EFFECTS OF PERIPHERALITY AND HYBRIDIZATION ON RANGE-WIDE GENETIC STRUCTURE OF THE ENDANGERED COASTAL DUNE PLANT *ABRONIA UMBELLATA* (NYCTAGINACEAE)

by

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Abstract

The pattern of genetic variation across a species range may give insight into geographic variation in adaptive capacity and hence, conservation priority. For this reason, determining the factors that may influence range-wide genetic structure is vital, but remains relatively understudied. Demography has long been viewed as the dominant factor influencing species' genetic structure, but non-demographic processes must be considered as well. In this study, we investigated the effects of peripherality, hybridization and mating system on the range wide genetic structure of *Abronia umbellata*, a pink-flowered Pacific coastal dune endemic and that ranges from San Quintin, Baja California to Coos Bay, Oregon and occurs as disjunct populations in Washington and on Vancouver Island in Canada. The species exhibits striking mating system differentiation across the range with obligately outcrossing populations south of San Francisco and highly selfing populations to the north. *Abronia umbellata* also has two congeners that it co-occurs and creates putative phenotypic hybrids with, yellow-flowered *A. latifolia* and magenta-flowered *A. maritima*. Genetic analyses of sequence variation at nine single-copy genes revealed that outcrossing and selfing populations of *A. umbellata* are genetically differentiated from each other and northern disjunct populations of *A. umbellata* are genetically similar to selfing populations. Furthermore, the northern disjunct populations contain little unique variation and thus, likely arose through a recent long-distance dispersal event or fragmentation of a recent expansion. Southern edge populations in Baja California, on the other hand, are genetically unique and significantly higher in genetic variation than other outcrossing populations. Extensive searches in 37 populations across the range of *A. umbellata* in 2017 and 2018, revealed that phenotypic hybrids occurred in 100% of locations where outcrossing populations of *A. umbellata* were sympatric with a congener and sequence variation at five

single-copy genes indicate that phenotypic hybrids are in fact hybrids. However, during the same searches we did not find any phenotypic hybrids with selfing populations of *A. umbellata* and quantitative analyses showed that hybrids were only found in 2.6% of 695 3-m radius plots across all 37 populations.

Co-Authorship

This thesis conforms to the manuscript format as outlined by the School of Graduate Studies and Research. The manuscripts that are directly a result of this thesis and its co-authors are:

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Chapter 1

General Introduction

Understanding range-wide genetic structure can bring insight into a species' adaptive capacity, demographic and evolutionary history, and possible conservation strategies. Many factors can influence species' genetic structure, but demography has long been viewed as the dominant factor. Hence, predictions concerning how genetic structure might vary across species' ranges derive from demographic models such as the Abundant-Centre Hypothesis (ACH). The long-standing ACH posits that a species should have the highest abundance and fitness in the centre of its range, where the environment is conducive to highest individual fitness, and abundance should decrease with fitness towards the range edges (Lawton, 1993; Vucetich and Waite, 2003). Because lower abundance and fitness should reduce genetic effective population size and levels of gene flow between populations, the ACH indirectly suggests that genetic variation within populations should decrease and genetic differentiation among populations should increase towards range edges.

The demographic and genetic ramifications of the ACH have led to controversy surrounding the conservation value of peripheral populations. Some argue that peripheral populations are not worth conserving because they are small, isolated, low in genetic variation and more vulnerable to extinction through demographic and environmental stochasticity (García and Arroyo, 2001; Williams et al., 2003). Others argue that peripheral populations could be locally adapted to extreme environments at range edges, making them genetically unique from populations throughout the rest of the range. In this way, peripheral populations are an important

component of the evolutionary potential of the species (Rehm et al., 2015). As an extension of the second argument, some contend that geographically disjunct populations are particularly worth conserving because they are most likely to be genetically unique owing to infrequent gene flow from populations in the core of the range (Bunnell et al., 2004). Conservation of peripheral populations is especially relevant in Canada as ~ 75% of plant species federally listed as "at-risk" are peripheral populations of species that are otherwise common south of the Canada-USA border (Klemet-N'Guessan et al., 2019).

Straightforward extension of the ACH does not consider the influence of factors other than demography on range-wide genetic structure. For instance, hybridization with closely related congeners can have a variety of impacts on genetic structure, especially of peripheral populations, and may strongly affect a species' capacity for range expansions. When a species is rare at its range edge, hybridization, and subsequent introgression, with more abundant congeners may increase the standing genetic variation or beneficial mutations needed for adaptation to beyond range conditions (Pfennig et al., 2016). Alternatively, hybridization at the range edge could impose a demographic cost on peripheral populations if hybrids suffer relatively low fitness (Prentis et al., 2007) or may lead to genetic assimilation of the rare species by the more common one (Hufford and Mazer, 2003).

Shifts in mating system can also influence range-wide genetic structure, especially in plants. Approximately 10–15% of all angiosperms are predominantly self-fertilizing, while another 40–45% have mating system involving a broad mix or selfing and outcrossing (Goodwillie et al., 2005). Self-fertilization may be selected at range edges if peripheral

populations chronically suffer from a lack of mates and/or pollinators owing to their low density (Baker, 1955). Because selfing dramatically reduces effective population size and opportunities for gene flow, full self-fertilization is expected to be accompanied by a 50% reduction in genetic variation and higher among-population genetic differentiation (Charlesworth and Wright, 2001). Thus, a comprehensive understanding of how genetic structure varies across species ranges and the underlying factors influencing this structure requires an assessment of more than just demographic variation.

In this study, we investigated variation in genetic structure and its underlying influences across the geographic range of the Pacific coastal dune endemic *Abronia umbellata* Lam. (Nyctaginaceae), with a focus on its range edges. *Abronia umbellata* is a good case study for range-wide genetic structure because its range is restricted to a nearly one-dimensional band of dune habitat running up the Pacific coast of western North America. Moreover, populations are easily accessed, and it is an attractive pink-flowered and conspicuous member of the dune flora that is well-represented among herbarium specimens. *Abronia umbellata* ranges from Baja California, Mexico to southern Oregon, USA and occurs as disjunct populations in southern Washington, USA and on Vancouver Island in British Columbia, Canada (Tillett, 1967). It is designated as endangered in the states of Oregon and Washington and, because there is only one natural population on Vancouver Island, it is federally listed as endangered in Canada (ORNHIC, 2004; Washington Natural Heritage Program, 1997; Douglas, 2004). In the northern part of its range (north of San Francisco, California), *A. umbellata* shifts from self-incompatible and obligately outcrossing to self-compatible and very highly selfing. Outcrossing and selfing populations have been designated different varieties, variety *umbellata* and variety *breviflora*,

respectively. *Abronia umbellata* also grows sympatrically with two dune-forming, maritime *Abronia* species, magenta-flowered *A. maritima* and yellow-flowered *A. latifolia* and creates putative hybrids with intermediate flower colour with both species. These two congeners are, themselves, parapatric thereby simplifying the provenance of hybrids with *A. umbellata*.

We used analysis of DNA sequences along with field surveys and historical records to quantify the geographic pattern of genetic variation in *A. umbellata* and the processes underlying that pattern. In Chapter 2, I describe how we used sequence variation at nine single-copy, nuclear genes assayed for 94 individuals from 25 populations to determine the range wide patterns of genetic variation and differentiation in *A. umbellata* and whether peripheral populations of *A. umbellata* should be a conservation priority. In Chapter 3, I describe how we used sequence variation at five single-copy genes assayed for 261 individuals (94 *A. umbellata,* 67 *A. latifolia,* 57 *A. maritima* and 42 putative hybrids) from 37 populations and morphological data of 840 individuals (377 *A. umbellata,,* 238 *A. latifolia,* 170 *A. maritima,* 6 var. *umbellata* x *A. latifolia*, 38 var. *umbellata* x *A. maritima* and 11 *A. latifolia* x *A. maritima*) to investigate if hybridization and introgression occurs between maritime *Abronia* species and whether this might be more frequent at range edges. In both studies we consider how genetic patterns have been influenced by a geographic shift in the mating system.

Chapter 2

Genetics and conservation significance of peripheral populations of *Abronia umbellata* (Nyctaginaceae)

Abstract

Whether geographically peripheral populations are worth conserving has been hotly debated over several decades. This debate is especially relevant in high-latitude countries where, within their political jurisdictions, many species reach their northern range limits as peripheral isolates and, even if the species is common elsewhere, require conservation attention. For example, in Canada, ~77% of plant species "at-risk" are at their northern range limit in southern Canada but much more common south of the Canada-USA border. On one hand, peripheral populations may have low genetic variation, low fitness and be prone to extinction. Alternatively, they might be adapted to extreme range-edge environments and thus well-poised for range shifts during climate change. *Abronia umbellata* is endemic to coastal dunes from Baja California, Mexico to Oregon, USA but also occurs as disjunct populations in Washington, USA and on Vancouver Island in British Columbia, Canada that are designated "at risk". Based on sequence variation at nine single-copy genes assayed for 94 individuals from 25 populations across the species range, these disjunct populations were very similar to range edge populations in Oregon, and likely arose through recent, long-distance dispersal or fragmentation of a recently expanded range. Southern-edge populations in Baja, on the other hand, were genetically unique and diverse but nine populations located previously in 2003, have disappeared. Records from iNaturalist indicate that since then, populations have re-appeared but to what population census size is unknown. Nevertheless, populations in Baja are of high conservation value and show evidence of demographic declines but receive no conservation protection. In this case, the

conservation significance of range edge populations depends on which edge and perhaps, the unprotected low-latitude populations seem a higher priority than those benefitting from special status at the northern range limit.

Introduction

Changes in species' distributions caused by large-scale climate change are thought to be largely determined by the responses of peripheral populations (Hampe and Petit, 2005; Rehm et al., 2015). Many conservationists believe they may be adapted to marginal range edge conditions and could facilitate range expansions and movements, including poleward shifts expected with climate change (Gibson et al., 2009; Rehm et al., 2015). If so, geographically peripheral populations should be a conservation priority. On the other hand, some argue that they are likely small and isolated, and thus more vulnerable to extinction through environmental and demographic stochasticity (García and Arroyo, 2001; Williams et al., 2003) which arose from the Abundant Centre Hypothesis (ACH), that claims that a species should have the highest abundance and fitness in the centre of its range and lowest abundance and fitness towards range edges (Lawton, 1993; Vucetich and Waite, 2003). Peripheral populations are a large focus of conservation in high-latitude countries because many species reach their northern range limits within the southern edges of their jurisdictions. For example, in Canada, 77% of plant species "at-risk" are peripheral populations of species more widely distributed south of the Canada-USA border (Klemet-N'Guessan et al., 2019). Other high-latitude countries that have significant numbers of peripheral populations listed as "at-risk" include the United Kingdom, Sweden and Finland (Rassi et al., 2001; Cheffings et al., 2005; Gärdenfors, 2005). Studies of these high-latitude peripheral populations rarely analyze them in the context of the entire geographic range, which may restrict findings to one geographic region (Eckert et al., 2008).

Plants exhibit marked geographical differentiation in key life history and reproductive traits that can also strongly influence the genetic distinctness of peripheral populations. For

instance, a shift from outcrossing to selfing towards geographic range edges has been repeatedly observed, especially in short lived plant species (Vasek, 1964; Schoen, 1982; Barrett *et al.*, 1989; Wyatt, 1988; Schueller, 2004; Moeller and Geber, 2005; Busch, 2005). Selfing may facilitate long-distance dispersal and also persistence after range fragmentation, which often occurs at range edges, as it lowers the limitations of reduced mates and/or pollinators (Baker, 1955; Cheptou, 2004). However, declines in effective population size and opportunities of gene flow associated with selfing may make peripheral populations more prone to the loss of genetic variation through drift.

Peripheral populations that are geographically disjunct from the core of the species range are thought to be more genetically differentiated than those at the edge of a continuous distribution. In fact, Bunnell et al. (2004) claim that, as a general rule, disjunct populations should receive the highest conservation priority because they likely harbour unique genetic variation and have a higher chance of being locally adapted because they are not subject to the influx of maladaptive alleles from the range centre (Kirkpatrick and Barton, 1997). However, much of this depends on the evolutionary history of disjunct populations. If they are relicts of a once continuous range that was long established but has since fragmented, then they are likely to be genetically unique. The same may occur through long-distance dispersal from the range core that happened long enough ago that peripheral populations had acquired novel genetic variation. Disjunct peripheral populations would be less unique if, in contrast, they were the result of range fragmentation after a recent range expansion or recent long-distance dispersal. In this case they would only contain a much-reduced subset of genetic variation found in central populations (Kropf et al., 2006).

In high-latitude populations, the scope of the genetic differentiation of disjunct peripheral populations is limited by glacial history (Afzal-Rafii and Dodd, 2007; Reisch, 2008). For example, in a study of disjunct alpine populations of *Carex atrofusca,* they found that disjunct populations were not genetically unique and likely arose through immigration from Central Asia after the last glacial maximum (Schönswetter et al., 2006). Conversely, the equatorial range margins may not have experienced recent glaciation, so low latitude disjunct population may have had the time required for divergence in the manner envisioned by Bunnell et al. (2004). Yet, low-latitude populations are rarely studied, often because research on this is limited in countries with lower-economic standing (Hampe and Petit, 2005).

In this study, we investigate variation in population genetic diversity and differentiation across the geographic range of the short-lived Pacific coastal dune endemic *Abronia umbellata* Lam. (Nyctaginaceae). The species is continuously distributed from San Quintin, Baja California to Coos Bay, Oregon, USA but occurs as putative disjunct populations in Washington, USA and on Vancouver Island in British Columbia, Canada (Tillett, 1967). It is designated as endangered in Canada and in the states of Oregon and Washington (Washington Natural Heritage Program, 1994; Douglas, 2004) because populations have been declining over the past several decades (ORNHIC, 2004). *Abronia. umbellata* provides a good case study for understanding the genetic uniqueness of peripheral populations because the species has a simple, near one-dimensional geographical range that is easily accessible, it is a conspicuous member of the dune flora with a long history of natural history, and herbarium records. Additionally, a previous study has located almost all extant populations across the geographic range and quantified geographic variation in

population demography and key reproductive traits (Samis and Eckert, 2007). *Abronia. umbellata* has two varieties; self-incompatible *A. umbellata* var. *umbellata* (hereafter var. *umbellata*) that occurs south of San Francisco, California and predominately selfing *A. umbellata* var. *breviflora* (hereafter var. *breviflora*) that occurs north of San Francisco (Tillett 1967; Darling et al. 2008; Doubleday et al. 2013).

We (1) analyzed historical herbarium records of *A. umbellata* to determine the degree to which range edge populations are disjunct and infer their contemporary persistence. Next, we determined (2) to what extent genetic variation declines towards range edges and (3) if peripheral populations are genetically differentiated from those in the range core. Following measures of differentiation, we inferred (4) what the nature of this differentiation is. Does it derive from peripheral populations containing unique variation or is it simply a subset of variation found in the core of the range? Finally, we suggested (5) if the genetic properties of peripheral populations indicate that they should be of high priority for conservation.

Materials and methods

Analysis of historical distribution using herbarium records (Objective 1)

Location data of *Abronia umbellata* was collected using 571 herbarium specimens from 24 herbaria and 973 records from iNaturalist (Appendix 2A; Fig. 2.1; Samis and Eckert 2007; iNaturalist.org 2019). Edge populations were considered to be populations at or near the range edge and to determine which of these edge populations were to be analyzed as disjunct, we subjected the herbarium and iNaturalist records to graphical analysis.

Population Sampling Strategy

In summers of 2017 and 2018, we sampled *A. umbellata* at 25 sites across the entire geographic range, from Punta Colonet, Baja California, Mexico (30°57'57.1"N 116°19'47.2"W) to Vancouver Island, British Columbia, Canada (46°38'02.0"N 124°04'03.2"W; Fig. 2.2, Table 2.1) and collected leaf material from 20 randomly chosen individuals at each site, which we flash dried in silica gel. We also assessed presence/absence of *A. umbellata* at 14 more sites between 2017 and 2019 to determine if populations still existed at those locations (Table 2.2). All sites were selected based on presence/absence data from surveys in 2002 and 2003 in Samis and Eckert (2007). Individuals sites were separated from other sites by at least 1 km, usually much more.

Sequencing

Five samples from each site were randomly chosen and DNA was extracted from leaf material using the CTAB protocol of Doyle and Doyle (1987). Nine primers were designed by Greer (2016) using PrimerQuest (Integrated DNA Technologies) based on assembled transcripts sequences of *A. umbellata*, which BLAST analyses indicated were single-copy exons. Samples were amplified using PCR by an Applied Biosystems SimpliAmp Thermal Cycler (Applied Biosystems LSA37835), with initial denaturation at 95°C for 2 minutes; followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at a primer-optimized temperature (55–57°C) for 40 seconds, extension at 72°C for 60 seconds and final extension at 72°C for 10 minutes (Appendix 2B). Resulting amplicons were assayed for yield and size on a 50% agarose gel. High- yield PCR products of uniform size were submitted to GénomeQuébec (Montréal, Québec, Canada) for Sanger sequencing of the forward or reverse strand (Appendix 2B) using the same PCR primers on an Applied Biosystems DNA analyzer (Applied Biosystems 3730XL).

Chromatograms were visualized in Geneious Prime 2019.0.4 software (https://www.geneious.com) and low-quality sequences with Phred scores < 40 were excluded. Remaining sequences were aligned using a MUSCLE alignment with a maximum of eight refinement iterations (Edgar, 2004). Sequences were then manually edited to trim low-quality sequence ends and identify heterozygous sites. We sequenced a mean of 453bp/locus for each individual. Invariant sites were excluded to obtain an alignment of all single nucleotide polymorphisms (mean = 28 SNPs per locus). Resulting sequences were exported as FASTA files.

Statistical Analyses

Estimating genetic variation **(Objective 2)**

To determine if within-population genetic diversity varies across the geographic range of *A. umbellata* and if it is lower in edge than core populations, taking into account known variation in the mating system, we calculated mean gene diversity (expected heterozygosity H_{exp}), a commonly used measure of genetic variation (Frankham, 1995) calculated for each population. Raw FASTA files were converted to genepop files using the Convert function in PGD Spider (version 2.1.1.5, Lischer and Excoffier, 2012), which were then converted to genind objects using the df2genind function in adegenet (version 2.0.0, Jombart, 2008; Jombart and Ahmed, 2011) in the R statistical environment (version 3.5.3, R Core Team, 2018). Mean gene diversity within populations was calculated using the seppop and summary functions in the adegenet package on the resulting genind object. We then fit population levels of gene diversity to a general additive model, using the gam function in the mgcv package (version 1.8- 31;Wood, 2011), with latitude as a predictor, and compared this model to a simple linear model and null model using AICc ,which we calculated using the AICc function in the MuMIn package (version 1.42.1; Bartoń, 2018).

We calculated the private alleles for each population and group of populations (core, peripheral) using the pophelper function in the poppr package (version 2.3.0; Kamvar, 2015).

Estimating genetic differentiation **(Objective 3 and 4)**

To determine the extent to which edge populations were genetically differentiated from core populations, we conducted a Discriminant Analysis of Principal Components (DAPC) of SNP frequencies. DAPC are commonly used to infer population structure and are especially useful when populations vary dramatically in the mating system, as they do in *A. umbellata*, because the analysis does not make any genetic assumptions like Hardy-Weinberg equilibrium (Jombart et al., 2010). Additionally, DAPC summarises results from Principal Components Analysis (PCA) and unlike PCA, maximizes the separation between populations by minimizing variation within them (Jombart et al., 2010). The DAPC was run using the dapc function in adegenet on the same genind object used to calculate mean gene diversity, and DA scores for the first two discriminant axes for each individual were extracted from the DAPC object.

We used Bayesian clustering implemented in the program InStruct to infer genetic groups among individuals to determine if peripheral populations make up a unique genetic cluster. InStruct is similar to the program STRUCTURE as it uses the Markov Chain Monte Carlo (MCMC) algorithm to infer genetic groups but does not assume Hardy-Weinberg equilibrium, which would be violated in selfing populations of *A. umbellata* (Gao et al., 2007; Pritchard et al., 2000). We ran simulations with a model that assumed population structure with admixture from 1 to 10 genetic clusters and used 250,000 burn-in chains and 500,000 MCMC iterations for 10 independent replicates. We determined the most likely number of clusters using the ΔK and DIC methods (Evanno et al., 2005) and aligned the 10 independent outputs for the best K using CLUMPP (Jakobsson et al., 2007).

We ran an isolation by distance analysis to determine if edge populations are particularly differentiated when controlling for their geographic isolation. We fit a linear model to pairwise genetic distances as the response variable, and pairwise geographic distance and population comparison group and their interaction as predictors. Comparison groups were low-latitude edge populations, core populations, and high-latitude disjunct edge populations, since we were interested if the edge populations were more genetically unique than core populations. To test for differences in slopes, we included an interaction between predictor variables and to test for differences in intercepts, we excluded the interaction. Next, to test post-hoc pairwise differences among population groups, we took out one group at a time for all combinations and ran the models again. Finally, we did a permutation test for both models to confirm our results, as points were not independent. Permutation tests were conducted by running the same models 1000 more times but randomizing the response variable each time and determining what proportion of the 1000 models had a p-value was less than the p-value from the original model. We calculated pairwise great circle geographic distances using the GeoDistanceInMetresMatrix function created by Rosenmai (2014) and pairwise genetic distances (measured as Jost's D) using the PopGenReport function in the PopGenReport package (version 2.2.2, Adamack and Gruber, 2014; Gruber and Adamack, 2015). We used Jost's D in our analysis because unlike G'_{ST} and F_{ST} it is not biased by very low within-population heterozygosity that are likely in highly selfing populations (Jost, 2008).

Results

Analysis of herbarium records

Samis and Eckert (2007) determined that the range of *Abronia umbellata* is from San Quintin, Baja California, Mexico (30°21'58.9"N 116°00'17.5"W) to Coos Bay, Oregon, USA (43°22'08.8"N 124°19'40.5"W) and our graphical analyses with updated herbarium data and iNaturalist records (iNaturalist.org, 2019) are consistent with this (Fig. 2.3). There are also two disjunct areas where *A. umbellata* has been regularly collected: Willapa Bay National Wildlife Refuge, Washington, USA (WWA; 46°38'02.0"N 124°04'03.2"W) 350 km from the northern limit and Pacific Rim National Seashore Reserve, British Columbia, Canada (SCH; 49°01'33.0"N 125°40'49.0"W), 650 km from the northern limit. These two records are the only populations currently existing outside the range of *A. umbellata*, and in addition to one other record in Washington, are the only locations beyond the range that have yielded more than one record (herbarium and iNaturalist combined).

Patterns of genetic variation across the range of Abronia umbellata

Among 94 individuals, we found 251 SNPs (93.6% di-allelic, 6.4% tri-allelic) across 4077 bp of sequence from 9 genes (mean ± 1SD = 2.06 ± 0.24 alleles/SNP). Mean population gene diversity (H_{exp}) across all populations of outcrossing var. *umbellata* was 0.13 (n_{pop} = 13; n_{ind} = 59; Table 2.3) and 0.0096 across selfing var. *breviflora* (n_{pop} = 12; n_{ind} = 35). A generalized additive model with latitude as the predictor did not fit the pattern of geographic variation of H_{exp}

(AICc = -55.01) better than a simple linear regression (AICc = -59.75) which was a significantly better fit than the null model (AICc = -48.76). In the simple linear model, we found that H_{exp} correlated negatively with increasing latitude ($R^2 = 0.42$, $F_{1,23} = 16.6$, $P = 0.0005$; Fig. 2.4). Additionally, similar to northern var. *breviflora* populations, the two northern disjunct populations had near zero gene diversity (mean ± 1SD = 0.0013 ± 0.002).

Among outcrossing core populations, the number of private variants range 0–10 and averaged 3 (1SD ± 2.86; Table 2.3). Among all core populations, none of the selfing populations had any private variants. The northern disjunct populations in WA and BC had 3 and 0 private variants respectively (Table 2.3). The southern edge population had 106 private variants, which was significantly higher than the mean of the other outcrossing populations (1-sample *t*-test, $t = 3.28$, df = 4, $P = 0.03$). Within var. *breviflora* populations, only three populations had private variants (CDB, CLR and PRNS) and all only had one.

***Patterns of genetic differentiation across the range of* Abronia umbellata**

Northerly selfing and southerly outcrossing *A. umbellata* are genetically differentiated from each other along the first DAPC axis (Fig. 2.5; DA1, 68% of variation, $R^2 = 0.60$, $F_{1,23} = 34.34$, $P < 0.0005$) but not DA2 (15% of variation, $R^2 = 0.008$, $F_{1,23} = 0.18$, $P = 0.67$). The northern disjunct populations in Washington and on Vancouver Island clustered closely with northern selfing populations of var. *breviflora* from Oregon and northern California. Selfing populations and the most northerly outcrossing populations had similar DA1 scores. Among var. *umbellata* populations, there was no latitudinal trend with DA1 ($R^2 = 0.14$, $F_{1,10} = 1.69$, $P =$

0.22). The southernmost population in Baja California, Mexico (BSC) was the most differentiated along DA1. There was little geographical pattern for population scores on DA2.

Based on deviance information criterion (DIC) and ΔK (Appendix 2C), Bayesian clustering analysis (InStruct) detected two geographically organized clusters of genotypes (Fig. 2.6). For all genotypes from selfing populations and two outcrossing populations just to the south of the selfing populations, ancestry coefficients in the first cluster were ~ 1. Genotypes from the other outcrossing populations, except Santa Cruz Island (CSC), had much higher ancestry coefficients for the other cluster. Notably, all genotypes from the two northern disjunct populations, like the other selfing populations, had ancestry coefficients = 1 for cluster 1. Genotypes from the southern edge population in Baja California had coefficients near 1for cluster 2, the highest among all outcrossing populations.

Genetic distance covaried positively with geographic distance among pairs of populations (Fig. 2.7; $R^2 = 0.13$, $P_{perm} < 0.0001$). The three classes of comparisons (northern disjunct vs. core populations, southern edge vs. core populations and core vs. core populations) differed in the regression intercept but not slopes (Table 2.4). Per unit distance, northern edge disjunct populations were less differentiated, and the southern edge population was more differentiated from core populations than core populations were from each other.

Discussion

The nature of genetic differentiation of northern disjunct populations of Abronia umbellata

Using herbarium and iNaturalist data, we determined that there are two, disjunct populations of *A. umbellata* occurring in Washington, USA and on Vancouver Island, British Columbia, Canada (iNaturalist. org, 2019; Fig. 2.3). Because they are geographically isolated and experience little if any gene flow from core populations, we might expect them to be genetically unique (Bunnell et al., 2004), so why aren't they?

The population in Washington (WWA) is on the northern tip of the Long Beach Peninsula in Leadbetter Point of Willapa National Wildlife Refuge and was first discovered in summer 2006 (two individuals) during an effort to remove the invasive grass *Ammophila arenaria* in winter 2006 (personal correspondence, William Ritchie). Since then, there has been limited plantings from seed of the original two plants and recruitment from natural germination after further *A. arenaria* removal released additional *A. umbellata* seed. Although the peninsula itself was formed ~ 10,000 years ago (Schuster, 2005), the dunes at Leadbetter Point apparently only developed in the last 70-90 years due to the construction of a jetty at the mouth of the Columbia River (University of Washington Burke Museum, 2019), suggesting that the population of *A. umbellata* was recently colonized.

The population on Vancouver Island (SCH) is currently managed by Parks Canada as it occurs in Pacific Rim National Seashore Reserve. Two individuals were found on the shores of

Clo-oose Bay in 2000 and were not seen again after 2001 (Douglas, 2004). There are several other records of *A. umbellata* on Vancouver Island, including Clo-oose Bay in 1941, two records in Bamfield in 1909 and 1927 and one in Tofino in 1915, but the lack of records after that time suggests that the species was extirpated in the intervening decades. The contemporary population at Clo-oose Bay has been maintained by a planting program initiated in 2007. However extant plants were derived from only two individuals grown from seed collected from the original plants in 2000. Since the propagation program, there has been regular (mostly annual/bi-annual) plantings at five sites between 2008 and 2018.

Beside what we know about the short-term history of the disjunct populations of *A. umbellata*, a recent origin is suggested by their strong similarity to the selfing populations in Oregon. Moreover, the selfing var. *breviflora* as a whole constitutes a small subset of genetic variation found in outcrossing var. *umbellata*, with very few exclusive SNP variants. Based on this pattern, modeling by Greer (2016) suggests that the selfing lineage diverged from the outcrossing lineage via a population bottleneck ~ 30,000 years ago. Whether the disjunct populations resulted from long-distance dispersal from Oregon or fragmentation of a recent range expansion cannot be distinguished using the data at hand. This inability to distinguish dispersal from fragmentation has also been found in a study of *Pilosella* in eastern Europe where, although its low-level dispersal capacity makes a scenario of natural dispersion seem unlikely, they found a drop in genetic variation in disjunct populations, indicative of founder effect from long-distance dispersal (Šingliarová et al., 2008). Similarly, a study of *Comastoma* in the Alps found low genetic variation in disjunct populations, again, indicative of long-distance dispersal,

but unlike Šingliarová et al. (2008), also found strong phylogeographical structure, suggesting old vicariance (Schönswetter et al., 2004).

A reasonable explanation for long-distance dispersal in *A. umbellata* is the movement of seed in a shallow ocean current, known as the Davidson current, that moves poleward up the Pacific coast in winter (Reid and Schwartlose, 1962). This is further plausible as Darling et al. (2008) found that the seeds of *A. umbellata* are able to float in saltwater for at least 14 days. The Davidson current is thought to be responsible for the long-distance dispersal of *Spartina* (Morgan and Sytsma, 2013) and *Nereocystis* (Saunders, 2014) as well.

Exceptional genetic variation of southern range edge populations

The low-latitude edge population in Baja California, Mexico is genetically distinct from the rest of the range at the nine single-copy genes we sequenced as southern edge populations have the highest ancestry coefficient for the cluster of southern populations of *A. umbellata* and DAPC analyses separate the southern edge population from all other populations on the first axis (Fig. 2.5; Fig. 2.6). Additionally, in the isolation by distance analysis pairwise genetic distances were higher per unit geographic distance between this southern edge population and core populations than between core populations and high-latitude disjunct populations (Table 2.4). Furthermore, the southern edge population had an exceptional number of private alleles, almost 10-fold more than the next highest number of private alleles in a population.

An interesting finding that contrasts the Abundant Centre Hypothesis, was that we found a decreasing trend in genetic diversity (measured as mean gene diversity, H_{exp}) as latitude increased, with the low-latitude edge population having the highest genetic variation. Edge populations not having lower genetic variation is not uncommon, as Eckert et al.'s (2008) review found that approximately 36% of the 134 studies assessed did not find a decrease. However, studies detecting increasing variation towards range edges were very rare and the mean increase in H_{exp} was only 0.022. In our study, the mean difference between the southern edge population, consisting of only six individuals, and the rest of the populations was 0.16 for other outcrossing populations.

Populations in Baja California appear to be collapsing as we failed to detect *Abronia umbellata* at any of the 9 sites in Baja California where Samis and Eckert (2007) found the species in 2003. We only found plants at a population located using the only iNaturalist record from 2018 (Fig. 2.8; iNaturalist.org, 2019). Because the southern site we used in our analysis was almost as isolated as one of our northern disjunct population (~200 km, ~300 km to closest population, respectively) it could be considered recently disjunct. Moreover, there were 11 locations on iNaturalist in the year following our survey (Fig. 2.8). Perhaps conditions for recruitment were poor during 2018 which is possible because this year was part of a decade-long drought on the southern Pacific coast of North America (Kam, 2019). Though, this is not to say that populations are not in decline as we do not know the demography (census/effective population size, fitness, etc.) of the populations posted on iNaturalist in 2019. Additionally, as its existence continues, iNaturalist postings have increased due to an increase in users (40,000 in 2018; iNaturalist.org, 2019) so perhaps the increase in sightings in Baja California could be

attributed to increased website use. Furthermore, the number of iNaturalist posts in Baja California in 2019 has been more than the number of sites Samis and Eckert (2007) surveyed in 2003 but most of the iNatualist sightings were in the same location (Fig. 2.8). Overall, there have been no reports of declines in coastal plants in this part of Baja in the same time period.

Implications for conservation

Many species at risk in Canada are peripheral isolates of species that are more common south of the Canada-US border, and there has been considerable debate over whether limited resources should be used to conserve these populations (García and Arroyo; Gibson et al., 2009). Some argue that if these high-latitude edge populations are genetically unique then they are worth conserving because they may be adapted to more northern conditions and could 'lead' a range shift poleward in periods of climate change (Bunnell et al., 2004; Gibson et al., 2009). The northern disjunct populations of *A. umbellata* do not appear to be genetically unique from populations of var. *breviflora* much further south, which may suggest they may not deserve much conservation effort. However, populations of var. *breviflora* show striking variation in mating system relative to outcrossing var. *umbellata* populations and selfing may provide reproductive assurance in times of limited mates and/or pollinators and thus, have the potential to aid in range expansion (Baker, 1955).

We found that the low-latitude edge population in Baja California is genetically differentiated from the core of the range, as assumptions based on the Abundant-Centre hypothesis would predict, but not lower in genetic diversity. Contrarily, the low-latitude edge

population actually contains a large portion of the species overall SNP variation and therefore, may be an important contributor to the overall evolutionary potential of *A. umbellata*. In general, low-latitude range edge populations remain largely understudied but are often disproportionately important for the survival and evolution of species (Hampe and Petit, 2005). Findings from our study are concordant with this and highlights the importance of broad range-wide sampling when evaluating the geographic pattern of ecological and genetic variation of relevance to conservation (Yakimowski and Eckert, 2007).

Table 2.1 Twenty-five populations of *Abronia umbellata* spanning the species' geographic range used in this study. Population code is presented along with, state or province, mating system, latitude and longitude.

Population	State/province	Mating system	Latitude (°N)	Longitude (°W)
BSC	Baja California	Outcrossing	30.9898	116.3333
CSS	California	Outcrossing	32.6408	117.1436
CTP	California	Outcrossing	32.9289	117.2591
CCP	California	Outcrossing	33.2351	117.4178
CSO	California	Outcrossing	33.3808	117.577
CSC1	California	Outcrossing	34.0571	119.9221
CPM	California	Outcrossing	34.1145	119.1494
CBV	California	Outcrossing	34.2679	119.2783
CCO	California	Outcrossing	34.412	119.8829
CSP	California	Outcrossing	35.3658	120.8623
CAS	California	Outcrossing	36.6189	121.941
CMN	California	Outcrossing	36.9175	121.8528
CWR	California	Outcrossing	36.9541	122.0799
CSD	California	Selfing	37.907	122.6794
PRNS	California	Selfing	38.0309	122.9502
CDB	California	Selfing	38.3141	123.0438
CMC	California	Selfing	38.9827	123.7058
CBR	California	Selfing	39.3047	123.7966
CMA	California	Selfing	40.8476	124.0075
CLR	California	Selfing	41.0274	124.1106
CGB	California	Selfing	41.3618	124.0748
OPR	Oregon	Selfing	42.2709	124.405
OPO	Oregon	Selfing	42.7412	124.4989
WWA	Washington	Selfing	46.6339	124.0676
SCH	British Columbia	Selfing	49.0258	125.6803

Table 2.2 Sites sampled by Samis and Eckert (2007) in 2002 and 2003 that we resurveyed for presence/absence of *Abronia umbellata* in 2017, 2018 and 2019. Dashed lines represent a location we did not survey that year. We present population code, along with state/province, mating system, latitude, longitude, and presence when surveyed.

*Samples used in genetic analysis when populations were surveyed in more than one year

					Year of survey		
Population	**State**	**Mating system**	**Latitude (°N)**	**Longitude (°W)**	**2017**	**2018**	**2019**
BBR	Baja California	Outcrossing	30.0478	115.7864	--	No	--
BES	Baja California	Outcrossing	30.3187	115.8257	--	No	--
BBQ4	Baja California	Outcrossing	30.3801	115.9904	--	No	--
BCH	Baja California	Outcrossing	30.4639	116.0426	--	No	--
BBF2	Baja California	Outcrossing	30.4765	116.0462	--	No	--
BBF1	Baja California	Outcrossing	30.4843	116.0027	--	No	--
BQW	Baja California	Outcrossing	30.6154	116.0273	--	No	--
BSA	Baja California	Outcrossing	31.1068	116.3112	--	No	--
BPE	Baja California	Outcrossing	31.7257	116.6482	--	No	--
CSS	California	Outcrossing	32.6408	117.1436	Yes*	Yes*	Yes
CTP	California	Outcrossing	32.9289	117.2591	Yes*	Yes	Yes
CCP	California	Outcrossing	33.2351	117.4178	Yes	--	--
CSN3	California	Outcrossing	33.2572	119.5617	--	Yes	--
CSO	California	Outcrossing	33.3808	117.5769	Yes*	Yes	Yes
CHB	California	Outcrossing	33.6325	117.9604	Yes	Yes	Yes
CSC1	California	Outcrossing	34.0571	119.9221	--	Yes	--
CBV	California	Outcrossing	34.2679	119.2783	Yes*	--	Yes
CCO	California	Outcrossing	34.4119	119.8829	Yes*	--	Yes
CSP	California	Outcrossing	35.3658	120.8623	Yes*	Yes	Yes

CMS	California	Outcrossing	35.3964	120.8662	No	--	--
CAS	California	Outcrossing	36.6181	121.9422	Yes*	Yes	Yes
CST	California	Outcrossing	36.8766	121.8252	Yes	--	Yes
CWR	California	Outcrossing	36.9541	122.0799	Yes	Yes*	Yes
CCF	California	Selfing	37.8112	122.4877	--	No	Yes
CSD	California	Selfing	37.9071	122.6794	--	Yes*	Yes
PRNS	California	Selfing	38.0309	122.9502	--	Yes*	Yes
CMC	California	Selfing	38.9827	123.7058	--	Yes	
CBR	California	Selfing	39.3047	123.7966	Yes	--	
CMK	California	Selfing	39.5033	123.7838	--	Yes	Yes
CMA	California	Selfing	40.8476	124.0075	--	Yes	
CLA	California	Selfing	40.8923	124.1492	--	--	Yes
CLR	California	Selfing	41.0274	124.1106	Yes*	--	Yes
CGB	California	Selfing	41.3618	124.0748	--	Yes*	Yes
OPR	Oregon	Selfing	42.2709	124.4050	Yes*	No	No
OOP	Oregon	Selfing	42.4482	124.4295	No	--	--
OPO	Oregon	Selfing	42.7407	124.4984	Yes*	Yes	Yes
ONS	Oregon	Selfing	43.3669	124.3253	Yes	Yes	Yes
WWA	Washington	Unknown	46.6339	124.0676	Yes*	Yes	Yes
SCH	British Columbia	Unknown	49.0258	125.6803	Yes*	Yes	Yes

Table 2.3 Genetic diversity within 25 populations of *Abronia umbellata* across the geographic range. Population code and mating system are presented along with geographic location, sample size (n_{ind}), the number of private SNP variants exclusive to each population and within-population genetic diversity (H_{exp}) with standard error around the mean.

Population	Mating system	Location	n_{ind}	Private variants	H_{exp} (Standard error)
BSC	Outcrossing	Southern edge	5	106	0.28 (0.26 — 0.29)
CSS	Outcrossing	Core	7	6	0.11 (0.09 — 0.12)
CTP	Outcrossing	Core	4	1	0.08 (0.07 — 0.09)
CCP	Outcrossing	Core	4	3	0.13 (0.11 — 0.14)
CSO	Outcrossing	Core	4	4	0.09 (0.08 — 0.10)
CSC1	Outcrossing	Core	3	1	0.05 (0.04 — 0.06)
CPM	Outcrossing	Core	5	1	0.15 (0.13 — 0.16)
CBV	Outcrossing	Core	4	2	0.12 (0.11 — 0.14)
CCO	Outcrossing	Core	4	1	0.11 (0.10 — 0.12)
CSP	Outcrossing	Core	5	10	0.22 (0.20 — 0.24)
CAS	Outcrossing	Core	5	0	0.05 (0.04 — 0.06)
CMN	Outcrossing	Core	5	2	0.26 (0.24 — 0.29)
CWR	Outcrossing	Core	4	1	0.04 (0.03 — 0.05)
CSD	Selfing	Core	4	0	0.00
PRNS	Selfing	Core	2	0	0.11 (0.09 — 0.13)
CDB	Selfing	Core	3	0	0.00
CMC	Selfing	Core	3	0	0.00
CBR	Selfing	Core	2	0	0.00
CMA	Selfing	Core	2	0	0.002 (0 — 0.003)
CLR	Selfing	Core	4	0	0.002 (0 — 0.004)
CGB	Selfing	Core	2	0	0.00
OPR	Selfing	Core	2	0	0.00
OPO	Selfing	Core	3	0	0.00
WWA	Unknown	Northern edge/ disjunct	4	3	0.003 (0.001 — 0.004)
SCH	Unknown	Northern edge/ disjunct	4	0	0.00

Table 2.4 Summary of results from our isolation by distance analysis. We ran two models, one comparing slopes of three regressions corresponding to pairwise comparisons with populations from three geographic groups (southern edge, core, northern edge- disjunct) and the other comparing intercepts. Geographic groups used in each analysis are presented along with variation in slope and intercept.

Comparison	**Variation in slopes**	**Variation in intercepts**
All geographic groups	$P_{perm} = 0.31$	$P_{perm} = < 0.001$
Pairwise comparisons between groups		
Southern edge vs. Core	$P_{perm} = 0.99$	$P_{perm} < 0.001$
Southern edge vs. Northern edge - disjunct	$P_{perm} = 0.30$	$P_{perm} < 0.001$
Northern edge – disjunct vs. Core	$P_{perm} = 0.12$	$P_{perm} = < 0.001$

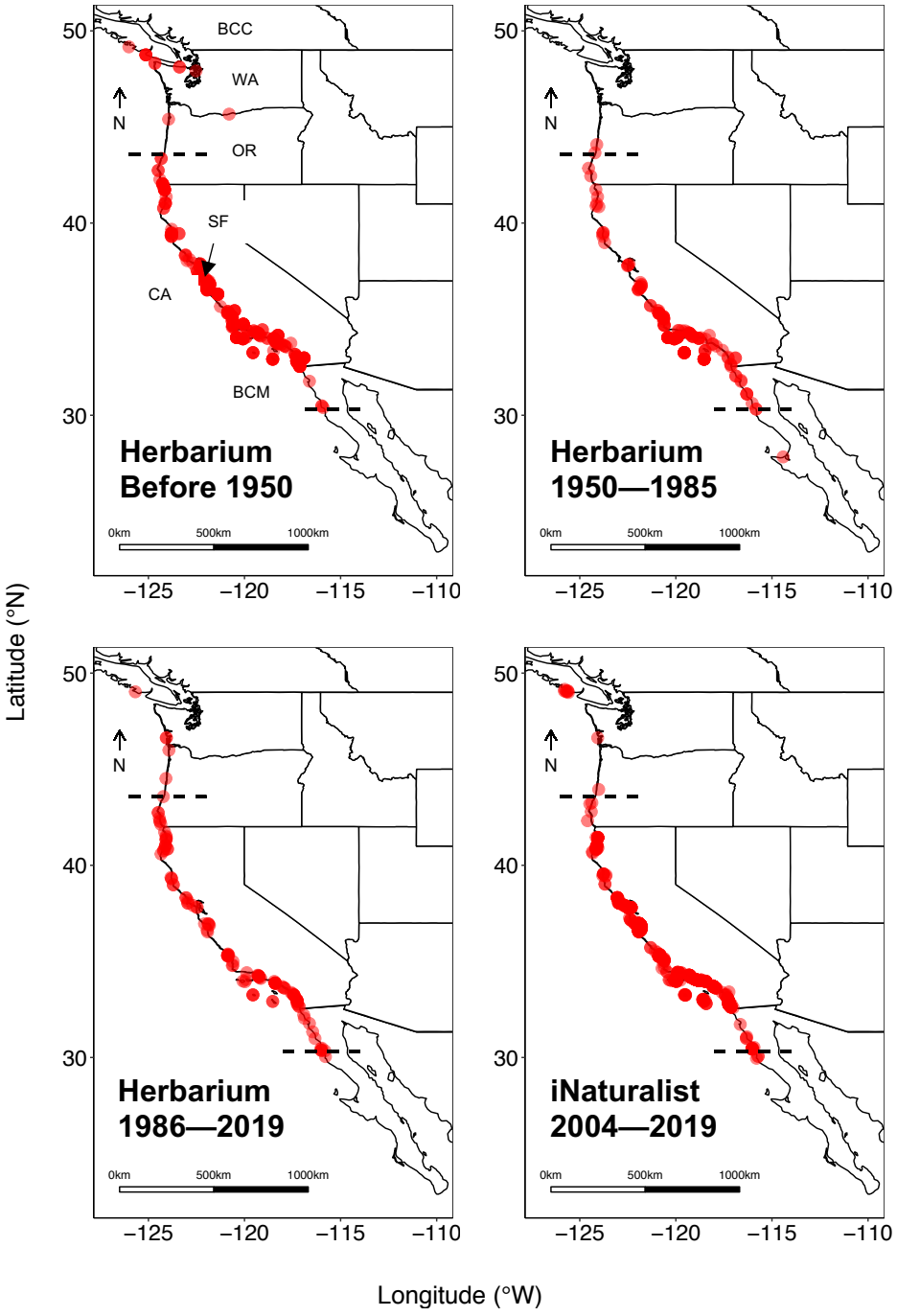

Fig. 2.1 Distribution of locations from herbarium records of *Abronia umbellata* separated into three timescales for easier visualization in the top two and the bottom left panels: before 1950 (left panel), 1950—1985 (centre) and 1986—2019 (right). Distribution of iNaturalist records (2004—2019; iNaturalist.org, 2019) are in the bottom right panel. The northern and southern

limits to the bulk of the geographic range of *A. umbellata* are indicated by black dashed lines. States and provinces are indicated as follows: BCC= British Columbia, WA= Washington, OR= Oregon, CA=California, BCM= Baja California, SF= San Francisco.

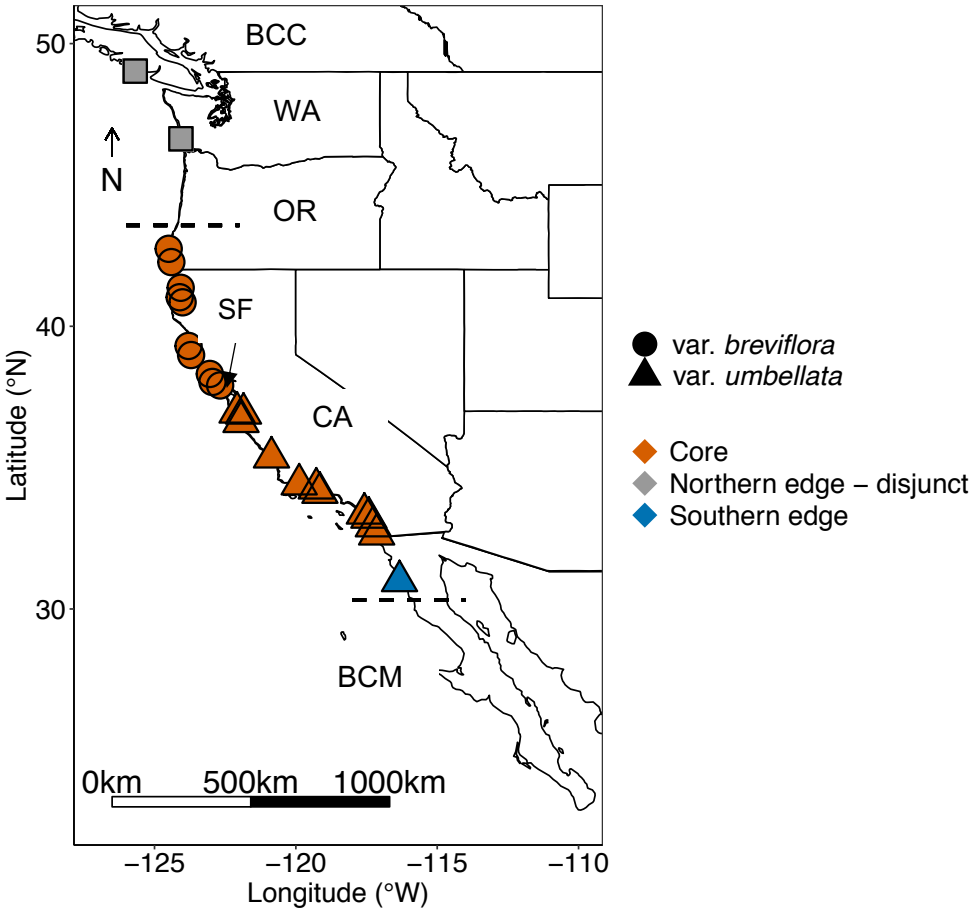

Fig. 2.2 Distribution of 25 sampled populations of *Abronia umbellata* with range limits, determined from herbarium and iNaturalist records (iNaturalist.org 2019) represented by black dashed lines. Populations of *A. umbellata* var. *umbellata* and *A. umbellata* var. *breviflora* are triangles and circles respectively. Northern disjunct populations are represented by squares as it is unknown whether they are var. *umbellata,* var. *breviflora* or neither. Colours correspond to the geographic location with grey points representing northern disjunct populations, orange points representing core populations and the blue point representing the southern edge population. States and provinces are indicated as follows: BCC= British Columbia, WA= Washington, OR= Oregon, CA=California, BCM= Baja California, SF= San Francisco.

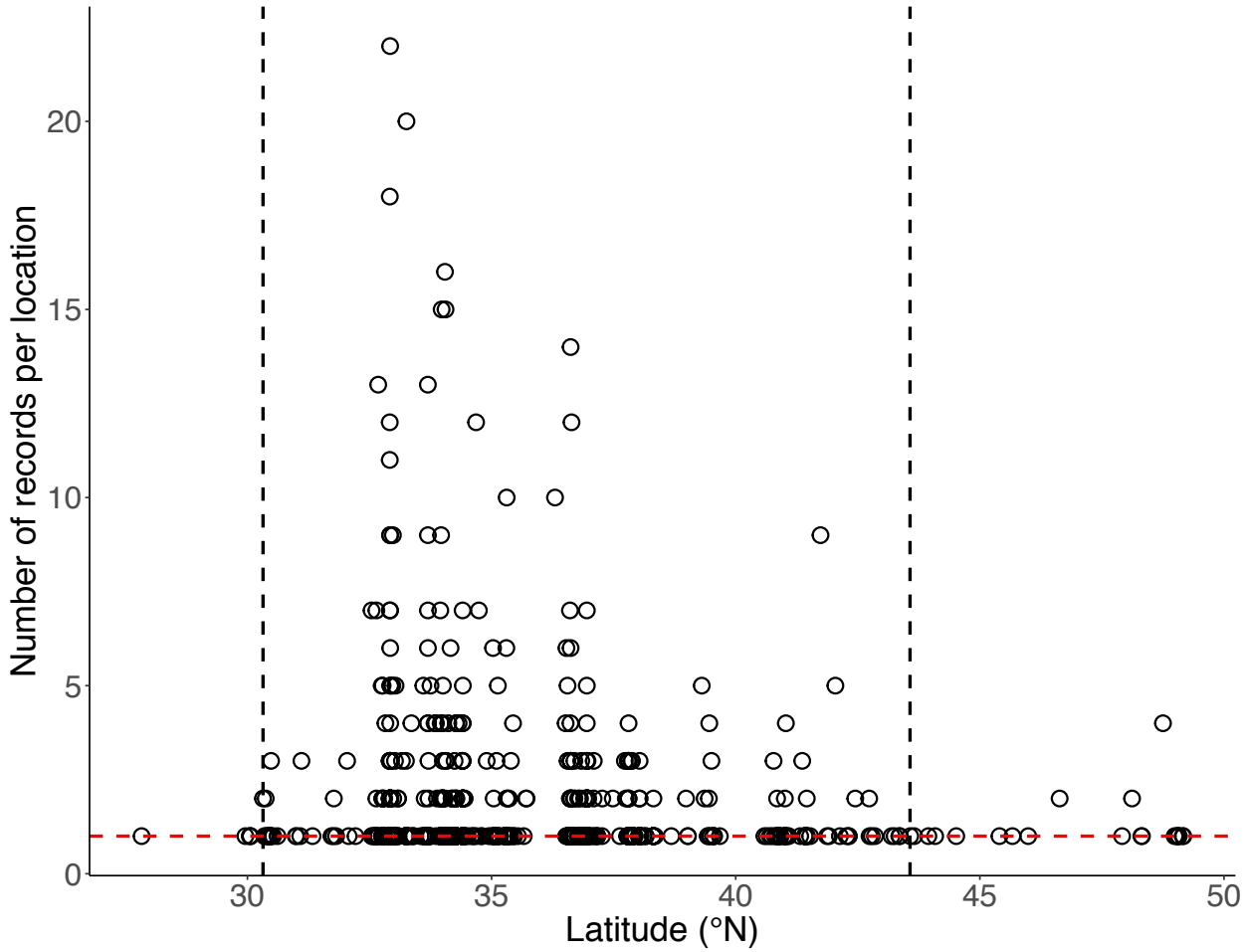

Fig. 2.3 Number of herbarium and iNaturalist records per unique location of *Abronia umbellata*. Black dotted lines represent the range limits of *A. umbellata* inferred by Samis and Eckert (2007). The red dotted line intercepts locations that only have one record. Of 853 unique locations, 824 (96.6%) fall within the range limits.

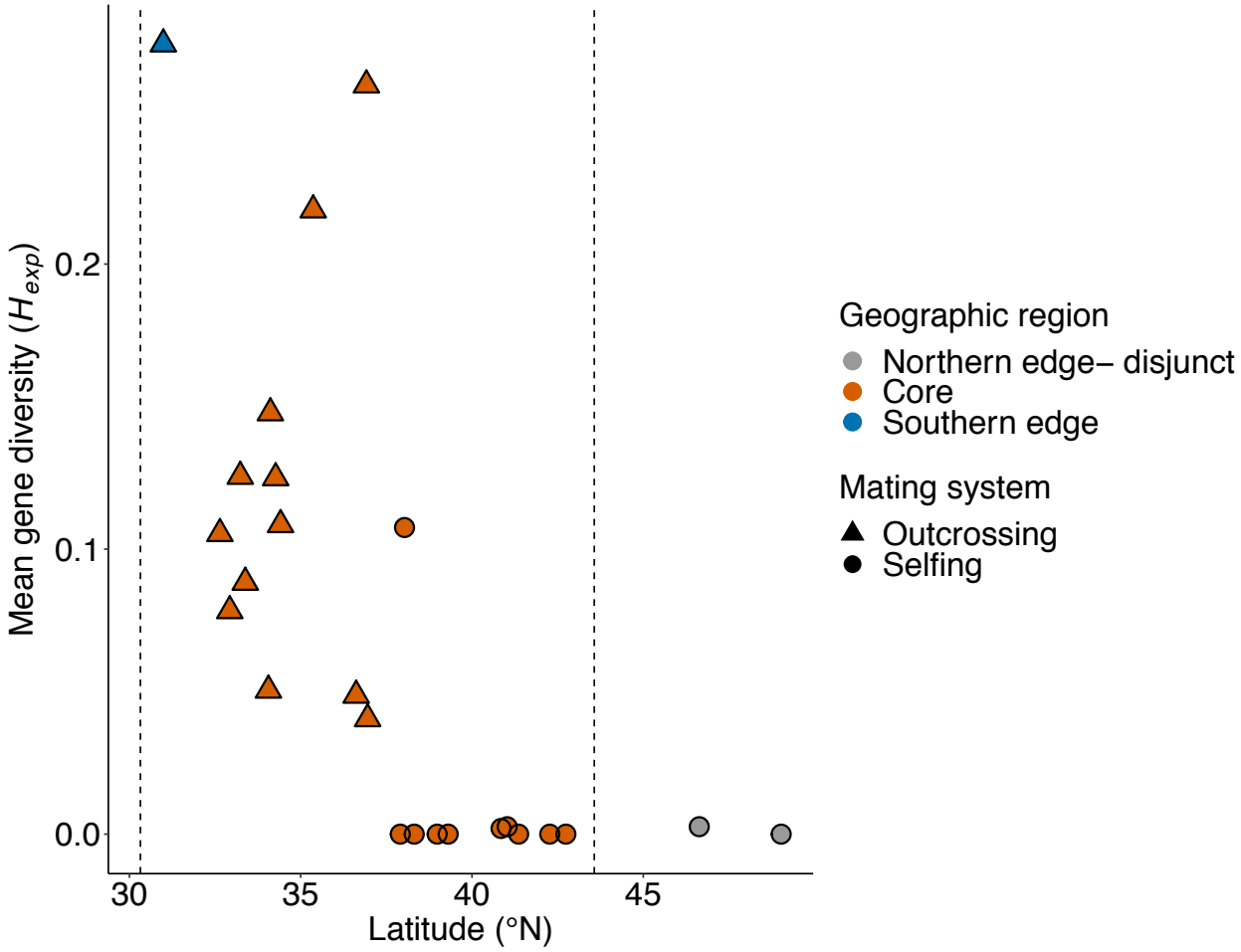

Fig 2.4 Within-population genetic diversity (measured as H_{exp}) decreases with increasing latitude (°N) among 25 populations of *Abronia umbellata.* Circles are self-incompatible, outcrossing *A. umbellata* var. *umbellata* populations and triangles are self-compatible, selfing *A. umbellata* var. *breviflora* populations. Grey points represent northern edge disjunct populations, orange points represent core populations and the blue point represents the southern edge population. Range limits are represented by dashed black lines. Number of individuals assayed from each population can be found in Table 2.3.

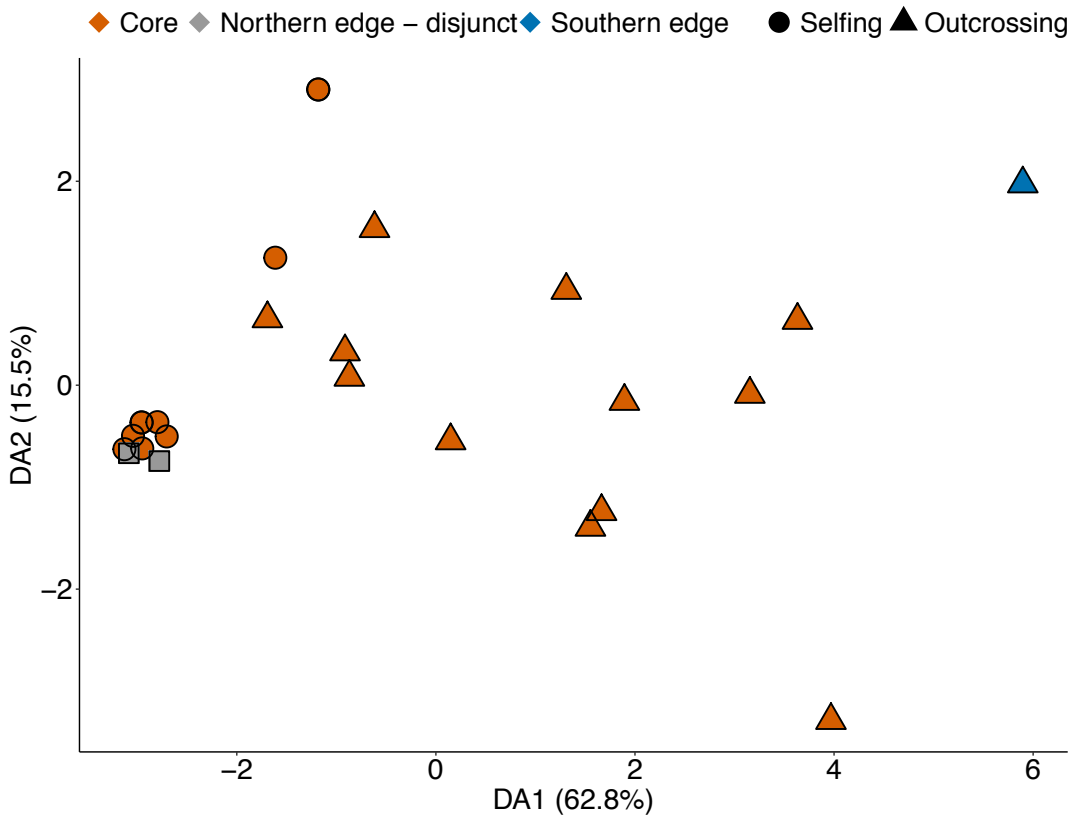

Fig 2.5 Relative genetic distance among populations estimated by the first two discriminant axes (DA) of a Discriminant Analysis of Principal Components (DAPC) based on frequencies at 251 SNPs among 94 individuals and 25 populations across the range of *Abronia umbellata*. Four PCs went into the DAPC and the first two axes account for 78.3% of the genetic variation among individuals. Each point is a population mean based on 2-7 individuals per population. Shape of the point corresponds to mating system: circle points are selfing *A. umbellata* var. *breviflora,* triangle points are outcrossing *A. umbellata* var. *umbellata* and square points are northern disjunct populations in Washington and on Vancouver Island, which are of unknown origin and mating system. Point colour corresponds to the populations' geographic location: orange points are populations in the core of the range of *A. umbellata,* grey points are the northern disjunct populations and the blue point is the southern edge population in Baja California.

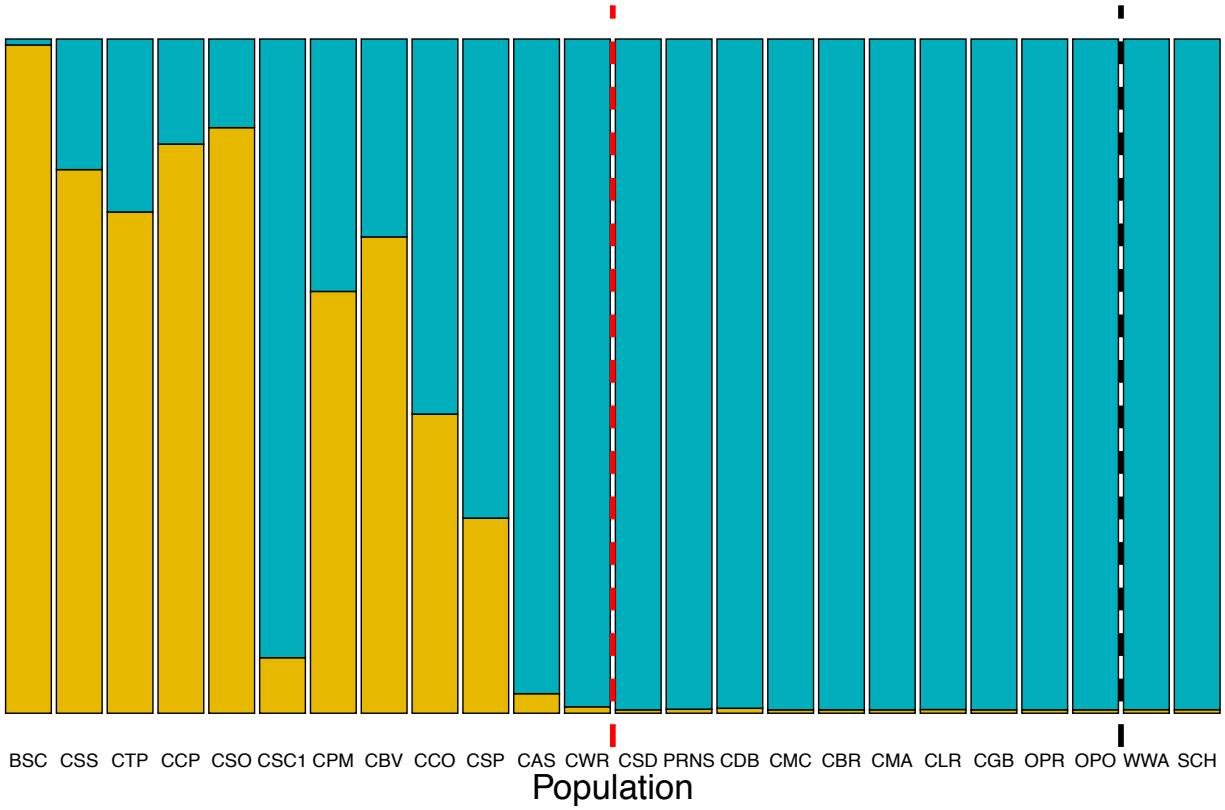

Fig. 2.6 Bayesian clustering of 94 multilocus SNP genotypes sampled from 25 populations across the range of *Abronia umbellata.* The optimal number of genetic clusters was $K = 2$ (see Appendix 2C). Populations, indicated by 3-letter codes, are ordered left to right by increasing latitude and separated by thin black lines. Northern disjunct populations are to the right of the dotted black line. Outcrossing *A. umbellata* var. *umbellata* populations are to the left of the red dotted line and selfing *A. umbellata* var. *breviflora* populations are to the right. Each colour represents estimated ancestry proportion to each genetic cluster averaged over 2 to 7 individuals per population.

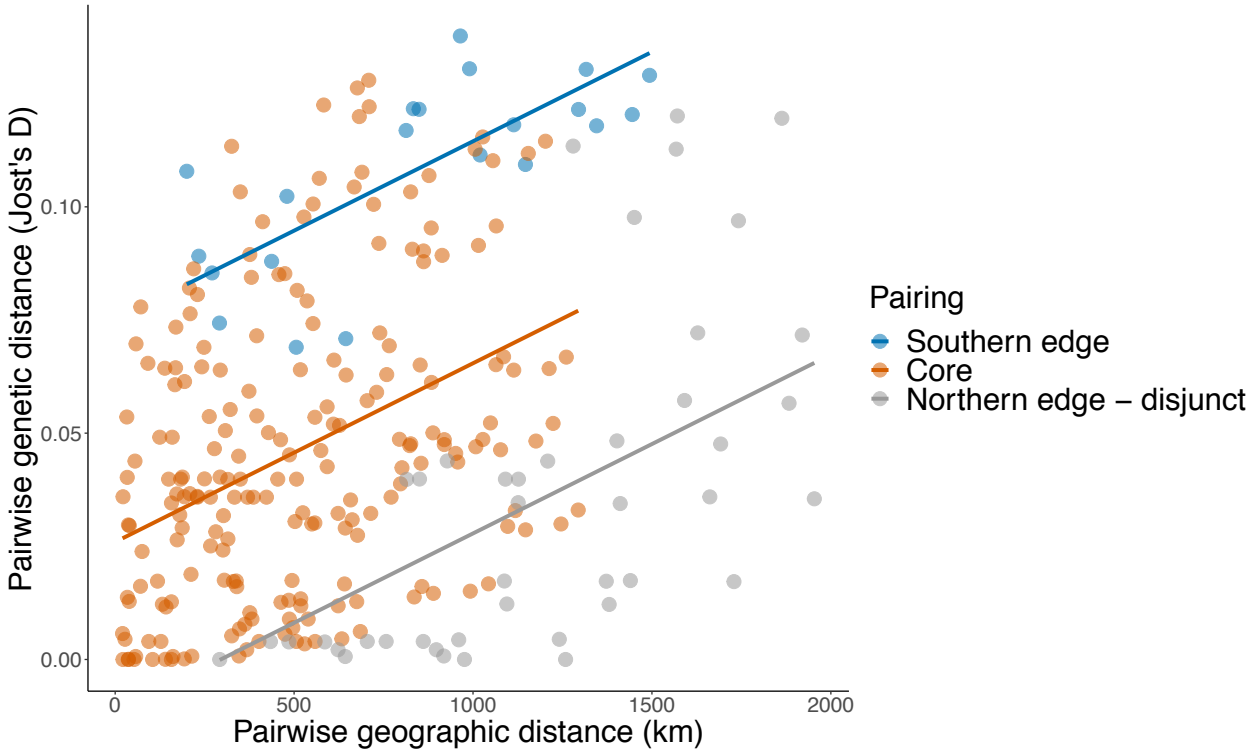

Fig. 2.7 Regression of genetic distance (measured as Jost's D) on geographic distance (kilometres) between pairs of *Abronia umbellata* populations among three geographic regions (southern edge, core and northern disjunct edge). Blue points are the southern edge population compared with all other populations (n = 21 pairwise comparisons), grey points are the two northern disjunct populations compared with all other populations (n = 43) and the orange points are the core populations compared with all other core populations (n = 210). Regression lines were created using predicted values from a model comparing intercepts among the three regions because there was no difference in slope among the groups (Table 2.4).

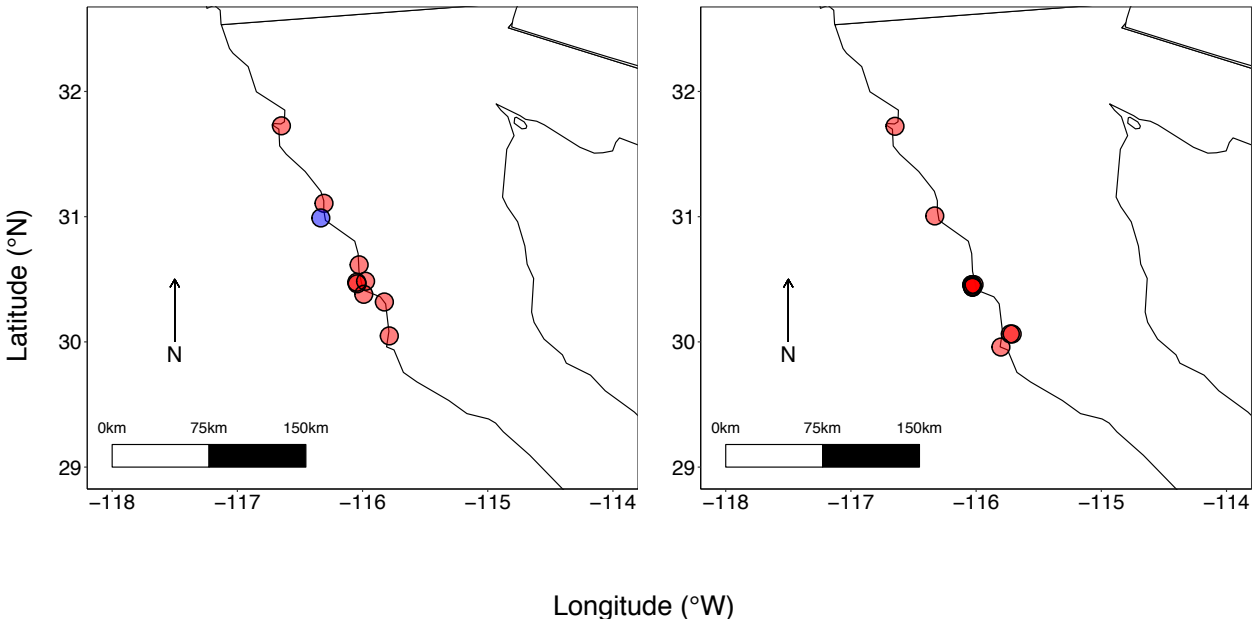

Fig. 2.8 Temporal variation in the occurrence and abundance of *Abronia umbellata* in Baja California, Mexico. The left-hand panel shows populations of *A. umbellata* sampled by Samis and Eckert (2007) in 2003 (red points; $n = 9$). None of these sites contained plants in 2018. Instead the species was only found at a single location posted on iNaturalist.org in 2018 (blue point). The right-hand panel shows the 11 iNaturalist records posted 2019 (iNaturalist.org, 2019).

Chapter 3

Hybridization varies geographically in *Abronia umbellata* (Nyctaginaceae)

Abstract

A long-standing goal in evolutionary ecology is determining the sources of genetic variation required for adaptation in natural populations. One source of genetic variation may be through hybridization, but very few studies have quantified the impact of hybridization across a species range. *Abronia umbellata* is a coastal dune endemic that ranges from San Quintin, Baja California, Mexico to Coos Bay, Oregon, USA that co-occurs and potentially hybridizes with two other coastal *Abronia* species, *A. latifolia* and *A. maritima*, which themselves are parapatric. *Abronia umbellata* exhibits striking differentiation in its mating system with obligately outcrossing *A. umbellata* var. *umbellata* populations occurring south of San Francisco and predominantly selfing *A. umbellata* var. *breviflora* populations occurring in the north. In this study, we quantified geographical variation in hybrid occurrence frequency across the range of *A. umbellata*. Additionally, using morphology from 840 individuals and sequence variation at five single-copy genes assayed for 261 individuals from 37 populations, we determined if introgression, required for the transfer of interspecific alleles, was occurring, and if so, if it played a part in the origin of *A. umbellata* var. *breviflora*. Exhaustive searches detected hybrid phenotypes in 43.4% of populations where *A. umbellata* co-occurred with a congener but these hybrids only contributed to hybrid occurrence frequency greater than zero in 18.9% of populations and 2.4% of 695 plots and only in populations sympatric with *A. maritima*. NewHybrids and DAPC analyses reveal that introgression is likely occurring almost anywhere where *A. umbellata* var. *umbellata* hybridizes with another species, but likely did not contribute to the origin of selfing *A. umbellata* var. *breviflora,* as they are genetically similar to outcrossing

populations of *A. umbellata* and not intermediate between proposed parental species. Overall, the findings from our study show that hybridization has the potential to be a source of genetic variation for natural outcrossing populations, including populations at the southern edge, but likely not selfing populations as selfing may be acting as a reproductive barrier preventing opportunities for hybridization.

Introduction

Species expand their range into novel habitat through incremental local adaption which, at the onset, relies primarily on beneficial allelic variants within the standing genetic variation, rather than new variants by mutation (Blows and Hoffmann, 2005; Bijlsma and Loeschcke, 2012). Thus, range limits are expected to occur where adaptation fails due to a lack of genetic variation for traits or combinations of traits that allow persistence in the habitat beyond (Bridle and Vines, 2007). Therefore, to begin to understand the adaptive capacity of a natural population, we must first investigate factors determining the sources and limits of genetic variation, especially for populations at the range edge.

Genetic variation within populations is dictated by the balance between the loss of variation through genetic drift and directional selection and its replenishment through gene flow from other populations. It is generally thought that most gene flow involves gene movement between populations of the same species. However, hybridization can transfer alleles between closely related species. This requires that species occur in sympatry or very close parapatry and will be facilitated if i) there is a demographic asymmetry between species (ie. sympatry occurs at the range centre of one species and the range edge of the other) and; (ii) if trans-specific alleles confer high fitness (ie. if fitness is highest in the range-centre for both species). In this way, hybridization may facilitate the expansion of a species into new habitat at its range edge. Of course, hybridization may have the opposite effect, range contraction, if hybrids or advanced generation backcrossed individuals have poor fitness (demographic swamping; Prentis et al., 2007), or if hybridization and backcrossing is frequent enough and hybrid viability high enough

that the rare species becomes genetically assimilated into the common species (genetic swamping; Hufford and Mazer, 2003).

It has been suggested that hybridization may be an impetus for a shift to selfing as hybrids, especially allopolyploid hybrids, show weakened self-incompatibility (Husband et al., 2008). Selfing is known to allow dispersal into habitats with limited mates and/or pollinators (Baker, 1955) and therefore, if hybridization is facilitating a shift to selfing, it may indirectly facilitate colonization of new habitats and possible range expansion.

In this study, we investigate the effects of hybridization on the range-wide genetic structure of the coastal dune endemic plant *Abronia umbellata* Lam. (Nyctaginaceae) which co-occurs and putatively hybridizes with two other coastal *Abronia* species, *Abronia latifolia* and *Abronia maritima* (Tillett, 1967). *Abronia umbellata* is continuously distributed from San Quintin, Baja California, Mexico to Coos Bay, Oregon, USA and occurs as disjunct populations in Washington, USA and on Vancouver Island in British Columbia, Canada. It has two varieties, self-incompatible *A. umbellata* var. *umbellata* (hereafter var. *umbellata*) that occurs south of San Francisco, California and predominantly selfing *A. umbellata* var. *breviflora* (hereafter var. *breviflora*) that occurs north of San Francisco (Tillett, 1967; Darling et al., 2008; Doubleday et al., 2013). *Abronia umbellata* var. *breviflora* is relatively rare, with population numbers drastically declining in the past quarter-century (Giles-Johnson et al., 2011; Van Natto and Eckert, *unpublished*), and is listed as endangered in Oregon, Washington and Canada (ORNHIC, 2004; Washington Natural Heritage Program, 1997 Douglas, 2004). It also appears to be in decline in Mexico (Van Natto and Eckert, *unpublished*) but is not on Mexico's Species-at-Risk

List (Norma Oficial Mexicana, 2002). *Abronia latifolia* and *A. maritima* are both common perennial dune-formers that grow parapatrically (except for two locations where they coexist and create putative hybrids) and extend past the northern and southern range limits, respectively, of *A. umbellata* (Tillett,1967). The shift from of *A. maritima* (in the south) to *A. latifolia* (in the north) occurs at Morro Bay, California, within the range of var. *umbellata* (Fig. 3.1).

Hybrids between pink-flowered *A. umbellata*, yellow-flowered *A. latifolia* and magenta-flowered *A. maritima* have previously been identified by Tillett (1967) based on intermediate flower colour. Tillett (1967) assessed the frequency and degree of hybridization across the range of *A. umbellata* using additional floral and fruit morphological traits and concluded that hybridization and bi-directional introgression occurred frequently throughout the range of *A. umbellata* but did not evaluate whether hybrid occurrence frequency varied geographically. Additionally, they claimed that the selfing variety of *A. umbellata,* var. *breviflora,* likely arose through introgression between var. *umbellata* and *A. latifolia.* Previous work by Van Natto and Eckert (*unpublished*) indicates that introgression may be occurring between *A. umbellata* and its congeners because there is a genetic subdivision in populations of *A. umbellata* that corresponds to the transition from *A. latifolia* to *A. maritima.* Here, we quantify geographic variation in hybrid occurrence frequency based on both morphology and sequence variation at five single-copy nuclear genes. We also determined whether there was evidence for introgression, required for the transfer of genetic material between species, and whether such introgression contributed to the origin of var. *breviflora*.

Materials and methods

Population surveys

In the summers of 2002, 2003, 2017 and 2018 , we surveyed 37 sites across the geographic range of *Abronia umbellata* known to be previously occupied by at least one maritime *Abronia* species (Samis and Eckert, *unpublished*; Fig. 3.2; Table 3.1) and recorded the presence of each *Abronia* species and their hybrids, which we identified by their flower colour as they are intermediate between parental species (e.g. hybrids between yellow *A. latifolia* and pink *A. umbellata* have salmon coloured flowers; Mentiady and Eckert *unpublished*).

In 2017 and 2018, we collected leaves from 20–24 individuals from each pure species present and all putative hybrids and flash dried the leaf material in silica gel. For each sampled individual, we recorded the presence/absence of pure and hybrid individuals within a 1-m and 3-m radius. We also measured seven morphological traits on each individual (Table 3.2) following Tillett (1967), but excluded any subjective traits (eg. reflexion of perianth limb) because of the lack of consistency among multiple observers, and the fruit traits (ie. anthocarp diameter) because not enough plants were producing fruits at each site when visited. Flower colour was recorded qualitatively because time constraints prevented us from measuring it quantitatively on such a large sample of individuals.

Sequencing

For each site surveyed in 2017 and 2018, we randomly chose five samples from each of the species present (var. *breviflora, var*. *umbellata*, *A. latifolia* and *A. maritima*, $n = 277$) plus all putative hybrids (total $n = 25$ hybrids). For each sample, we extracted DNA using a modified CTAB protocol (Doyle and Doyle, 1987) and amplified target sequences for five single-copy nuclear genes (Greer, 2016) and 500bp of the chloroplast gene MaturaseK (matK), using PCR performed on an Applied Biosystems SimpliAmp Thermal Cycler (Applied Biosystems LSA37835). For the PCR reaction, initial denaturation was set to 95°C for 2 minutes followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at a primer optimized temperature (55–57°C; Appendix 3A) for 40 seconds, extension at 72°C for 60 seconds and final extension at 72°C for 10 minutes. Resulting amplicons were visualized for quality on a 50% agarose gel and high-yield PCR products of uniform size were submitted to GénomeQuébec (Montreal, Québec, Canada) for Sanger sequencing of the forward strands on an Applied Biosystems DNA analyzer (Applied Biosystems 3730XL) using the same PCR primers used for amplification (Appendix 3A).

We visualized chromatograms in GeneiousPrime version 2019.0.4 (https://www.geneious.com), removed low-quality sequences with Phred scores < 40, aligned sequences using a MUSCLE alignment with a maximum of eight refinement iterations (Edgar, 2004) and manually edited to trim low-quality ends and identify heterozygous sites. This left an average of 407bp of sequence per locus per individual. We removed invariant sites to obtain an alignment of single nucleotide polymorphisms (mean = 41 per locus) and exported those sequences as FASTA files.

Statistical Analyses

To determine how hybrid occurrence frequency varies across the range of *A. umbellata,* we calculated the proportion of 3-m radius plots around phenotypically pure species that contained a hybrid, for each population. We also calculated the mean proportion of plots occupied across all populations, and then just populations with at least two *Abronia* species, to determine range wide hybrid occurrence frequency. Finally, we compiled presence/absence data of parental *Abronia* species and their hybrids for the 37 sites from the 2002, 2003, 2017 and 2018 (Table 3.1). For each sampling period (2002/2003 and 2017/2018), we calculated the proportion of sites with hybrids present within the ranges of *A. latifolia* and *A. maritima*. Overall, we predicted that hybridization would be less common in the range of var. *breviflora* because its selfing mating system would act as a reproductive barrier.

To evaluate differentiation among the three species in the morphological traits we measured, we ran a Discriminant Analysis of Principal Components (DAPC) on four floral and three leaf traits using the dapc function in the adegenet package (version 2.1.1; Jombart, 2008; Jombart and Ahmed, 2011) in the R statistical environment (version 3.5.2; R Core Team, 2018). All parental species and putative hybrids were included in the analysis. *Abronia umbellata* was divided into its two varieties, outcrossing var. *umbellata* and selfing var. *breviflora,* because their floral traits are known to vary strongly due to differences in mating system (Doubleday et al., 2013). We then used these DA scores from the first two discriminant axes as response variables in linear models with species/hybrid type as a predictor. Multiple comparisons among species were performed using the emmeans and pairs functions in the emmeans package (version 1.3.3.;

Lenth, 2019). We did the same analysis using nuclear SNPs to evaluate differentiation at the genetic level. However, for this analysis we divided var. *umbellata* into populations that are within the range of *A. latifolia* (northern var. *umbellata*) and populations within the range of *A. maritima* (southern var. *umbellata*). This divide is based on results from Van Natto and Eckert (*unpublished*) in which they found genetic differentiation of var. *umbellata* at the transition zone between *A. latifolia* and *A. maritima*. Raw FASTA files of nuclear data were converted to genepop files using the Convert function in the PGD Spider package (version 2.1.1.5; Lischer and Excoffier, 2012) which were then converted to genind objects using the df2genind function in the adegenet package and run with the dapc function in the same package.

Next we determined if there was a correlation between morphology (Table 3.2) and nuclear SNPs. We ran the analysis in two groupings; one with var. *umbellata,* var. *breviflora* and *A. latifolia* and their putative hybrids (n = 167 individuals) and the other with var. *umbellata, A. maritima* and their putative hybrids (n = 137 individuals). We correlated the DA scores from the first axis of the DAPCs of nuclear SNP frequencies and morphological characteristics.

To determine if introgression was occurring, we first analyzed SNP sequences from the chloroplast gene, MaturaseK (matK). Since organellar genomes are maternally inherited, we predicted that hybrid lineages that have deeply introgressed with one parent species may produce individuals that are identified as one species based on morphology and nuclear SNPs but carry the distinct chloroplast haplotype of another species. We used a minimum spanning network to illustrate the genealogical relation among matK haplotypes. To do this, we converted the raw FASTA file of matK sequences to a nexus file (using Convert function), which we then used to

make a minimum spanning network using the Minimum Spanning Network tool in PopArt software (version 1.7; Bandelt et al., 1999).

We used NewHybrids (version 1.1 beta 3; Anderson and Thompson, 2003) to identify hybrids independently of morphological data and determine if introgression was occurring. NewHybrids classifies individuals as parental species, F_1 or F_2 hybrids, or backcrossed individuals based on their genotypes at nuclear SNPs using a Bayesian algorithm to calculate the posterior probability that an individual falls into each category. The model assumes Hardy-Weinberg equilibrium and linkage equilibrium for the markers being analyzed, thus we excluded individuals of selfing *A. umbellata* var. *breviflora* from this analysis. We ran NewHybrids in parallel using the parallelnewhybrid R package (version 0.0.0.9002; Wringe et al., 2016) with the parallelnh function with 1000 iterations and 1000 burn-ins.

Relative to outcrossing individuals, selfers are expected to have reduced levels of heterozygosity and early generation hybrids are expected to have exceptionally high levels of heterozygosity (Fitzpatrick 2012). Therefore, since we were not able to use NewHybrids on predominantly selfing var. *breviflora* individuals, we inferred if there were F_1 or F_2 hybrids using observed heterozygosity, which we calculated using the summary function in adegenet, because we would expect early generation hybrids to stand out among selfing individuals. However, this method would not work to detect introgression from var. *breviflora* into *A. latifolia* if *A. latifolia* is predominantly outcrossing, because higher levels of heterozygosity would not be abnormal.

Results

Field surveys and hybrid occurrence frequency

Extensive field searches of 37 populations in 2017 and 2018, revealed hybrid phenotypes in 100% of populations where outcrossing var. *umbellata* co-occurred with a congener (n_{pop} = 10; Table 3.1). Conversely, no hybrids were found between selfing var. *breviflora* and *A. latifolia.* In 2002 and 2003, we found phenotypic hybrids with var. *umbellata* at only 40% of the populations it was sympatric with a congener, but did find hybrids at two locations where var. *umbellata* was allopatric, and found phenotypic var. *breviflora* x *A. latifolia* hybrids at 75% of populations where the parental species were sympatric

Analyses of hybrid occurrence frequency revealed that only 2.7% of 695 3-m radius plots from the 37 populations surveyed contained a hybrid. When allopatric populations were removed, 4.4% of the remaining 491 plots from 22 populations contained a hybrid. Additionally, all populations with a proportion of plots occupied greater than zero were within the range of *A. maritima* (Table 3.1).

Analyses of morphology and SNP variation

From linear models of estimated marginal means of DA scores from the DAPC of morphological data from 840 individuals (140 var. *breviflora*, 237 var. *umbellata,* 238 *A. latifolia,* 170 *A. maritima,* 6 var. *umbellata* x *A. latifolia*, 38 var. *umbellata* x *A. maritima* and 11

A. latifolia x *A. maritima*; Fig. 3.3) with species/hybrid as the predictor variable, we found significant differentiation among the three parental *Abronia* species and their hybrids on DA1 ($R^2 = 0.77$, $F_{6, 833} = 462.4$, $P < 0.001$; Table 3.2) and DA2 ($R^2 = 0.70$, $F_{6, 833} = 319.4$, $P < 0.001$). Additionally, from visual inspection, we found that hybrid phenotypes are intermediate between parental species but that both var. *umbellata* x *A. latifolia* and var. *umbellata* x *A. maritima* hybrids clustered slightly closer to var. *umbellata.*

From 261 individuals (42 var. *breviflora*, 59 var. *umbellata,* 67 *A. latifolia,* 57 *A. maritima,* 9 var. *umbellata* x *A. latifolia*, 23 var. *umbellata* x *A. maritima* and 4 *A. latifolia* x *A. maritima*), we detected 205 SNPs across 2034 bp of sequence from five nuclear loci (mean ± 1SD = 2.11 ± 0.33 SNPs/locus). Similar to morphological analysis, the DAPC of SNP frequencies (Fig. 3.4) shows that there is significant differentiation in DA1 scores among the three parental *Abronia* species and their hybrids ($R^2 = 0.95$, $F_{7, 249} = 657.8$, $P < 0.001$; Table 3.2) and significant differentiation among DA2 scores ($R^2 = 0.89$, $F_{7, 249} = 292.8$, $P < 0.001$). Also similar is that visual inspection reveals that hybrids cluster intermediately between parental species but hybrids of var. *umbellata* and *A. latifolia* and var. *umbellata* and *A. maritima* cluster more closely to var. *umbellata*. For this DAPC analysis, we originally divided var. *umbellata* populations within the range of *A. maritima* (southern var. *umbellata*) and *A. latifolia* (northern var. *umbellata*), as findings by Van Natto and Eckert (*unpublished*) suggest that these two groups may be genetically differentiated, but found no difference between groups (var. *breviflora* – var. *umbellata;* $t = -0.45$, $df = 249$, $P = 0.99$). Thus, for simpler comparisons to morphological data, we ran the analysis again but amalgamated all var. *umbellata* populations into a single group.

We found a significant correlation between morphology and genetics in the two groups of *Abronia* species (Group 1: var. *umbellata, A. latifolia* and their hybrids; Group 2: var. *umbellata*, *A. maritima* and their hybrids; Fig. 3.5). Scores on the DAPC axis based on morphology correlated strongly with scores based on nuclear SNPs for both species comparisons (var. *umbellata* and *A. maritima,* $r = –0.48$ (CI= -0.61 — -0.34, $P < 0.001$; var. *umbellata* and *A. latifolia* $r = 0.88$ (CI= 0.84 — 0.91), $P < 0.001$; Fig. 3.5). However, from visual inspection we can see that the correlation among var. *umbellata, A. maritima* and their hybrids does not appear strong as morphology DA1 scores are very similar among all groups.

We detected four distinct haplotypes at matK involving 10 SNPs across 623 bp of sequence of from 114 individuals (35 *A. umbellata,* 33 *A. latifolia,* 25 *A. maritima,* 6 var. *umbellata* x *A. latifolia* hybrids, 13 var. *umbellata* x *A. maritima* hybrids and 2 *A. maritima* x *A. latifolia* hybrids). Each haplotype was found only in individuals thought to be of one pure species and putative hybrids involving that species. Each class of putative hybrids included individuals bearing either haplotype from the pure species involved suggesting that hybridization occurs in both directions (i.e. both species acting as the maternal parent, Fig. 3.6). However, there was no clear evidence of introgression as all individuals identified as a pure parental species had the chloroplast haplotype associated with that species.

Posterior probabilities from NewHybrids analyses revealed that zero putative var. *umbellata* x *A. latifolia* hybrids and 30.4% of putative var. *umbellata* x *A. maritima* hybrids were F_1's. Additionally, we found no F_2 hybrids in either group but did find that there is likely backcrossing to var. *umbellata* in var. *umbellata* x *A. maritima* and var. *umbellata* x *A. latifolia*

hybrids. There was no indication of backcrossing to *A. maritima* or *A. latifolia* showing that introgression is likely unidirectional. From visual inspection, backcrossed individuals also clustered more closely with var. *umbellata* on PCAs with var. *umbellata, A. latifolia* and their hybrids and var. *umbellata, A. maritima* and their hybrids (Fig. 3.7).

Mean population observed heterozygosity was zero or near zero in all selfing populations of *A. umbellata* indicating that no pure phenotypes are early generation hybrids (Table 3.3). Additionally, we found that observed heterozygosity was significantly different between selfing and outcrossing populations of *A. umbellata* ($R^2 = 0.59$, $F_{1,23} = 32.97$, $P < 0.001$).

Discussion

Hybrid frequency occurrence across the range of A. umbellata

We aimed to quantify the geographic variation in hybrid occurrence frequency across the range of *Abronia umbellata* and determine if there is evidence for introgression between *A. umbellata* and its congeners, as would be required for the transfer of genetic material between species. Our analysis of presence/absence of hybrids within 3-m radii of focal plants revealed that hybridization, in general, does not appear to be frequent in *A. umbellata* as hybrid phenotypes were detected in only 2.2% of plots in all populations and 4.4% of plots in populations where more than one *Abronia* species co-occurred. In all, we found hybrid phenotypes in only seven of thirty-five populations and all were in the range of *A. maritima,* indicating that hybrid occurrence frequency is much higher with *A. maritima*. However, in our extensive searches for hybrid phenotypes in 2017 and 2018, we found phenotypic hybrids at all sites where var. *umbellata* co-occurred with a congener, whether it was *A. maritima* or *A. latifolia*.

Contrary to hybridization surveys in 2002 and 2003, we did not identify any hybrid phenotypes between var. *breviflora* and *A. latifolia* in the 2017 and 2018 field seasons. A lack of hybridization between var. *breviflora* and *A. latifolia* may partially be explained by the selfing mating system of var. *breviflora* because in general, selfing reduces opportunities for cross-fertilization of any kind, including hybridization. For example, mating system divergence has been found to prevent hybridization between *Mimulus guttatus* and *M. nasutus,* likely because of

the reduced competitive abilities of pollen tubes of selfing *M. nasutus* (Martin and Willis 2007). However, this does not explain why hybrids were found between var. *breviflora* and *A. latifolia* in 2002 and 2003. Perhaps the decline in var. *breviflora* populations over the past several decades (Giles-Johnson et al., 2011) has lead to less frequent hybrid matings.

At the southern range limit of *A*. *umbellata,* we only found a single extant population in 2018 that was not sympatric with *A. maritima*, contained only 6 *A. umbellata* individuals and did not contain any hybrid phenotypes. We suspect that the lack of phenotypic hybrids is partially due to the apparent disappearance var. *umbellata* populations toward the southern range limit (Van Natto and Eckert, *unpublished*).

***Degree of hybridization across the range of* A. umbellata**

Tillett (1967) used floral and fruit morphology indices to distinguish putative early-generation and introgressed hybrids from parental species and claimed that introgression was frequent across the range of var. *umbellata*, albeit more common with *A. maritima*. They also stated that introgression was bidirectional but more often in the direction of *A. latifolia* and *A. maritima* because introgressed hybrids clustered with them in morphological analyses, rather than *A. umbellata.* Only one population of var. *breviflora* was included in their analysis but they claimed that var. *breviflora* is likely the result of introgression between var. *umbellata* and *A. latifolia.*

Similar to Tillett (1967), our morphological analyses showed that parental species are largely distinct from one another and putative hybrids are intermediate between their respective parental species. Genetic SNP data was partially congruent with morphological data as several phenotypic hybrid individuals were, again, intermediate between parental species and there was a weak but significant correlation between morphology and SNP data. However, from visual inspection, both var. *umbellata* x *A. latifolia* and var. *umbellata* x *A. maritima* hybrids clustered more closely with outcrossing *A. umbellata* in a DAPC of SNP frequencies, suggesting that if introgression was occurring, it was likely from backcrossing to *A. umbellata*. NewHybrids analysis supported this hypothesis as it detected introgressed hybrids, but contrary to Tillett (1967) only ones backcrossed to var. *umbellata* rather than bidirectionally

We found that selfing var. *breviflora* is likely not the result of introgression between outcrossing var. *umbellata* and *A. latifolia* as var. *breviflora* clustered significantly closer with northern populations of var. *umbellata* in a DAPC, rather than intermediately between var. *umbellata* and *A. latifolia,* as we would expect with introgressed hybrids. Additionally, all var. *breviflora* individuals had low genetic variation, as expected with selfing, whereas we would expect the signature of hybridization (increased heterozygosity) to persist for several generations. However, it is not completely implausible if enough time has passed, because the genetic signature of introgression may be lost as 50% of genetic variation is lost with each generation of full selfing (Charlesworth and Wright, 2001).

NewHybrids analysis revealed that two of the individuals from the Baja California population (BSC) were backcrossed hybrids to var. *umbellata* which indicates that hybridization

with *A. maritima* has occurred at the southern range edge, likely during some point of sympatry or close parapatry. Across the rest of the range of var. *umbellata*, NewHybrids analysis revealed that early-generation hybridization was occurring anywhere var. *umbellata* was sympatric with another species, and introgression was present at seven of the eight populations with *A. maritima* x var. *umbellata* hybrids.

Pfennig et al. (2016) claim that gene exchange through introgression can prevent extinction of vulnerable range edge populations and may even facilitate range expansion by providing genetic material needed for adaptation. This pattern has been detected in a study of *Anopheles* mosquitos where introgression between *A. gambiae* and *A. arabiensis* may have allowed the expansion of *A. gambiaea* into more arid environments (Besansky et al., 2003). In *A. umbellata*, adaptive introgression is unlikely to occur at the northern range edge because hybridization does not appear frequent between var. *breviflora* and *A. latifolia* based on extensive field surveys for hybrids. Conversely, introgression appears to be occurring at the southern edge, based on NewHybrids analysis, and therefore, adaptive introgression may be possible.

Why is introgression unidirectional to **Abronia umbellata***?*

An interesting finding is that we detected introgression in nuclear SNPs but could not detect backcrossing back to the maternal parent of hybrids in chloroplast haplotypes. Introgression could be more easily be detected in the minimum spanning network of SNPs from the chloroplast gene (matK) if parental species shared haplotypes, as it would suggest extensive

backcrossing (Palme et al., 2004; Hufbauer and Sforza, 2008). We found the opposite, as all parental phenotypes had distinct non-shared haplotypes. However, DAPC and NewHybrids analyses of nuclear SNPs we detected introgression. These findings suggest that there may be nucleocytoplasmic incompatibilities between var. *umbellata* and its congeners, which would result in introgressed hybrids that are only viable if they cross with their maternal parent of the original F_1 which mentioned previously, would not be detected in analyses of chloroplast haplotypes. Notably, nucleocytoplasmic incompatibilities may also completely prevent backcrossing if F_1 hybrids are sterile. For example, in a study of hybridization between a wild pea accession of *Pisum sativum* subsp. *elatius* and cultivated *P. sativum* representatives, they found that F_1 hybrids were nearly sterile and chlorophyll deficient when *P. sativum* subsp. *elatius* was the maternal parent.

Nucleocytoplasmic incompatibilities may also explain why we found apparent unidirectional introgression to var. *umbellata.* Our analysis of chloroplast haplotypes revealed that both var. *umbellata* x *A. latifolia* and var. *umbellata* x *A. maritima* F_1 hybrids had haplotypes of either parental species but NewHybrids analysis only detected backcrosses to var. *umbellata*. If F_1 hybrids with *A. latifolia* or *A. maritima* as the maternal parent were infertile, it would explain a lack of backcrosses in that direction. This hypothesis could be tested in a pollination experiment but was not in the scope of this study.

Conclusion

Climate-change induced range shifts are expected to increase secondary contact of previously allopatric species which could increase the opportunity for hybridization (Garcia-Elfring et al., 2017; Heppenheimer et al., 2018). Increased opportunities for hybridization may be beneficial in some species if introgression provides standing genetic variation required for adaptation to novel conditions (Pfennig et al., 2016; Hamilton and Miller, 2015). Conversely, some species, including many endangered species, are thought to be at risk of genetic or demographic swamping with increased hybridization (Genovart, 2009). Thus, determining how hybridization varies across species ranges and how it may influence genetic structure, whether it be beneficial, neutral or of detriment, is a vital component in understanding species evolutionary responses to contemporary conditions.

Table 3.1 Populations surveyed for the presence of hybrids in the summers of 2017 and 2018 and 2002 and 2003. Populations above the line-break are within the range of the outcrossing variety of *Abronia umbellata,* var. *umbellata* (n= 23)*,* and populations below the line-break are within the range of the predominantly selfing variety, var. *breviflora* (n=14). Several populations were not surveyed in 2002 and 2003 but were surveyed in 2017 and 2018. Populations BSC, CDB, OBS, and OFS were found with iNaturalist records (iNaturalist.org, 2019), CMN was used in a study by Doubleday et al. (2013) in an analysis of floral volatiles and CSC1, CSC2 and CSC3 were found surveying Santa Cruz Island, California. Species are represented as follows: *M= Abronia maritima, L = Abronia latifolia, U= Abronia umbellata* var. *umbellata, B = Abronia umbellata* var. *breviflora.*

Population	Latitude (°N)	Longitude (°W)	Taxon Present/ Hybrid Present – 2017/2018	n_{plots} / Proportion Occupied	Taxon Present/ Hybrid Present – 2002/2003
BBR	30.0478	115.7864	*M*/--	24 (0)	*U*/ --
BBQ4	30.3801	115.9904	*M*/--	20 (0)	*U*/ --
BPA	30.3904	115.9854	*M*/--	24 (0)	*M*/ --
BSC	30.9898	116.3333	*U*/--	6 (0)	n/a
BSA	31.1068	116.3087	*M*/--	1 (0)	*U*/ --
CSS	32.6408	117.1436	*M, U*/ *M* x *U*	32 (0.06)	*U*/ --
CTP	32.9296	117.2591	*U*/--	23 (0)	*U*/ --
CCP	33.2351	117.4178	*M, U*/ *M* x *U*	33 (0.18)	*M, U*/ *M* x *U*
CSO	33.3808	117.5776	*M, U*/ *M* x *U*	11 (0.27)	*M, U*/ --
CSC1	34.0570	119.9220	*M, U*/ *M* x *U*	27 (0.19)	n/a
CSC3	34.0375	119.8843	*M*/ --	24 (0)	n/a
CSC4	34.0028	119.8847	*M*/ --	9 (0)	n/a
CPM	34.1145	119.1494	*M, U*/ *M* x *U*	15 (0)	*U*/ --
CBV	34.2679	119.2783	*M, U*/ *M* x *U*	23 (0.09)	*U*/ *M* x *U*
CCO	34.4122	119.8829	*M, U*/ *M* x *U*	24 (0.16)	*M, U*/ *M* x *U*
CJB	34.5117	120.5023	*M*/ --	6 (0)	*M*/ --
CSU	34.6829	120.6062	*L, M*/ *L* x *M*	43 (0.05)	*L, M*/ *L* x *M*
CSP	35.3658	120.8623	*L, M, U*/ *L* x *M, L* x *U*	59 (0)	*L, U*/ --
CMS	35.3964	120.8662	*M*/ --	20 (0)	*M, U*/ --

CAS	36.6189	121.9411	*L, U*/ *L* x *U*	39 (0)	*U*/ *L* x *U*
CMN	36.9175	121.8528	*U*/ --	24 (0)	n/a
CWR	36.9541	122.0799	*L, U*/ *L* x *U*	42 (0)	*L, U*/ *L* x *U*
CCF	37.8112	122.4877	*L*/ --	6 (0)	*U*/ --
CSD	37.907	122.6794	*B, L*/ --	2 (0)	*B, L*/ *B* x *L*
PRNS	38.0309	122.9502	*B, L*/ --	9 (0)	*B, L*/ *B* x *L*
CDB	38.3141	123.0438	*B, L*/ --	3 (0)	n/a
CMC	38.9827	123.7058	*B, L*/ --	24 (0)	*L*/ --
CBR	39.3047	123.7966	*B, L*/ --	24 (0)	*B, L*/ *B* x *L*
CMA	40.8476	124.0075	*B, L*/ --	23 (0)	*B*/ --
CLR	41.0274	124.1106	*B, L*/ --	6 (0)	*B*/ *B* x *L*
CGB	41.3618	124.0748	*B, L*/ --	17 (0)	*B, L*/ *B* x *L*
OPR	42.2709	124.4059	*B, L*/ --	8 (0)	*L*/ --
OPO	42.7412	124.4989	*B, L*/ --	8 (0)	*B, L*/ *B* x *L*
OBS	45.1841	123.9685	*L*/ --	16 (0)	n/a
OFS	46.1973	123.9899	*L*/ --	1 (0)	n/a
WWA	46.6339	124.0675	*B, L*/ --	3 (0)	*B, L*/ --
SCH	49.0258	125.6802	*B, L*/ --	16 (0)	*B, L*/ --

Table 3.2 Morphological variation between three species of maritime *Abronia* and their putative hybrids. Mean and standard deviation of seven morphological traits measured from 20–24 individuals (or all individuals if < 20) of each parental species and all hybrids present at each population. Each leaf trait was measured on three different leaves, umbel angle and flowers per umbel on five inflorescences (or all inflorescences if less than five) and floral tube length and face diameter on one flower twice to account for variation within individual. Hybrids are represented as follows: *U* x *M*= var. *umbellata* x *A. maritima* hybrid, *U* x *L*= var. *umbellata* x *A. latifolia* hybrid and *L* x *M*= *A. latifolia* x *A. maritima* hybrid.

		Morphological Trait						
Species/ Hybrid	n_{inds}/n_{sites}	Leaf thickness (mm)	Leaf length (mm)	Leaf width (mm)	Umbel angle (degrees)	Flowers per umbel	Floral tube length (mm)	Floral face diameter (mm)
A. umbellata var. *umbellata*	247/13	1.00 (± 0.29)	17.62 (± 5.24)	10.60 (± 3.44)	114.17 (± 27.68)	13.11 (± 2.97)	10.98 (± 2.35)	8.63(± 1.79)
A. umbellata var. *breviflora*	214/14	0.92 (± 0.20)	22.36 (± 5.77)	12.51 (± 4.09)	91.52 (± 14.55)	13.01 (± 2.42)	7.38 (± 2.22)	5.98 (± 1.38)
A. latifolia	274/20	1.88 (± 0.44)	23.40(± 5.12)	24.64 (± 5.74)	105.47 (± 22.90)	17.92 (± 4.39)	9.50 (± 2.28)	7.38 (± 1.41)
A. maritima	214/17	2.29 (± 0.61)	26.42 (± 5.39)	17.85 (± 4.19)	60.02 (± 10.42)	11.53 (± 1.82)	7.93 (± 1.61)	5.62 (± 1.37)
U x *M*	38/7	1.36 (± 0.52)	18.00 (± 4.63)	12.95 (± 3.21)	83.93 (± 13.70)	11.9 (± 2.34)	8.91 (± 2.48)	6.58 (± 1.43)
U x *L*	9/3	1.41 (± 0.96)	21.09 (± 7.80)	14.74 (± 5.79)	118.89 (± 21.86)	15.84 (± 2.07)	10.81 (± 2.29)	8.83 (± 1.73)
L x *M*	11/2	2.12 (± 0.57)	25.56 (± 5.16)	20.00 (± 4.49)	90.74 (± 17.72)	15.92 (± 2.44)	7.75 (± 2.02)	7.00(± 1.33)

Table 3.3 Pairwise comparisons of estimated marginal means of DA1 scores from Discriminant Analysis of Principal Components (DAPC) of sequence variation (SNP DA1 Scores) and morphology (Morphology DA1 Scores). Comparisons being tested were run with the Tukey method. Hybrids are represented by the following three-digit codes: LUH = var. *umbellata* x *A. latifolia,* MUH = var. *umbellata* x *A. maritima* and LMH = *A. latifolia* x *A. maritima* hybrids.

Contrast	SNP DA1 Scores	Morphology DA1 Scores
var. *breviflora* - *A. latifolia*	t = 27.12, df = 250, $P < 0.0001$	t = 42.26, df = 833, $P < 0.0001$
var. *breviflora* - LMH	t = -2.51, df = 250, $P = 0.16$	t = 8.91, df = 833, $P < 0.0001$
var. *breviflora* - LUH	t = 2.96, df = 250, $P = 0.05$	t = 3.47, df = 833, $P = 0.0097$
var. *breviflora* - *A. maritima*	t = -32.65, df = 250, $P < 0.0001$	t = 14.88, df = 833, $P < 0.0001$
var. *breviflora* - MUH	t = -10.21, df = 250, $P < 0.0001$	t = 5.39, df = 833, $P < 0.0001$
var. *breviflora* - var. *umbellata*	t = -3.89, df = 250, $P = 0.0024$	t = 2.65, df = 833, $P = 0.11$
A. latifolia - LMH	t = -13, df = 250, $P < 0.0001$	t = -5.55, df = 833, $P < 0.0001$
A. latifolia - LUH	t = -11.32, df = 250, $P < 0.0001$	t = -7.39, df = 833, $P < 0.0001$
A. latifolia - *A. maritima*	t = -66.76, df = 250, $P < 0.0001$	t = -27.91, df = 833, $P < 0.0001$
A. latifolia - MUH	t = -32.86, df = 250, $P < 0.0001$	t = -20.12, df = 833, $P < 0.0001$
A. latifolia - var. *umbellata*	t = -34.54, df = 250, $P < 0.0001$	t = -45.98, df = 833, $P < 0.0001$
LMH - LUH	t = 4.01, df = 250, $P = 0.0015$	t = -2.65, df = 833, $P = 0.11$
LMH - *A. maritima*	t = -10.43, df = 250, $P < 0.0001$	t = -3.51, df = 833, $P = 0.0084$
LMH - MUH	t = -2.55, df = 250, $P = 0.15$	t = -5.27, df = 833, $P < 0.0001$
LMH - var. *umbellata*	t = 1.01, df = 250, $P = 0.95$	t = -8.13, df = 833, $P < 0.0001$
LUH - *A. maritima*	t = -20.78, df = 250, $P < 0.0001$	t = 0.6, df = 833, $P = 0.99$
LUH - MUH	t = -9.31, df = 250, $P < 0.0001$	t = -1.05, df = 833, $P = 0.94$
LUH - var. *umbellata*	t = -5.14, df = 250, $P < 0.0001$	t = -2.82, df = 833, $P = 0.073$
A. maritima - MUH	t = 15.95, df = 250, $P < 0.0001$	t = -3.97, df = 833, $P = 0.0015$
A. maritima - var. *umbellata*	t = 31.73, df = 250, $P < 0.0001$	t = -14.09, df = 833, $P < 0.0001$
MUH - var. *umbellata*	t = 7.64, df = 250, $P < 0.0001$	t = -4.03, df = 833, $P = 0.0012$

Table 3.4 Observed heterozygosity within 25 population of *Abronia umbellata* across the geographic range. Population code and mating system are presented along with sample size (n_{inds}), within population mean observed heterozygosity and standard error around means.

Population	n_{inds}	Mating System	H_{obs} (Standard error)
BSC	5	Outcrossing	0.31 (0.29 — 0.33)
CSS	7	Outcrossing	0.13 (0.11 — 0.14)
CTP	4	Outcrossing	0.10 (0.08 — 0.11)
CCP	4	Outcrossing	0.16 (0.14 — 0.18)
CSO	4	Outcrossing	0.09 (0.07 — 0.10)
CSC1	3	Outcrossing	0.06 (0.05 — 0.07)
CPM	5	Outcrossing	0.12 (0.11 — 0.13)
CBV	4	Outcrossing	0.15 (0.14 — 0.17)
CCO	4	Outcrossing	0.15 (0.13 — 0.17)
CSP	5	Outcrossing	0.15 (0.14 — 0.17)
CAS	5	Outcrossing	0.05 (0.04 — 0.06)
CMN	5	Outcrossing	0.05 (0.04 — 0.06)
CWR	4	Outcrossing	0.05 (0.03 — 0.06)
CSD	4	Selfing	0.00
PRNS	2	Selfing	0.00
CDB	3	Selfing	0.00
CMC	3	Selfing	0.00
CBR	2	Selfing	0.00
CMA	2	Selfing	0.00
CLR	4	Selfing	0.00
CGB	2	Selfing	0.00
OPR	2	Selfing	0.00
OPO	3	Selfing	0.00
WWA	4	Selfing	0.00
SCH	4	Selfing	0.00

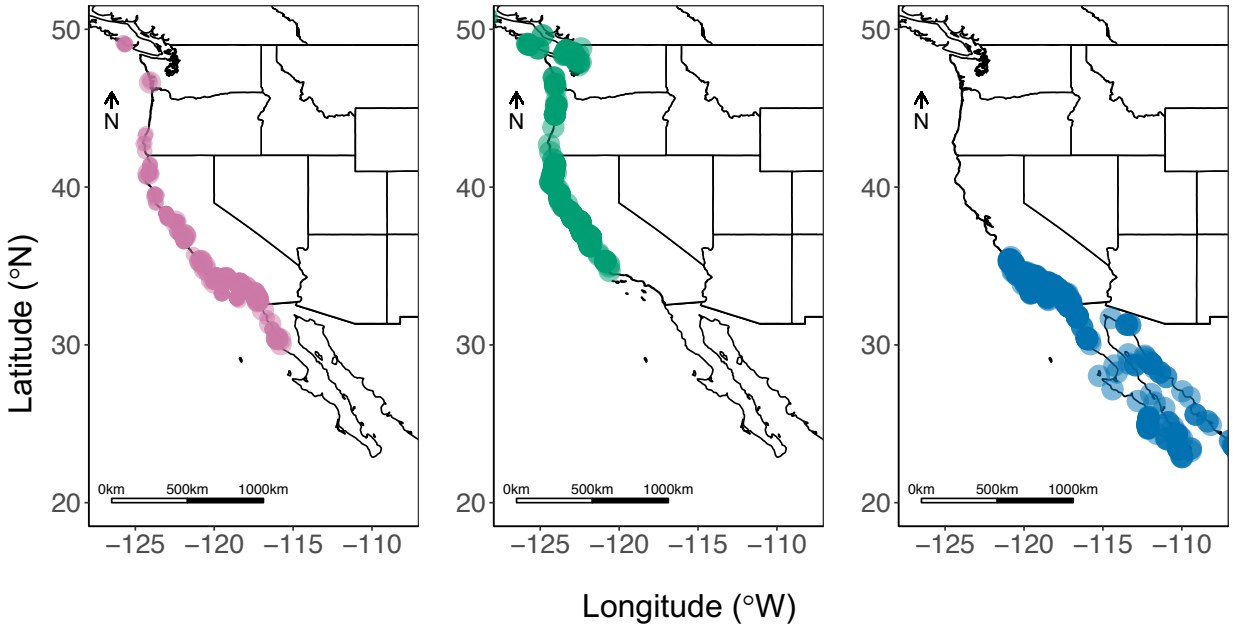

Fig. 3.1 Distribution of locations of *Abronia umbellata* (pink points, left), *A. latifolia* (green points, centre) and *A. maritima* (blue points, right). Locations of all three species are from iNaturalist records collected between 2004 and 2019 (iNaturalist.org 2019).

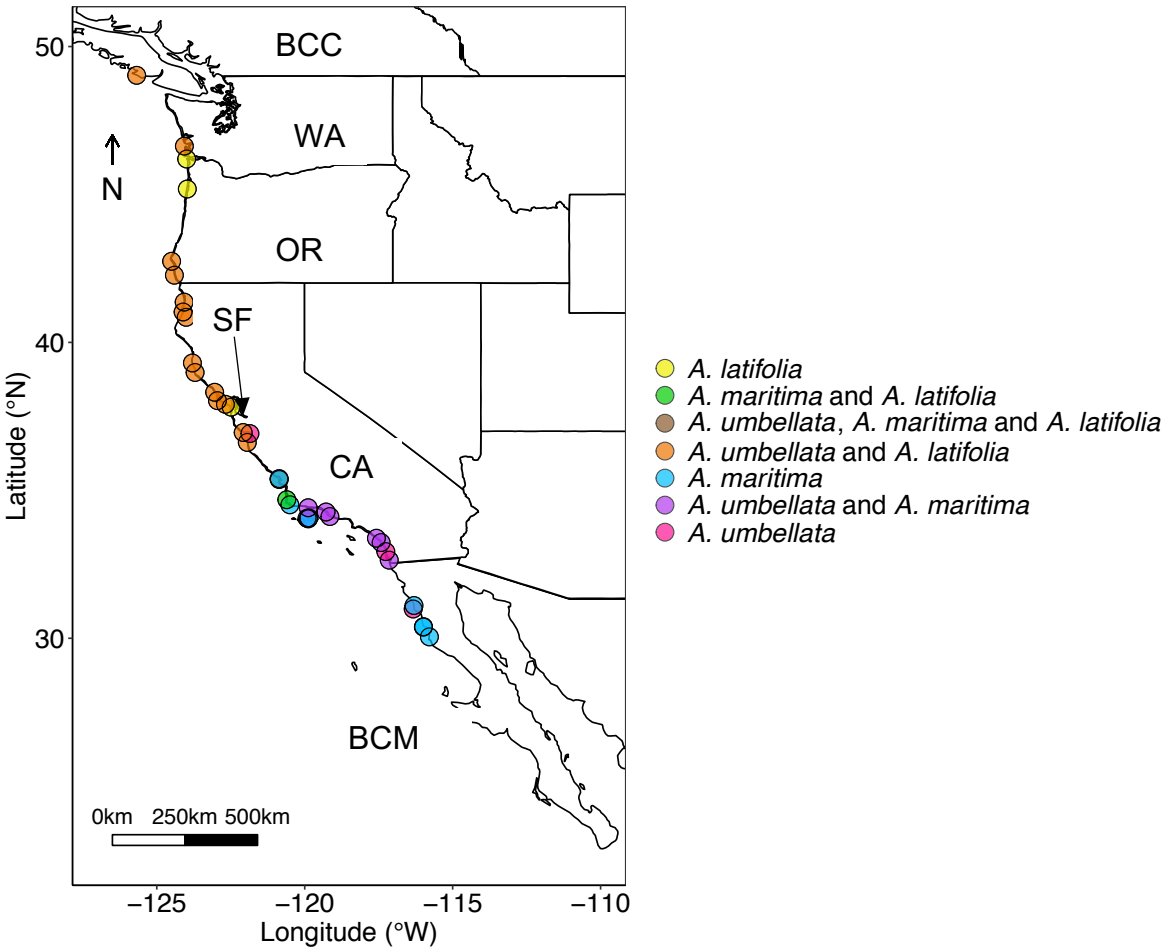

Fig. 3.2 Distribution of sampled populations of *Abronia umbellata, A. latifolia, A. maritima* and hybrids in summers of 2017 and 2018. Colours correspond to what *Abronia* species were present at each population site with yellow, blue and pink points representing pure populations of *A. latifolia, A. maritima* and *A. umbellata,* respectively, and green, orange, purple and brown points representing mixed populations of *A. maritima* and *A. latifolia, A. umbellata* and *A. latifolia, A. umbellata* and *A. maritima* and all three species, respectively. States and provinces are indicated as follows: BCC= British Columbia, WA= Washington, OR= Oregon, CA=California, BCM= Baja California, SF= San Francisco.

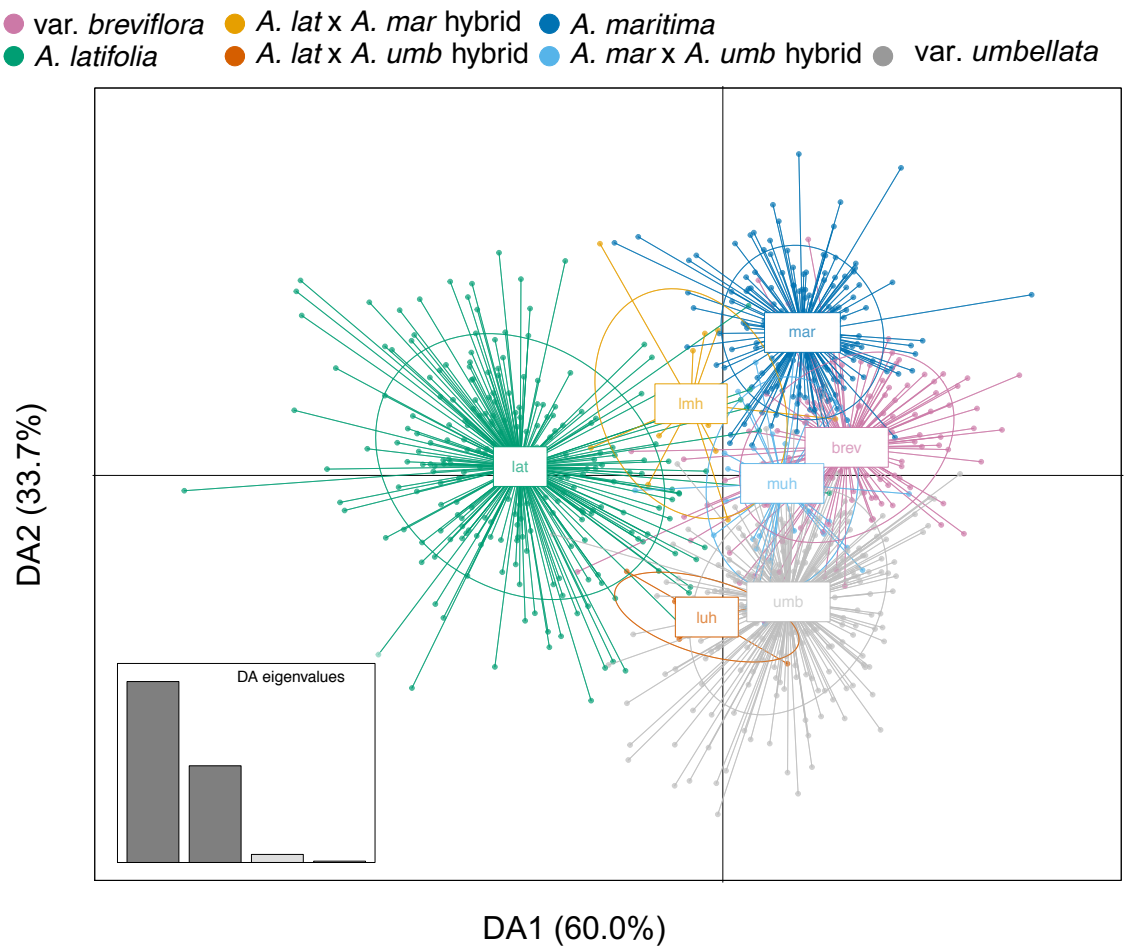

Fig. 3.3 Discriminant analysis of principal components (DAPC) of morphology, showing the differentiation among *Abronia umbellata, A latifolia* and *A. maritima* and their hybrids. *Abronia umbellata* was split into two groups for this analysis: selfing var. *breviflora* and outcrossing var. *umbellata.* Each point represents an individual (n_{ind} = 840) and the colour corresponds to its phenotypic identification: var. *breviflora* is represented by pink points, var. *umbellata* by grey points, *A. latifolia* by green points, *A. maritima* by blue points, var. *umbellata* x *A. latifolia* hybrids by orange points, var. *umbellata* x *A. maritima* by light blue points and *A. latifolia* x *A. maritima* by yellow points. Circles around clusters are intertia ellipses that enclose 67% of genotypes within each group. The proportion of variance captured by the first two discriminant axes is represented as a percentage.

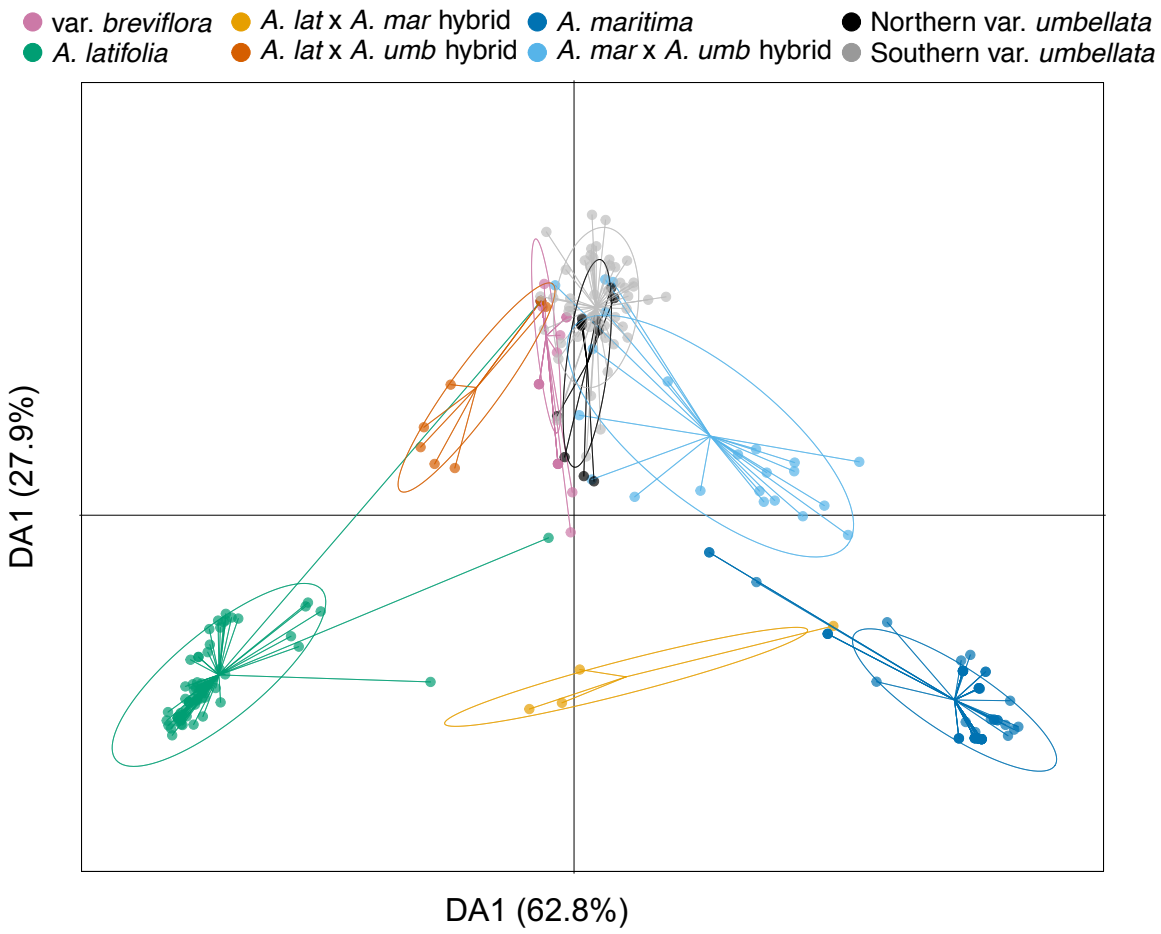

Fig. 3.4 Discriminant Analysis of Principal Components (DAPC) of SNP frequencies, generated from the sequences of five single-copy genes ($n_{SNP} = 205$), showing the differentiation among *Abronia umbellata, A latifolia* and *A. maritima* and their hybrids. *Abronia umbellata* was split into three groups for this analysis: var. *breviflora,* var. *umbellata* within the range of *A. maritima* (southern var. *umbellata*) and var. *umbellata* within the range of *A. latifolia* (northern var. *umbellata*). Each point represents an individual ($n_{ind} = 261$) and the colour corresponds to its phenotypic identification: var. *breviflora* is represented by pink points, northern var. *umbellata* by black points, southern var. *umbellata* by grey points, *A. latifolia* by green points, *A. maritima* by blue points, var. *umbellata* x *A. latifolia* hybrids by orange points, var. *umbellata* x *A. maritima* by light blue points and *A. latifolia* x *A. maritima* by yellow points. Circles around

clusters are intertia ellipses that enclose 67% of genotypes within each group. The proportion of variance captured by the first two discriminant axes is represented as a percentage.

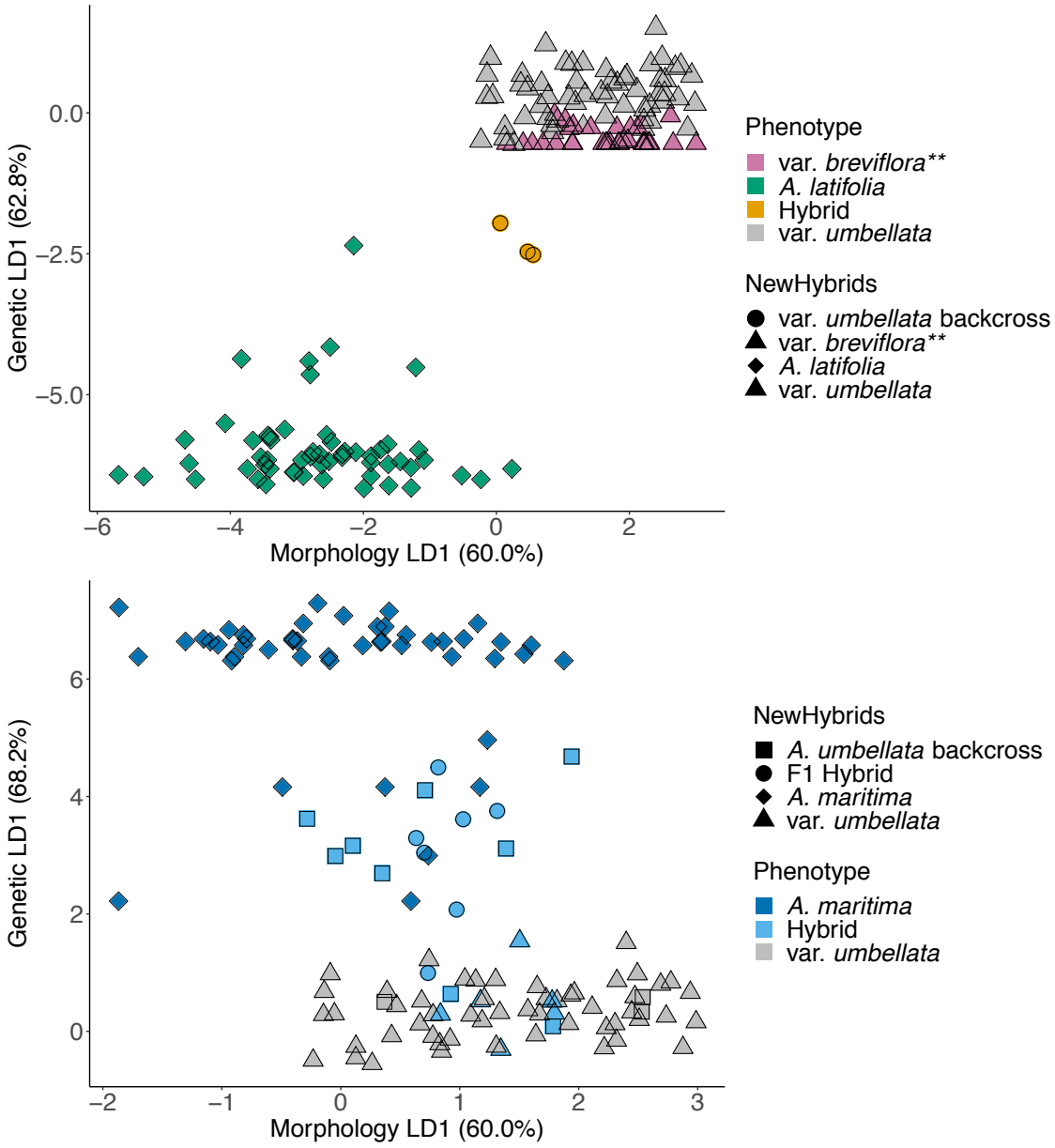

Fig. 3.5 Correlations based on DA1 scores of Discriminant Analysis of Principal Components (DAPC) for sequence variation in 205 SNPs in five-single copy nuclear genes (n_{ind} = 261; Fig. 3.4) and morphological variation in three leaf and four floral traits (n_{ind} = 840; Fig 3.3; Table 3.2). Colours correspond to individual phenotype and shape corresponds to posterior probabilities that an individual belongs to a particular hybrid group designated by NewHybrids (pure parental 1, pure parental 2, F_1, F_2, backcross to parental 1, backcross to parental 2). The top

panel is the correlation between *Abronia umbellata* var. *umbellata, A. umbellata* var. *breviflora, A. latifolia* and hybrids with var. *breviflora* represented by pink points, var. *umbellata* by grey points, *A. latifolia* by green points and hybrids by yellow points. Backcrossed hybrids to var. *umbellata* are represented circles, pure *A. latifolia* by diamonds, pure var. *umbellata* by triangles and var. *breviflora* also by triangles. Note that selfing var. *breviflora* was not included in NewHybrids analyses and thus, the shape does not correspond to the analysis. The bottom panel is the correlation between var. *umbellata, A. maritima* and their hybrids with var. *umbellata* being represented by grey points, *A. maritima* by dark blue and hybrids by light blue. Pure var. *umbellata* is represented by triangles, pure *A. maritima* by diamonds, F_1 hybrids by circles and backcrossed hybrids to var. *umbellata* by squares.

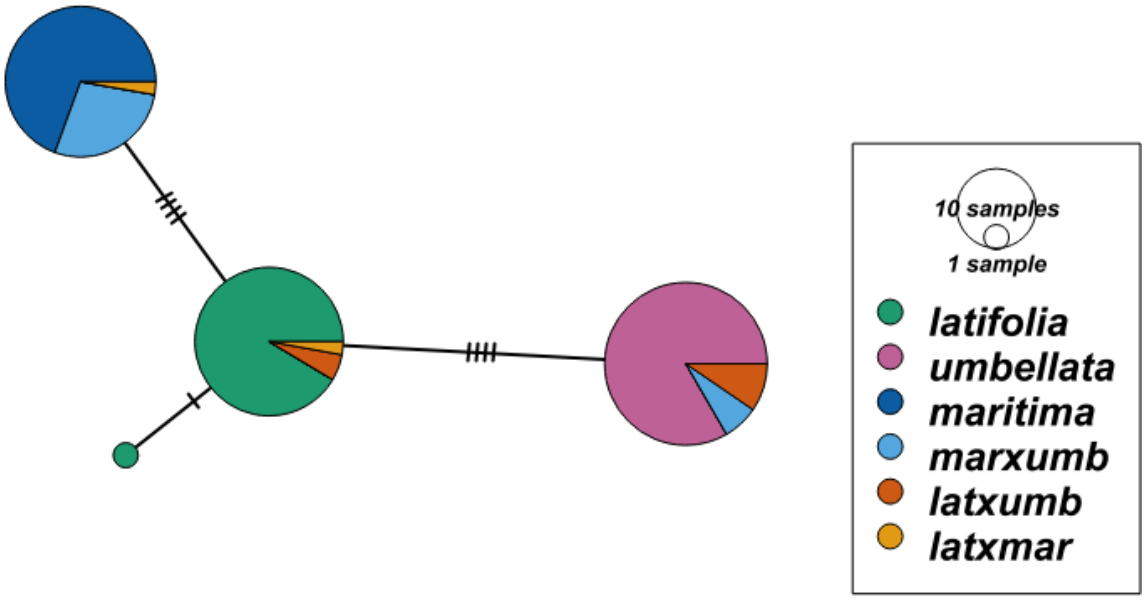

Fig. 3.6 Minimum spanning haplotype network of SNPs from 623 bp of the chloroplast gene MaturaseK (matK). Mutational differences are represented by hatch marks and the diameter of the circles is proportional to the number of individuals that belong to the haplotype group. Colours indicate the phenotypic classification of individuals with pink, dark blue, green, light blue, orange and yellow representing *Abronia umbellata* (umbellata), *A. maritima* (maritima) and *A. latifolia* (latifolia), var. *umbellata* x *A. maritima* hybrids (marxumb), var. *umbellata* x *A. latifolia* hybrids (latxumb) *A. latifolia* x *A. maritima* hybrids, respectively.

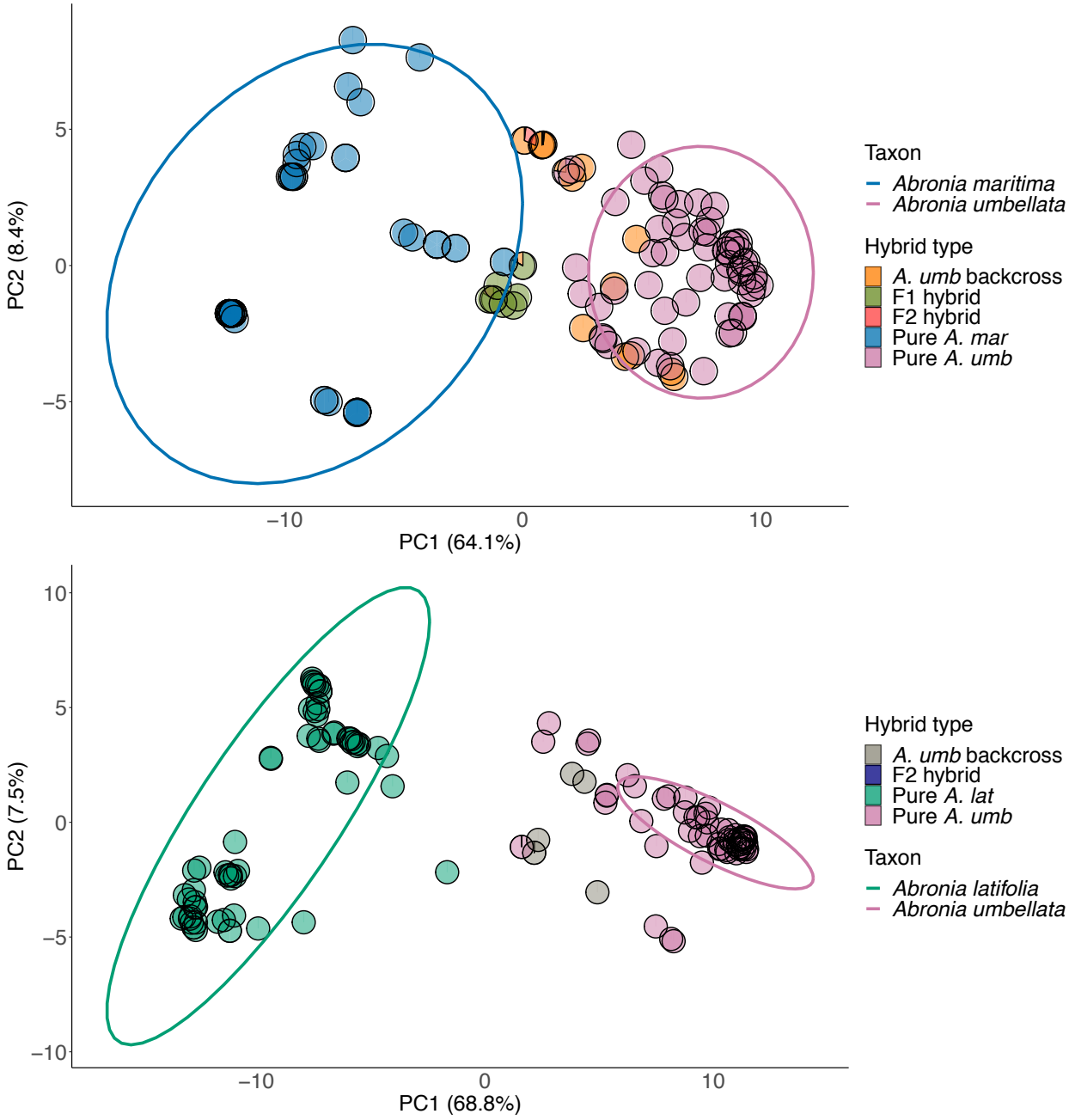

Fig. 3.7 Principal component analysis (PCA) based on frequencies of 110 SNPs in five single-copy nuclear genes for var. *umbellata, A. maritima* and their hybrids (n_{ind} = 189; top panel) and 134 SNPs for var. *umbellata, A latifolia* and their hybrids (n_{ind} = 139; bottom panel). Each point is a pie-chart of posterior probabilities that an individual belongs to a particular hybrid group designated by NewHybrids (pure parental 1, pure parental 2, F_1, F_2, backcross to parental 1,

backcross to parental 2). Ellipses are within-taxon 95% concentration ellipses. The proportion of variance captured by the first two principal components is represented as a percentage.

Chapter 4

General Discussion

We investigated the effects of peripherality, hybridization and mating system on the range wide genetic structure of the coastal dune endemic *Abronia umbellata.* In Chapter 2, we used sequence variation at nine single copy genes to determine patterns of genetic variation and differentiation throughout the range of *A. umbellata* and inferred the nature of this differentiation in peripheral populations, including two geographically disjunct populations north of the species' range. In Chapter 3, we quantified hybrid occurrence frequency with *A. maritima* and *A. latifolia* across the range of *A. umbellata* and, using sequence variation at five single-copy genes and one chloroplast gene, determined if introgression was occurring. In both chapters, we accounted for the shift in mating system in *A. umbellata* and how this may influence genetic structure and demographic processes.

Our analysis of peripheral populations did not completely conform with theoretical predictions that range edge populations should be lower in genetic variation and more differentiated relative to core populations. Instead, we found a latitudinal trend in genetic variation, with the southern edge population containing the highest variation and northern selfing populations and northern disjunct populations containing the least variation. Additionally, we found that the southern edge population was genetically unique from the rest of the range, while the northern disjunct populations were genetically similar to other northern selfing populations, indicating that they likely arose through recent long-distance dispersal or fragmentation after a recent range expansion. Thus, the low genetic variation of the northern disjunct populations may

be explained by their selfing mating system without invoking demographic processes unique to disjunct populations.

Extensive hybrid searches in 2017 and 2018 revealed that hybridization was occurring at 100% of locations where outcrossing *A. umbellata* var. *umbellata* was sympatric with a congener and zero locations where selfing *A. umbellata* var. *breviflora* was sympatric with a congener. In our analysis of hybrid occurrence frequency, based on presence in a 3-m radius around focal plants, we found that phenotypic hybrids were only frequent within the range of *A. maritima* which overlaps outcrossing *A. umbellata*. From genetic analyses, we determined that introgression is occurring between var. *umbellata* and its congeners and is present at every sympatric site, except for one in the range of *A. maritima,* and is occurring in the southern-most population of *A*. *umbellata* where only phenotypic var. *umbellata* were found.

The results from Chapters 2 and 3 may have important conservation implications for *A. umbellata. Abronia umbellata* is designated as endangered in Canada and in the states of Oregon and Washington because northern populations have been declining over the past several decades (ORNHIC, 2004; Kaye, 2015). Disjunct populations in Canada and Washington appear to be genetically similar to northern selfing populations of *A. umbellata* and thus, perhaps not worth conserving. However, *A. umbellata* var. *breviflora'*s marked differentiation in mating system may provide reproductive assurance in times of limited mates and/or pollinators and thus, have the potential to aid in range expansion (Baker, 1955). Populations at the southern edge on the other hand, are genetically unique and high in genetic variation relative to the rest of the range and thus, of high conservation value, but seem to be disappearing and currently receive no

conservation attention (Norma Oficial Mexicana, 2002). Therefore, our findings highlight the importance of considering the southern range edge when creating management strategies for species at-risk.

There has also been concern surrounding hybridization with rare species as there may be risks of genetic and demographic swamping (Hufford and Mazer 2003; Prentis et al. 2007). However, there does not seem to be a risk of these consequences at the northern edge of *A. umbellata'*s range because hybridization and introgression is likely not occurring. Hybridization and introgression is, however, occurring at the southern edge population in Baja California, Mexico. Introgression may provide southern edge populations with genetic variation required for adaptation to changing environments expected with climate change (Pfennig et al. 2016) or put them at risk of extinction from aforementioned swamping but whether either of these scenarios is more likely is unknown. Overall, our study demonstrates that patterns of genetic structure may be missed if the entire geographic range is not considered.

Summary

1. The pattern of genetic variation across a species range may give insight into geographic variation in adaptive capacity and hence, conservation priority

2. Determining the factors that may influence range-wide genetic structure is vital, but remains relatively understudied

3. Demography has long been viewed as the dominant factor influencing species' genetic structure, but non-demographic processes must be considered as well

4. We investigated the effects of peripherality, hybridization and mating system on the range wide genetic structure of *Abronia umbellata*, a pink-flowered Pacific coastal dune endemic and that ranges from San Quintin, Baja California to Coos Bay, Oregon and occurs as disjunct populations in Washington and on Vancouver Island in Canada

5. The species exhibits striking mating system differentiation across the range with obligately outcrossing populations south of San Francisco and highly selfing populations to the north

6. *Abronia umbellata* has two congeners that it co-occurs and creates putative phenotypic hybrids with, yellow-flowered *A. latifolia* and magenta-flowered *A. maritima*

7. Genetic analyses of sequence variation at nine single-copy genes revealed that outcrossing and selfing populations of *A. umbellata* are genetically differentiated from each other and northern disjunct populations of *A. umbellata* are genetically similar to selfing populations

8. Furthermore, the northern disjunct populations contain little unique variation and thus, likely arose through a recent long-distance dispersal event or fragmentation of a recent expansion

9. Southern edge populations in Baja California, on the other hand, are genetically unique and significantly higher in genetic variation than other outcrossing populations

10. Extensive searches in 37 populations across the range of *A. umbellata* in 2017 and 2018, revealed that phenotypic hybrids occurred in 100% of locations where outcrossing populations of *A. umbellata* were sympatric with a congener and sequence variation at five single-copy genes indicate that phenotypic hybrids are in fact hybrids

11. During the same searches we did not find any phenotypic hybrids with selfing populations of *A. umbellata* and quantitative analyses showed that hybrids were only found in 2.6% of 695 3-m radius plots across all 37 populations

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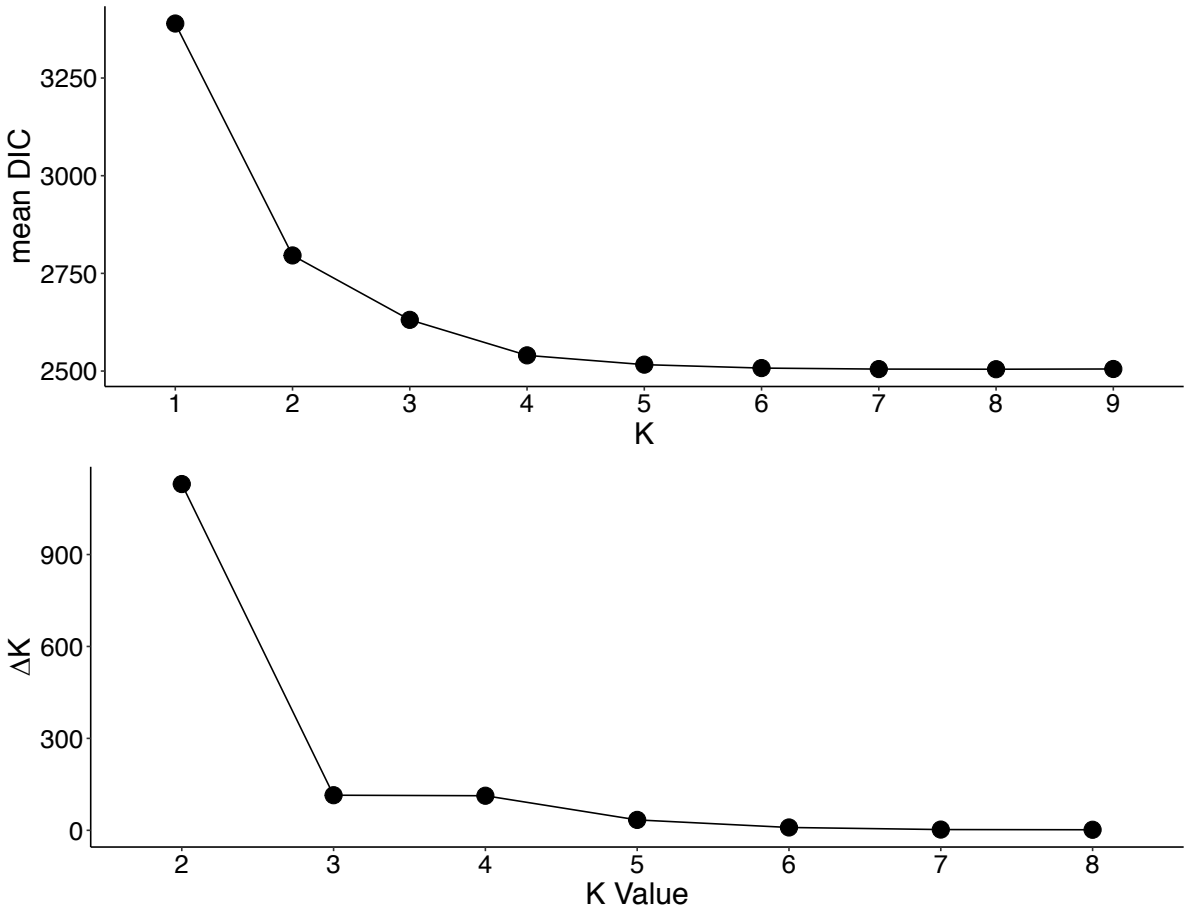
Appendix

Appendix 2A The 24 herbaria location data of *Abronia umbellata* was collected from are as follows: Bailey Hortorium (BH), California Academy of Sciences (CAS), Carnegie Museum of Natural History (CM), California State University Chico (CHSC), Cornell University (CU), Instituto PolitŽcnico Nacional (ENCB), Harvard University (GH), Instituto de Ecolog'a (IE-BAJiO), Jepson (JEPS), Missouri Botanic Garden (MO), New York Botanic Garden (NY), Academy of Natural Sciences (PH), Oregon State University (OSC), Santa Barbara Botanic Garden (SBBG), San Diego Natural History Museum (SD), San Diego State University (SDSU), Universidad Autonoma De Baja California (UABC), University of California (UC), UC Riverside (UCR), UC Davis (DAV), Smithsonian Institution (US), Willamette University (WILLU), Washington State University (WS) and University of Washington (WTU).

Appendix 2B Forward (F) and reverse (R) primers for sequencing nine single-copy, nuclear loci of *Abronia umbellata.* The PCR mix for all primers had a $MgCl_2$ concentration of 20nM. PCR amplification was run on a Applied Biosystems SimpliAmp Thermal Cycler (Applied Biosystems LSA37835) with the following reaction protocol: initial denaturation was set to 95°C for 2 minutes followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at a primer optimized temperature (55–57°C) for 40 seconds, extension at 72°C for 60 seconds and final extension at 72°C for 10 minutes. Sequence length is the length of sequences for each locus after alignment and trimming of low-quality ends. Sequencing primer was the primer direction that was used for Sanger sequencing. Primers were designed by Greer (2016) using transcriptome sequences of *A. umbellata.*

Primer	Primer sequence	Sequence length (bp)	Annealing temperature (°C)	Sequencing primer
1F 1R	5'-CAATGGGCTGTGCATTTAACC-3' 5’-GACCCGAACCCGAAATCAAT-3’	527	56	R
2F 2R	5’-CGGGCTACTCATGTCGATATTT-3’ 5’-ATCTTCACACCTTCCCATTTCT-3’	555	55	F
3F 3R	5'-TAGTCTTGCTCTTTGGGTTGG-3' 5’-AATCCGTCGGAGTTCCATAAAG-3’	418	56	F
4F 4R	5'-ATCAAGGGTGTTGGGAAATCTA-3' 5’-CGCTTACGGCTCCTCTTATC-3’	442	56	F
5F 5R	5'-ATTCCGGTGTCAATGCTAGG-3' 5’-CATGCCAAGGTCTCTGAATGTA-3’	493	56	F
6F 6R	5'-CATCTCCAATCACCTTCCTCTC-3' 5’-CTGGATCATCTGGATGGATTTCT-3’	406	57	R
7F 7R	5’-GAGGTACGAGGAAGAACAAACC-3’ 5’-TACTCCCAGGAGAAAGCATAGA-3’	515	57	F
9F 9R	5’-CCCGCCTTTATTATGCCAATG-3’ 5’-TCCAGCACAACATGGAAAGA-3’	451	55	F

10F 10R	5’-GCAACAAAGCTTCATCAGAGAC-3’ 5’-CTCACCAATCACGTACACCTT-3’	407	56	F



Appendix 2C The optimal number of clusters in a Bayesian clustering analysis. Graphical representations of mean DIC (top panel) and ΔK (bottom panel) analyses after Bayesian clustering of 94 multilocus SNP genotypes sampled from 25 populations across the range of *Abronia umbellata.* Better models have lower mean DIC values and higher ΔK values.

Appendix 3A Forward (F) and reverse (R) primers for sequencing five single-copy, nuclear loci of *Abronia umbellata, A. latifolia, A. maritima* and hybrids. The PCR mix for all primers had a $MgCl_2$ concentration of 20nM. PCR amplification was run on a Applied Biosystems SimpliAmp Thermal Cycler (Applied Biosystems LSA37835) with the following reaction protocol: initial denaturation was set to 95°C for 2 minutes followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at a primer optimized temperature (55–57°C) for 40 seconds, extension at 72°C for 60 seconds and final extension at 72°C for 10 minutes. Sequence length is the length of sequences for each locus after alignment and trimming of low-quality ends. Sequencing primer was the primer direction that was used for Sanger sequencing. Primers were designed by Greer (2016) using transcriptome sequences of *A. umbellata.*

Primer	Primer sequence	Sequence length (bp)	Annealing temperature (°C)	Sequencing primer
2F	5’-CGGGCTACTCATGTCGATATTT-3’	555	55	F
2R	5’-ATCTTCACACCTTCCCATTTCT-3’			
5F	5'-ATTCCGGTGTCAATGCTAGG-3'	493	56	F
5R	5’-CATGCCAAGGTCTCTGAATGTA-3’			
7F	5’-GAGGTACGAGGAAGAACAAACC-3’	515	57	F
7R	5’-TACTCCCAGGAGAAAGCATAGA-3’			
9F	5’-CCCGCCTTTATTATGCCAATG-3’	451	55	F
9R	5’-TCCAGCACAACATGGAAAGA-3’			
10F	5’-GCAACAAAGCTTCATCAGAGAC-3’	407	56	F
10R	5’-CTCACCAATCACGTACACCTT-3’			