multiple

regression analysis was conducted to examine the effects of latitude, longitude, and mean population size on the fixation indices of populations.

# CHAPTER 3. RESULTS

## 3.1. Genetic variability

Considerable evidence was found of allelic diversity within the seven populations sampled. A total of 52 alleles were found among the eight microsatellite loci examined. The total number of alleles per population ranged from 9.8 to 30 (Table 3.1). The mean number of alleles per locus ranged from 4.0 in Dinesen to 2.2 in Lake Louise, the smallest population. The number of private alleles per population was also lowest in Lake Louise with only 1.4 and highest in Lake Bronson with 2.2. Lake Louise had the lowest number of alleles over all loci with only 18.

In all populations, the observed heterozygosity was lower than expected. The values ranged from 0.183 in Lake Louise to 0.488 in Lake Bronson. All P-values associated with a chi-square test of HW expectations are less than 0.05 indicating that all populations had an excess of homozygotes. There was evidence of linkage disequilibrium based on analyses of data by Genepop.

Based on the results of the Microchecker analysis, it is possible that null alleles at three of the loci could explain the measured levels of homozygosity. Those were removed and $F_{is}$ was recalculated. In the absence of those three loci, inbreeding measurements were not significant (Figure 3.1).

**Table 3.1.** By population estimates of allelic and genotypic variation.

| Populations | | N | Na | Ne | I | Ho | He | $F_{is}$ |
|---|---|---|---|---|---|---|---|---|
| **Manitoba** | **Mean** | 30.000 | 3.400 | 1.941 | 0.700 | 0.373 | 0.363 | 0.165 |
| | **SE** | 0.000 | 1.030 | 0.471 | 0.273 | 0.163 | 0.134 | 0.255 |
| **Lake Bronson** | **Mean** | 28.800 | 3.800 | 2.183 | 0.802 | 0.488 | 0.421 | -0.168 |
| | **SE** | 0.200 | 0.860 | 0.495 | 0.266 | 0.161 | 0.140 | 0.108 |
| **Blue Mound** | **Mean** | 15.000 | 2.600 | 1.906 | 0.605 | 0.373 | 0.357 | -0.084 |
| | **SE** | 0.000 | 0.678 | 0.389 | 0.253 | 0.169 | 0.147 | 0.239 |
| **Rose Dell** | **Mean** | 9.800 | 2.800 | 1.915 | 0.610 | 0.327 | 0.318 | -0.011 |
| | **SE** | 0.200 | 0.917 | 0.551 | 0.295 | 0.183 | 0.149 | 0.187 |
| **Lake Louise** | **Mean** | 11.800 | 2.200 | 1.406 | 0.361 | 0.183 | 0.201 | 0.078 |
| | **SE** | 0.200 | 0.800 | 0.251 | 0.222 | 0.113 | 0.123 | 0.087 |
| **Dinesen** | **Mean** | 23.400 | 4.000 | 2.165 | 0.771 | 0.458 | 0.398 | 0.067 |
| | **SE** | 0.6000 | 1.304 | 0.521 | 0.309 | 0.191 | 0.151 | 0.312 |

### 3.2. Among-population comparisons

A significant Jost's D statistic comparing all populations at all loci (D = 0.0939 $p<0.05$) indicated that there is significant structure among populations. An AMOVA indicated that 11% of the variation in allele frequencies was among populations (Table 3.2). Pairwise $R_{st}$ comparison indicated that there are significant genetic differences at all pair-wise comparisons except for two (Table 3.4). In addition, the Mantel test was significant (R=0.195, P< 0.001, df=119) supporting the hypothesis of isolation by distance (IBD) as a mechanism of genetic structure.

**Table 3.2.** D-jost & Gst

| | Loci | gst | Gst | GGst | D |
|---|---|---|---|---|---|
| **1** | 02VIC | 0.0860 | 0.3120 | 0.3236 | 0.2472 |
| **2** | 14FAM | NaN | NaN | NaN | 0.0000 |
| **3** | 27NED | 0.1005 | 0.3683 | 0.3808 | 0.2977 |
| **4** | 12PET | 0.1791 | 0.8778 | 0.8820 | 0.8511 |
| **5** | 07VIC | 0.1861 | 0.3953 | 0.4170 | 0.2571 |
| **6** | 16NED | 0.1015 | 0.1778 | 0.1942 | 0.0849 |
| **7** | 17PET | 0.0178 | 0.0194 | 0.0229 | 0.0016 |
| **8** | 05NED | 0.0465 | 0.1408 | 0.1487 | 0.0989 |
| **9** | Global | 0.1177 | 0.2396 | 0.2571 | 0.0939 |

**Table 3.3.** Summary of Analysis of Molecular Variance on allele frequency data for all populations.

| Source | df | SS | MS | Est. Var. | % |
|---|---|---|---|---|---|
| **Among Populations** | 5 | 59.312 | 11.863 | 0.242 | 11% |
| **Among Individuals** | 114 | 284.246 | 2.493 | 0.495 | 22% |
| **Within Individuals** | 120 | 180.500 | 1.504 | 1.504 | 67% |
| **Total** | 239 | 524.058 | | 2.241 | 100% |

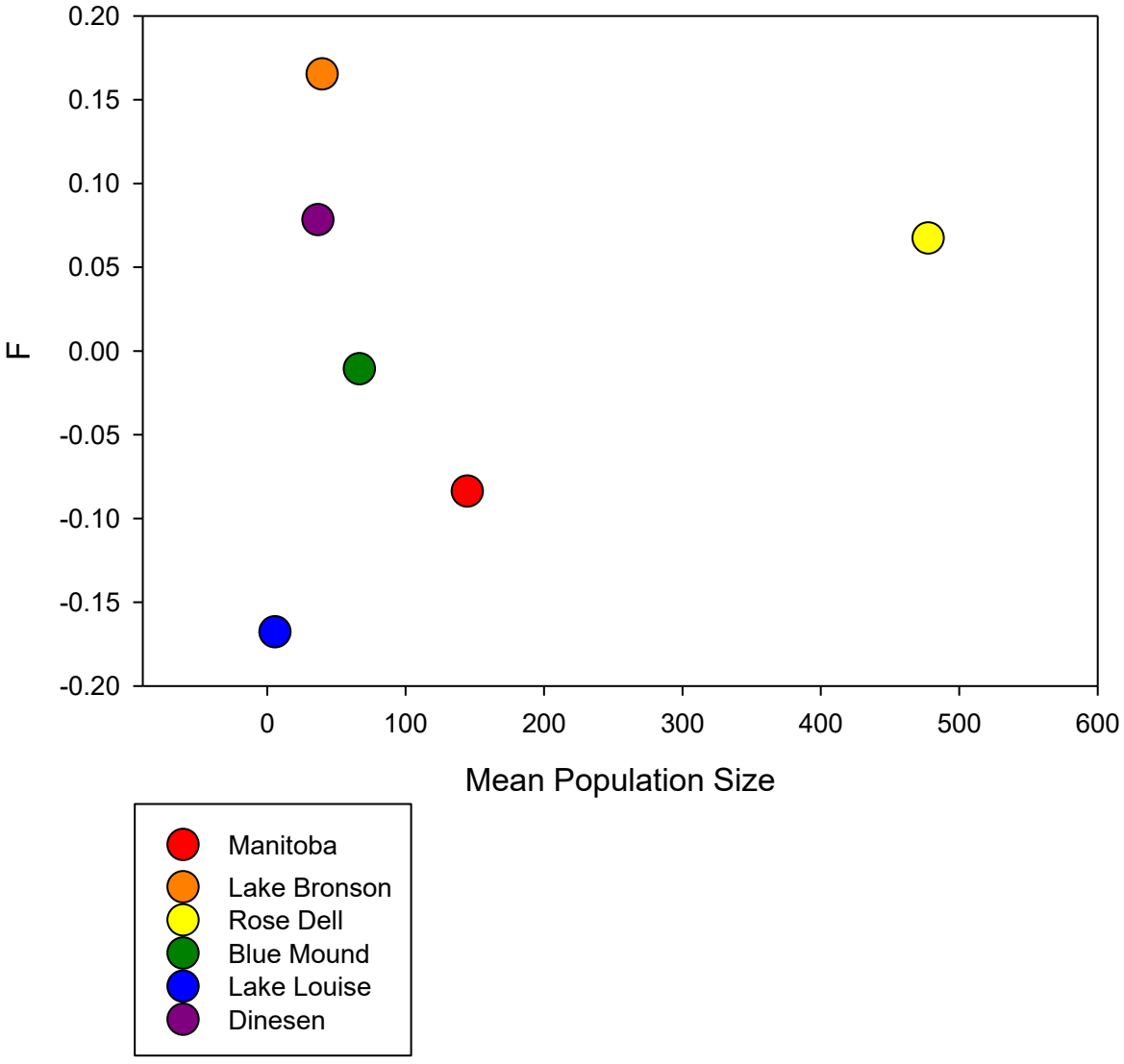


**Figure 3.1.** $F_{st}$ values with the removal of three loci possibly affected by null alleles.

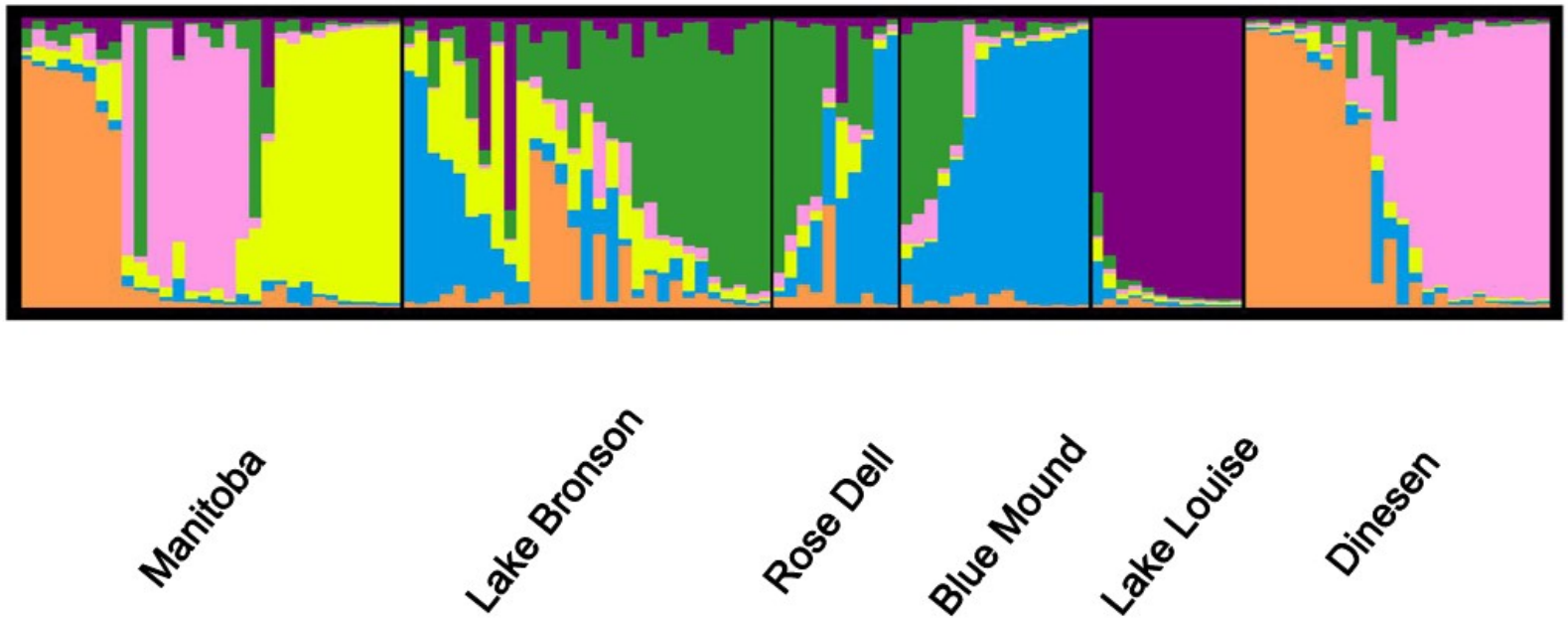


**Figure 3.2.** The results of Structure analysis of six populations (D = 6).

**Table 3.4.** Pairwise Population $F_{st}$ Values (below the diagonal). P-values are above the diagonal.

| | **Manitoba** | **Lake Bronson** | **Rose Dell** | **Blue Mound** | **Lake Louise** | **Dinesen** |
|---|---|---|---|---|---|---|
| **Manitoba** | --- | 0.010 | 0.020 | 0.020 | 0.010 | 0.010 |
| **Lake Bronson** | 0.028 | --- | 0.030 | 0.170 | 0.010 | 0.010 |
| **Rose Dell** | 0.043 | 0.055 | --- | 0.030 | 0.020 | 0.010 |
| **Blue Mound** | 0.061 | 0.014 | 0.063 | --- | 0.010 | 0.040 |
| **Lake Louise** | 0.112 | 0.091 | 0.011 | 0.174 | --- | 0.010 |
| **Dinesen** | 0.123 | 0.040 | 0.091 | 0.038 | 0.132 | --- |

**Table 3.5.** Results of multiple linear regression testing the following model:
Fixation index = longitude + latitude + population size + ε. The fixation indices for six orchid populations were fit to a model with the above three independent variables.

| **Source** | **Coefficient** | **T value** | **P value** |
|---|---|---|---|
| **Longitude** | -0.04 | -0.483 | 0.68 |
| **Latitude** | -0.007 | -0.151 | 0.90 |
| **Population size** | -0.0003 | -0.415 | 0.72 |
| F = 0.326 | Df = 3, 2 | P value = 0.812 | $R^2$ = 0.328 |

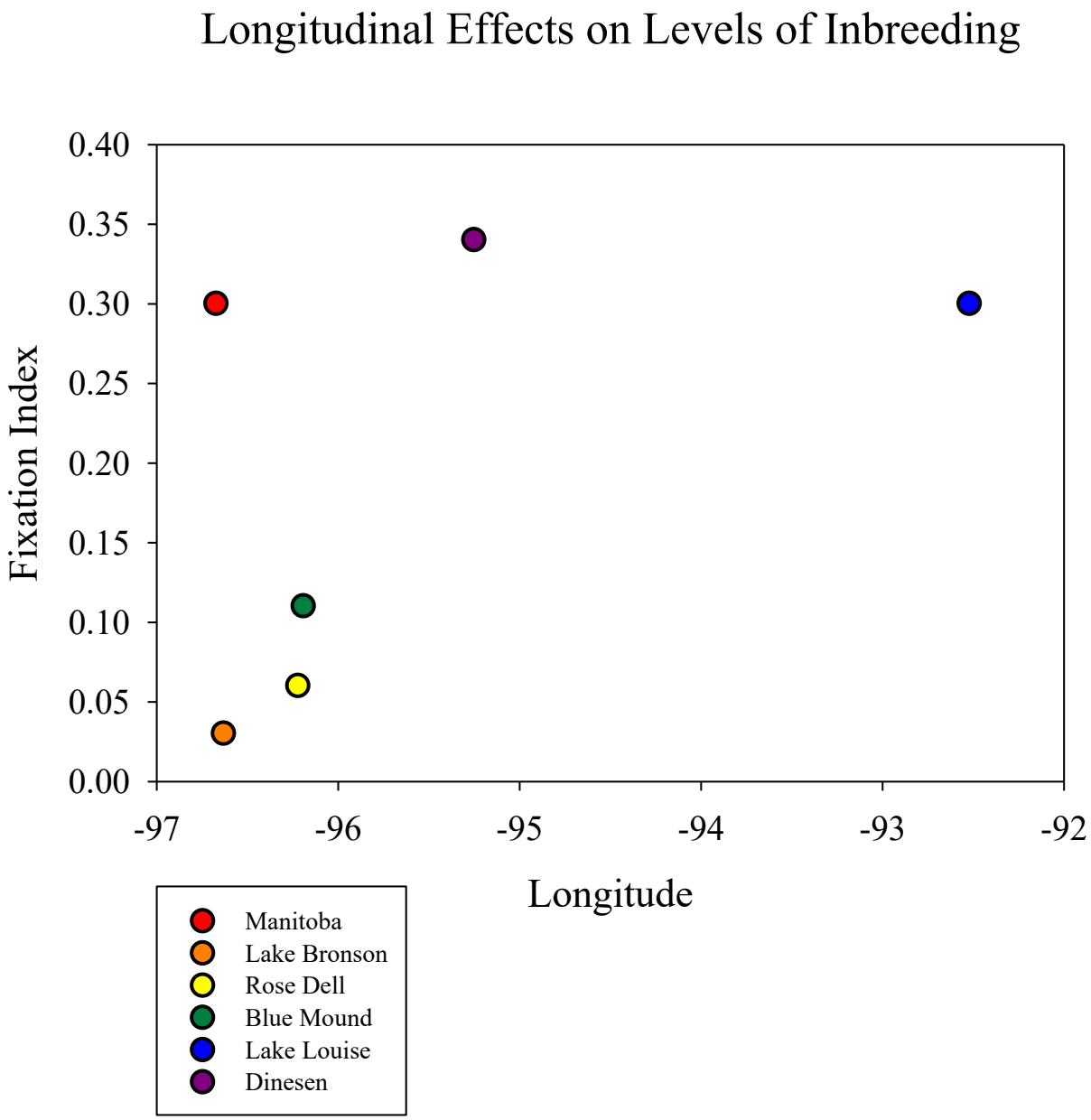


**Figure 3.3.** Fixation Index as a function of longitude. Longitude was chosen because it is thought that the sister species of the WPFO, the eastern prairie fringe orchid, evolved from the WPFO (Alexander, 2006).

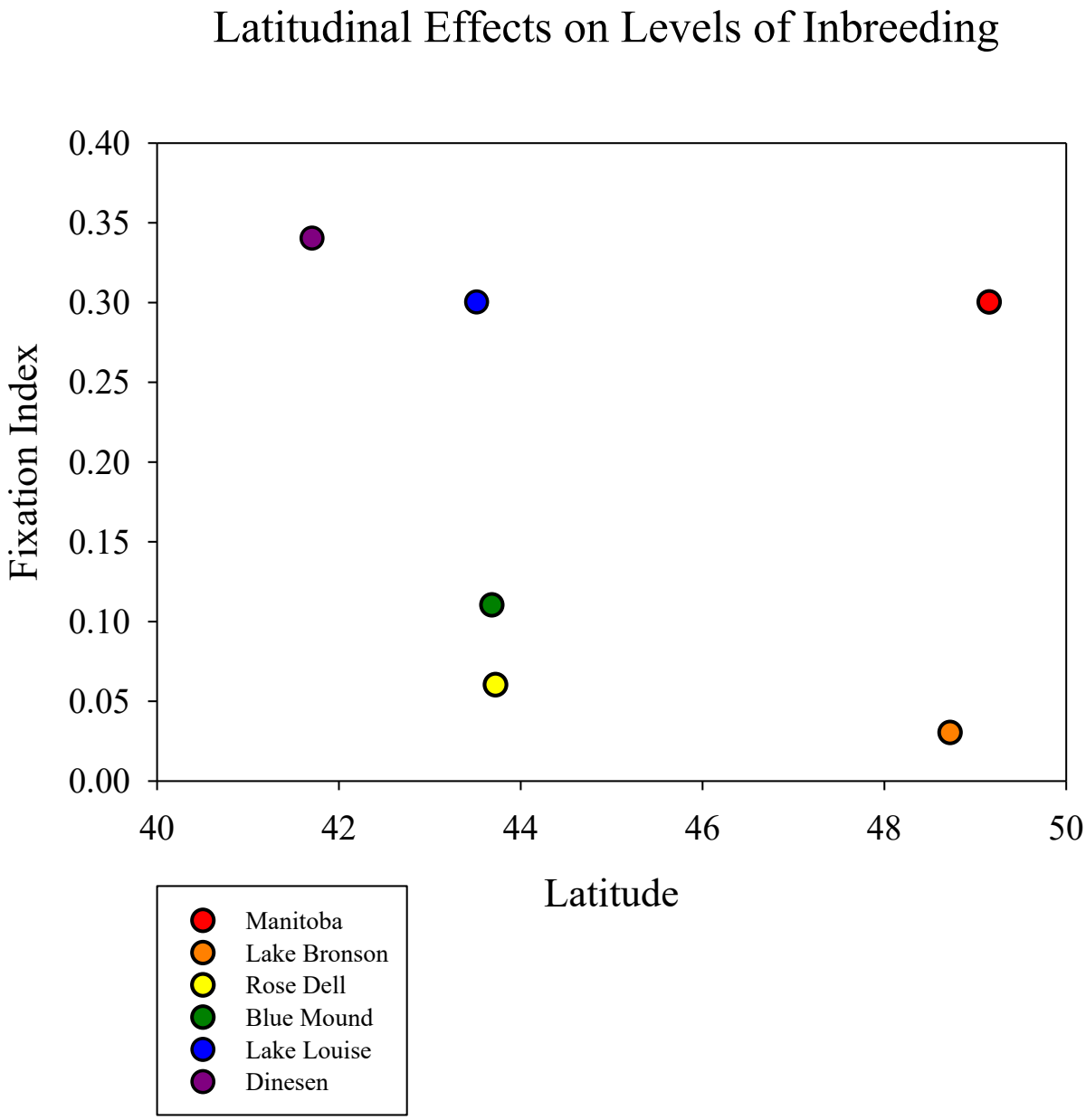


**Figure 3.4.** Fixation index as a function of latitude. Latitude was chosen because of the trend we are seeing with the disappearance of the WPFO from the southern states and eco regions.

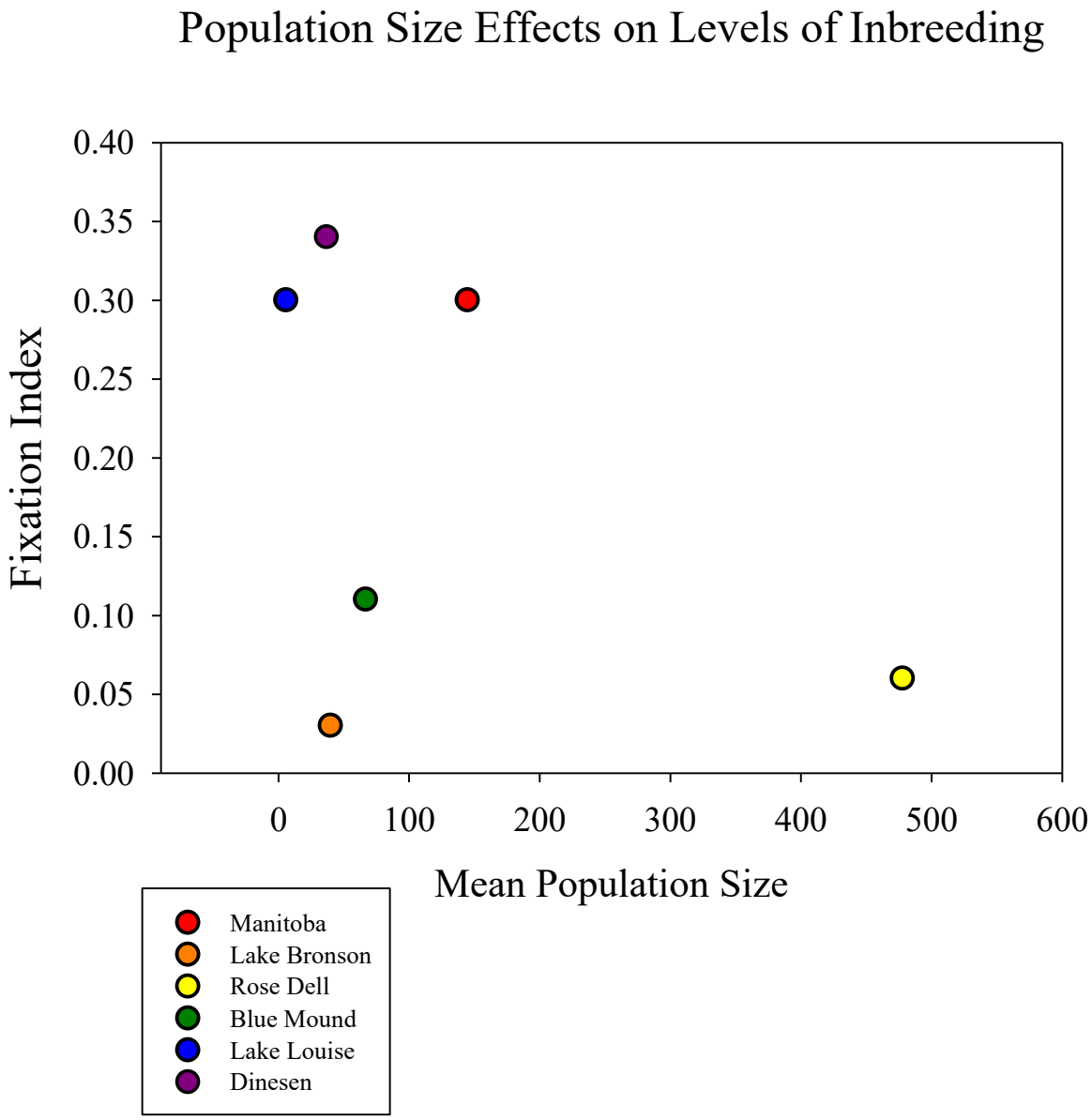


**Figure 3.5.** Fixation index as a function of mean population size.

The data is consistent with larger populations have less inbreeding. Even in small population you have low levels of inbreeding. The extreme populations have the highest fixation index (Figure 3.5).

Structure analysis identified six statistical clusters based on Bayesian analysis (D = 6). There is not complete overlap of population identity and statistical cluster (Figure 3.2). However,

three populations stand out as unique. As expected Lake Louise is largely composed of a unique cluster. Blue Mound is also largely composed of a single cluster. The Manitoba population is composed of three different clusters one of which is largely unique to this population.

Multiple regression analysis of latitude, longitude and population size versus fixation index indicates that latitudinal shifts from the core populations to the fringe populations are not statistically related to the estimated degree of inbreeding. The northern-most population (Manitoba), a central population (Lake Louise), and the southern-most populations (Dinesen) all have high levels of inbreeding. The northern-most population (Manitoba), the southern-most population (Dinesen), and the eastern-most population all have high levels if inbreeding. A regression analysis of mean populations size and fixation index indicated no apparent correlation between population size and level of inbreeding either. The data are consistent with larger populations having lower levels of inbreeding although there is a wide range of fixation indices for large populations. However, even small populations have low levels of inbreeding. The highest fixation indices were in the populations the farthest to the east, north and south.

# CHAPTER 4. DISCUSSION

## 4.1. Genetic diversity

Genetic diversity can impact fitness and ability of the WPFO to prosper. The maintenance of genetic diversity of the WPFO is a conservation priority for this rare and declining species. This is particularly true in fragmented populations where natural gene flow is interrupted and populations become small and isolated. Understanding the WPFO's diversity and divergence of remaining populations is important for designing management strategies. The populations (N=6) analyzed in this study face genetic and demographic challenges due to the decline of suitable habitat. Understanding the genetic differences is essential for writing an effective conservation plan.

The results do not support a null hypothesis model of extensive gene flow in a panmictic metapopulation. If the populations in this study were part of a well-connected metapopulation with high levels of outbreeding it would expected that populations to have high levels of genetic variation and high levels of heterozygosity within populations, and low levels of genetic divergence among populations (Wallace, 2003). In contrast, population-level analysis of allele frequencies in the populations (N=6) showed evidence of inbreeding in all populations (Table 3.1). The fixation indices per population ranged from 0.03 to 0.34 (Table 3.1). Tests of Hardy-Weinberg equilibrium indicated that unexpectedly low levels of heterozygosity were common in our study populations. Low levels of heterozygosity decrease the WPFO ability to adapt to changing environments, such as these changes can include increased frequency of droughts, flooding and increased habitat disturbances from humans, by decreasing the availability of alleles for future generations (Ross and Travers, 2016). Our results also show evidence of genetic divergence among populations. The estimated $D_{jost}$, which compares allele frequencies among all

populations, was significant ($D_{jost} = 0.0939$, $p < 0.05$), indicating that significant genetic structure exists at the regional scale of this study. This result is confirmed by the output from our Structure analysis. The Structure analysis shows relative separation of populations into six distinct genotype clusters (Figure 3.2). There is no evidence that any one population is completely isolated from the others, but the structure analysis shows an overall trend of populations diverging from each other. This is particularly true for the Lake Louise population; its cluster is dominated by a single genotype indicating a low level of genetic diversity and high levels of inbreeding. The pairwise $F_{st}$ comparisons of populations indicated significant divergences of allele frequencies between 11 of the 15 possible pairs of populations (Table 3.4). These results suggest significant levels of isolation among the populations (N=6) in this study.

**4.2. Population divergence and inbreeding**

A possible explanation for inbreeding within populations and divergence among population is that pollinator movement is restricted to a relatively small scale. This movement is restricted because WPFO's pollinator, the hawk moth, may not be traveling the large distances between the fragmented populations (Sparks *et al*., 2007). Though there is some evidence that the hawkmoth is increasing its migration in response to climate change (Sparks *et al*., 2007). Because they are at greater risk for the genetic problems associated with selfing and inbreeding, rare plants would benefit most from outcross pollination. Increased pollinator migration would increase pollination from different populations adapted to very different local environments and increase the overall diversity within the gene pool of a species (Travers *et al*., 2011). The ability of seeds to be dispersed could also be a limiting factor in outbreeding. If seeds do not disperse far then pollen transfer between near neighbors could result in inbreeding.

Little is known about pollinator interactions with WPFO species. The questions that remain are 1. Are the pollinators' visits to orchid populations promoting outcross pollination or self-pollination? 2. Is the degree of outcross-pollination related to population size? and 3. Which environmental conditions increase or suppress the efficiency of migratory pollinators?

Insect pollinators are essential to maintaining population of threatened and endangered plants in native habitats (Kearns *et al*., 1998). Six hawk moth species carry orchid pollinia: *Eumorpha achemon, Sphinx drupiferarum, Hyles gallii, Lintneria eremitus, H. Lineata,* and *H. euphorbiae L*. (Fox *et al*., 2013). However, limited research is available on WPFO pollinators. The hawkmoth has one of the longest proboscises of any of the sphynx moths, ranging from 28-40 mm (Travers *et al*., 2011). A long proboscis allows a pollinator to acquire nectar concealed in a nectar spur; however, it also forces the pollinator to become aligned with the flower's reproductive structure to either remove the pollinaria or deposit pollen grains on the stigma (Pijl and Dodson, 1966). If the pollinaria does not get placed in the correct spot on the pollinator, reproductive output is reduced. Because of their relatively long migration, hawk moths have an increased potential for transferring pollen between isolated populations, thus increasing the percent of out-breeding. The moths respond most strongly to aromatic esters such as benzyl acetate and methyl salicylate as well as oxygenated monoterpenoids including linalool and linalool oxides, all significant components for flower scents (Borkowsky, 2006). Within a pollinator-limited population or species, the recognized pollen vectors, if present in the habitat at all, fail to remove the viable pollen during the flowering season of the orchid and/or fail to deposit viable pollen grains on receptive stigmas (Bernhardt and Edens-Meier, 2010). This trend of low pollination rates could be caused by the specialization of pollinators to specific orchid species.

Why does the degree of inbreeding vary among populations? We hypothesized that longitude might be a correlate of inbreeding. There is a change in climate across a longitudinal gradient. The region in the west is relatively dry due to a rain shadow effect while the population at Lake Louise in the east gets the most precipitation. This trend could also be a result of the change in climate across gradient the farther you move away from the Rocky Mountain and its rain shadow.

However, there was no clear pattern in the inbreeding from east to west, mainly because inbreeding varied enormously along the narrow range of longitude where most of the western populations are located. Longitude was chosen because of the speciation pattern of the eastern and western prairie fringed orchids, with the Eastern prairie fringed orchid only found east of the Mississippi river and the western prairie fringed orchid only found to the west. Instead, the populations farthest east were found to be the most inbred, and the core populations are different from outlying populations.

The regression analysis of mean population size and fixation index indicated no apparent correlation between population size and level of inbreeding as was found in the study by Ross and Travers (Ross and Travers, 2016). However, the lack of a significant effect of population size on inbreeding may be due to low statistical power. Most populations were relatively small. Larger populations consistently had less inbreeding but even in some small populations there were low levels of inbreeding. Sharma (2002) did observe a significant correlation between populations size and genetic diversity. It was predicted that the largest populations will have significant levels of genetic diversity.

There is evidence that core populations differ from fringe populations. Manitoba, Dinesen, and Lake Louise, the most extreme northern, southern and eastern populations, had the

highest levels if inbreeding (high fixation index). One possible explanation is that the moths are traveling in a narrow, centralized range during orchid flowering season., This pattern of pollinator movement would lead to a decreased number of moths encountering smaller more isolated populations. However, this hypothesis assumes that fewer moth visitors leads to more self-pollination which has not been demonstrated. However, *P. praeclara* is self-compatible and visiting pollinators are likely to visit multiple flowers on the same plant (Fox *et al*., 2013). In 2006, Bokowsky found that pollinators with increased feeding activity were more likely to travel between geographically distant populations. Long distance travel may increase the level of out breeding and reproductive success. Pollinators with lower feeding rates are less likely to travel great distances between visits, and thus might ultimately pollinate sibling plants, leading to increased levels of inbreeding.

These results suggest that either mating between close relatives or selfing through geitonogamy are more common at the edges of the range compared to the center. Given, that inbreeding leads to reduced seed quality in this genus (Wallace, 2003; Travers *et al*. unpublished data) conservation and management efforts should focus on the edges of the species' range.

**4.3. Conservation implications**

USFWS considerers loss of tallgrass prairie habitat in central North America to be the leading cause for the decline of the WPFO (USFWS, 1996). Tallgrass prairies are considered the most endangered ecosystem in North America. It is estimated that between 1 and 4 percent of the original tallgrass prairies remain in small fragments across the Midwest (USFWS, 1996).

The WPFO has long been used as a symbol of the disappearing prairie lands as they are being converted into crop and range lands. Current U.S. Fish and Wildlife recovery plans (USFWS, 1996) identified 4 objectives that need to be met to maintain the WPFO: 1) increase

public knowledge of WPFO and other endangered prairie species; 2) protect current habitats; 3) identify new suitable locations; and 4) monitor and research all populations. The findings of this study suggest that an additional objective needs to be added to the current conservation plan.

The results of this study suggest that monitoring and reducing levels of inbreeding should be an additional conservation objective. The problems associated with inbreeding include increased homozygosity, accumulation of deleterious alleles, decreased fitness, and ultimately an inbreeding depression. The negative effects of inbreeding observed in both outcrossing and selfing species for a variety of traits with consequences for offspring fitness (Charlesworth and Charlesworth, 1987; Keller and Waller, 2002).

Based on the levels of inbreeding observed in this study the optimal habitat for this species may be the habitat associated with the central part of its range. Our research suggests that the Mower, Dinesen, and Manitoba populations warrant the greatest effort in recovery; these are the populations located on the fringe of the known range. These populations have the highest levels of inbreeding and are most likely to experience an extinction vortex. The reintroduction of more distantly related genetic material is essential in the preservation of these three populations sites. This effort can be in the form of hand pollination, increased support for the known pollinator species, and an increase in federally protected land that is suitable to the WPFO's needs.

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## APPENDIX A. SUMMARY OF THE MICROSATELLITE LOCI USED IN *P. PRAECLARA* POPULATIONS

| Fluorescent Tag | Primer | Forward Sequence | Reverse Sequence | Repeat Type | Range in Base Pairs |
|---|---|---|---|---|---|
| VIC | 02 | ATGAGGGTCTTCACGCATGT | CCACGGGATCTCCTTCCAAT | CT | 177-199 |
| FAM | 14 | GAGTGCCAAAGTCCATCGTG | AGCCTCGTATGGTTCCATCT | ACAG | 275 |
| NED | 27 | CAATGGTTGTGCTCTGAATGAC | CCGGTTCCAACAAAGTGC | | 433-456 |
| PET | 12 | GGTGCCGGTCACTAACTTTGA | GGCGCAACCCACATTGATT | AC | 316-328 |
| VIC | 07 | ACCCTCGTAGATCGTTTCGG | GTGGATTTCGTGTGCCTT | AG | 229-245 |
| NED | 16 | TCGAGGTGCTTCAACGATCC | TCCGGGTTTCCTTTGACGTA | AAAC | 283 |
| PET | 17 | GCATGTCTCAAGCTCTCACG | TCGCTCTCATTTCCACCG | GTTT | 311-325 |
| NED | 05 | TACCCGAGTTCCTTGCTGAC | CCTCTGACAACAACCAGT | CT | 202-214 |
| | | | | | |

# APPENDIX B. ELECTROPHEROGRAM PEAKS

| Population | ID # | 02 VIC | 02 VIC | 14 FAM | 14 FAM | 27 NED | 27 NED | 12 PET | 12 PET | 07 VIC | 07 VIC | 16 NED | 16 NED | 17 PET | 17 PET | 05 NED | 05 NED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lake_Bronson | LB-02 | 181 | 189 | 287 | 287 | 443 | 443 | 0 | 0 | 241 | 241 | 290 | 298 | 322 | 322 | 209 | 211 |
| Lake_Bronson | LB-03 | 181 | 189 | 287 | 287 | 437 | 437 | 287 | 309 | 241 | 241 | 290 | 298 | 322 | 322 | 209 | 209 |
| Lake_Bronson | LB-04 | 187 | 187 | 287 | 287 | 435 | 435 | 0 | 0 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |
| Lake_Bronson | LB-05 | 181 | 187 | 287 | 287 | 435 | 443 | 293 | 293 | 241 | 241 | 298 | 298 | 314 | 322 | 209 | 209 |
| Lake_Bronson | LB-06 | 185 | 195 | 287 | 287 | 445 | 445 | 289 | 309 | 241 | 243 | 290 | 298 | 322 | 326 | 205 | 209 |
| Lake_Bronson | LB-07 | 187 | 187 | 287 | 287 | 435 | 435 | 0 | 0 | 241 | 241 | 290 | 298 | 322 | 322 | 203 | 205 |
| Lake_Bronson | LB-08 | 187 | 187 | 287 | 287 | 0 | 0 | 291 | 291 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 207 |
| Lake_Bronson | LB-09 | 195 | 195 | 287 | 287 | 445 | 445 | 289 | 309 | 243 | 243 | 290 | 298 | 322 | 326 | 205 | 209 |
| Lake_Bronson | LB-10 | 185 | 185 | 287 | 287 | 445 | 445 | 293 | 293 | 241 | 241 | 294 | 298 | 322 | 322 | 207 | 209 |
| Lake_Bronson | LB-11 | 181 | 185 | 287 | 287 | 435 | 445 | 267 | 311 | 241 | 243 | 290 | 298 | 322 | 322 | 209 | 209 |
| Lake_Bronson | LB-12 | 181 | 185 | 287 | 287 | 445 | 445 | 311 | 311 | 239 | 241 | 290 | 298 | 322 | 322 | 209 | 209 |
| Lake_Bronson | LB-13 | 183 | 187 | 287 | 287 | 433 | 443 | 309 | 309 | 239 | 239 | 298 | 298 | 322 | 322 | 207 | 209 |
| Lake_Bronson | LB-14 | 185 | 187 | 287 | 287 | 445 | 445 | 291 | 293 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |
| Lake_Bronson | LB-15 | 187 | 189 | 287 | 287 | 435 | 445 | 291 | 293 | 241 | 241 | 290 | 298 | 314 | 322 | 205 | 209 |
| Lake_Bronson | LB-16 | 185 | 187 | 287 | 287 | 443 | 443 | 287 | 295 | 239 | 241 | 298 | 298 | 322 | 322 | 0 | 0 |
| Lake_Bronson | LB-17 | 185 | 185 | 287 | 287 | 443 | 443 | 291 | 291 | 241 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Lake_Bronson | LB-18 | 185 | 195 | 287 | 287 | 433 | 443 | 283 | 291 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 209 |
| Lake_Bronson | LB-19 | 185 | 187 | 287 | 287 | 443 | 443 | 287 | 311 | 241 | 241 | 290 | 298 | 314 | 322 | 205 | 209 |
| Lake_Bronson | LB-20 | 185 | 187 | 287 | 287 | 445 | 445 | 289 | 289 | 239 | 241 | 290 | 298 | 322 | 322 | 207 | 207 |
| Lake_Bronson | LB-21 | 181 | 185 | 287 | 287 | 433 | 443 | 311 | 325 | 241 | 243 | 290 | 298 | 322 | 324 | 205 | 211 |
| Lake_Bronson | LB-22 | 185 | 187 | 287 | 287 | 443 | 443 | 279 | 293 | 239 | 241 | 298 | 298 | 322 | 322 | 205 | 209 |
| Lake_Bronson | LB-23 | 181 | 185 | 287 | 287 | 433 | 443 | 283 | 309 | 241 | 241 | 294 | 298 | 322 | 322 | 211 | 211 |
| Lake_Bronson | LB-24 | 185 | 187 | 287 | 287 | 435 | 445 | 0 | 0 | 241 | 241 | 290 | 298 | 322 | 322 | 207 | 209 |
| Lake_Bronson | LB-25 | 181 | 185 | 287 | 287 | 445 | 445 | 279 | 311 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |
| Lake_Bronson | LB-26 | 185 | 185 | 287 | 287 | 433 | 443 | 279 | 279 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |

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| Lake_Bronson | LB-27 | 185 | 185 | 287 | 287 | 433 | 445 | 291 | 321 | 239 | 241 | 294 | 298 | 322 | 322 | 207 | 209 |
| Lake_Bronson | LB-28 | 187 | 187 | 287 | 287 | 435 | 445 | 279 | 283 | 241 | 241 | 294 | 298 | 322 | 322 | 205 | 207 |
| Lake_Bronson | LB-29 | 185 | 187 | 287 | 287 | 443 | 443 | 285 | 303 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |
| Lake_Bronson | LB-30 | 187 | 189 | 287 | 287 | 443 | 443 | 269 | 269 | 239 | 241 | 290 | 298 | 322 | 322 | 209 | 211 |
| Blue_Mound | BM-01 | 185 | 187 | 287 | 287 | 0 | 0 | 0 | 0 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 207 |
| Blue_Mound | BM-02 | 187 | 187 | 287 | 287 | 447 | 447 | 259 | 259 | 239 | 243 | 290 | 298 | 322 | 322 | 207 | 209 |
| Blue_Mound | BM-03 | 185 | 187 | 287 | 287 | 445 | 445 | 281 | 281 | 239 | 241 | 290 | 298 | 322 | 322 | 207 | 207 |
| Blue_Mound | BM-04 | 187 | 189 | 287 | 287 | 0 | 0 | 0 | 0 | 241 | 243 | 290 | 298 | 0 | 0 | 205 | 211 |
| Blue_Mound | BM-05 | 185 | 187 | 287 | 287 | 443 | 443 | 259 | 259 | 239 | 243 | 290 | 298 | 322 | 322 | 209 | 209 |
| Blue_Mound | BM-06 | 187 | 189 | 287 | 287 | 445 | 445 | 0 | 0 | 241 | 243 | 290 | 298 | 322 | 322 | 205 | 205 |
| Blue_Mound | BM-07 | 187 | 187 | 287 | 287 | 443 | 443 | 259 | 321 | 241 | 241 | 290 | 298 | 322 | 322 | 207 | 207 |
| Blue_Mound | BM-08 | 187 | 189 | 287 | 287 | 445 | 451 | 259 | 289 | 241 | 243 | 290 | 298 | 322 | 322 | 207 | 209 |
| Blue_Mound | BM-09 | 185 | 187 | 287 | 287 | 445 | 445 | 291 | 337 | 243 | 243 | 290 | 298 | 322 | 322 | 207 | 207 |
| Blue_Mound | BM-10 | 185 | 187 | 287 | 287 | 445 | 445 | 337 | 337 | 243 | 243 | 290 | 298 | 322 | 322 | 205 | 209 |
| Blue_Mound | BM-11 | 185 | 187 | 287 | 287 | 443 | 443 | 259 | 307 | 239 | 243 | 298 | 298 | 322 | 322 | 209 | 209 |
| Blue_Mound | BM-13 | 185 | 185 | 287 | 287 | 443 | 443 | 259 | 279 | 243 | 243 | 290 | 298 | 322 | 322 | 209 | 209 |
| Blue_Mound | BM-14 | 183 | 187 | 287 | 287 | 443 | 443 | 305 | 321 | 241 | 243 | 290 | 298 | 322 | 322 | 207 | 207 |
| Blue_Mound | BM-15 | 185 | 189 | 287 | 287 | 443 | 443 | 259 | 337 | 241 | 243 | 290 | 298 | 322 | 322 | 209 | 209 |
| Blue_Mound | BM-16 | 185 | 185 | 287 | 287 | 443 | 443 | 281 | 287 | 239 | 241 | 290 | 298 | 322 | 322 | 205 | 207 |
| Blue_Mound | BM-17 | 185 | 185 | 287 | 287 | 443 | 443 | 281 | 315 | 241 | 243 | 294 | 298 | 322 | 322 | 207 | 209 |
| Blue_Mound | BM-18 | 185 | 189 | 287 | 287 | 443 | 443 | 259 | 287 | 243 | 243 | 298 | 298 | 322 | 322 | 209 | 211 |
| Blue_Mound | BB-01 | 181 | 187 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 294 | 294 | 322 | 322 | 205 | 209 |
| Blue_Mound | BB-03 | 185 | 187 | 0 | 0 | 0 | 0 | 0 | 0 | 241 | 243 | 298 | 298 | 322 | 322 | 205 | 207 |
| Blue_Mound | BB-04 | 0 | 0 | 0 | 0 | 443 | 445 | 0 | 0 | 0 | 0 | 298 | 298 | 322 | 322 | 209 | 209 |
| Blue_Mound | BB-05 | 0 | 0 | 0 | 0 | 443 | 445 | 0 | 0 | 0 | 0 | 298 | 298 | 0 | 0 | 207 | 207 |
| Blue_Mound | BB-06 | 0 | 0 | 0 | 0 | 443 | 443 | 0 | 0 | 0 | 0 | 298 | 298 | 0 | 0 | 207 | 211 |
| Blue_Mound | BB-07 | 181 | 185 | 0 | 0 | 0 | 0 | 0 | 0 | 243 | 243 | 298 | 298 | 322 | 322 | 205 | 205 |
| Blue_Mound | BB-08 | 185 | 185 | 0 | 0 | 443 | 443 | 0 | 0 | 0 | 0 | 298 | 298 | 0 | 0 | 209 | 209 |
| Blue_Mound | BB-09 | 181 | 185 | 0 | 0 | 443 | 443 | 0 | 0 | 0 | 0 | 294 | 294 | 0 | 0 | 205 | 205 |
| Rose_Dell | H-02 | 185 | 189 | 287 | 287 | 443 | 443 | 0 | 0 | 241 | 243 | 298 | 298 | 322 | 322 | 207 | 209 |
| Rose_Dell | H-03 | 185 | 189 | 287 | 287 | 437 | 443 | 303 | 303 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 205 |
| Rose_Dell | H-04 | 183 | 189 | 287 | 287 | 437 | 443 | 259 | 259 | 241 | 243 | 290 | 298 | 322 | 322 | 207 | 209 |
| Rose_Dell | H-05 | 187 | 189 | 287 | 287 | 435 | 435 | 283 | 293 | 239 | 243 | 298 | 298 | 322 | 322 | 205 | 207 |
| Rose_Dell | H-06 | 185 | 189 | 287 | 287 | 435 | 435 | 283 | 309 | 241 | 243 | 0 | 0 | 322 | 322 | 209 | 209 |
| Rose_Dell | H-08 | 189 | 191 | 287 | 287 | 437 | 445 | 281 | 303 | 241 | 243 | 294 | 298 | 322 | 322 | 207 | 207 |

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| Rose_Dell | H-09 | 183 | 193 | 287 | 287 | 445 | 445 | 283 | 283 | 239 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Rose_Dell | H-10 | 185 | 189 | 287 | 287 | 445 | 445 | 281 | 281 | 239 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Rose_Dell | H-11 | 189 | 191 | 287 | 287 | 435 | 445 | 295 | 309 | 239 | 243 | 290 | 298 | 322 | 322 | 209 | 209 |
| Rose_Dell | H-12 | 185 | 187 | 287 | 287 | 445 | 445 | 281 | 321 | 239 | 243 | 298 | 298 | 322 | 322 | 209 | 209 |
| Rose_Dell | H-19 | 181 | 189 | 0 | 0 | 443 | 443 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 209 | 211 |
| Rose_Dell | H-20 | 181 | 187 | 0 | 0 | 443 | 443 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 205 | 205 |
| Rose_Dell | H-25 | 185 | 187 | 0 | 0 | 0 | 0 | 0 | 0 | 241 | 243 | 294 | 298 | 0 | 0 | 205 | 209 |
| Rose_Dell | H-26 | 181 | 183 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 294 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-01 | 183 | 197 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 294 | 298 | 0 | 0 | 207 | 209 |
| Rose_Dell | H2-02 | 183 | 185 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 290 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-03 | 183 | 185 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 243 | 298 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-04 | 185 | 185 | 287 | 287 | 0 | 0 | 323 | 323 | 241 | 243 | 298 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-05 | 185 | 189 | 287 | 287 | 0 | 0 | 0 | 0 | 241 | 243 | 298 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-06 | 183 | 193 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 243 | 290 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-07 | 183 | 185 | 287 | 287 | 0 | 0 | 319 | 321 | 239 | 241 | 298 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-08 | 185 | 189 | 287 | 287 | 0 | 0 | 323 | 323 | 241 | 243 | 290 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-09 | 189 | 193 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 298 | 298 | 0 | 0 | 205 | 209 |
| Rose_Dell | H2-10 | 183 | 189 | 287 | 287 | 0 | 0 | 0 | 0 | 239 | 239 | 298 | 298 | 0 | 0 | 207 | 209 |
| Rose_Dell | H2-11 | 183 | 193 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 239 | 290 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-12 | 183 | 189 | 287 | 287 | 0 | 0 | 321 | 321 | 239 | 241 | 294 | 298 | 0 | 0 | 205 | 209 |
| Rose_Dell | H2-13 | 185 | 189 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 239 | 298 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-14 | 185 | 189 | 287 | 287 | 0 | 0 | 323 | 323 | 243 | 243 | 298 | 298 | 0 | 0 | 207 | 209 |
| Rose_Dell | H2-15 | 185 | 185 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 298 | 298 | 0 | 0 | 207 | 209 |
| Rose_Dell | H2-16 | 183 | 193 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 243 | 298 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-17 | 185 | 185 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 298 | 298 | 0 | 0 | 207 | 207 |
| Rose_Dell | H2-18 | 185 | 185 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 243 | 0 | 0 | 0 | 0 | 205 | 209 |
| Rose_Dell | H2-19 | 195 | 197 | 287 | 287 | 0 | 0 | 321 | 321 | 241 | 241 | 294 | 298 | 0 | 0 | 205 | 209 |
| Rose_Dell | H2-20 | 187 | 189 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 0 | 0 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-21 | 189 | 191 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 0 | 0 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-22 | 183 | 185 | 287 | 287 | 0 | 0 | 0 | 0 | 241 | 243 | 298 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-23 | 183 | 185 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 298 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-24 | 181 | 195 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 298 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-25 | 0 | 0 | 287 | 287 | 0 | 0 | 321 | 321 | 243 | 243 | 298 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-26 | 0 | 0 | 0 | 0 | 0 | 0 | 323 | 323 | 243 | 243 | 298 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-27 | 0 | 0 | 287 | 287 | 0 | 0 | 323 | 323 | 241 | 243 | 298 | 298 | 0 | 0 | 0 | 0 |

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| Rose_Dell | H2-28 | 0 | 0 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 241 | 298 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-29 | 0 | 0 | 287 | 287 | 0 | 0 | 323 | 323 | 241 | 243 | 298 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-30 | 0 | 0 | 287 | 287 | 0 | 0 | 323 | 323 | 241 | 241 | 298 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-31 | 0 | 0 | 287 | 287 | 0 | 0 | 323 | 323 | 241 | 243 | 298 | 298 | 0 | 0 | 209 | 209 |
| Rose_Dell | H2-32 | 0 | 0 | 287 | 287 | 0 | 0 | 323 | 323 | 241 | 243 | 298 | 298 | 0 | 0 | 0 | 0 |
| Rose_Dell | H2-33 | 0 | 0 | 287 | 287 | 0 | 0 | 323 | 323 | 239 | 243 | 298 | 298 | 0 | 0 | 209 | 209 |
| Lake_Louise | M-01 | 185 | 185 | 287 | 287 | 445 | 445 | 0 | 0 | 241 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Lake_Louise | M-02 | 185 | 185 | 287 | 287 | 443 | 443 | 275 | 285 | 241 | 241 | 0 | 0 | 322 | 322 | 205 | 209 |
| Lake_Louise | M-03 | 187 | 189 | 287 | 287 | 445 | 445 | 275 | 287 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 205 |
| Lake_Louise | M-04 | 185 | 185 | 287 | 287 | 445 | 445 | 0 | 0 | 239 | 239 | 0 | 0 | 322 | 322 | 209 | 209 |
| Lake_Louise | M-05 | 185 | 191 | 287 | 287 | 445 | 445 | 0 | 0 | 241 | 241 | 298 | 298 | 322 | 322 | 209 | 211 |
| Lake_Louise | M-06 | 185 | 191 | 287 | 287 | 445 | 445 | 275 | 275 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 205 |
| Lake_Louise | M-07 | 185 | 185 | 287 | 287 | 445 | 445 | 275 | 275 | 241 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Lake_Louise | M-08 | 185 | 185 | 287 | 287 | 445 | 445 | 275 | 299 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 205 |
| Lake_Louise | M-09 | 185 | 191 | 287 | 287 | 445 | 445 | 299 | 299 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 209 |
| Lake_Louise | M-10 | 181 | 185 | 287 | 287 | 445 | 445 | 285 | 299 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 209 |
| Lake_Louise | M-11 | 185 | 187 | 287 | 287 | 443 | 445 | 285 | 285 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 209 |
| Lake_Louise | M-12 | 185 | 185 | 287 | 287 | 443 | 443 | 275 | 275 | 241 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Lake_Louise | M-13 | 185 | 185 | 287 | 287 | 443 | 443 | 275 | 299 | 241 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Pembina | P-03 | 185 | 185 | 0 | 0 | 443 | 443 | 0 | 0 | 0 | 0 | 298 | 298 | 0 | 0 | 0 | 0 |
| Pembina | P-05 | 0 | 0 | 0 | 0 | 443 | 445 | 0 | 0 | 0 | 0 | 298 | 298 | 0 | 0 | 209 | 209 |
| Pembina | P-06 | 185 | 185 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 298 | 298 | 0 | 0 | 209 | 209 |
| Pembina | P-07 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 298 | 298 | 0 | 0 | 209 | 209 |
| Pembina | P-19 | 185 | 187 | 0 | 0 | 0 | 0 | 0 | 0 | 243 | 243 | 290 | 298 | 322 | 322 | 209 | 209 |
| Pembina | P-23 | 181 | 187 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 322 | 322 | 207 | 209 |
| Pembina | P2-11 | 177 | 185 | 0 | 0 | 439 | 449 | 295 | 295 | 239 | 241 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pembina | P2-15 | 185 | 193 | 0 | 0 | 433 | 435 | 265 | 283 | 241 | 241 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pembina | P2-17 | 181 | 185 | 0 | 0 | 435 | 435 | 277 | 289 | 241 | 241 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pembina | P2-18 | 185 | 187 | 0 | 0 | 435 | 435 | 299 | 331 | 241 | 243 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pembina | P2-21 | 181 | 185 | 0 | 0 | 435 | 435 | 285 | 299 | 241 | 241 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pembina | P2-25 | 185 | 187 | 0 | 0 | 437 | 449 | 285 | 313 | 239 | 243 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pembina | P2-27 | 185 | 189 | 0 | 0 | 435 | 437 | 0 | 0 | 241 | 241 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dinesen | D-01 | 181 | 185 | 287 | 287 | 435 | 435 | 323 | 323 | 241 | 243 | 298 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-02 | 185 | 187 | 287 | 287 | 447 | 447 | 323 | 323 | 241 | 243 | 290 | 298 | 326 | 326 | 205 | 207 |
| Dinesen | D-03 | 183 | 185 | 287 | 287 | 443 | 443 | 323 | 323 | 241 | 243 | 290 | 298 | 322 | 322 | 205 | 207 |

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| Dinesen | D-04 | 181 | 185 | 287 | 287 | 0 | 0 | 319 | 319 | 241 | 243 | 290 | 298 | 322 | 322 | 205 | 207 |
| Dinesen | D-05 | 181 | 195 | 0 | 0 | 0 | 0 | 0 | 0 | 239 | 243 | 298 | 298 | 318 | 318 | 209 | 209 |
| Dinesen | D-06 | 181 | 181 | 287 | 287 | 0 | 0 | 0 | 0 | 239 | 243 | 298 | 298 | 322 | 322 | 209 | 211 |
| Dinesen | D-07 | 181 | 185 | 287 | 287 | 0 | 0 | 0 | 0 | 243 | 243 | 290 | 298 | 322 | 322 | 205 | 205 |
| Dinesen | D-08 | 185 | 187 | 287 | 287 | 445 | 445 | 323 | 323 | 243 | 243 | 290 | 298 | 322 | 322 | 205 | 207 |
| Dinesen | D-09 | 185 | 185 | 287 | 287 | 435 | 435 | 0 | 0 | 239 | 239 | 290 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-10 | 181 | 187 | 287 | 287 | 447 | 447 | 323 | 323 | 239 | 239 | 290 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-11 | 181 | 185 | 287 | 287 | 435 | 435 | 323 | 323 | 243 | 243 | 298 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-12 | 181 | 185 | 287 | 287 | 0 | 0 | 0 | 0 | 243 | 243 | 298 | 298 | 322 | 322 | 207 | 211 |
| Dinesen | D-13 | 183 | 185 | 287 | 287 | 447 | 447 | 323 | 323 | 241 | 243 | 290 | 298 | 322 | 322 | 209 | 209 |
| Dinesen | D-14 | 183 | 185 | 0 | 0 | 435 | 435 | 323 | 323 | 243 | 243 | 298 | 298 | 322 | 322 | 207 | 213 |
| Dinesen | D-15 | 183 | 185 | 0 | 0 | 435 | 435 | 0 | 0 | 241 | 241 | 290 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-16 | 185 | 185 | 0 | 0 | 0 | 0 | 0 | 0 | 241 | 241 | 290 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-17 | 177 | 179 | 287 | 287 | 435 | 445 | 321 | 321 | 243 | 243 | 290 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-18 | 177 | 183 | 287 | 287 | 437 | 451 | 0 | 0 | 243 | 243 | 290 | 298 | 322 | 322 | 207 | 207 |
| Dinesen | D-19 | 181 | 187 | 0 | 0 | 445 | 447 | 323 | 323 | 243 | 243 | 290 | 298 | 322 | 322 | 205 | 207 |
| Dinesen | D-20 | 185 | 195 | 287 | 287 | 437 | 445 | 321 | 321 | 241 | 243 | 290 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-21 | 185 | 185 | 0 | 0 | 445 | 445 | 323 | 323 | 239 | 239 | 298 | 298 | 322 | 322 | 207 | 207 |
| Dinesen | D-22 | 181 | 185 | 287 | 287 | 0 | 0 | 0 | 0 | 239 | 239 | 290 | 298 | 322 | 322 | 205 | 207 |
| Dinesen | D-23 | 181 | 181 | 287 | 287 | 435 | 445 | 321 | 321 | 239 | 239 | 290 | 298 | 322 | 322 | 201 | 207 |
| Dinesen | D-24 | 181 | 185 | 287 | 287 | 445 | 445 | 321 | 321 | 239 | 239 | 290 | 298 | 322 | 322 | 201 | 209 |
| Dinesen | D-25 | 181 | 185 | 287 | 287 | 435 | 435 | 321 | 321 | 239 | 239 | 290 | 298 | 322 | 322 | 207 | 211 |
| Dinesen | D-27 | 185 | 185 | 287 | 287 | 437 | 437 | 323 | 323 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 205 |
| Dinesen | D-28 | 181 | 185 | 287 | 287 | 435 | 435 | 0 | 0 | 0 | 0 | 294 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-29 | 181 | 181 | 287 | 287 | 0 | 0 | 321 | 321 | 239 | 243 | 294 | 298 | 322 | 322 | 209 | 211 |
| Dinesen | D-30 | 181 | 181 | 0 | 0 | 445 | 445 | 0 | 0 | 239 | 239 | 290 | 298 | 322 | 322 | 205 | 207 |
| Dinesen | D-31 | 185 | 185 | 287 | 287 | 435 | 435 | 321 | 321 | 241 | 241 | 290 | 298 | 322 | 322 | 207 | 209 |
| Dinesen | D-32 | 191 | 191 | 287 | 287 | 0 | 0 | 323 | 323 | 243 | 243 | 290 | 298 | 322 | 322 | 205 | 207 |
| Dinesen | D-33 | 185 | 185 | 287 | 287 | 445 | 449 | 321 | 321 | 239 | 239 | 290 | 298 | 322 | 322 | 205 | 209 |
| Dinesen | D-34 | 185 | 185 | 287 | 287 | 447 | 447 | 321 | 321 | 239 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |
| Dinesen | D-35 | 185 | 191 | 0 | 0 | 445 | 445 | 0 | 0 | 241 | 243 | 290 | 298 | 322 | 322 | 205 | 205 |
| Manitoba | C-2951 | 183 | 185 | 287 | 287 | 435 | 435 | 323 | 323 | 241 | 243 | 294 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2952 | 187 | 189 | 287 | 287 | 425 | 425 | 323 | 323 | 241 | 243 | 294 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2953 | 187 | 187 | 287 | 287 | 445 | 445 | 323 | 323 | 239 | 241 | 298 | 298 | 322 | 322 | 207 | 207 |
| Manitoba | C-2955 | 185 | 187 | 287 | 287 | 443 | 443 | 325 | 325 | 241 | 241 | 294 | 298 | 322 | 322 | 209 | 209 |

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| Manitoba | C-2956 | 185 | 189 | 287 | 287 | 449 | 449 | 325 | 325 | 241 | 241 | 294 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2957 | 187 | 187 | 287 | 287 | 0 | 0 | 325 | 325 | 241 | 241 | 294 | 298 | 322 | 322 | 209 | 211 |
| Manitoba | C-2959 | 185 | 187 | 287 | 287 | 437 | 437 | 323 | 323 | 239 | 239 | 298 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2961 | 187 | 187 | 287 | 287 | 445 | 445 | 323 | 323 | 241 | 243 | 294 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2962 | 185 | 187 | 287 | 287 | 435 | 449 | 285 | 321 | 239 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2965 | 181 | 187 | 287 | 287 | 0 | 0 | 285 | 321 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2969 | 185 | 187 | 287 | 287 | 445 | 449 | 291 | 321 | 239 | 239 | 290 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2971 | 187 | 195 | 287 | 287 | 437 | 437 | 285 | 321 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2972 | 187 | 187 | 287 | 287 | 433 | 451 | 325 | 325 | 239 | 241 | 294 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2973 | 181 | 185 | 287 | 287 | 447 | 449 | 323 | 323 | 241 | 243 | 298 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2974 | 185 | 187 | 287 | 287 | 451 | 451 | 325 | 325 | 241 | 241 | 290 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2975 | 187 | 189 | 287 | 287 | 0 | 0 | 325 | 325 | 241 | 241 | 294 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2976 | 181 | 187 | 287 | 287 | 437 | 437 | 325 | 325 | 241 | 241 | 294 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2977 | 185 | 195 | 287 | 287 | 449 | 449 | 325 | 325 | 241 | 243 | 298 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2979 | 185 | 187 | 287 | 287 | 443 | 449 | 323 | 323 | 241 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2980 | 187 | 195 | 287 | 287 | 435 | 445 | 0 | 0 | 241 | 243 | 298 | 298 | 318 | 318 | 205 | 209 |
| Manitoba | C-2983 | 183 | 185 | 287 | 287 | 437 | 447 | 321 | 321 | 241 | 241 | 298 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2985 | 187 | 195 | 287 | 287 | 445 | 445 | 321 | 321 | 239 | 241 | 298 | 298 | 322 | 322 | 205 | 209 |
| Manitoba | C-2986 | 187 | 189 | 287 | 287 | 435 | 449 | 321 | 321 | 239 | 241 | 298 | 298 | 322 | 322 | 205 | 205 |
| Manitoba | C-2988 | 189 | 195 | 287 | 287 | 447 | 447 | 321 | 321 | 241 | 241 | 294 | 298 | 322 | 322 | 205 | 207 |
| Manitoba | C-2989 | 187 | 187 | 287 | 287 | 435 | 435 | 323 | 323 | 243 | 243 | 298 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-2991 | 185 | 197 | 287 | 287 | 449 | 449 | 323 | 323 | 239 | 241 | 290 | 298 | 322 | 322 | 207 | 207 |
| Manitoba | C-2992 | 187 | 189 | 287 | 287 | 433 | 433 | 325 | 325 | 239 | 243 | 298 | 298 | 322 | 322 | 205 | 207 |
| Manitoba | C-2995 | 187 | 197 | 287 | 287 | 433 | 433 | 0 | 0 | 241 | 243 | 298 | 298 | 318 | 318 | 205 | 207 |
| Manitoba | C-2996 | 185 | 189 | 287 | 287 | 433 | 435 | 325 | 325 | 239 | 241 | 298 | 298 | 322 | 322 | 209 | 209 |
| Manitoba | C-3000 | 187 | 189 | 287 | 287 | 445 | 445 | 285 | 325 | 241 | 243 | 298 | 298 | 322 | 322 | 205 | 209 |