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Speciation in arctic and alpine diploid plants

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”A cryptic species is one that is commonly described in literature but never found in nature.”

Galina Gussarova, Brooks Range, Atigon pass, 2009-07-19, after multiple attempts to find the “common” *Silene uralensis*, during our fieldtrip in Alaska.

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1. SUMMARY

The main objectives of this thesis are to study patterns and processes of plant speciation in arctic and alpine diploid plants. Cryptic species are here referred to as morphologically similar individuals belonging to the same taxonomic species but that are unable to produce fertile offspring (i.e. 'sibling' species).

The arctic flora is considered as one of the most species-poor floras of the world, and the latitudinal gradient with decreasing diversity from low to high latitudes is likely the oldest recognised pattern in ecology. However, these estimates are usually based on morphological differentiation into taxonomically recognizable species and may not provide accurate numbers of biological species. Previous intraspecific crossing experiments in three diploid circumpolar species of *Draba* (Brassicaceae) revealed the presence of numerous cryptic biological species within each taxonomic species. The present study expands the knowledge based on these previously published results and suggests that frequent formation of cryptic biological species may be a general pattern in the arctic flora. Intraspecific crossing experiments including several distantly related circumpolar diploid plant species revealed that intrinsic postzygotic isolation has developed multiple times, even at small geographical scales. This was shown for all five selfing species investigated, whereas crosses within one outcrossing species generated fully fertile F_1 hybrids. This suggests that a selfing mating system may accelerate the accumulation of hybrid incompatibilities. The barriers have in addition developed very rapidly, apparently within a few millennia, suggesting that speciation rates are unexpectedly high in the arctic flora. Cryptic biological species, although not yet recognisable morphologically, are thought to represent starting points for new evolutionary lineages that given sufficient time may develop into full-fledged new taxa. Other factors may thus account for the low diversity of the contemporary arctic flora in terms of taxonomic species. It is likely that high extinction rates rather than low speciation rates have played an important role in shaping the extent diversity in the arctic flora, possibly associated with climatic shifts during the Pleistocene glacial cycles.

The genetic mechanisms involved in the build-up of reproductive isolation are of central importance in understanding the evolution of new species. This thesis presents further insights into the mechanisms underlying reproductive isolation in *Draba nivalis* (Brassicaceae) – a small, circumpolar, predominantly selfing diploid herb that demonstrates numerous cryptic biological speciation events. By performing genetic linkage mapping and

searching for quantitative trait loci (QTL) associated with reproductive isolation more knowledge about the mechanisms involved in the evolution of intrinsic postzygotic reproduction in this system has been gathered. The linkage map was produced by combining both codominant and dominant markers and resolved eight linkage groups that most likely correspond to the eight chromosomes of *D. nivalis*. Observed patterns of inheritance were consistent with the influence of both nuclear-nuclear interactions and chromosomal changes. In particular, all seed set QTLs and one pollen fertility QTL displayed underdominant effects, matching expectations of chromosomal speciation models. Theory struggles to account for the establishment of large and strongly underdominant chromosomal translocations. *Draba nivalis* may however be an exception as a selfing mating system, is conducive for the establishment of chromosomal rearrangements through genetic drift. Overall this study confirms that multiple genetic mechanisms are involved in the build-up of reproductive isolation in *D. nivalis*, suggesting the involvement of both nuclear-nuclear interactions and structural chromosomal changes.

As plants are sessile organisms, they depend largely on adapting to locally changing climatic conditions such as temperature, aridity, and day length. Natural selection acting on traits that respond to such changes has likely played an important role in the evolution of plants. Climatic cycles of the Pleistocene caused drastic changes to species' ranges. For example, the Japanese alpine endemic plant *Cardamine nipponica* (Brassicaceae) probably diverged into northern and central populations during the Pleistocene climatic oscillations. The northern and central populations present highly diverged alleles of a particular photoreceptor gene *phytochrome E* (*PHYE*). Phytochromes such as *PHYE* monitor the surrounding light environment, and likely play an important role in the regulation of plant life cycles. The present study infers the evolutionary history of the *PHYE* in *C. nipponica* and its close relatives using maximum likelihood models. The resulting genealogical relationship suggested that standing genetic variation of *PHYE*, which diverged under positive selection prior to speciation, resulted in the selective differentiation between the northern and central Japanese populations of *C. nipponica.* This further suggests the importance of standing genetic variation in regard to quick responses to climatic changes.

2. LIST OF PAPERS

I. A. Lovisa S. Gustafsson, Galina Gussarova, Liv Borgen, Hajime Ikeda, Jan Suda, Loren H. Rieseberg, Christian Brochmann. High speciation rates in arctic plants. Manuscript.

II. A. Lovisa S. Gustafsson, Inger Skrede, Heather C. Rowe, Galina Gussarova, Liv Borgen, Loren H. Rieseberg, Christian Brochmann, Christian Parisod. Genetics of cryptic speciation within an arctic mustard, *Draba nivalis*. Submitted.

III. Hajime Ikeda, A. Lovisa S. Gustafsson, Christian Brochmann, Hiroaki Setoguchi. Pre-speciation origin of selective divergence and balancing selection in a plant photoreceptor gene, *phytochrome E*. Submitted.

3. INTRODUCTION

3.1 Speciation in plants

The species concept is a heavily debated issue in evolutionary biology, and per date more than 25 concepts have been proposed (Coyne and Orr 2004). The biological species concept may be the most generally accepted one, and was defined by Mayr as "species are groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr 1995), and represents the species concept discussed in this thesis. Furthermore, cryptic species are referred to as morphologically similar individuals belonging to the same taxonomic species but that are unable to produce fertile offspring.

Speciation in plants is characterized by the evolution of reproductive barriers preventing (or drastically reducing) genetic interchange between previously interbreeding populations (Rieseberg and Willis 2007). Plants vary dramatically in mating system, ploidy level, mode of dispersal, as well as life history, which gives us better understanding of how various ecological and evolutionary factors contribute to speciation (Brochmann et al. 1993, Levin 2000).

Plant species are typically isolated by multiple reproductive barriers (Rieseberg and Willis 2007). The genetically based traits that prevent gene exchange can act before fertilization (prezygotic mechanisms: prepollination or postpollination) and/or after fertilization (postzygotic extrinsic or intrinsic mechanisms related to habitat or genetic background, respectively). Intrinsic postzygotic barriers may be caused either by changes in functional genes or chromosomal rearrangements. The Bateson-Dobzhansky-Muller (BDM) model accounts for the accumulation of genic incompatibilities among isolated populations without loss of fitness (Lexer and Widmer 2008), whereas chromosomal rearrangements result in reduced fitness in heterozygotes leading to 50% inviable gametes (Rieseberg 2001). Structural divergence that results from fixation of chromosomal rearrangements reduces gene exchange between lineages by interfering with meiosis or reducing the level of recombination (Stebbins 1971, Rieseberg 2001, Levin 2002, Butlin 2005). Cytonuclear interactions may also be important in raising reproductive barriers. Such interactions create asymmetric reproductive isolation (Lowry et al. 2008, Leppälä and Savolainen 2011) because of dysfunctional interactions between nuclear and cytoplasmic factors (Levin 2003).

Whether the origin of reproductive barriers is predominantly due to selection or drift is an unresolved question among evolutionary biologists. Selection has generally been considered the main evolutionary force, but drift may be more important in small inbreeding populations (Levin 2000). BDM incompatibilities have largely been favoured over chromosomal rearrangements for the general origin of instrinsic postzygotic isolation (Orr et al. 2004), but chromosomal rearrangements may be more important in plant genomes (Chester et al. 2012). Indeed, genome doubling which is prevalent in plants, is expected to restore the fertility of hybrids with chromosomal rearrangements, while not alleviating genetic incompatibilities due to BDM (Rieseberg 2001, Rieseberg and Willis 2007). Recent theoretical developments have, however, highlighted selection in heterogeneous environments as an efficient promoter of the establishment of chromosomal rearrangements (Rieseberg 2001, Kirkpatrick and Barton 2006, Faria and Navarro 2010).

3.2 Cryptic speciation in the Arctic: the Draba *case*

Contrary to expectations, completely sterile F_1 hybrids were obtained in intraspecific crosses of the diploid *Draba fladnizensis* Wulfen after crossing plants from Svalbard and mainland Norway (Brochmann et al. 1993). To follow up these unexpected results, intraspecific crossing experiments were conducted on the full circumpolar scale of three diploid and circumpolar *Draba* species: *D. fladnizensis*, *D. nivalis* Lilj., and *D. subcapitata* Simmons (Grundt et al. 2006). Within all three species, crosses between individuals from different geographic areas (Alaska, Greenland, Svalbard and mainland Norway) produced mostly sterile F_1 hybrids, revealing the presence of numerous cryptic biological species within each taxonomic species. For *D. fladnizensis* and *D. nivalis* as many as 92% of the within- and among-region crosses resulted in sterile or semisterile F_1 hybrids, despite fully fertile parental plants. Furthermore, there was a positive correlation between the genetic distance among parents and the sterility of the resulting hybrids. The development of such reproductive barriers in other plant species is often associated with ecological and/or morphological divergence. In contrast, the genetic divergence and reproductive isolation in *Draba* were correlated with neither morphological nor ecological differentiation, suggesting that incipient speciation was caused by recent formation of intrinsic postzygotic isolation. The reproductive barriers must indeed have accumulated very rapidly as molecular data suggest that these three taxonomic species have arisen recently, most likely during the Pleistocene. The accumulation

of hybrid incompatibilities may have been facilitated by the predominant selfing mating system of all three species, possibly through genetic drift (Grundt et al. 2006).

Grundt et al. (2006) concluded that "although the Arctic is comparatively poor in morphological species, it may be rich in cryptic, biological species as demonstrated here for three species of *Draba*".

3.3 Postzygotic isolation in Draba nivalis

To better understand the genetic mechanisms underlying the discovery of recent intrinsic postzygotic isolation in *Draba nivalis*, linkage mapping and quantitative trait loci (QTL) analyses, searching for traits associated with reproductive isolation, was conducted (Skrede et al. 2008b). A large F_2 population was raised by selfing a semi-fertile F_1 hybrid generated from a cross performed by Grundt et al. (2006; paternal lineage originated from Norway and the maternal lineage from Alaska), and several traits related to hybrid incompatibility (pollen fertility, seed set, flowering time, number of flowers) were measured. In total, 383 F_2 individuals were genotyped with 50 microsatellite markers, and linkage mapping followed by QTL analysis was conducted. It was concluded that multiple genetic mechanisms were underlying intrinsic postzygotic reproductive barriers in this system, and QTL analysis identified five loci underlying seed fertility and two underlying pollen fertility. Average seed and pollen fertility was lower in the F_2 population than in the parental species, but higher than in the F_1 population, suggesting that under-dominant loci underlie hybrid sterility. However, some F_2 individuals had lower fertility than any of the F_1 individuals, suggesting that also BDM incompatibility could be involved in the origin of sterility barriers. Maternal alleles for pollen fertility QTLs were in addition consistently associated with higher hybrid fertility than paternal alleles, suggesting the possible involvement of cytonuclear incompatibilities.

In summary, seed fertility was affected by under-dominant loci, most probably due to microchromosomal rearrangements since no obvious disruption was observed during meiosis, in addition to epistatic interactions due to reciprocal translocations and/or BDM incompatibilities. Pollen fertility was affected by BDM incompatibilities and possibly cytonuclear incompatibilities.

The linkage map was nevertheless produced with 50 microsatellites only. Evidencing more linkage groups than the number of chromosomes, Skrede et al. (2008b) suggested that

less than half of the *Draba* genome was covered, thus possibly underestimating important fertility QTLs and epistatic interactions.

3.4 Aims of the present thesis

The present thesis aims to provide further insights into the patterns and processes involved in the evolution of new species in arctic and alpine regions.

Paper I focuses on intraspecific crossing barriers in arctic diploid plants, elaborating on the possibility of the arctic flora containing numerous cryptic biological species. Including distantly related species, with contrasting mating system in circumpolar, intraspecific crossing experiments will allow generalisations. The hypothesis is that selfing species will reveal more cryptic biological species than outcrossing species.

Paper II focuses on gaining further insights into the genetic mechanisms involved in the build up of intrinsic postzygotic reproductive isolation. The aim is to increase the genome coverage of *Draba nivalis* by adding a number of molecular markers to be mapped on reciprocal F_2 populations, followed by QTL analysis searching for traits associated with reproductive isolation. This will increase the certainty to what extent chromosomal rearrangements, nuclear-nuclear interactions and cytonuclear incompatibilities are involved in the rapid accumulation of hybrid incompatibilities in this system.

Paper III focuses on a particular gene: the photoreceptor *phytochrome E* (*PHYE*). This gene presents highly diverged alleles between northern and central populations of the Japanese endemic plant *Cardamine nipponica.* The present study address whether the selective differentiation in this species originated from alleles that coalesced prior to speciation (i.e. standing genetic variation) or from newly accumulated mutations.

4. SUMMARY OF PAPERS

Paper I: High speciation rates in arctic plants.

The Arctic is considered to be one of the most species-poor regions of the world, and the latitudinal gradient with decreasing species diversity from low to high latitudes is considered as the oldest recognized pattern in ecology. These estimates are, however, based on morphological differentiation and may not provide accurate numbers of biological species diversity. The present study followed up the previously demonstrated crossing barriers found within three circumpolar plant species, trying to elucidate if the formation of cryptic biological species is a common pattern in the arctic flora. After performing intraspecific crossing experiments in several distantly related circumpolar, diploid plant species, F_1 hybrid fertility was measured.

Living plant material was collected of 22 species in three main geographical regions (Alaska/Yukon, the North Atlantic archipelago of Svalbard, and mainland Norway). Crosses were successful in five selfing species and one outcrossing species. The results indicate that sterility barriers have formed frequently within single taxonomic species, suggesting that the formation of cryptic biological species is a general pattern in the arctic flora. All five selfing species demonstrated hybrid incompatibilities, whereas the hybrids in the one outcrossing species were fully fertile. This suggests that a selfing mating system accelerates the accumulation of hybrid incompatibilities. In addition, the barriers appear to have evolved very rapidly as investigated populations were genetically very similar in spite of being more or less reproductively isolated, suggesting surprisingly high speciation rates in the arctic flora. Thus, the results contradict previous explanations for the latitudinal diversity gradient that assume lower evolutionary rates towards the poles.

A recent study of New World birds and mammals using birth-death models suggest that both speciation and extinction rates increase at higher latitudes. High extinction rates rather than low speciation rates may indeed account for the low species diversity in the arctic flora, possibly associated with climatic shifts during the Pleistocene glacial cycles.

Paper II: Genetics of cryptic speciation within an arctic mustard, *Draba nivalis*.

The origin and build-up of reproductive isolation is of central interest in evolutionary biology and has been the subject of considerable debate and discussion for decades. Here the focus was to get further insights into the mechanisms underlying intraspecific reproductive isolation in the diploid, circumpolar herb *Draba nivalis* (Brassicaceae). Multiple genetic mechanisms,

including nuclear-nuclear and cyto-nuclear incompatibilities as well as structural chromosomal changes, have previously been reported in the rapid evolution of postzygotic reproductive isolation in this system. Genetic linkage mapping of a large F_2 population was conducted followed by quantitative trait loci (QTL) analysis searching for traits associated with reproductive isolation. The linkage map was produced by combining a dataset of 31 co-dominant microsatellites with 63 dominant markers, including 52 amplified fragment length polymorphisms (AFLPs) and 11 sequence-specific amplified polymorphisms (SSAPs). The map resolved eight linkage groups that most likely correspond to the eight chromosomes of *D. nivalis*. The QTL-analysis revealed four QTLs associated with pollen fertility, three with seed set, three with flowering time and four with number of flowers. Among the 14 detected QTLs, patterns of inheritance reported for those QTLs associated with postzygotic isolation were consistent with the influence of both nuclear-nuclear interactions and chromosomal changes. In particular, all seed set QTLs and one pollen fertility QTL displayed underdominant effects, matching expectations of chromosomal speciation models. The establishment of underdominant chromosomal rearrangements may be facilitated in species such as *Draba nivalis* that are predominantly self-fertilizing. Selfing is likely to reduce gene flow and effective recombination between populations, as well as possibly increasing the speed of fixation of adaptive loci. The present study suggests that multiple genetic mechanisms are indeed involved in the build-up of reproductive isolation in *D. nivalis*, highlighting the importance of both nuclear-nuclear interactions and structural chromosomal changes, although no evidence of cyto-nuclear incompatibilities was demonstrated.

Paper III: Pre-speciation origin of selective divergence and balancing selection in a plant photoreceptor gene, *phytochrome E.*

Climatic oscillations during Pleistocene invariably caused drastic changes to species' ranges. For species to respond to such shifts in their local environment, standing genetic variation would play a more important role than newly accumulated mutations. The focus in this paper was to investigate the importance of standing genetic variation in relation to genetic differentiation following Pleistocene climatic oscillations in *Cardamine nipponica*, a perennial herb endemic to the high mountains in the Japanese archipelago. The photoreceptor *phytochrome E* (*PHYE*) presents strong genetic differentiation between northern and central populations of *C. nipponica* and has evolved under balancing selection. A previous study revealed a firm sister relationship between *C. nipponica* and the arctic-alpine *C. bellidifolia,*

with *C. alpina* and *C. resedifolia* included in the same clade. Using this phylogenetic framework, the entire coding region of *PHYE* was sequenced along with two additional photoreceptor genes; *phytochrome A* (*PHYA*) and *cryptochromes 1* (*CRY1*), used as reference loci. The genealogies for the three phytochromes were inferred using maximum likelihood models, and were consistent with previous phylogenetic studies for both *PHYA* and *CRY1,* where northern and central populations of *C. nipponica* formed a monophyletic group with *C. bellidifolia* as sister. In contrast, the genealogy for *PHYE* presented a robust paraphyletic relationship, with northern populations of *C. nipponica* forming a clade with *C. bellidifolia*, presenting central populations of *C. nipponica* as sister to this clade. Tests of natural selection further supported a model assuming positive selection on divergence for both clades. Accordingly, the differentiation of *PHYE* between northern and southern populations of *C. nipponica* was most likely caused by alleles under natural selection that diverged prior to speciation. This highlights the possible importance of standing genetic variation in regard to quick responses to climatic changes.

5. METHODS

5.1 Crossing experiments

In paper I the aim was to perform extensive crossing experiments to potentially reveal a general pattern of cryptic biological speciation in the arctic flora. The choice of species to be investigated was critical, and the following five criteria had to be fulfilled. The plants should:

i) be diploid to avoid introducing additional complexity because of polyploidy. There are currently too few named diploid species in the arctic flora to account for the high diversity seen at the polyploid level adding to the interest to study diploids in regard to cryptic speciation (Brochmann et al. 2004).

ii) have a full circumpolar range to maximize the possibility for detecting cryptic biological species, which may be most likely at large spatial scales.

iii) be more or less common, to facilitate the field work.

iv) represent divergent phylogenetic lineages, to investigate whether formation of cryptic biological species is common across diverse genera and families in the arctic flora, and not only confined to the three *Draba* species studied by Grundt et al. (2006).

v) vary in mating system to test for potential differences between selfing and outcrossing species, as previous studies (Grundt et al. 2006, Skrede et al. 2008b) indicate that a selfing mating system might accelerate the formation of cryptic biological species.

Twenty-two species representing ten plant families were selected, and a total of 1722 specimens were collected (Supplementary Information, Table S1). Plant material was collected from three main geographical regions: Alaska/Yukon, the arctic archipelago of Svalbard, and mainland Norway in 2009. One population was defined as plants occurring within an area of 100 m × 100 m, and individual plants were (if possible) collected at least 10 m apart. For each population the aim was to collect a minimum of ten living plants, one plant as a voucher and leaves from five plants as silica samples. The living plants were cleaned for soil and wrapped in moist paper and plastic bags before shipped to Norway. In Alaska/Yukon this was performed twice, as widely separated sampling areas were visited, with intermediate stops in Fairbanks, where the living plants were replanted in the green house at the University of Alaska Fairbanks. Upon arrival in Norway the plants were once again replanted in a phytotron free from pollinating insects, at the University of Oslo (cultivation conditions as

specified in Brochmann et al. 1992). The plants were cultivated for two flowering seasons per year with three months of summer conditions and three months of winter conditions (vernalization).

The crossing program was designed to cover three main spatial scales: within-population crosses, within-region crosses and between-region crosses. The within-region crosses were performed among four subregions in Alaska/Yukon (Seward Peninsula, Brooks Range, Central Alaska along Denali Highway, and Yukon Territory). The between-region crosses were performed among the three main geographic regions Alaska/Yukon, Svalbard, and mainland Norway. For logistical reasons, collections in Svalbard were made only around Longyearbyen, and crosses performed among these closely located populations are referred to as Svalbard-population crosses. In each crossing experiment, flower buds were emasculated on the maternal plant long before anthesis to avoid self-fertilization. Pollen was transferred 2-9 days later, depending on stigma receptivity. Whenever possible, reciprocal crosses were performed. Many of the species were, however, either difficult to cultivate or did not flower regularly under the specific phytotron conditions. Thus large portions of the crossing experiments failed. Ploidy level of parental populations was verified using DNA flow cytometry and type of breeding system was assessed based on ability to set seed after spontaneous self-pollination (paper II).

F_1-seeds were harvested and vernalized before sowing. Five F_1 seedlings (if available) from each cross were raised to maturity. F_1 hybrid fertility was estimated as percent stainable pollen and as percent seed set. Pollen stainability was estimated by counting the proportion of fully stained pollen grains after adding lactophenol in cotton blue on pollen transferred to a microscope slide, and about 200 pollen grains (Fig. 1) were counted for each plant (Radford et al. 1974). This is a commonly used method to estimate pollen fertility (see e.g. Brochmann 1993, Kelly et al. 2002, Stucky et al. 2012), but it should however be noted that it only measures the pollen *stainability* and might not necessary reflect the actual pollen fertility. For the selfing species, seed set was measured as percent fully developed seed set after spontaneous selfing, relative to total number of ovules. Also here it should be noted that the seed set does not necessarily reflect the actual number of fertile seeds as no germination tests were conducted. For simplicity, however, pollen stainability and seed set is referred to as pollen and seed fertility. Based on the high correlation between pollen and seed fertility estimates, it was possible to classify the F_1 hybrids (and parents) as fertile (fertility $\geq$ 70%), semisterile (fertility $\geq$30% to <70%) and sterile (fertility < 30%).

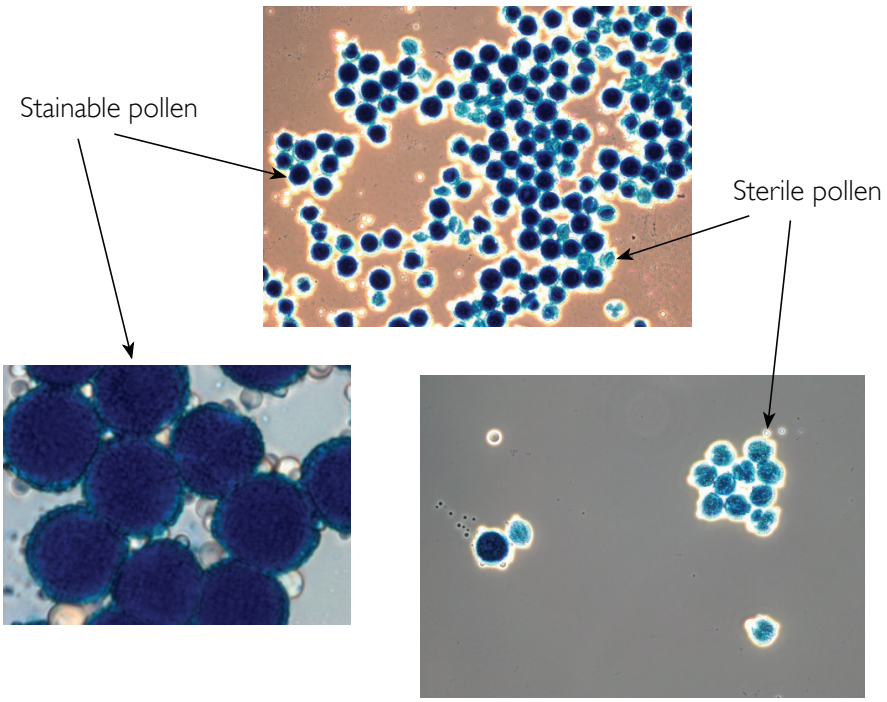


Figure 1. Pictures of pollen grains of *Cardamine bellidifolia* stained by the addition of lactophenol in cotton blue on pollen transferred to a microscope slide. Showing stainable (potentially fertile) and sterile pollen grains. PHOTO: A. Lovisa S. Gustafsson

Successful crosses (resulting in viable F_1 hybrids) were obtained for six species out of which five were predominantly selfing; *Cardamine bellidifolia* L., *Cochlearia groenlandica* L., *Saxifraga hyperborea* R. Br., *Ranunculus pygmaeus* Wahlenb. and *Silene uralensis* (Rupr.) Bocquet, and one species predominantly outcrossing; *Silene acaulis* (L.) Jacq. (Fig. 2). It is worth noting that the successful crosses thus included two species from the same plant genus, but with different mating systems (*S. uralensis* and *S. acaulis*; Caryophyllaceae).

Figure 2. The six species for which successful intraspecific crosses were obtained (resulting in viable F_1 hybrids): *Cardamine bellidifolia, Cochlearia groenlandica, Saxifraga hyperborea, Ranunculus pygmaeus, Silene uralensis* and *Silene acaulis*. POTO: A. Lovisa S. Gustafsson: *Cardamine bellidifolia, Cochlearia groenlandica, Ranunculus pygmaeus, Silene acaulis*; Bjørn Erik Sandbakk (www.svalbardflora.net): *Silene uralensis*; Aud Else Berglen Eriksen: *Saxifraga hyperborea.*

5.2 Molecular analysis of Cardamine bellidifolia *and* Ranunculus pygmaeus

In paper I, molecular analyses were conducted for two species, i.e. *Cardamine bellidifolia* and *Ranunculus pygmaeus*. Eight nuclear genes (*CHS, CO, COP1, DET1, DFR, F3H, FRI,* and *GA1*) were sequenced to infer the level and timing of evolutionary divergence between the crossed populations of *C. bellidifolia*. The Japanese alpine endemic *C. nipponica* was inferred

as sister species of *C. bellidifolia* (Ikeda et al. 2012), and used as outgroup along with plants of *C. alpina, C. resedifolia,* and *C. glauca*. Maximum likelihood methods were used to assess phylogenetic relationships, and the isolation with migration (IM, Nielsen and Wakeley 2001, Hey and Nielsen 2004) model was used to infer the demographic history. To complement the IM results, additional divergence time estimates using *BEAST (Heled and Drummond 2010) were calculated.

A range-wide genetic analysis was conducted for *R. pygmaeus* using Amplified Fragment Length Polymorphism AFLP (Vos et al. 1995). Very little AFLP variation was observed even though primer combinations with only two selective nucleotides also were tested. The final AFLP dataset included 34 polymorphic markers and reproducibility was very high (99.16%). To visualize the main structure in the data, Principal Coordinate Analysis (PCoA) was conducted and a neighbor-joining tree was produced using PAUP* 4.0b10 (Swofford 2002). For details, see paper I.

5.3 Phylogenetic analysis in Cardamine nipponica *and its relatives*

A previous phylogenetic study revealed a robust sister relationship between the Japanese alpine endemic *Cardamine nipponica* and *C. bellidifolia* based on internal transcribed spacer (ITS) sequences and 10 nuclear genes (Ikeda et al. 2012). Among other material, this study used collections of *C. bellidifolia* initially gathered for crossing experiments (paper I). The study in paper III takes advantage of this previously demonstrated sister relationship in order to unravel the evolutionary history of a photoreceptor gene; *phytochrome E* (*PHYE*), determining whether standing genetic variation or newly accumulated mutations were involved in the selective differentiation of *PHYE*. Plants are highly dependent on being able to adapt to surrounding climatic conditions such as changes in temperature, aridity, and day length, and natural selection acting on traits that respond to such changes has likely played an important role in plant evolution. Accordingly, several studies have focused on photoreceptor genes such as phytochromes, which sense red and far-red light. *PHYE* is particularly important for germination and flowering at low temperature conditions (Halliday and Whitelam 2003, Heschel et al. 2007). *Cardamine nipponica* grows at high altitudes ranging from 2000-3000 m and is exposed to cool temperatures, suggesting an important role for the *PHYE*. Ikeda et al. (2009) reported that alleles of *PHYE* were highly diverged in populations from northern and central Japan, a pattern congruent with other Japanese plant species (e.g.

Fujii and Senni 2006, Ikeda et al. 2008b, a). The previous studies focused on intraspecific variation only, leaving the question of whether standing genetic variation or newly accumulated mutations formed the basis for the presented divergence between northern and central populations unanswered. The present study aims to determine the divergence history of the *PHYE* alleles in *C. nipponica*, investigating the importance of standing genetic variation in relation to genetic differentiation following Pleistocene climatic oscillations.

Cardamine nipponica was analyzed together with its close relatives *C. bellidifolia, C. alpina, C. residifolia,* and *C. glauca* was used as outgroup. The entire *PHYE* gene was sequenced, along with two additional phytochromes (used as reference loci); *phytochrome A* (*PHYA*) and *cryptochrome 1* (*CRY1*), and added to previously published sequences of *C. nipponica* and *C. resedifolia* (Ikeda et al. 2009, 2011). The genealogical relationships for each gene were estimated using maximum likelihood models and implemented in TREEFINDER (Jobb et al. 2004). To confirm the significance of the obtained topology, the Approximately Unbiased (AU) test (Shimodaira and Hasegawa 1999) was conducted testing three alternative topologies (paper I). To examine non-neutral divergence for *PHYE* in *C. nipponica*, likelihood analysis was conducted based on the ratio of nonsynonymous to synonymous substitutions (d_N / d_S) using CODEML in PAML4.0 (Yang 2007).

5.4 Linkage mapping and QTL analysis in Draba nivalis

To investigate the impact of cytonuclear incompatibilities on reproductive isolation in *D. nivalis*, the aim was to raise a new mapping population, by crossing the same individuals as in Skrede et al. (2008b), but in the opposite direction (i.e. reciprocal cross). Furthermore, the strategy to characterize speciation loci was to increase the density of the linkage map produced in the previous study (Skrede et al. 2008b) genotyping reciprocal F_2 populations with additional genetic markers. To compare traits on reciprocal F_2 populations, quantitative trait loci (QTL) analysis would be conducted, further taking advantage of *D. nivalis* being a close phylogenetic relative to model plant species such as *Arabidopsis* spp. and *Brassica* spp. Investigating relatives in an evolutionary perspective offers ample opportunities to share knowledge and molecular tools (Mitchell-Olds 2001, Schranz et al. 2007). It is suggested that the compact genome of *Arabidopsis thaliana* (L.) Heynh. and its chromosome number $n = 5$ are derived characters that evolved from close relatives (Schranz et al. 2006). It is likely that $n = 8$ is the ancestral chromosome number for the tribe Camelinae, to which *Arabidopsis*

belongs, and also potentially for most of the Brassicaceae. Integrating the search of co-linear portions of chromosomes across Brassicaceae into the concept of an ancestral genome with *n* = 8, Schranz et al. (2006) proposed a set of 24 conserved genomic blocks (Brassicaceae Building Blocks; BBB) that are mainly reshuffled to produce the different Brassicaceae genomes. This new paradigm represents an important step towards a unified comparative genomic system across the Brassicaceae. Using this genomic block system in a physical mapping context, (Schranz et al. 2007) demonstrated that the *Boechera stricta* Graham genome (*n* = 7) evolved from the ancestral genome (*n* = 8).

By comparing the *D. nivalis* genome with the ancestral Brassicaceae genome the aim was to reconstruct the *Draba* genome evolution. Furthermore, since BBB-markers have been shown to be conserved and co-linear among Brassicaceae species, QTL characterization with such markers of known location in the *Arabidopsis* genome would provide outstanding information about their location and putative content, and would allow specific focus for investigation on candidate portions of the *Draba* genome. In addition to the BBB-markers, AFLPs and Sequence-Specific Amplification Polymorphism (SSAPs, specifically marking insertions of Transposable Elements (TEs, Syed and Flavell 2006) would further increase the map density. SSAPs are typically more polymorphic than AFLPs and would possibly determine whether or not TEs are involved in the origin of sterility barriers in *D. nivalis*. Three particular TEs showing evidence of recent transpositional activity in Brassicaceae were chosen; TRIM-Br, SB2 and AtC10 (paper II).

Performing linkage mapping on reciprocal F_2 populations, including this wide variety of genetic markers (i.e. microsatellites, BBB-markers, AFLPs, SSAPs), followed by QTL analysis of fertility traits such as seed set, pollen fertility, and number of flowers, should result in a dense QTL map. However, finding markers polymorphic between the two parental lineages proved very difficult. Initial analyses of the BBB- markers were very promising and amplification in the available F_2 population was successful: 25% of the initial screening of 300 markers (primers provided by Eric Schranz, Amsterdam) amplified well. To test for polymorphism, the markers that amplified well in the F_2 population were sequenced for the two parental lineages, but no polymorphism was observed. Thus the BBB-markers had to be excluded from further analyses. Unfortunately, the raising of a reciprocal F_2 population also failed.

Difficulties in finding polymorphic markers were encountered for the AFLPs and SSAPs as well, but after screening 72 AFLP and 36 SSAP primer combinations in eight

individuals (i.e. the two parents, three F_1 hybrids and three F_2 hybrids), 13 AFLP and seven SSAP of the most informative primer combinations were chosen for further analysis. Together with the microsatellites, a total of 128 loci were genotyped in 359 F_2 individuals (Paper II). Markers presenting high transmission ratio distortion (TRD) were removed prior to linkage analysis as they might hinder accurate estimation of the genomic location and effects of QTLs. The linkage map was produced by pairwise linkage of estimated recombination fraction and minimum LOD (Logarithm of Odds) score, i.e. two markers were placed in the same linkage group if the estimated recombination fraction was ≤ 0.35 and LOD score ≥ 5, retained the marker order associated with a maximized likelihood score (error probability 0.01) and minimized number of crossover events.

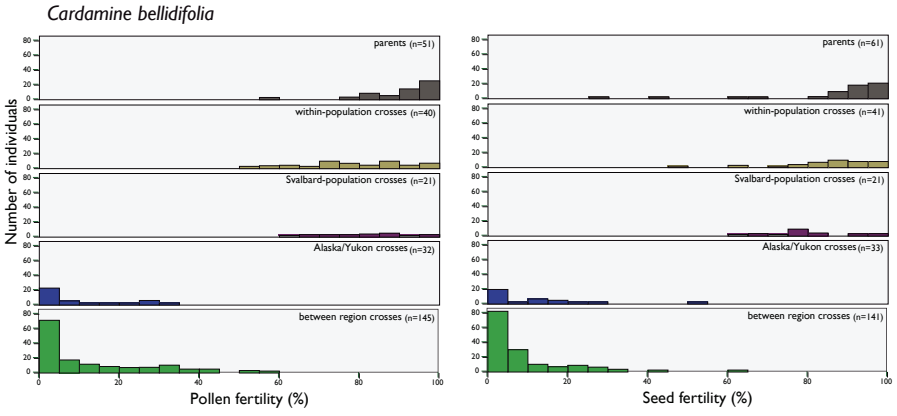
QTL analyses identify loci that are linked to genes underlying traits. Composite interval mapping of the four phenotypic traits (i.e. pollen fertility, seed set, flowering time and number of flowers) was performed in R/qtl (Broman et al. 2003). Combining codominant (microsatellites) and dominant markers (AFLPs and SSAPs) allow genotypes of dominant markers to be inferred using the information from the codominant markers (i.e., Hidden Markov models (HMMs) estimated QTL genotype probabilities as a function of the genotypes at the nearest markers, assuming no crossover interference). A genome-wide LOD significance threshold for each trait was assessed with 1000 permutations (alpha=0.05).

The previous study (Skrede et al. 2008b) suggested that structural chromosomal rearrangements might have contributed to the rapid build up of reproductive isolation in this system. If chromosomal rearrangements were responsible for reproductive isolation, then genome duplication could restore the fertility, as two identical copies of one chromosome are produced. Thus, experiments to double the chromosome set of F_1 hybrids by treating seedlings with colchicine (that induces genome duplication) was conducted. The F_1 seedlings were exposed to colchicine in many different concentrations and for variable periods of time, but unfortunately either died, or their genomes had not been doubled (ploidy level was investigated based on flow cytometry). Comparative chromosomal painting to highlight chromosomal changes was also attempted, in collaboration with Martin Lysak and Terezie Mandakova at the Masaryk University, Czech Republic, but without success.

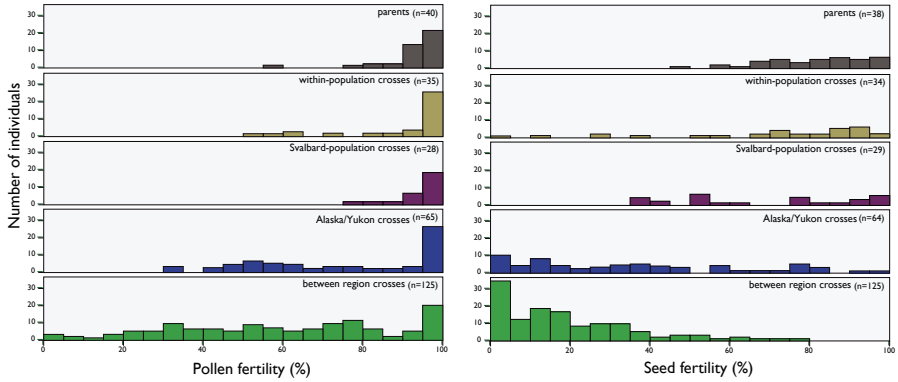
6.1 Crossing experiments

The parental plants of all species were (with a few exceptions) fully fertile (Fig. 3). In total, 742 F_1 hybrids were analysed for pollen fertility and 709 F_1 hybrids for seed fertility. The within-population crosses as well as the Svalbard-population crosses generated highly fertile F_1 hybrids in all species, except for a few F_1 hybrids in *Cochlearia groenlandica* that showed reduced seed fertility, and a few F_1 hybrids in *Saxifraga hyperborea* that showed highly reduced pollen and seed fertility. Both the within-region crosses (Alaska/Yukon crosses) and the between-region crosses in all selfing species mainly generated F_1 hybrids with pollen and seed fertility that was strongly reduced compared to parental plants. In the single outcrossing species, *Silene acaulis*, highly fertile F_1 hybrids were generated from the within-population crosses, the Svalbard-population crosses, and the between-region crosses (Fig. 3).

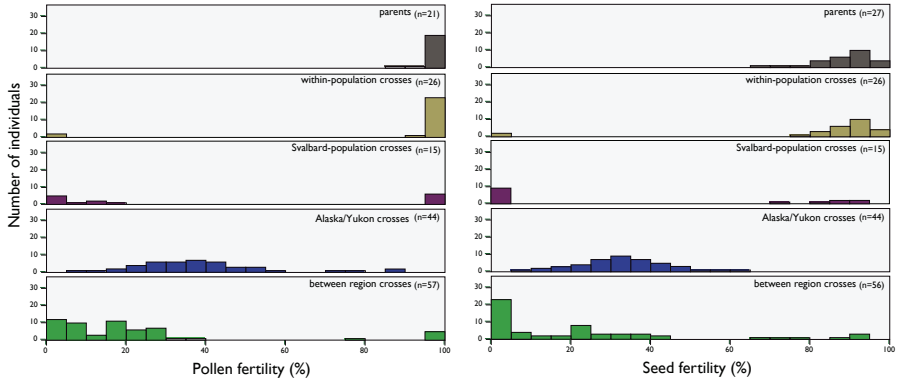
In *Cardamine bellidifolia*, 63 crosses were successful, and 238 F_1 hybrids were analysed for pollen fertility and 236 F_1 hybrids for seed fertility. The within-population crosses and the Svalbard-population crosses resulted in F_1 hybrids almost as fertile as the parental plants (mean pollen fertility 78-85%, mean seed fertility 82-85%). All within- and between-region crosses resulted in F_1 hybrids with strongly reduced pollen and seed fertility (mean pollen fertility 7-15%, mean seed fertility 7-8%). The reciprocal crosses resulted in similar hybrid fertility. In this species, not only the fertility but also the quantity of the pollen was reduced in the F_1 hybrids (Fig. 4). In addition, the fruit set was also reduced (Fig. 4).



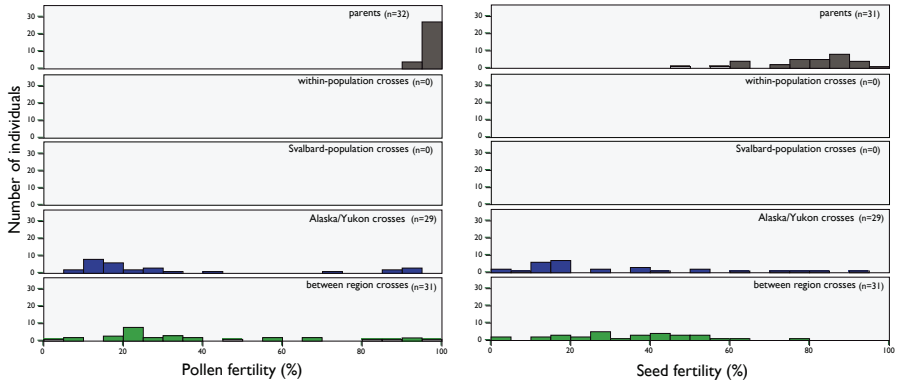
Cochlearia groenlandica



Saxifraga hyperborea



Ranunculus pygmaeus



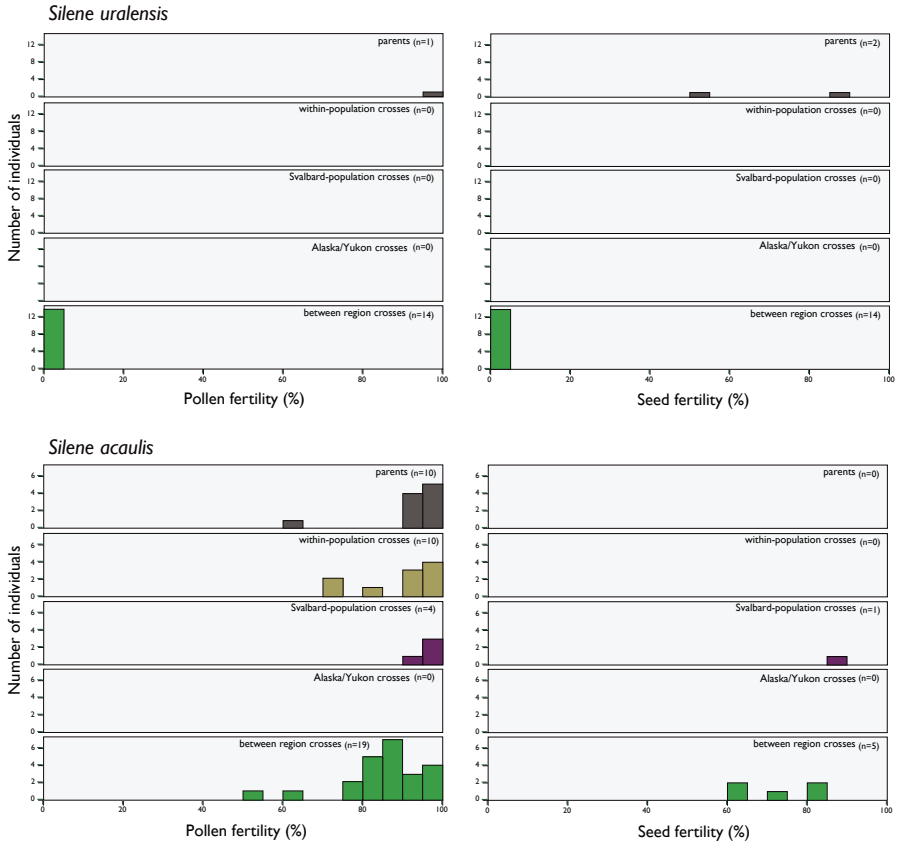


Figure 3. Fertility data for parental plants and intraspecific F_1 hybrids in *Cardamine bellidifolia, Cochlearia groenlandica, Saxifraga hyperborea, Ranunculus pygmaeus, Silene uralensis* and *S. acaulis*. Fertility was estimated as % fully stainable pollen grains (out of ca. 200 pollen grains) and as % developed seeds after spontaneous selfing, relative to total number of ovules.

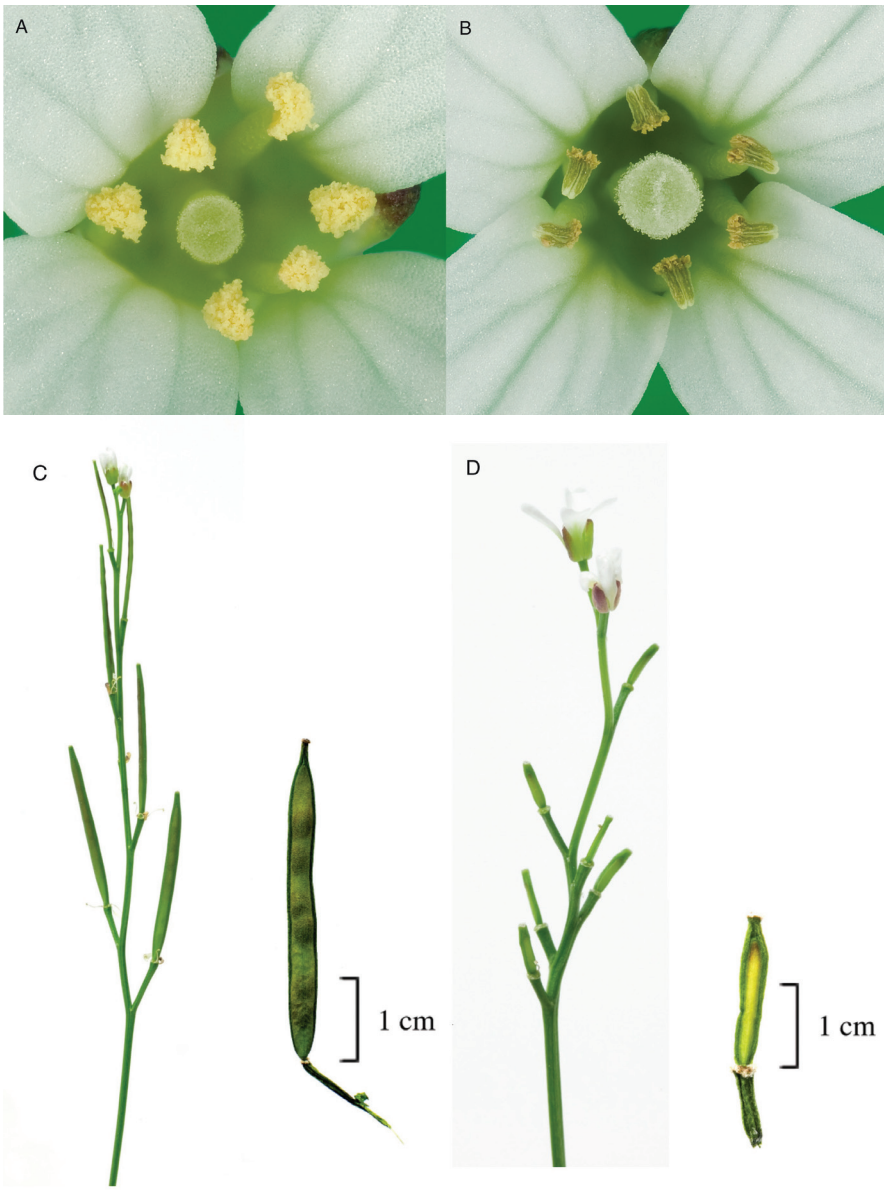


Figure 4. Pollen and fruit production in F_1 hybrids of *Cardamine bellidifolia*. A and C shows pollen and fruit from a fertile F_1 hybrid generated from a within-population cross (Alaska). B and D shows pollen and fruit from a sterile F_1 hybrid generated from a between-region cross (Alaska-Svalbard). POTO: Ulla Schildt (inflorescence in C, D) and Karsten Sund (A, B and fruit in C, D)

In *Cochlearia groenlandica*, 55 crosses were successful, and 253 F_1 hybrids were analysed for pollen fertility and 252 F_1 hybrids for seed fertility (Fig. 3). The within-population crosses and the Svalbard-population crosses resulted mostly in F_1 hybrids with similar fertility as the parental plants (mean pollen fertility 92-95%, mean seed fertility 73-69%). The within- and between-region crosses resulted in F_1 hybrids with reduced fertility, in particular seed fertility (mean pollen fertility 79-60%, seed fertility 38-18%). The reciprocal crosses resulted in similar hybrid fertility.

In *Saxifraga hyperborea*, 48 crosses were successful, and 142 F_1 hybrids were analysed for pollen fertility and 141 F_1 hybrids for seed fertility (Fig. 3). Both the within-population crosses and the Svalbard population crosses resulted primarily in fully fertile F_1 hybrids, although a handful of hybrids had strongly reduced pollen and seed fertility (mean pollen fertility 88-44%, mean seed fertility 80-34%). The within- and between-region crosses resulted in F_1 hybrids with reduced fertility (mean pollen fertility 38-22%, seed fertility 32-21%). All reciprocal crosses except for one cross in population 23 resulted in similar hybrid fertility.

In *Ranunculus pygmaeus*, 17 crosses were successful and 60 F_1 hybrids were analysed for pollen and seed fertility (Fig. 3). No within-population crosses or Svalbard-population crosses were successful. Most within- and between-region crosses resulted in F_1 hybrids with reduced fertility (mean pollen fertility 33-37%, seed fertility 32-36%). No reciprocal crosses were successful in this species.

In *Silene uralensis*, only one cross was successful and 14 F_1 hybrids were analysed for pollen and seed fertility (Fig. 3). The between-region cross generated completely sterile F_1 hybrids (mean pollen and seed fertility 0%).

In *Silene acaulis*, 17 crosses were successful, and 33 F_1 hybrids were analysed for pollen fertility and six F_1 hybrids for seed fertility (Fig.3). The within-population crosses, the Svalbard-population crosses and the between-region crosses generated highly fertile F_1 hybrids (mean pollen fertility 85-97%). Seed fertility was only tested for a few between-region crosses (mean seed fertility 70%). No reciprocal crosses were performed.

6.2 Molecular analysis of Cardamine bellidifolia

Consistent with Ikeda et al. (2012), *C. bellidifolia* was resolved as sister to the Japanese alpine endemic *C. nipponica* (paper I). The Maximum likelihood (ML) tree had poor resolution,

however, with virtually no geographic structure observed within *C. bellidifolia*, presenting plants from Alaska and Svalbard intermingled in the tree (paper I).

In the isolation with migration (IM) analyses the estimated demographic parameters for each pair of regions were consistent among three independent replicates and an unambiguous peak of posterior probability was obtained for each parameter. Divergence between Alaska and Svalbard was estimated to be most recent (2600 yr. before present [BP], 95% HPD (Highest Posterior Density) 0 - 35600 yr. BP), while divergence between Alaska and Scandinavia was estimated as the oldest (22000 yr. BP, 95% HPD 3000 - 186600 yr. BP). Although the HDP intervals were quite large, divergence among the contemporary populations of *C. bellidifolia* across the entire circumpolar region seems to have occurred during the last glacial cycle, possibly even after the last glaciation.

In the *BEAST analysis, the oldest divergence was estimated between Scandinavia (Norway) and the other regions (Svalbard, Alaska and Yukon; 38800 yr. BP, 95% HPD = 14800-59600 yr. BP). The most recent divergence was inferred between Yukon and Alaska, but this estimate was not significant due to poor geographic structure within *C. bellidifolia* in the gene trees.

6.3 Molecular analysis of Ranunculus pygmaeus

Very little genetic variation was observed in *Ranunculus pygmaeus*. The final AFLP dataset included only 34 polymorphic markers despite the large number (41) of primer combinations initially tested. The reproducibility was very high (99.16%). Virtually no variation was observed in the northern North Atlantic area (Scandinavia, Svalbard and Greenland). The neighbour-joining tree revealed two major groups: one Central European group consisting of the populations from the Alps and the Tatra Mountains, and one arctic group (paper I). The arctic group obtained 90% bootstrap support but virtually no support for internal branches. The PCoA plot revealed some geographic structure that was largely consistent with the NJ tree, with the Central European and Russian Taymyr populations placed at one extreme of the first axis and the Scandinavian/Svalbard/Greenland populations at the other extreme of the axis (paper I).

6.4 Phylogenetic analysis in Cardamine nipponica *and its relatives*

The lengths of the aligned sequences of *CRY1*, *PHYA*, and *PHYE* were 2,247-2,304 bp, 3,691-3,720 bp and 3,623-3,671 bp, respectively (paper III). The ML trees revealed monophyly for all three genes for the northern Japanese populations of *C. nipponica*, as well as for *PHYA* and *PHYE* in central Japanese populations. Among these photoreceptor genes, no alleles were shared between northern and southern populations of *C. nipponica.* In the *CRY1* and *PHYA* trees, *C. nipponica* was retrieved as monophyletic (i.e. including both central and northern populations). In contrast to *PHYA* and *CRY1*, *PHYE* resolved northern populations of *C. nipponica* together with *C. bellidifolia* as sister to the central populations of *C. nipponica*. The likelihood ratio tests examining non-neutral divergence for *PHYE* indicated that positive selection had been involved in amino acid replacements accumulating on the basal branches of *C. nipponica* and *C. bellidifolia.*

6.5 Linkage mapping and QTL analysis in Draba nivalis

A total of 128 loci were genotyped in 359 F_2 individuals. Twenty-nine markers (22.6%) were excluded from the map construction because of TRD. The final map (see paper II) was constructed using 94 markers (31 microsatellites, 52 AFLPs and 11 SSAPs), with a total map length of 894 cM, forming eight linkage groups (LG1-LG8) that most likely correspond to the eight chromosomes of *D. nivalis*. A total of 14 significant QTLs were detected, of which four were associated with pollen fertility, three with seed set, three with flowering time and four with number of flowers.

Pollen fertility QTLs were detected on LG2, LG3, LG4 and LG7 and showed considerable variation in gene action (as assessed based on the marker closest to the LOD peak). This includes intermediate dominance (LG2; marker AFLP41), additivity (LG3; marker D11), dominance (LG4; AtC10_7), and underdominance (LG7; AtC10_17; Fig. 5). The maternal allele was dominant to the paternal allele for the QTLs on LG2 and LG4. Seed set QTLs were detected on LG1, LG2 and LG7. All three QTLs were underdominant (marker closest to LOD peak on LG1; AFLP21, LG2; A214, LG7; AtC10_17; Fig. 5). Flowering time QTLs were detected on LG2, LG4 and LG5. The QTLs on LG2 and LG4 had intermediate dominance effects (marker closest to LOD peak; AFLP33 and AFLP69 respectively), whereas the QTL on LG5 displayed additive gene action (marker closest to LOD peak; AFLP5; Fig. 5). Number of flower QTLs were detected on LG2, LG3 and LG5. The QTLs on LG2, LG5,

and LG7 had additive effects, whereas for the LG3 QTL, the paternal allele was dominant to the maternal allele (Fig. 5).

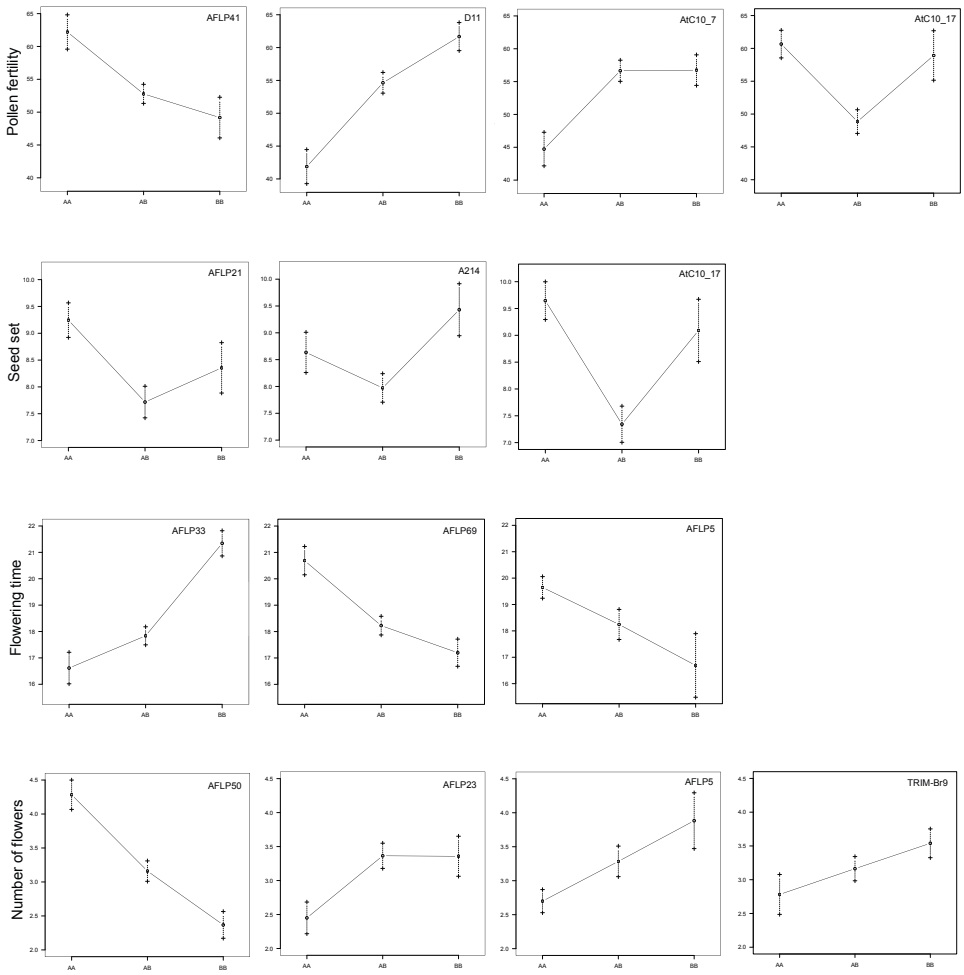


Figure 5. Effect plots for fertility QTLs as assessed based on the marker closest to the LOD peak for each trait. AA is homozygote for the maternal allele and BB is homozygote for the paternal allele.

7. DISCUSSION

The present study proposes that the formation of cryptic biological species is a general pattern in the arctic flora, and indicate that hybrid incompatibilities arise very rapidly, even at small spatial scales, suggesting high speciation rates in the arctic flora. All five selfing species investigated produced sterile or semisterile hybrids whereas hybrids from the one outcrossing species were fully fertile, further suggesting that a selfing mating system appears to accelerate accumulation of hybrid incompatibilities.

Highlighting the importance of both nuclear-nuclear interactions and structural chromosomal changes, the present study suggests that multiple genetic mechanisms are involved in the rapid build-up of reproductive isolation in *Draba nivalis.* All traits associated with seed fertility and one pollen fertility QTL demonstrated underdominant effects. The selfing mating system of this species may be an important factor in the fixation of chromosomal changes, which in addition is the main reproductive mode of many arctic diploid plants.

The major advance in paper III was that the evolutionary origin of intra-specific divergence in the Japanese endemic *Cardamine nipponica* was elucidated. The differentiation of *PHYE* between northern and southern populations of *C. nipponica* was most likely inferred from alleles under natural selection that diverged prior to speciation, i.e. in the ancestor of *C. nipponica* and *C. bellidifolia.*

7.1 Cryptic speciation in the Arctic

Despite the full fertility of parental populations, all selfing species mainly produced sterile or semisterile F_1 hybrids on the circumpolar scale, usually also at the regional scale (Alaska/Yukon), and mostly fertile F_1 hybrids at the local scale. These results are based on large data sets (>200 crosses and >750 F_1 hybrids), and include many populations both at local, regional and circumpolar scale, indicating that numerous cross-incompatible cryptic biological species have developed within single taxonomic arctic plant species. In total, 98% of the crosses between geographic regions and 96% of the crosses between sites in the Alaska/Yukon region produced sterile or semisterile hybrids. The one outcrossing species generated highly fertile hybrids both at the local and circumpolar scale. No apparent morphological or ecological differences between the populations that were isolated by postzygotic reproductive barriers were found. Thus, supporting previous work (Grundt et al.

2006) suggesting that a selfing mating system may accelerate the accumulation of hybrid incompatibilities both by reducing gene flow between diverging lineages, and by increasing the fixation rate of hybrid incompatibilities, possibly via genetic drift.

In the two selfing species investigated for genetic diversity (*Cardamine bellidifolia* and *Ranunculus pygmaeus*), investigated populations were genetically very similar in spite of being more or less reproductively isolated. Molecular analysis of *C. bellidifolia* suggests a very recent divergence, possibly after the last glaciation. This implies that hybrid incompatibilities have arisen very rapidly, even among closely related populations. Accordingly, the present study not only show that intrinsic postzygotic isolation has developed multiple times and at small geographic scales within single named taxonomic species, but also that postzygotic isolation may develop very rapidly, apparently within a few millennia.

The arctic flora is one of the most polyploid-rich floras on earth, with many species formed via reticulate rather than divergent evolution (Brochmann et al. 2004, Brochmann and Brysting 2008, Brochmann et al. 2013). This type of speciation occurs via hybridization and genome doubling and may take only a single or a few generations for completion. The present study provides additional evidence that rapid speciation may also take place via divergent evolution in arctic diploid plants, extending the previous finding of cryptic biological speciation events in the three *Draba* species (Brochmann et al. 1993, Grundt et al. 2006, Skrede et al. 2008a) to other genera and plant families.

The results obtained in this study indicate that speciation rates are surprisingly high in the arctic flora, suggesting that other factors should account for the low species diversity in the Arctic. It is tempting to suggest that high extinction rates, rather than low speciation rates, possibly related to Pleistocene climatic oscillations, account for the present low species diversity. Results presented here are in agreement with those obtained for New World birds and mammals (Weir and Schluter 2007) that, based on birth-death models, suggest that both speciation and extinction rates increase at higher latitudes. There are not many studies on how extinction rates vary with latitude and quantifying extinction rates is problematic (Dowle et al. 2013). However, a study investigating marine bivalve genera based on fossil data over the past 11 million years indicate higher extinction rates at higher latitudes (Jablonski et al. 2006).

The origin of the latitudinal diversity gradient has long been the subject of lively discussions and many different hypotheses have been proposed. While a generally accepted explanation for the latitudinal diversity gradient remains elusive (Mittelbach et al. 2007),

ecologists have speculated that elevated temperatures may influence speciation rates via higher mutation rates, shorter generation times and/or faster physiological processes (Davies et al. 2004a, Davies et al. 2004b, Wright et al. 2006, Rohde 2013). However, changes in diversification rates could result from variation in both speciation and extinction rates. Many estimates of tropical diversity are based on net diversification, which could result from differences in either speciation and/or extinction rates (Mittelbach et al. 2007), highlighting the importance of studies incorporating both factors, such as the study of New World birds and mammals by Weir and Schluter (2007).

7.2 Reproductive isolation in Draba nivalis

The genetic linkage map is based on robust linkage analysis combining 99 codominant and dominant markers, resolving eight linkage groups, possibly corresponding to the eight chromosomes of *D. nivalis* (2*n*=16).

The higher fitness of selfed F_2 hybrids as compared to F_1 hybrids, as well as the mapping of underdominant QTLs for pollen fertility and seed set, suggest the importance of chromosomal rearrangements in the build-up of reproductive isolation (RI) in this system. In particular, the underdominant seed set QTLs detected on LG1, LG2 and LG7 are consistent with multiple restructuring events among lineages, promoting RI in *D. nivalis* following chromosomal models of speciation. These results are congruent with the previous study of Skrede et al. (2008b) that also highlight the importance of chromosomal speciation in this system. Only a few recent studies have examined the processes underlying the fixation of chromosomal rearrangements among plant lineages and their impact on RI. Adaptive QTLs underlying prezygotic isolation between *Mimulus lewisii* and *M. cardinalis* mapped to regions of suppressed recombination corresponding to reciprocal translocations and inversions, suggest that chromosomal rearrangements have a crucial impact on the build-up of RI (Fishman et al. 2013). Other studies indicate that selection may drive the fixation of chromosomal rearrangements and thus lead to chromosomal speciation (Rieseberg 2001, Faria and Navarro 2010, Lowry and Willis 2010, Glemin and Ronfort 2013). No morphological or ecological differentiation between the populations in *D. nivalis* was observed, suggesting that selection has not been a primary force in the build-up of RI in this system.

Earlier studies suggest an important role of genetic drift in speciation (Wright 1941, Mayr 1963, Key 1968, Grant 1971), but theoretical studies in the latter part of the 20th century

imply that fixation of chromosomal rearrangements by genetic drift was unlikely to effectively drive RI (Hedrick 1981, Walsh 1982). The probability of maintaining chromosomal rearrangements with large underdominant effects was shown to be extremely low, except in very small, inbred populations, leading to a broad consensus that BDM incompatibilities predominate over chromosomal rearrangements in the origin of intrinsic postzygotic isolation (Orr et al. 2004).

Draba nivalis is a predominantly self-fertililizing plant (Brochmann and Steen 1999). Populations examined here have in addition a small effective population size, with low levels of molecular diversity, that most likely have experienced repeated periods of extinction and recolonization events during the Pleistocene climatic oscillations (Grundt et al. 2004). These are all characteristics that may promote establishment of chromosomal rearrangements (Hedrick 1981, Walsh 1982, Lande 1985, Levin 2002, Gavrilets 2004). The predominantly selfing strategy of *D. nivalis* most likely contributed to the rapid build up of RI, possibly by reducing gene flow and effective recombination between populations. Despite recent speciation literature that emphasize the importance of BDM incompatibilities (Coyne and Orr 2004), chromosomal speciation may very well be more important in highly selfing plants such as the majority of arctic diploid plants.

The two QTLs on LG7 (i.e. near AtC10_17) showing underdominant effects for both pollen fertility and seed set, were associated with a polymorphic insertion of the 5000 bp LTR retrotransposon AtC10. Previous work has shown that retrotransposons may contribute significantly to genome evolution (Kidwell and Lisch 2001, Bennetzen 2005, Biemont and Vieira 2006). However, plant genomes usually contain hundreds of such insertions (Wicker et al. 2007, Gaut and Ross-Ibarra 2008) and QTLs may map to such intervals without any significant effect on focal phenotypes. The association revealed here is, however, not necessarily coincidental. Polymorphic insertions may indeed modify local recombination rates by disrupting co-linearity and/or inducing heterochromatinization (Dooner and He 2008, Colome-Tatche et al. 2012, Melamed-Bessudo and Levy 2012). Accordingly, such microchromosomal change or the resulting linkage between previously segregating BDM loci would behave as an underdominant locus (Hoffmann and Rieseberg 2008) without the involvement of disproportionately strong genetic drift for their fixation (Rieseberg 2001, Levin 2002).

BDM incompatibilities is largely favoured over chromosomal rearrangements as they account for the accumulation of genetic incompatibilities among isolated populations, without

loss of fitness (Rieseberg and Willis 2007, Lexer and Widmer 2008). The present study also shows evidence of nuclear-nuclear incompatibilities. QTLs underlying pollen fertility displayed additive to dominant effects in addition to underdominance. In contrast to Skrede et al. (2008b) and other studies suggesting the importance of cytonuclear incompatibilities in RI (Lowry et al. 2008, Leppälä and Savolainen 2011), maternal alleles for pollen QTLs were not consistently associated with higher fertility. Maternal alleles were indeed associated with increased fertility in F_2 hybrids for the QTL on LG3 (i.e. near D11) and LG4 (i.e. near AtC10_7), but the QTL on LG2 (i.e. near AFLP41) showed the opposite pattern and most likely represents a nuclear-nuclear BDM incompatibility. This is consistent with the importance of BDM incompatibilities in the evolution of new species (Coyne and Orr 2004, Rieseberg and Willis 2007).

7.3 Evolutionary history of PHYE *in* Cardamine nipponica

The genealogical analysis of *PHYE* in *C. nipponica* and its close relatives revealed that the northern populations of *C. nipponica* formed a monophyletic group together with those of *C. bellidifolia*, whereas the alleles observed in the central populations of *C. nipponica* formed a monophyletic sister group. This paraphyletic relationship of *PHYE* is discordant with the previously demonstrated monophyly of *C. nipponica* based on ten nuclear loci (Ikeda et al. 2012). The reference loci *PHYA* and *CRY1*, supported populations of *C. nipponica* as monophyletic, even though *CRY1* did not differentiate between northern and southern populations. Furthermore, the topology constraining paraphyly of northern and central Japanese populations of *C. nipponica* was significantly rejected both for *CRY1* and *PHYA* as well as for the concatenated data of the eight nuclear genes. Accordingly, the paraphyletic relationships among the *PHYE* alleles in *C. nipponica* likely represent the gene specific evolutionary history, suggesting divergence prior to speciation, i.e. in the ancestor of *C. nipponica* and *C. bellidifolia.* It is also possible that introgression occurred after speciation. However, the genealogy of *PHYE* showed reciprocal monophyly of the northern Japanese clade of *C. nipponica* and *C. bellidifolia*, without sharing any alleles. In addition, the speciation history of *C. nipponica* and *C. bellidifolia* indicated that gene flow after speciation occurred solely from the former to the latter (Ikeda et al. 2012). It is therefore unlikely that the present genetic similarity at *PHYE* between *C. bellidifolia* and northern Japanese populations of *C. nipponica* is the result of introgression.

Tests of natural selection significantly supported the model assuming positive selection on the divergence of central Japanese populations of *C. nipponica*, northern Japanese populations of *C. nipponica* and *C. bellidifolia*. This indicates that positive selection was involved in the divergence of *PHYE* in the ancestral species, potentially reflecting functional differences that could be involved in adaptation. For instance, the photoperiod in the growing season is different between northern and central Japan (~0.5-1 hour in the summer). *Cardamine bellidifolia* grows in habitats with longer photoperiod and at higher latitudes as compared to *C. nipponica*. This suggests that selection in regard to different photoperiods may have been important in the divergence of *PHYE*. Previous studies have indeed shown that natural variation in phytochromes is associated with latitudinal clines of ecologically important traits such as flowering time (Balasubramanian et al. 2006) and timing of bud-set (Ingvarsson et al. 2008). The involvement of *PHYE* in local adaptation to environmental changes along latitudes has also been suggested for another alpine plant, *Arcterica nana* (Maxim.) Makino (Ikeda and Setoguchi 2010). Thus, *PHYE* probably played an important role in local adaptation of northern and central populations of *C. nipponica*, resulting in the present genetic differentiation.

Although genetic drift following climate oscillations also might have been important in inducing the observed genetic differentiation of *PHYE* between northern and central populations of *C. nipponica*, natural selection was most likely important for shaping the extant differentiation. Because local or temporal adaptation following climate change requires immediate response to the changing environment, standing genetic variation would contribute more to adaptation than newly accumulated mutations. The present study suggests that standing genetic variation of *PHYE*, which diverged under positive selection prior to speciation, resulted in the selective differentiation between the northern and central Japanese populations of *C. nipponica*, further suggesting the importance of standing genetic variation in regard to quick responses to climate changes.

7.4 Strengths and limitations

The conclusions in paper I are based on results from a very large data set, and present direct evidence of reproductive isolation within several phylogenetically distantly related plant species. This is contrary to many other studies that only rely on indirect measurements of reproductive isolation using molecular methods. In addition, including results from one

predominant outcrossing species allowed interpretations of underlying evolutionary forces (such as drift and selection). In paper II the linkage map and following QTL-analysis of the F_2 population in *Draba nivalis* was conducted using a large set of markers, a large F_2 population and performed using solid analytical tools. The resulting eight linkage groups likely represent the eight chromosomes of *D. nivalis* suggesting that substantial parts of the genome have been covered. Combining both codominant and dominant markers allowed genotypes of the dominant markers to be inferred, generating more information from the dominant markers than otherwise possible. In addition, the present map was constructed after removing distorted markers, enabling a more accurate estimation of the genomic location and effects of QTLs as compared to the previous published map (Skrede et al. 2008b). In paper III the major advance was that the evolutionary origin of intra-specific divergence in *Cardamine nipponica* was elucidated. The present study revealed that the selective divergence in the photoreceptor gene *PHYE*, was inferred from ancestral polymorphisms. Previous works on population genetics found evidence for natural selection within species, but did little to clarify its evolutionary origin i.e., ancestral polymorphisms or newly accumulated mutations.

A major limitation in paper I was that no direct calculations of speciation rates were conducted. Furthermore, no direct estimates of extinction rates could be performed to test the hypothesis of high extinction rates (in addition to high speciation rates). A selfing mating system appeared to aid the accumulation of hybrid incompatibilities, with the outcrossing species investigated presenting fully fertile F_1 hybrids. However, results were only obtained from one outcrossing species (due to difficulties with cultivation and crossing experiments of the other outcrossing species initially collected). No obvious morphological differences were observed for the different populations showing hybrid incompatibilities, but no morphometric analysis was conducted to provide firm evidence of this. A major limitation in paper II was that no mapping of parental populations was conducted. The probable involvement of chromosomal rearrangements in the evolution of hybrid incompatibilities in this system could have been corroborated if the genetics of parental lineages were known. In addition, genome duplication and chromosomal painting could have shown the importance of chromosomal rearrangements. However these experiments failed. Difficulties with finding polymorphisms between the parents limited the number of markers included in the genetic linkage mapping. With more markers, the *Draba* genome could have been covered even further. A primary aim was to produce a reciprocal mapping population to compare traits between them, and possibly elucidate the involvement of cytonuclear incompatibilities. Unfortunately this failed, leaving

the question of the importance of cytonuclear incompatibilities largely unanswered. In paper III it would have been interesting to include studies on the functions of *PHYE* alleles and confirm their ecological and evolutionary importance, but no such studies were conducted.

8. PERSPECTIVES

The presence of numerous cryptic biological species in the Arctic strongly calls for further studies. Could the same pattern be found in other regions with similar climatic conditions, or perhaps in other systems with many selfing species or even in other organism groups? Arctic regions are characterized by low temperatures, short growing seasons, and drought. Traditionally, these extreme environmental constraints, especially low temperatures, have been postulated to cause very low evolutionary rates in the arctic ecosystem. The present study contradicts this view, suggesting that speciation rates are in fact exceptionally high. Crossing experiments for other areas with similar climatic conditions, such as mountainous regions, should be conducted to investigate if evolutionary rates are in fact high in these regions as well.

Selfing is the main reproduction mode for a large proportion of the arctic flora. The present study suggests that a selfing mating system facilitates the accumulation of hybrid incompatibilities. All selfing species generated fully sterile or semisterile F_1 hybrids, in contrast to the one outcrossing species that generated fully fertile F_1 hybrids. Including more species with contrasting reproductive modes would enable better generalizations. To see whether similar patterns are observed in other regions containing large proportions of selfing species, it would further be interesting to perform crossing experiments in other areas where selfing is a main reproduction mode. One such habitat could be semideserts.

The present study does not provide any direct calculations of speciation rates, something that definitely should be conducted to confirm that speciation rates are exceptionally high in the Arctic. Estimating speciation rates among a wide range of populations representing a broad taxonomic sampling will provide generalizations, and such analyses are presently being conducted in our group. Providing information about past extinction rates would also be very interesting, but harder do perform as data for such calculations does not exist.

Weir and Schluter (2007) suggested that both speciation and extinction rates increase at higher latitudes in New World birds and mammals. However, in contrast to the present study, the results were based on birth-death models and not empirical crossability data. Performing crossing experiments including other organism groups could provide additional support for the latitudinal correlation.

Numerous genetic mechanisms are involved in the rapid accumulation of hybrid incompatibilities in *Draba nivalis*. With the improved access to conducting whole genome

sequencing, the parental lineages in this species should be sequenced. This would provide information about to what extent chromosomal rearrangements are important in the up-rise of reproductive isolation in this system, including the potential involvement of transposable elements. Furthermore, genome sequencing of parental lineages would yield additional polymorphic markers that could be used in genetic mapping, thereby increasing the coverage of the *Draba* genome even further.

With the present study more knowledge about the genetic mechanisms involved in the evolution of reproductive isolation in *Draba nivalis* has been gained. Including more species in genetic mapping would provide useful comparisons. *Cardamine bellidifolia* would be an interesting candidate. This species clearly demonstrated cross-incompatibilities at large and small spatial scales and is a suitable study plant as it is easily cultivated and generating reciprocal mapping populations should be feasible.

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11. SUPPLEMENTARY INFORMATION

Family, Species (Pre-dominant mating system) Pop. ID	Geographic Origin	n	Corema Accession no. From	To	Coordinates Latitude	Longitude	Altitude (m.a.s.)
Brassicaceae							
Cardamine bellidifolia L. (S)							
LG09-S-01	Norway, Svalbard, Spitsbergen: Nybyen	18	45729	45746	N78° 12' 2.58"	E15° 35' 26.4"	137
LG09-S-27	Norway, Svalbard, Spitsbergen: Todalen	21	46179	46199	N78° 11' 3.9"	E15° 51' 27.9"	48
LG09-S-32	Norway, Svalbard, Spitsbergen, Longyearbyen: Sarkofagen	15	46256	46270	N78° 9' 59.9"	E15° 32' 0.1"	513
LG09-A-42	U.S.A., Alaska, Seward Peninsula: Nome	11	46418	46428	N65° 2' 53.64"	W164° 48' 45.96"	237
LG09-A-52	U.S.A., Alaska, Seward Peninsula: Wooly Lagoon	7	46498	46504	N64° 51' 18.7"	W166° 24' 6.7"	42
LG09-A-63	U.S.A., Alaska, Brooks Range: Near Atigun Pass	19	48946	48963	N68° 13' 45.48"	W149° 24' 43.68"	954
			54904	-			
LG09-A-68	U.S.A., Alaska, Brooks Range: Atigun Pass	15	48986	48999	N68° 8' 10.44"	W149° 28' 37.56"	1447
			54905	-			
LG09-A-83	U.S.A., Alaska, Denali Highway: Near Downwind Lake	18	49386	49401	N63° 4' 23.1"	W146° 13' 42.3"	1194
			49412	-			
			53163	-			
LG09-A-104	U.S.A., Alaska, Denali Highway: Near Clearwater Airport	6	49567	49572	N63° 2' 48.84"	W147° 9' 46.08"	1170
LG09-A-109	Canada, Yukon Territory, Tombstone Territorial Park: Goldensides Trail	16	49643	49658	N64° 31' 43.74"	W138° 11' 57.42"	1552
LG09-N-130	Norway, Oppland, Lom, Bøverdalen: Near Juvasshytta	13	25850	25861	N61° 40' 31.2"	E8° 22' 0.2"	1520
			25901	-			
Cochlearia groenlandica L. (S)							
LG09-S-04	Norway, Svalbard, Spitsbergen: Nybyen	16	45779	45794	N78° 12' 20.7"	E15° 35' 53.4"	79
LG09-S-24	Norway, Svalbard, Spitsbergen: Bjørndalen	13	46134	46146	N78° 13' 25.4"	E15° 19' 42.9"	31
LG09-S-34	Norway, Svalbard, Spitsbergen: Blomsterdalen	16	46282	46297	N78° 14' 16.4"	E15° 30' 43.0"	41
LG09-A-48	U.S.A., Alaska, Seward Peninsula: Nome	14	46447	46460	N64° 29' 4.48"	W165° 25' 49.9"	2
LG09-A-51	U.S.A., Alaska, Seward Peninsula: Wooly Lagoon	19	46480	46497	N64° 51' 18.7"	W166° 24' 6.7"	42
			56044	-			
LG09-A-54	U.S.A., Alaska, Seward Peninsula: Teller	16	48670	48684	N65° 15' 52"	W166° 21' 57.1"	6
			56045	-			
Caryophyllaceae							
Minuartia biflora (L.) Schintz & Thell. (S)							
LG09-S-09	Norway, Svalbard, Spitsbergen: Longyearbyen	16	45879	45894	N78° 13' 18.3"	E15° 36' 50.8"	66
LG09-S-15	Norway, Svalbard, Spitsbergen: Endalen	16	45975	45990	N78° 11' 41"	E15° 47' 32.9"	28
LG09-S-22	Norway, Svalbard, Spitsbergen: Bjørndalen	16	46103	46118	N78° 13' 25.4"	E15° 19' 42.9"	31
LG09-S-30	Norway, Svalbard, Spitsbergen: Todalen	17	46233	46249	N78° 10' 37.0"	E15° 50' 24.9"	150
LG09-N-131	Norway, Oppland, Lom, Bøverdalen: Near Juvasshytta	13	25862	25873	N61° 40' 31.2"	E8° 22' 0.2"	1520
			25902	-			
Minuartia rubella (Wahlenb.) Hiern (S)							
LG09-A-50	U.S.A., Alaska, Seward Peninsula: Wooly Lagoon	12	46468	46479	N64° 52' 41.3"	W166° 8' 53.3"	214
LG09-A-65	U.S.A., Alaska, Brooks Range: Near Atigun Pass	6	48980	48985	N68° 12' 46.86"	W149° 24' 55.98"	995

LG09-A-72	U.S.A., Alaska, Brooks Range: Atigun Pass	2	49227	49228	N68° 7' 41.34"	W149° 28' 47.94"	1383
LG09-A-100	U.S.A., Alaska, Denali Highway: Near Clearwater Airport	4	49525	49528	N63° 2' 39.18"	W147° 7' 59.16"	1576
LG09-N-143	Norway, Oppland, Lom, Bøverdalen: Along road to Juvasshytta	12	26057	26067	N61° 43' 34.298"	E8° 23' 49.2"	1087
			54902	-			
Silene acaulis (L.) Jacq. (O)							
LG09-S-06	Norway, Svalbard, Spitsbergen: Longyearbyen	16	45811	45826	N78° 13' 2.6"	E15° 36' 39.6"	53
LG09-S-11	Norway, Svalbard, Spitsbergen: Endalen	16	45911	45926	N78° 11' 10.9"	E15° 46' 10.2"	52
LG09-S-26	Norway, Svalbard, Spitsbergen: Bjørndalen	16	46163	46178	N78° 14' 55.5"	E15° 29' 1.1"	7
LG09-A-40	U.S.A., Alaska, Seward Peninsula: Nome, towards Pilgrim Hot Springs	17	46340	46356	N64° 53' 38.1"	W165° 12' 52.56"	262
LG09-A-56	U.S.A., Alaska, Seward Peninsula: Nome, road to Jensen Camp	6	48884	48889	N64° 37' 16.399"	W165° 27' 0.799"	22
LG09-A-71	U.S.A., Alaska, Brooks Range: Atigun Pass	27	49200	49226	N68° 7' 41.34"	W149° 28' 47.94"	1383
LG09-A-98	U.S.A., Alaska, Denali Highway: Near Clearwater Airport	16	49509	49524	N63° 2' 39.18"	W147° 7' 59.16"	1576
LG09-A-116	Canada, Yukon Territory, Tombstone Territorial Park: Angelcomb Peak	11	49752	49762	N64° 34' 49.68"	W138° 14' 29.4"	1496
LG09-A-125	Canada, Yukon Territory, Tombstone Territorial Park: Along Demster Highway	5	55948	55952	N64° 36' 18.06"	W138° 19' 14.58"	1210
LG09-N-132	Norway, Oppland, Lom, Bøverdalen: Along road to Juvasshytta	16	25874	25883	N61° 41' 56.5"	E8° 23' 18.1"	1520
			25903	25908			
Silene uralensis (Rupr.) Bocquet (S)							
LG09-S-14	Norway, Svalbard, Spitsbergen: Endalen	16	45953	-	N78° 11' 10.9"	E15° 46' 10.2"	52
			45960	45974			
LG09-S-31	Norway, Svalbard, Spitsbergen: Todalen	6	46250	46255	N78° 10' 34.7"	E15° 53' 56.9"	44
LG09-S-36	Norway, Svalbard, Spitsbergen: Bolterdalen	16	46306	46321	N78° 9' 56.4"	E15° 57' 27.6"	13
LG09-A-70	U.S.A., Alaska, Brooks Range: Atigun Pass	21	49169	49189	N68° 7' 41.34"	W149° 28' 47.94"	1383
LG09-A-79	U.S.A., Alaska, Brooks Range: Near Galbraith Lake Campground	1	49363	-	N68° 27' 6.54"	W149° 28' 37.56"	853
LG09-A-126	Canada, Yukon Territory, Tombstone Territorial Park: Towards Auston Pass	23	49826	49848	N64° 37' 13.26"	W138° 27' 2.28"	417
Diapensiceae							
Diapensia lapponica L. (O)							
LG09-A-44	U.S.A., Alaska, Seward Peninsula: Nome	15	46379	46393	N64° 59' 42.12"	W164° 42' 37.8"	256
LG09-A-78	U.S.A., Alaska, Brooks Range: Near Galbraith Lake Campground	16	49347	49362	N68° 27' 6.54"	W149° 28' 37.56"	853
LG09-A-84	U.S.A., Alaska, Denali Highway: Near Downwind Lake	16	49413	49428	N63° 4' 23.1"	W146° 13' 42.3"	1194
Ericaceae							
Arctous alpinus L. Nied. [O/S)							
LG09-A-58	U.S.A., Alaska, Seward Peninsula: Salmon Lake Campground	14	48890	48903	N64° 55' 4.5"	W164° 57' 41.76"	166
LG09-A-76	U.S.A., Alaska, Brooks Range: Near Galbraith Lake Campground	13	49319	49331	N68° 27' 5.1"	W149° 28' 27.96"	842
LG09-A-105	U.S.A., Alaska, Denali Highway: Near Glacier Gap Lake	16	49595	49610	N63° 7' 51.78"	W146° 15' 8.52"	1162
LG09-A-111	Canada, Yukon Territory, Tombstone Territorial Park: Goldensides Trail	16	49670	49685	N64° 31' 23.64"	W138° 14' 45.24"	1216
LG09-N-142	Norway, Oppland, Lom, Bøverdalen: Near Jotunheim fjellstue	16	26041	26056	N61° 39' 15.098"	E8° 8' 23.5"	1130
Cassiope tetragona (L.) D.Don (O/S)							
LG09-S-13	Norway, Svalbard, Spitsbergen: Endalen	15	45944	45952	N78° 11'10.9"	E15° 46' 10.2"	52
			45954	45959			
LG09-S-19	Norway, Svalbard, Spitsbergen: Bjørndalen	16	46040	46055	N78° 13' 25.4"	E15° 19' 42.9"	31
LG09-S-28	Norway, Svalbard, Spitsbergen: Todalen	16	46200	46215	N78° 11' 3.9"	E15° 51' 27.9"	48

LG09-A-45	U.S.A., Alaska, Seward Peninsula: Nome	15	46394	46408	N65° 2' 53.64"	W164° 48' 45.96"	237
LG09-A-64	U.S.A., Alaska, Brooks Range: Near Atigun Pass	16	48964	48979	N68° 12' 46.86"	W149° 24' 55.98"	995
LG09-A-82	U.S.A., Alaska, Denali Highway: Near Downwind Lake	15	49364	49378	N63° 4' 23.1"	W146° 13' 42.3"	1194
LG09-A-108	Canada, Yukon Territory, Tombstone Territorial Park: Goldensides Trail	16	49627	49642	N64° 31' 43.74"	W138° 11' 57.42"	1552
LG09-A-115	Canada, Yukon Territory, Tombstone Territorial Park: Near Angelcomb Peak	11	49741	49751	N64° 33' 41.22"	W138° 14' 34.86"	1284
Kalmia procumbens (L.) Spreng (O/S)							
LG09-A-46	U.S.A., Alaska, Seward Peninsula: Nome	9	46409	46417	N65° 2' 53.64"	W164° 48' 45.96"	237
LG09-A-86	U.S.A., Alaska, Denali Highway: Near Downwind Lake	10	49445	49454	N63° 4' 23.1"	W146° 13' 42.3"	1194
LG09-A-88	U.S.A., Alaska, Denali Highway: Near Phalarope lake	7	49455	49461	N63° 5' 19.74"	W146° 24' 14.16"	1250
LG09-N-144	Norway, Oppland, Lom, Bøverdalen: Along Orma River	17	26069	26080	N61° 42' 23.9"	E8° 24' 54.2"	1200
			55943	55947			
Polemoniaceae							
Polemonium boreale Adams (O)							
LG09-S-35	Norway, Svalbard, Spitsbergen: Longyearbyen	10	46298	46305	N78° 13' 16"	E15° 37' 21.601"	37
			46322	46323			
Polygonaceae							
Oxyria digyna (L.) Hill (O/S)							
LG09-S-02	Norway, Svalbard, Spitsbergen: Nybyen	16	45747	45762	N78° 12' 20.7"	E15° 35' 53.4"	79
LG09-S-17	Norway, Svalbard, Spitsbergen: Endalen	17	46007	46023	N78° 11' 41"	E15° 47' 32.9"	28
LG09-S-29	Norway, Svalbard, Spitsbergen: Todalen	17	46216	46232	N78° 11' 3.9"	E15° 51' 27.9"	48
LG09-A-69	U.S.A., Alaska, Brooks Range: Atigun Pass	16	49153	49168	N68° 8' 10.44"	W149° 28' 37.56"	1447
LG09-A-89	U.S.A., Alaska, Denali Highway: Near Phalarope lake	16	49462	49476	N63° 5' 19.74"	W146° 24' 14.16"	1250
			49478	-			
LG09-A-118	Canada, Yukon Territory, Tombstone Territorial Park: Near Tombstone Range Viewpoint	11	49708	49718	N64° 2' 39.6"	W138° 13' 57.36"	1225
LG09-A-120	Canada, Yukon Territory: Keno Sumit	17	49793	49809	N63° 56' 28.86"	W135° 13' 5.28"	1696
LG09-N-134	Norway, Oppland, Lom, Bøverdalen: Along road to Juvasshytta	16	25918	25933	N61° 41' 56.5"	E8° 23' 18.1"	1520
Ranunculaceae							
Ranunculus pygmaeus Wahlenb. (S)							
LG09-S-08	Norway, Svalbard, Spitsbergen, Longyearbyen: Huset	27	45843	-	N78° 12' 29"	E15° 35' 30.5"	72
			45846	-			
			45848	-			
			45850	-			
			45852	45857			
			45861	45875			
			45877	45878			
			53162	-			
LG09-S-16	Norway, Svalbard, Spitsbergen: Endalen	16	45991	46006	N78° 11' 41"	E15° 47' 32.9"	28
LG09-S-21	Norway, Svalbard, Spitsbergen: Bjørndalen	31	46072	46102	N78° 13' 25.4"	E15° 19' 42.9"	31
LG09-A-47	U.S.A., Alaska, Seward Peninsula: Nome	18	46429	46446	N64° 27' 22.2"	W165° 5' 46.3"	2
LG09-A-67	U.S.A., Alaska, Brooks Range: Atigun Pass	16	49128	49143	N68° 8' 10.44"	W149° 28' 37.56"	1447
LG09-A-102	U.S.A., Alaska, Denali Highway: Near Clearwater Airport	10	49537	49546	N63° 2' 48.84"	W147° 9' 46.08"	1170
LG09-A-119	Canada, Yukon Territory, Tombstone Territorial Park: Near Tombstone Range Viewpoint	10	49719	49728	N64° 2' 39.6"	W138° 13' 57.36"	1225
LG09-N-135	Norway, Oppland, Lom, Bøverdalen: Along road to Juvasshytta	16	25934	25949	N61° 41' 51.5"	E8° 23' 36.9"	1481
Thalictrum alpinum L. (O)							
LG09-N-136	Norway, Oppland, Lom, Bøverdalen: Along road to Juvasshytta	11	25951	25961	N61° 41' 51.5"	E8° 23' 36.9"	1481

Rosaceae

Dryas octopetala L. (O)

LG09-S-07	Norway, Svalbard, Spitsbergen: Longyearbyen	15	45827	45841	N78° 13' 2.6"	E15° 36' 39.6"	53
LG09-S-12	Norway, Svalbard, Spitsbergen: Endalen	17	45927	45943	N78° 11'10.9"	E15° 46' 10.2"	52
LG09-S-20	Norway, Svalbard, Spitsbergen: Bjørndalen	16	46056	46071	N78° 13' 25.4"	E15° 19' 42.9"	31
LG09-A-59	U.S.A., Alaska, Seward Peninsula: Salmon Lake Campground	16	48904	48919	N64° 55' 4.5"	W164° 57' 41.76"	166
LG09-A-77	U.S.A., Alaska, Brooks Range: Near Galbraith Lake Campground	15	49332	49346	N68° 27' 5.1"	W149° 28' 27.96"	842
LG09-A-94	U.S.A., Alaska, Denali Highway: Near Clearwater Airport	16	49477 - 49493 - 49495	49508	N63° 2' 39.18"	W147° 7' 59.16"	1576
LG09-A-129	Canada, Yukon Territory, Tombstone Territorial Park: Goldensides Trail	11	49853	49863	N64° 31' 30.6"	W138° 12' 45.18"	1370
LG09-N-139	Norway, Oppland, Lom, Bøverdalen: Near Bøvetun turiststation - Snipen	18	25993	26010	N61° 38' 29.7"	E08° 03' 37.7''	950

Sibbaldia procumbens L. (O/S)

LG09-A-55	U.S.A., Alaska, Seward Peninsula: Nome, road to Jensen Camp	10	48874	48883	N64° 33' 46.6"	W165° 24' 28.6"	123
LG09-A-85	U.S.A., Alaska, Denali Highway: Near Downwind Lake	16	49429	49444	N63° 4' 33.06"	W146° 13' 28.2"	1154
LG09-A-90	U.S.A., Alaska, Denali Highway: Near Phalarope lake	15	49479 - 49494	49492	N63° 5' 19.74"	W146° 24' 14.16"	1250
LG09-A-110	Canada, Yukon Territory, Tombstone Territorial Park: Goldensides Trail	11	49659	49669	N64° 31' 43.74"	W138° 11' 57.42"	1552
LG09-A-114	Canada, Yukon Territory, Tombstone Territorial Park: Dempster Highway	11	49730	49740	N64° 32' 46.74"	W138° 14' 7.56"	1223
LG09-N-133	Norway, Oppland, Lom, Bøverdalen: Along road to Juvasshytta	22	25884 - 25912	25899 - 25917	N61° 41' 56.5"	E8° 23' 18.1"	1520

Saxifragaceae

Saxifraga hyperborea R.Br. (S)

LG09-S-23	Norway, Svalbard, Spitsbergen: Bjørndalen	15	46119	46133	N78° 13' 25.4"	E15° 19' 42.9"	31
LG09-S-33	Norway, Svalbard, Spitsbergen: Blomsterdalen	11	46271	46281	N78° 14' 16.4"	E15° 30' 43"	41
LG09-A-66	U.S.A., Alaska, Brooks Range: Atigun Pass	9	49144	49152	N68° 8' 10.44"	W149° 28' 37.56"	1447
LG09-A-103	U.S.A., Alaska, Denali Highway: Near Clearwater Airport	10	49557	49566	N63° 2' 48.84"	W147° 9' 46.08"	1170
LG09-A-113	Canada, Yukon Territory, Tombstone Territorial Park: Near Tombstone Range Viewpoint	11	49697	49707	N64° 2' 39.6"	W138° 13' 57.36"	1225

Saxifraga oppositifolia L. (O)

LG09-S-03	Norway, Svalbard, Spitsbergen: Nybyen	16	45763	45778	N78° 12' 20.7"	E15° 35' 53.4"	79
LG09-S-18	Norway, Svalbard, Spitsbergen: Endalen	16	46024	46039	N78° 11' 41"	E15° 47' 32.9"	28
LG09-S-25	Norway, Svalbard, Spitsbergen: Bjørndalen	16	46147	46162	N78° 14' 55.5"	E15° 29' 1.1"	7
LG09-A-43	U.S.A., Alaska, Seward Peninsula: Nome, towards Pilgrim Hot Springs	7	46372	46378	N64° 53' 38.1"	W165° 12' 52.56"	262
LG09-A-53	U.S.A., Alaska, Seward Peninsula: Wooly Lagoon	16	46505 - 48658	46508 - 48669	N64° 51' 18.7"	W166° 24' 6.7"	42
LG09-A-74	U.S.A., Alaska, Brooks Range: Near Galbraith Lake Campground	11	49239	49249	N68° 27' 10.56"	W149° 28' 27.6"	1447
LG09-A-117	Canada, Yukon Territory, Tombstone Territorial Park: Angelcomb Peak	14	49763	49776	N64° 34' 49.68"	W138° 14' 29.4"	1496
LG09-N-138	Norway, Oppland, Lom, Bøverdalen: Near Bøvertun Turiststation - Snipen	18	25975	25992	N61° 38' 29.7"	E08° 03' 37.7''	950

Orobanchaceae

Pedicularis dasyantha (Trautv.) Hadac (O)

LG09-S-05	Norway, Svalbard, Spitsbergen: Longyearbyen	16	45795	45810	N78° 13' 2.6"	E15° 36' 39.6"	53
LG09-S-10	Norway, Svalbard, Spitsbergen: Endalen	17	45842 - 45895	45910	N78° 11' 10.9"	E15° 46' 10.2"	52
LG09-A-122	Canada, Yukon Territory: Keno Sumit	16	49777	49792	N63° 56' 32.04"	W135° 13' 3.06"	1689

Pedicularis oederi Vahl. (O)							
LG09-A-101	U.S.A., Alaska, Denali Highway: Near Clearwater Airport	8	49529	49536	N63° 2' 26.1"	W147° 10' 57.84"	1062
LG09-A-124	Canada, Yukon Territory, Tombstone Territorial Park: Along Demster Highway	5	49821	49825	N64° 36' 18.06"	W138° 19' 14.58"	1210
LG09-N-141	Norway, Oppland, Lom, Bøverdalen: Øvre Bøvertuntj	11	26029	26039	N61° 39' 9.5"	E8° 6' 51.3"	927
Tofieldiaceae							
Tofieldia pusilla (Michx.) Pers. (S)							
LG09-A-41	U.S.A., Alaska, Seward Peninsula: Nome, towards Pilgrim Hot Springs	15	46357	46371	N64° 53' 38.1"	W165° 12' 52.56"	262
LG09-A-75	U.S.A., Alaska, Brooks Range: Near Galbraith Lake Campground	17	49302	49318	N68° 27' 5.1"	W149° 28' 27.96"	842
LG09-A-107	U.S.A., Alaska, Denali Highway: Near Glacier Gap Lake	16	49611	49626	N63° 8' 59.28"	W146° 14' 52.68"	1158
LG09-A-123	Canada, Yukon Territory, Tombstone Territorial Park: Along Demster Highway	11	49810	49820	N64° 36' 18.06"	W138° 19' 14.58"	1210
LG09-N-140	Norway, Oppland, Lom, Bøverdalen: Near Bøvertun Winter Parking	18	26011	26028	N61° 38' 45.701"	E8° 6' 1.901"	945
Violaceae							
Viola biflora L. (O)							
LG09-A-61	U.S.A., Alaska, Seward Peninsula: Salmon Lake Campground	21	48920	48940	N64° 55' 51.06"	W164° 59' 5.64"	222
LG09-N-137	Norway, Oppland, Lom, Bøverdalen: Along Orma River	10	25962	25971	N61° 42' 4.799"	E8° 24' 10.501"	1438
	TOTAL	1722					

Table S1.

Sampling data for species collected during the field season summer 2009, and intended for intraspecific crossing experiments. Pre-dominant mating system is system indicated as: O=outcrossing, S=selfing, and O/S=mixed (data from Molau 1993, Brochmann and Steen 1999, pers. com. Reidar Elven). Plant families are indicated in bold. Population ID includes collector and year (LG09 = Lovisa Gustafsson 2009), main geographic region (S = Svalbard, N = mainland Norway, A = Alaska/Yukon), followed by population number. Accession number refers to the unique ID number in the DNAbank database Corema at the Natural History Museum, University of Oslo. Vouchers deposited in O.

13. PAPERS I-III

I

II

III