



Frequent sexual reproduction and high intraspecific variation in *Salix arctica*: Implications for a terrestrial feedback to climate change in the High Arctic

Heidi Steltzer,¹ Ruth A. Hufbauer,² Jeffery M. Welker,³ Maxime Casalis,⁴ Patrick F. Sullivan,³ and Rodney Chimner⁵

Received 31 May 2007; revised 4 December 2007; accepted 29 January 2008; published 26 April 2008.

[1] Genetic variation at molecular loci may underlie important variation in the phenotypes of arctic plants. Such intraspecific variation may be a neglected but important component of biological diversity in the Arctic that could impact how arctic ecosystems respond to climate change. Here, we characterized genetic and phenotypic variation in *Salix arctica* and evaluated the effect of *S. arctica* on ecosystem CO₂ exchange, a process by which terrestrial ecosystems in the Arctic feedback to the global climate system. We found high genetic variation at microsatellite loci of *S. arctica* collected from an inland and a coastal site in Greenland that indicates sexual reproduction has occurred frequently as the ice sheet has retreated. Across the North American range of *S. arctica*, ten chloroplast DNA haplotypes were identified. Haplotype diversity and allelic richness were high overall and similar across regions with different glacial histories. Phenotypic variation in ecologically important traits varied substantially in a High Arctic population of *S. arctica*. In a widespread High Arctic ecosystem, a net loss of CO₂ to the atmosphere was observed except where *S. arctica* was present. We suggest that high genetic variation in *S. arctica* is in part a result of frequent sexual reproduction, and that the phenotypic variation we observed is likely to be at least partially genetic-based. This would enable a productive High Arctic species to adapt and potentially prosper as climate changes, and thus affect the terrestrial feedback of the Arctic to the climate system.

Citation: Steltzer, H., R. A. Hufbauer, J. M. Welker, M. Casalis, P. F. Sullivan, and R. Chimner (2008), Frequent sexual reproduction and high intraspecific variation in *Salix arctica*: Implications for a terrestrial feedback to climate change in the High Arctic, *J. Geophys. Res.*, 113, G03S10, doi:10.1029/2007JG000503.

1. Introduction

[2] Our understanding of the feedbacks between the Arctic and the global climate system has focused primarily on the dynamics of sea ice, shifts in freshwater export to the ocean, plant species shifts that change ecosystem structure and albedo, and changes in the rate of ecosystem CO₂ exchange [Chapin *et al.*, 2005; McGuire *et al.*, 2006; Peterson *et al.*, 2006; Zhuang *et al.*, 2006; Serreze *et al.*, 2007]. These oceanic and terrestrial feedbacks to the climate system, which are an inherent characteristic of biocomplexity in the environment, can be extremely strong and the

positive ones may accelerate climate warming in the Arctic and globally [ACIA, 2005; Chapin *et al.*, 2005; McGuire *et al.*, 2006; Serreze *et al.*, 2007]. In Arctic terrestrial systems, the underlying processes that regulate plant responses to climate change are still largely unknown, though we are beginning to develop a conceptual understanding of how fire, depth to permafrost, and nutrient cycling may control some of the observed shifts in species composition [Chapin *et al.*, 1995; Epstein *et al.*, 2000; Lloyd, 2005; Sturm *et al.*, 2005]. Absent, however, has been an appreciation for the evolutionary processes that also may control the response of arctic plants to climate change.

[3] It has been shown that genetic-based variation in ecologically important plant traits allows plants to respond evolutionarily to climate change [Rehfeldt *et al.*, 1999; Davis *et al.*, 2005; Jump and Penuelas, 2005; Franks *et al.*, 2007]. In a region such as the Arctic where plant species diversity is low, variation within species might be particularly important. It could contribute substantially to phenotypic diversity and thus affect processes such as ecosystem CO₂ exchange as climate changes [Norberg *et al.*, 2001]. Yet, genetic variation is not well studied in the Arctic compared to temperate and tropical floras in part because it is thought to be relatively low. There are two

¹Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, Colorado, USA.

²Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colorado, USA.

³Environment and Natural Resources Institute and Biological Sciences Department, University of Alaska, Anchorage, Alaska, USA.

⁴Les Millets, Dom Pierre sur Besbre, France.

⁵Ecosystem Science Center, School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, Michigan, USA.

Table 1. Locations of Sampling Sites, Number of Individuals Sequenced (and Genotyped), and Haplotypes Present

Site	Location	Country	Latitude, °	Longitude, °	n	Haplotypes ^a
1	Kap Atholl-coastal ^{b,c,d}	Greenland	76.55	68.57	3 (17)	2A, 1G
2	Kap Atholl-between ^b	Greenland	76.48	68.42	6	1A, 5B
3	Kap Atholl-inland ^{b,d}	Greenland	76.43	68.28	4 (18)	3A, 1G
4	Kap Russell-coastal ^b	Greenland	78.88	68.75	4	1B, 2E, 1J
5	Kap Russell-inland ^b	Greenland	78.47	69.83	6	2A, 1E, 1G, 1J
6	Kap Atholl-bird cliffs	Greenland	76.28	69.07	6	4A, 1B, 1G
7	Agpat Agpai - coastal	Greenland	76.08	68.39	5	2A, 1D, 1G, 1J
8	Bellot Strait - coastal	Canada	72.02	94.2	5	2A, 3J
9	Brooks Range - Alaska	U.S.	68.42	149.28	8	4A, 2B, 1G, 1J
10	Wrangell Mtns-Alaska	U.S.	61.53	142.86	1	1J
11	Banff-Alberta	Canada	52.19	117.15	3	1A, 1H, 1I
12	Front Range Mtns - Colorado	U.S.	40.42	105.75	6	1A, 1E, 4F
13	San Juan Mtns 1 - Colorado	U.S.	37.88	107.83	6	2A, 4C
14	San Juan Mtns 2 - Colorado	U.S.	37.90	107.73	4	1A, 3E

^aNumber of individuals harboring each haplotype is indicated.

^bSites where plant traits were sampled.

^cSite where plant cover, LAI and ecosystem CO₂ exchange were measured.

^dSites where microsatellite variation was measured.

mechanisms underlying this idea. First, sexual reproduction, an important means of generating genetic variation, is considered infrequent in tundra ecosystems relative to asexual reproduction [Billings and Mooney, 1968; Bliss, 1971; Callaghan and Collins, 1976; Bell and Bliss, 1980]. Many arctic plants produce viable seed [Wager, 1938; Bliss, 1971; Billings, 1987], but germination often requires open space on a soil surface [Gough, 2006]. Frequent disturbance by physical processes creates an abundance of these surfaces in the High Arctic compared to the Low Arctic, but the harsher climate may limit seedling establishment and thus reduce the success of sexual reproduction [Wager, 1938; Bell and Bliss, 1980]. Even infrequent bouts of sexual reproduction, however, could contribute to the creation of new variations.

[4] A second reason that genetic variation is thought to be low in the Arctic is that glaciation during the Pleistocene likely led to the loss of some populations and an initial reduction in the genetic variation of arctic plant species [Abbott et al., 2000; Abbott and Brochmann, 2003; Alsos et al., 2005; Skrede et al., 2006]. However, glaciation can also generate genetic variation through enabling the divergence of populations in isolated glacial refugia. Recent evidence on European temperate tree species suggests that glacial refugia are hot spots of genetic diversity and that where populations converge following migration from isolated refugia genetic diversity can be even greater [Petit et al., 2003]. Patterns of genetic variation for arctic species support a similar glacial history of isolation in refugia, divergence, and postglacial expansion leading to high genetic variation among glacial refugia and within convergence zones [Abbott et al., 2000; Alsos et al., 2005; Skrede et al., 2006]. Many arctic species are widely distributed across glacial refugia and convergence zones [Hulten, 1968; Molau and Mølgaard, 1996; Murray, 1997], and their phenotypes vary within and across these regions [Mooney and Billings, 1961; Teeri, 1973; Chapin and Chapin, 1981; McGraw and Antonovics, 1983; Dawson and Bliss, 1989a; Fetcher and Shaver, 1990; Philipp and Siegismund, 2003]. Thus, genetic variation and the consequent variation in plant phenotypes may not be low for at least some Arctic species,

and could affect plant production and ecosystem CO₂ exchange as climate changes.

[5] Deciduous shrubs may be particularly important in the response of vegetation to climate change across the Arctic [Tape et al., 2006]. They have increased in abundance in tundra ecosystems as the climate has warmed [Chapin et al., 1995; Sturm et al., 2001], and respond similarly to experimental warming [Walker et al., 2006]. In the High Arctic, where several experimental studies on plant response to climate change have been done [Jones et al., 1997; Welker et al., 1997; Robinson et al., 1998; Marchand et al., 2004; Welker et al., 2004; Sullivan et al., 2008], the deciduous dwarf-shrub *Salix arctica* may have the greatest potential to respond to climate change as its abundance and photosynthetic rate have been shown to increase in response to experimental warming [Jones et al., 1997; Robinson et al., 1998; Sullivan et al., 2008]. *S. arctica* has a circumpolar distribution [Hultén, 1937; Murray, 1995], and like many willows, has a high photosynthetic rate with correspondingly low cost leaves (i.e., low leaf mass per unit area) [Raven, 1992; Dawson and Bliss, 1993; Jones et al., 1999; Sullivan and Welker, 2007]. However, it is the only willow species that extends to the northern limit of land in the Arctic. Given its ability to fix carbon and respond to warming, its importance in the High Arctic, and its circumpolar distribution it may be a particularly important species in determining the terrestrial feedback to climate change in the High Arctic.

[6] The specific aims of our study were to: (1) Evaluate whether *S. arctica* primarily spreads asexually or sexually in a High Arctic landscape in northwestern Greenland by evaluating similarity among multilocus microsatellite genotypes; (2) Evaluate whether genetic variation at microsatellite loci differs between two sites within Greenland of different age and fertility; (3) Characterize diversity in chloroplast DNA (cpDNA) sequences in northwestern Greenland and determine the relationship of cpDNA sequences to a nearby Canadian location and more distant locations in Alaska and Colorado where arctic populations could have survived during the last glaciation; (4) Characterize the phenotypic variation in ecologically important

plant traits in a high arctic population; and (5) Determine whether *S. arctica* affects ecosystem CO₂ exchange.

2. Methods

2.1. Study Species

[7] *Salix arctica* Pall. is a dioecious, deciduous, prostrate shrub that occurs throughout the Arctic and in alpine tundra

in the Northern hemisphere [Hulten, 1968]. Its abundance is especially high in prostrate dwarf-shrub herb tundra, a widespread High Arctic ecosystem [CAVMteam, 2003], but it occurs in diverse tundra ecosystems, including young and old ecosystems that differ in fertility, and dry and wet environments [Dawson and Bliss, 1989b; Hodkinson et al., 2003; Cooper et al., 2004; Sullivan and Welker, 2007]. Individuals are long-lived [Wilson, 1964] and can reproduce sexually and vegetatively through the growth of attached and detached stems. There are three geographical races of *S. arctica*. Each race is morphologically distinct, but individuals often have blended morphological characteristics [Hulten, 1968; Murray, 1997]. *S. arctica* is tetraploid in eastern North America and hexaploid in western North America and Eurasia [Suda and Argus, 1969].

2.2. Sampling Sites

[8] Plants were sampled at seven sites in northwestern Greenland and at an additional seven sites across the species range in North America (Table 1 and Figure 1). The seven coastal and inland sites in northwestern Greenland differed in age and fertility as a result of the retreat of the Greenland Ice Sheet and increased nitrogen inputs at coastal sites with bird colonies. The additional seven sites across the species' North American range included regions with different glacial histories during the Pleistocene (Figure 1). Plants from all sites were genotyped using cpDNA. Microsatellite genotypes were characterized at two sites on Kap Atholl. Phenotypic variation was measured at five high arctic sites in northwestern Greenland (Table 1). Ecosystem CO₂ exchange was measured on Kap Atholl in prostrate dwarf-shrub herb tundra (Table 1).

2.3. Plant Collections and DNA Extraction

[9] Ten to twenty individuals were sampled at each site. In the prostrate dwarf-shrub herb tundra common in northwestern Greenland, a male and a female plant were sampled at each of five locations evenly spaced across a 100 m transect in 2003 and 2004. At the other sites, where *S. arctica* was not as abundant, plants were sampled by walking in a line until ten individuals at least 3 m apart could be located. Either fresh leaf tissue or woody stems

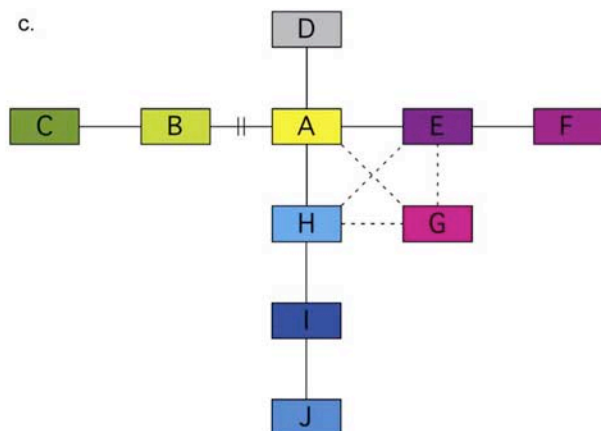
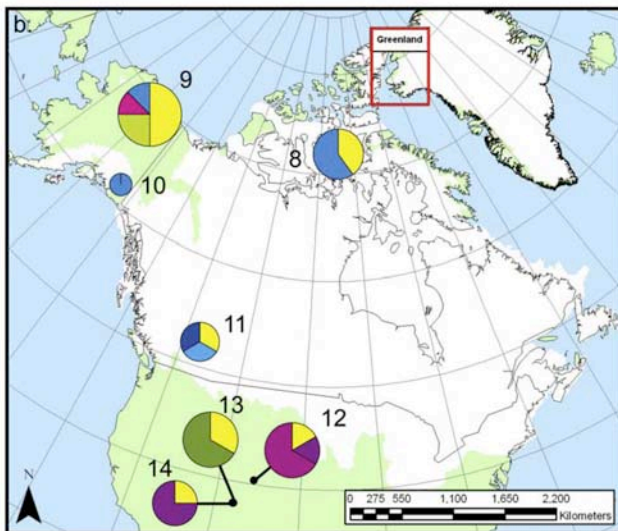
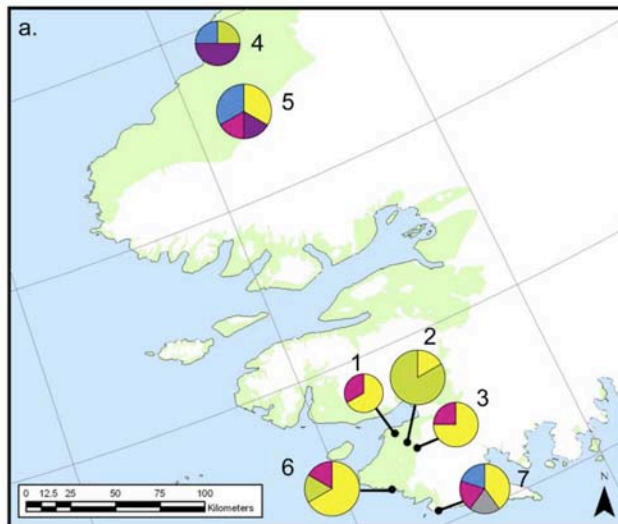


Figure 1. Locations sampled and frequencies of cpDNA haplotypes of *S. arctica* at each location in (a) northwestern Greenland and (b) at additional sites across its North American range. The area mapped in Figure 1a, is marked 'Greenland' in Figure 1b, and the current extent of the Greenland Ice Sheet is in white. In Figure 1b, the area in white is the maximum extent of ice across North America at the last glacial maximum [Frenzel, 1992]. Locations are numbered as in Table 1, the size of each pie is scaled by the number of individuals sampled, and the frequency of each haplotype is represented by a section of the pie. (c) Network showing relationships among haplotypes based on parsimony. Lines represent individual base-pair changes or insertion/deletion sites that differ between the haplotypes. One long insertion/deletion (25bp) is marked with a double hatch. Dashed lines indicate multiple alternate most-parsimonious connections between haplotypes. Haplotypes are lettered and color-coded to match the pie graphs in Figures 1a and 1b.

Table 2. Primer Sequences and Characteristics of the Four Microsatellite Loci Isolated From *Salix arctica*, Including Locus Name and GenBank Accession Number, Primer Sequences With Dye Indicated, PCR Annealing Temperature, Motif in Cloned Allele, and Size of the Sequenced Allele^a

Locus (Accession)	Primer Sequence (5' – 3')	T _a , °C	Repeat of Cloned Allele	Size, bp	Size Range, bp	Number of Alleles	H _E
S8	F: (VIC)(M-13)ATAAACAAGTGTGAAGGGGTGTG R: GATTAGGGCAATTGTCTTCATA	60	(GT) ₁₁	420	428 – 446	9	0.86
S147	F: (PET)(M-13)GGATCTACACGGGTACAGCATTAT R: ATTTGGAGTTCAATCAACACC	56	(GT) ₁₆	287	301 – 319	17	0.92
S28	F: (VIC)(M-13)TCTCTTGTGTACTCTTCCATTT R: TGATTACGAGTTCTGCTATTACAA	50	(CT) ₁₀	395	401 – 425	13	0.89
S37	F: (6-FAM)(M-13)AAGCCTTGATACGCGTCTGATT R: CAGAAGCCTGCTGCTGATGCTATC	65	(GAA) ₈	271	274 – 286	4	0.64

^aSummary information on the size range and numbers of alleles found in 35 individuals and expected heterozygosity for each locus.

were collected from individual plants. Leaf tissue was immediately stored in silica to dry, and stems were shipped to Colorado State University and planted. When stems leafed-out, leaves were collected. Genomic DNA was extracted from 1 to 18 individuals per site (Table 1) using approximately 300 mg of leaf tissue, dried, fresh or frozen at -80°C . DNA was extracted either using the CTAB method with reagents from AutoGen (Plant DNA, Ver. 1.01) or using Dneasy Plant Mini kits from Qiagen[®].

2.4. Development of Microsatellite Markers and Genotyping

[10] We focused our microsatellite genotyping on two locations. We genotyped 17 individuals from a coastal site on Kap Atholl approximately 21 km from the ice sheet, and 18 individuals from an inland site that is less than 1 km from the ice sheet (Table 1).

[11] Microsatellite cloning and sequencing was performed at the Evolutionary Genetics Core Facility of Cornell University following the protocol of *Hamilton et al.* [1999] with modifications following in *Marrs et al.* [2006]. Of 37 clones with possible microsatellites, four gave robust, relatively easy to score loci (Table 2). PCR amplifications for genotyping was performed in 10 μl reaction volumes with 1 μl extracted DNA, 1 μl 10 X buffer PCR, 0.8 μl MgCl₂ (25 mM), 0.08 μl dNTPs (100 mM), 0.2 μl Hotstart taq (5 U/ μl), 0.04 μl forward primer (10 nmole/ml), 0.17 μl reverse primer (10 nmole/ml), 0.17 μl M13 sequence labeled with dye (10 nmole/ml). After a preliminary denaturation step at 95°C for 8 min, PCR amplification was performed for 35 cycles of: 50 s denaturing at 95°C , 1min of annealing at locus specific temperatures (Table 2) and 1min 30 s of extension at 72°C , with a final 10 min extension step at 72°C . PCR products were stored at -20°C until genotyping. Diluted PCR products were mixed with 8.9 μl formamide and 0.1 μl GS 650 Liz Ladder, and denatured for 3 min at 90°C then separated on an ABI 3100 Genetic Analyzer and analyzed with the program GENE-MARKER 1.5 (SoftGenetics LLC[®], 2004).

2.5. cpDNA Sequencing

[12] An intergenic cpDNA region was amplified using primers trnQr and trnK2 from Dumolin-Lapegue et al. [1997]. PCR was performed in 10 μl reaction with 1 μl extracted DNA, 1 μl 10 X buffer PCR, 0.8 μl MgCl₂ (25mM), 0.08 μl dNTPs, 0.2 μl Hotstart taq, 0.2 μl forward and reverse primer. Cycling parameters were one denaturation cycle at 94°C for 8 min, followed by 30 cycles of 40 s

at 94°C to denature, 40 s at 50°C to anneal, 2 min at 72°C to extend and a final cycle at 72°C for 10 min to extend. PCR products were sequenced from both ends into the variable intergenic spacers using BigDye Terminator Cycle Sequencing (version 3.1) primed with the PCR primers. Sequencing reactions were cleaned up using ethanol precipitation, and separated on an ABI 3100 capillary sequencer. Sequences were aligned using the DNASTar package. We sequenced a single individual of each haplotype twice to verify our calls.

2.6. Identification of Asexually and Sexually Produced Individuals

[13] To infer whether sampled individuals were members of the same clone or were sexually reproduced, we compared multilocus microsatellite genotypes of individuals within sampling locations. Individuals that share common alleles at all four loci could be part of the same clonal individual, and therefore represent asexual reproduction, while individuals that have unique multilocus genotypes are likely to have originated from separate bouts of sexual reproduction. Somatic mutation in microsatellite loci could lead to variation among asexually produced plants which would inflate our estimate of sexual reproduction. Somatic mutation rates can vary widely by species and locus type [*Azaiez et al.*, 2006], but generally rates are low enough that this source of bias should be small.

2.7. Analysis of Genetic Variation

[14] For the microsatellite loci, the number of alleles and the number of private alleles for the two populations were tallied by hand. Expected heterozygosity corrected for sample size was estimated using SPAGeDi 1.2 [*Hardy and Vekemans*, 2002], a program that can accommodate data from polyploids such as *S. arctica*. We also used SPAGeDi to perform the equivalent of an AMOVA for polyploid data to determine whether the two populations differ significantly. A permutation test (20,000 permutations) was used to evaluate significance of the test.

[15] We compared cpDNA haplotype diversity and allelic richness controlling for sample size between Greenland, Alaskan, and Coloradan samples using CONTRIB [*Petit et al.*, 1998].

[16] We constructed haplotype networks from the sequence data using the network building software TCS 1.2.1 [*Clement et al.*, 2000], which uses statistical parsimony to construct an unrooted system of relationships between non-recombining sequences based on the genealogical reconstruction algorithm of *Templeton et al.* [1992]. In these analyses, individual

samples can be internal in the network. We treated indels as a 5th state [Giribet and Wheeler, 1999; Simmons and Ochoterena, 2000], and coded each indel as a single mutational event to prevent longer indels from overwhelming other signal in the data set [Simmons et al., 2001]. We evaluated population structure in cpDNA haplotypes by performing an AMOVA in Arlequin (version 2.0).

2.8. Phenotypic Variation in Plant Traits

[17] We measured three key plant traits that affect plant productivity: leaf mass per unit area (LMA), carbon isotope discrimination ($\Delta^{13}\text{C}$) and oxygen isotope discrimination ($\Delta^{18}\text{O}$). Our sampling scheme included five of the Greenlandic sites that had also been sampled for cpDNA in a crossed design of region by position from the Greenland Ice Sheet (Table 1). In mid-August 2003 at the end of the growing season, ten plants were sampled for LMA and leaf $\Delta^{13}\text{C}$ at sites 1–5 and for leaf $\Delta^{18}\text{O}$ at sites 1 and 2, where stem water $\Delta^{18}\text{O}$ data was available.

[18] To determine LMA, three leaves from one-year old vegetative stems were collected from each plant. Each leaf was scanned at 300 dpi, then oven-dried for 2 d to constant mass (60°C) and weighed to the nearest microgram. Leaf area was calculated from the scans using an unsupervised classification in ERDAS IMAGINE version 9.0 (Leica Geosystems Geospatial Imaging, LLC., Atlanta, GA). We calculated LMA for each leaf individually by dividing leaf mass by leaf area and then averaged the three values for each plant.

[19] An additional ten leaves were collected randomly from vegetative stems on each plant and pooled for the isotopic analyses. Samples were oven-dried for two days to constant mass (60°C), ground to a uniform texture, and analyzed using a Carlo Erba elemental analyzer (Thermo Electron Corp., Milan, Italy) interfaced with a continuous-flow Isochrom isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, UK) for leaf $\delta^{13}\text{C}$ and on a Euro-Vector Pyrolysis unit (Euro Vector, Milan, Italy) interfaced with VG-Optima stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, UK) for leaf $\delta^{18}\text{O}$ in the Natural Resource Ecology Laboratory at Colorado State University. Leaf carbon isotope discrimination ($\Delta^{13}\text{C}$) was calculated relative to $\delta^{13}\text{C}$ atmospheric CO_2 [O'Leary, 1993], with $\delta^{13}\text{C}$ atmospheric CO_2 held at -8.0‰ . A lower leaf $\Delta^{13}\text{C}$ corresponds with greater plant water use efficiency (WUE) or a higher amount of CO_2 fixed per unit water lost. Leaf $\delta^{18}\text{O}$ is reported as enrichment above source water [Barbour et al., 2000], using stem water $\delta^{18}\text{O}$ values for *S. arctica* at sites 1 (-14.4‰) and 2 (-15.1‰) [Sullivan and Welker, 2007]. A lower leaf $\Delta^{18}\text{O}$ corresponds with greater stomatal conductance or water loss. The data for each plant trait were analyzed using a one-way analysis of variance (ANOVA) to determine the effect of site and Tukey's HSD to determine differences among the means (SAS 9.1, SAS Institute, Cary, NC). A one-way ANOVA was appropriate, because the design was incomplete and without site-level replication.

2.9. Effect of *S. arctica* on LAI and Ecosystem CO_2 Exchange

[20] At one of the coastal sites on Kap Atholl (site 1), plant cover, the LAI and ecosystem CO_2 exchange were measured in 18 vegetated areas (sized 0.125 m^2), where

plant cover was greater than 70% at peak biomass, that varied in *S. arctica* abundance. Data were collected on eight dates during the growing season in 2004. Since these measurements were confined to vegetated areas that represent 50% or less of the area on Kap Atholl, we also measured plant cover and LAI at peak biomass in 82 randomly selected plots, in which plant cover ranged from 0 to 96%. Ecosystem CO_2 exchange was measured in a subset of these plots by selecting plots across the range of variation in the LAI. This sampling design assured that we could characterize spatial variation in plant cover and the LAI and relate this variation to ecosystem CO_2 exchange.

[21] Plant cover and the LAI were measured from multispectral images that were taken of each plot using a tripod-mounted, multispectral camera (Tetracam ADC, Chatsworth, CA). The camera is sensitive to green, red, and near-infrared (G, R, NIR) spectral bands at the following wavelength intervals: 520–620 nm (G), 620–750 nm (R), and 750–950 nm (NIR). The images were taken from nadir, under clear skies, near midday, at a set exposure. Images were also taken of a 99% reflectance white standard (Teflon), which was supplied with the camera and calibrated by Tetracam Inc. These images were used to normalize for incoming radiation and calculate reflectance. Visual estimates of plant cover and the proportion of plant cover composed of *S. arctica* to the nearest 10% were made from these images. The normalized difference vegetation index (NDVI) was calculated as the ratio of the difference between NIR and R reflectance to their sum. LAI was estimated from NDVI using an NDVI-LAI model developed for the ecosystem [Steltzer and Welker, 2006]. The seasonal change in LAI (ΔLAI) was calculated as the difference between the early season minimum LAI and maximum LAI during peak season. The season-long integration of LAI (iLAI) was calculated by multiplying the LAI by the number of days represented by each measurement and summing these products.

[22] Ecosystem CO_2 exchange was measured using a clear, Plexiglass chamber connected to a LiCor 6200 portable photosynthesis system (LiCor, Inc. Lincoln, NE) [Vourlitis et al., 1993]. The Plexiglass chamber was placed over a plot and weighted down with steel bars to create a closed system. Measurements of net ecosystem production (NEP) were made over a 30 s period under field conditions. The chamber was then covered to block all light for the measurement of ecosystem respiration (ER). Gross ecosystem production (GEP) was calculated as the difference between NEP and ER. Positive NEP characterizes a flux of CO_2 from the atmosphere into the ecosystem. Least linear squares regression was used to analyze the relationship between the proportion of plant cover composed of *S. arctica* and the LAI, the LAI and GEP, and GEP and NEP (SAS 9.1, SAS Institute, Cary, NC), which are the most relevant relationships for modeling the effect of *S. arctica* on ecosystem CO_2 exchange.

3. Results

3.1. Microsatellite Variation and Frequency of Sexual Reproduction

[23] All four microsatellite loci of *S. arctica* were highly variable (Table 2). The alleles from S8 and S37 conformed

Table 3. Haplotype Diversity, Number of Haplotypes, and Allelic Richness of *S. arctica*

Group ^a	<i>n</i>	Mean Haplotype Diversity (95% CI)	Number of Haplotypes	Allelic Richness
Alaska	9	0.78 (0.56–1.0)	4	3.0
Colorado	16	0.80 (0.72–0.88)	4	2.9
Greenland	39	0.77 (0.67–0.89)	6	3.3

^aSite 8 (Bellot Strait, Canada) and site 11 (Banff, Canada) were not included in this analysis.

well to models of microsatellite mutation given the repeat motifs (e.g., the allele sizes varied by twos for the dimer S8 and by threes for trimer S37). Although variable, the other two microsatellite loci did not conform as well. Most alleles from the dimer S28 followed a pattern consistent with insertions or deletions (indels) of a single repeat motif, with a few additional odd alleles suggesting indels of other sizes. S14, also a dimer, was highly variable, and had many alleles that varied by a single base pair, rather than the expected two. Evaluating all four loci, none of the individuals shared the exact multilocus microsatellite genotype. Even restricting the evaluation of multilocus genotypes to S8 and S37 to be conservative, only two individuals of 17 at the coastal site on Kap Atholl and two of 18 at the inland site nearest the ice sheet shared a multilocus genotype. Thus, it is likely that the individuals sampled were almost entirely produced by sexual reproduction. Average heterozygosity across alleles did not differ between the older, more fertile coastal site (mean \pm SD = 0.80 \pm 0.16) and younger, less fertile inland site on Kap Atholl (0.84 \pm 0.08). Additionally, pairwise F_{ST} was not significantly different (F_{ST} = 0.0039, P = 0.59).

3.2. Intraspecific Variation in cpDNA Haplotypes

[24] Chloroplast DNA haplotype diversity and allelic richness were high overall and similar among the regions sampled despite different glacial histories (Table 3). In all, we found six chloroplast haplotypes in only 39 samples in northwestern Greenland. Two to four cpDNA haplotypes were identified at each site (Table 1 and Figure 1a). There was one most common haplotype (A) that was found at all locations sampled except for the coastal site on Kap Russell. In 28 samples from across much of the North American range of *S. arctica*, eight cpDNA haplotypes were identified (Table 1 and Figure 1b). The most common haplotype was the same found to be most common within Greenland (A) and was found in all but one location (Site 10, at which only a single individual was sampled). Four new haplotypes were found in the additional samples across the range of *S. arctica* in North America (C, F, H, I), while two found in Greenland (D, E) were not present within these samples. The four, unique haplotypes all occurred in the alpine populations sampled in Colorado and in Banff, Canada. All haplotypes from Alaska were also found in Greenland. This geographical arrangement of haplotypes represents significant population structure (Table 4). Most of the variation was found

within populations, but there was significant structuring associated with individual sample locations within the geographical groupings. Somewhat surprisingly, there was no significant variation attributable to differences between Alaska, Colorado and Greenland.

3.3. Phenotypic Variation in Plant Traits

[25] Leaf mass area and two integrative measures of plant water relations (leaf $\Delta^{13}C$ and $\Delta^{18}O$) varied substantially among the *S. arctica* plants sampled in northwestern Greenland (Figure 2). Site explained 4.6% to 37.3% of the variance in the plant traits. The remaining variance, 62.7% to 95.4%, occurred within sites (Table 5). Variation in LMA was nearly continuous across its range from 62 g m⁻² to 109 g m⁻² with one value higher than this range (Figure 2a). Average LMA was lower at the inland sites on Kap Atholl and Kap Russell (Figure 2b). The range of variation was greater for leaf $\Delta^{13}C$ (18.6–22.8 ‰) than for leaf $\Delta^{18}O$ (27.5–30.6 ‰), but leaf $\Delta^{13}C$ was measured at more sites (Figures 2c and 2e). Average leaf $\Delta^{13}C$ was lower for plants sampled at the inland site on Kap Russell (Figure 2d). Average leaf $\Delta^{18}O$ did not vary between the two sites on Kap Atholl where it was measured (Figure 2f).

3.4. Effect of *S. arctica* on LAI and Ecosystem CO₂ Exchange

[26] The seasonal change in the leaf area index (Δ LAI) and the season-long integration of LAI (iLAI), which were measured in vegetated areas in the ecosystem, were both positively correlated to the proportion of plant cover composed of *S. arctica* leaves (Figures 3a and 3b). Since *S. arctica* is the most abundant deciduous species in this ecosystem, it was not surprising that the proportion of plant cover composed of *S. arctica* was correlated to Δ LAI. However, this seasonal increase in LAI was large enough and/or was sustained long enough that iLAI was also correlated to the abundance of *S. arctica*. GEP was correlated to LAI throughout the growing season (Figure 3c), and NEP was correlated to GEP (Figure 3d). These vegetated areas of the ecosystem were generally a source of CO₂ to the atmosphere during the growing season except where *S. arctica* was abundant and thus LAI and GEP were highest. Similarly, positive rates of NEP across bare and vegetated areas of the ecosystem were dependent on high rates of GEP, which only occurred where the LAI was high (Figures 4b and 4c). High values of the LAI were restricted to locations where *S. arctica* was abundant (Figure 4a). *S. arctica* was 58% of plant cover on average where it was present, but was not present in over half of the plots. Overall, the proportion of variation in LAI explained by the abundance of *S. arctica* was low (R^2 = 0.21 and 0.23 for seasonal and spatial variation, respectively). However, these

Table 4. Analysis of Molecular Variance of the Chloroplast DNA Sequences From Alaska, Colorado, and Greenland

Source of Variation	d.f.	Sum of Squares	Variance Components	Percent of Variation
Among groups	2	1.58	-0.002	-0.6
Among populations within groups	11	7.78	0.084	20.5 ^a
Within populations	53	17.38	0.328	80.0 ^a

^a $P < 0.00001$.

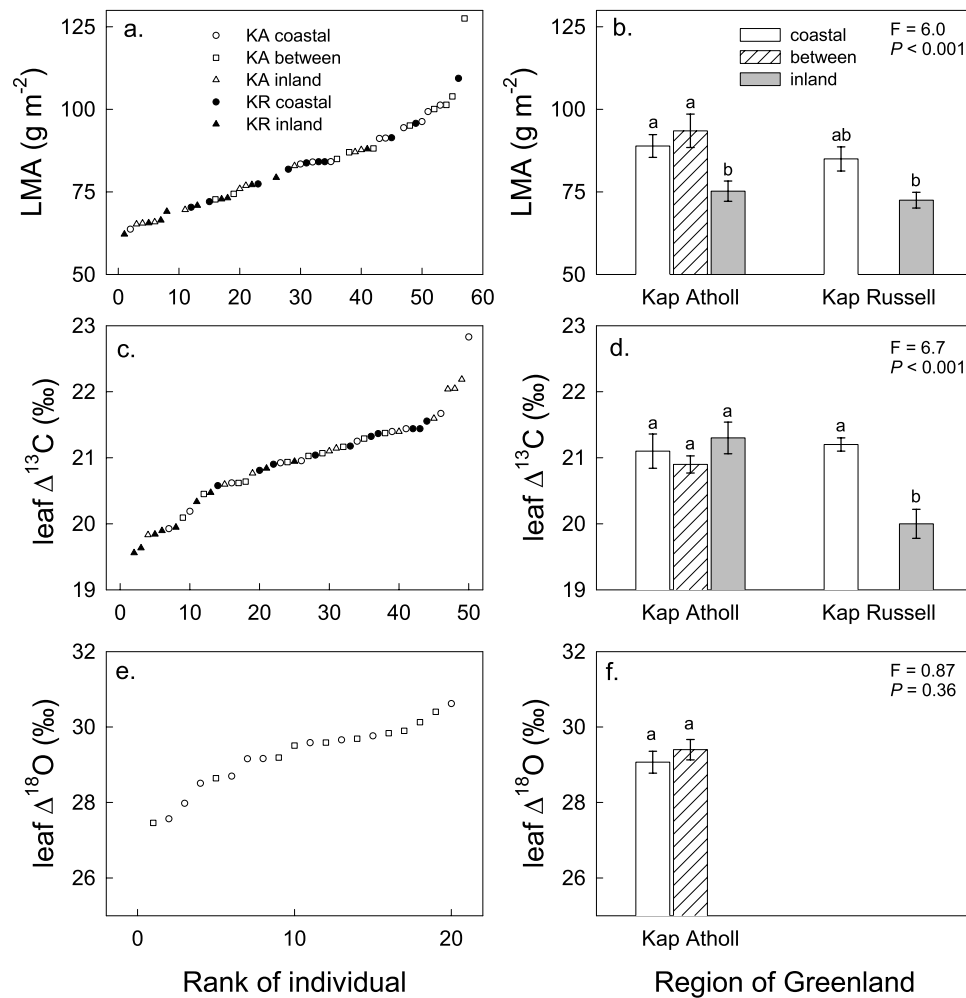


Figure 2. Phenotypic variation in plant traits of *S. arctica* in northwestern Greenland. (a, b) Leaf mass area (LMA); (c, d) leaf $\Delta^{13}\text{C}$; and (e, f) leaf $\Delta^{18}\text{O}$. Results of a one-way ANOVA are reported, and significant differences among sites are noted by different letters. Bars are means \pm 1 SE (n = 10).

relationships are independent of total plant cover, which in large part determines LAI and thus GEP and NEP.

4. Discussion

4.1. Frequent Sexual Reproduction and High Genetic Variation

[27] In northwestern Greenland, the genetic variation in *S. arctica* at microsatellite loci indicates that sexual repro-

duction has been common as the ice sheet has retreated. No more than two individuals of the 17 sampled at the coastal site on Kap Atholl and two of the 18 at the inland site near the ice sheet shared a multilocus genotype and could have been reproduced asexually. The high heterozygosities we observed reflect both sexual reproduction and polyploidy. Only one of the six copies of a microsatellite in a hexaploid needs to differ from the others for that individual to be labeled a heterozygote. Few microsatellite data from natural

Table 5. One-Way Analysis of Variance of Plant Traits in Greenland

Plant Trait	Source of Variation	df	Sum of Squares	Mean Squares	F	P Value	Percent of Variation
LMA	Site	4	3156	789	6.0	<0.001	35.3%
	Error	44	5776	131			
	Total	48	8932				
Leaf $\Delta^{13}\text{C}$	Site	4	10.49	2.62	6.70	<0.001	37.3%
	Error	45	17.62	0.39			
	Total	49	28.11				
Leaf $\Delta^{18}\text{O}$	Site	1	0.67	0.67	0.87	0.36	4.6%
	Error	18	13.92	0.77			
	Total	19	14.59				

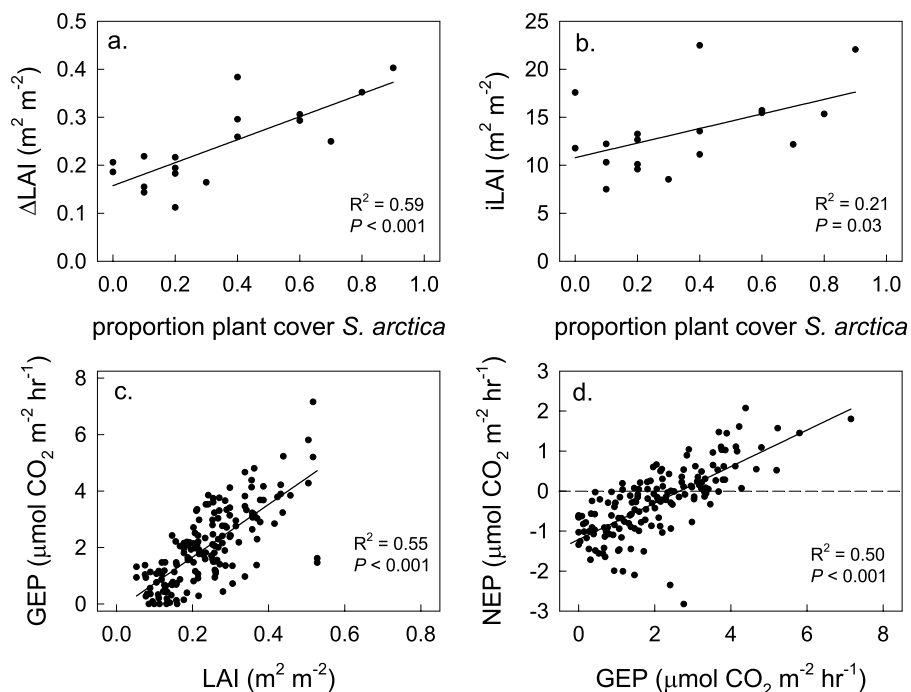


Figure 3. Relationships between (a, b) the proportion of plant cover composed of *S. arctica* and the LAI; (c) the LAI and GEP; and (d) GEP and NEP. Δ LAI is the seasonal maximum LAI minus the early season minimum LAI, and iLAI is the sum of LAI across the growing season. Data are for measurements throughout the growing season in vegetated areas where plant cover was greater than 70% at peak biomass. Least linear squares regressions are reported ($n = 18$ or $n = 162$).

populations of hexaploids are published, but one recent study of *Ranunculus cassubicus* shows that a hexaploid had heterozygosities of 1 across six different populations. Thus, by comparison our reported heterozygosities of 0.8 are modest. The lack of significant differentiation between the two sites suggests that similar colonization events have occurred over time, coastal sites were the source of seed for inland sites, and/or genetic exchange has occurred between these two sites.

[28] Rampant sexual reproduction in a High Arctic population of *S. arctica* challenges the long-held idea that sexual reproduction is rare in such an extreme environment [Wager, 1938; Bliss, 1971; Bell and Bliss, 1980; Billings,

1987]. Yet, the primary observation that led to this idea, high seedling mortality, may not be inconsistent with this result. In cold, dry environments, plant growth and thus clonal reproduction has likely been so slow that few seedlings may have needed to survive for sexually reproduced individuals to be common. Additionally, high seedling mortality is common to many environments and does not preclude sexual reproduction [Forbis, 2003]. Repeated demographic studies would be necessary to identify the frequency of sexual reproduction in a population, but have rarely been practical to do in the Arctic. Studies of seedling demography have rarely if ever exceeded two years [Wager, 1938; Mooney and Billings, 1961; Callaghan and Collins,

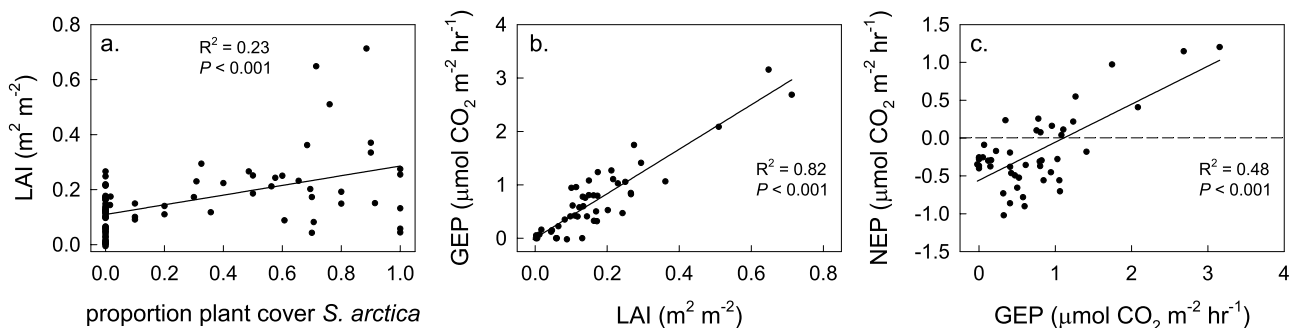


Figure 4. Relationships between (a) the proportion of plant cover composed of *S. arctica* and the LAI; (b) the LAI and GEP; and (c) GEP and NEP. Plant cover and LAI were measured at peak biomass in 82 randomly selected plots that varied in plant cover (0 to 96%). GEP and NEP were measured on a subset of 46 plots selected across the range of variation in LAI. Least linear squares regressions are reported ($n = 82$ or $n = 46$).

1976; Bell and Bliss, 1980; Bliss and Gold, 1999]. Genetic studies do not require multiple years of observation, produce more definitive results, and are becoming a common approach. Our study contributes to a growing body of genetic evidence confirming that frequent sexual reproduction can occur in arctic and alpine plants [Gabrielsen and Brochmann, 1998; Linhart and Gehring, 2003] and has led to the evolution of more species than previously thought [Grundt et al., 2006].

[29] Genetic variation in cpDNA haplotypes of *S. arctica* was high in northwestern Greenland and across its range in North America. Haplotype diversity was higher than that found in another Arctic species, *Saxifraga oppositifolia* [Abbott and Comes, 2003]. Dispersal of sexually reproduced propagules may contribute to this variation. Similarly, such dispersal is likely to account for the widespread distribution of some haplotypes of *S. arctica* within and across regions of the North American Arctic. In species that mainly reproduce clonally, widespread genotypes are relatively rare [Ellstrand and Roose, 1987]. Genetic variation can occur in predominantly clonal populations, because often they are comprised of multiple clones, a result of being founded through sexually reproduced individuals [Ellstrand and Roose, 1987]. However, such populations tend to have fewer genotypes, and these genotypes tend to be found in only one population [Holsinger, 2000]. Of the ten haplotypes identified across the North American range of *S. arctica*, four were found at only one site, and all four were at alpine sites in Colorado and southern Canada. This pattern may indicate that sexual reproduction in *S. arctica* occurs more frequently in the Arctic than in the alpine. However, we think it more likely that the alpine sites, as ecological islands, are simply more geographically isolated than most arctic sites [DeChaine and Martin, 2005].

[30] On Kap Atholl, common haplotypes of *S. arctica* were identified at younger sites (sites 2 and 3) near the Greenland Ice Sheet and at older, more fertile sites along the coast (sites 1 and 6) (Figure 1a). This suggests that plants at coastal sites on Kap Atholl may be the source of plants that have established at inland sites as the ice sheet has retreated and that these haplotypes can establish in diverse environments. Interestingly, haplotypes E and J, the two haplotypes common to both sites on Kap Russell, were both not found in our Kap Atholl samples.

[31] Overall, the pattern of variation in cpDNA haplotypes across North America suggests that glaciation did not lead to a permanent decrease in the genetic variation of *S. arctica*. Haplotype diversity and allelic richness were similar among the three regions despite their different glacial histories (Table 3), and many of the haplotypes found in Alaska and northern Canada were also found in northwestern Greenland. The genetic composition of alpine sites in the southern range of *S. arctica* was most distinct. Shared haplotypes between the alpine and Arctic regions of North America are consistent with an alpine origin for this species. The frequent presence of unique haplotypes at alpine sites suggests that divergence may have contributed to the genetic variation in the southern range of *S. arctica*, where it now only occurs in alpine environments. The patterns seen in *S. arctica* in North America are similar to *Saxifraga oppositifolia* and *Dryas integrifolia*, two other High Arctic species, in revealing relatively high genetic

variation [Tremblay and Schoen, 1999; Abbott et al., 2000; Abbott and Comes, 2003; Philipp and Siegismund, 2003]. Our findings differ from those of Abbott and Comes [2003] for *Saxifraga oppositifolia* with respect to population structure. We found most cpDNA variation was within and among populations of *S. arctica*, while they found variation at those levels and between broader geographical groupings. The lack of support for differentiation between Colorado, Alaska, and Greenland could stem from both our small sample sizes and high rates of gene flow between regions.

4.2. Implications for a Terrestrial Feedback to Climate Change in the High Arctic

[32] Gross ecosystem production in prostrate dwarf-shrub herb tundra was affected by variation in the seasonal and spatial abundance of *S. arctica*. The LAI was highest where *S. arctica* was abundant and could explain 55% of the temporal variation and 82% of the spatial variation in GEP. Across diverse tundra ecosystems, the LAI explains much of the variation in GEP [Shaver et al., 2007; Street et al., 2007], because GEP is more sensitive to LAI than to foliar N [Williams and Rastetter, 1999]. Thus, the abundance of species such as *S. arctica* that influence the LAI affect ecosystem CO₂ uptake and in part determine whether an ecosystem is a source or sink of CO₂ to the atmosphere. We suggest that frequent sexual reproduction and high genetic variation in *S. arctica* increase the likelihood that this species will be able to survive and potentially thrive as climate changes. Sexual reproduction could lead to the formation of new genotypes that are better adapted to the new climatic conditions [Davis et al., 2005; Jump and Penuelas, 2005; Parmesan, 2006], and high genetic variation increases the likelihood that successful genotypes already exist. Phenotypic variation on which selection can act was also high in *S. arctica*. Because the abundance of *S. arctica* was linked to carbon sequestration, adaptation to the changing climate could facilitate a negative feedback that could help counter the many positive feedbacks to climate change in the Arctic.

[33] **Acknowledgments.** We thank: W. Morris, P. Ray and K. Westergaard for assisting with the collection of *S. arctica*; M. Smith, J. Decant, D. Banks and M. Welker for field assistance; and E. Steltzer for the maps. D. Reuss provided assistance with the isotopic analyses. Thule Airbase and VECO Polar Resources provided logistical support in Greenland, and the Mountain Studies Institute and the Center for Snow and Avalanche Studies provided logistical support in the San Juan Mountains. Funding for this research included NSF-OPP grant 0221606 to J.M.W. and funding from the Colorado Agricultural Experiment Station to R.A.H.

References

- Abbott, R. J., and C. Brochmann (2003), History and evolution of the arctic flora: in the footsteps of Eric Hultén, *Mol. Ecol.*, *12*, 299–313.
- Abbott, R. J., and H. P. Comes (2003), Evolution in the Arctic: a phylogeographic analysis of the circumpolar plant, *Saxifraga oppositifolia* (Purple saxifrage), *New Phytol.*, *2003*, 211–224.
- Abbott, R. J., L. C. Smith, R. I. Milne, R. M. M. Crawford, K. Wolff, and J. Balfour (2000), Molecular analysis of plant migration and refugia in the Arctic, *Science*, *289*, 1343–1346.
- ACIA (2005), *Arctic Climate Impact Assessment*, Cambridge Univ. Press, New York.
- Alsos, I. G., T. Engelskjøn, L. Gielly, P. Taberlet, and C. Brochmann (2005), Impact of ice ages on circumpolar molecular diversity: insights from an ecological key species, *Mol. Ecol.*, *14*, 2739–2753.
- Azaiez, A., E. F. Bouchard, M. Jean, and F. J. Belzile (2006), Length, orientation, and plant host influence the mutation frequency in microsatellites, *Genome*, *49*, 1366–1373.

- Barbour, M. M., R. A. Fischer, K. D. Sayre, and G. D. Farquhar (2000), Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat, *Aust. J. Plant Physiol.*, *27*, 625–637.
- Bell, K. L., and L. C. Bliss (1980), Plant reproduction in a high arctic environment, *Arct. Alp. Res.*, *12*, 1–10.
- Billings, W. D. (1987), Constraints to plant growth, reproduction, and establishment in arctic environments, *Arct. Antarct. Alp. Res.*, *19*, 357–365.
- Billings, W. D., and H. A. Mooney (1968), The ecology of arctic and alpine plants, *Biol. Rev. Camb. Philos. Soc.*, *43*, 481–530.
- Bliss, L. C. (1971), Arctic and alpine plant life cycles, *Annu. Rev. Ecol. Syst.*, *2*, 405–438.
- Bliss, L. C., and W. G. Gold (1999), Vascular plant reproduction, establishment, and growth and the effects of cryptogamic crusts within a polar desert ecosystem, Devon N. W. T. Island, Canada, *Can. J. Bot.*, *77*, 623–636.
- Callaghan, T. V., and N. J. Collins (1976), Strategies of growth and population dynamics of tundra plants, *Oikos*, *27*, 383–388.
- CAVMteam (2003), *Circumpolar Arctic Vegetation Map. Conservation of Arctic Flora and Fauna (CAFF) Map No. 1*, U. S. Fish and Wildlife Serv., Anchorage.
- Chapin, F. S., and M. C. Chapin (1981), Ecotypic differentiation of growth processes in *Carex aquatilis* along latitudinal and local gradients, *Ecology*, *62*, 1000–1009.
- Chapin, F. S., III, G. R. Shaver, A. E. Giblin, K. J. Nadelhoffer, and J. A. Laundre (1995), Responses of Arctic tundra to experimental and observed changes in climate, *Ecology*, *76*, 694–711.
- Chapin, F. S., et al. (2005), Role of land-surface changes in Arctic summer warming, *Science*, *310*, 657–660.
- Clement, M., D. Posada, and K. A. Crandall (2000), TCS: a computer program to estimate gene genealogies, *Mol. Ecol.*, *9*, 1687–1699.
- Cooper, E. J., I. G. Alsos, D. Hagen, F. M. Smith, S. J. Coulson, and I. D. Hodkinson (2004), Plant recruitment in the High Arctic: Seed bank and seedling emergence on Svalbard, *J. Veg. Sci.*, *15*, 115–124.
- Davis, M. B., R. G. Shaw, and J. R. Etterson (2005), Evolutionary responses to changing climate, *Ecology*, *86*, 1704–1714.
- Dawson, T. E., and L. C. Bliss (1989a), Intraspecific variation in the water relations of *Salix arctica*, an arctic-alpine dwarf willow, *Oecologia*, *79*, 322–331.
- Dawson, T. E., and L. C. Bliss (1989b), Patterns of water use and the tissue water relations in the dioecious shrub, *Salix arctica*: The physiological basis for habitat partitioning between the sexes, *Oecologia*, *79*, 332–343.
- Dawson, T. E., and L. C. Bliss (1993), Plants as mosaics: leaf-, ramet-, and gender-level variation in the physiology of the dwarf willow, *Salix arctica*, *Funct. Ecol.*, *7*, 293–304.
- DeChaine, E. G., and A. P. Martin (2005), Marked genetic divergence among sky island populations of *Sedum lanceolatum* (Crassulaceae) in the Rocky Mountains, *Am. J. Bot.*, *92*, 477–486.
- Ellstrand, N. C., and M. L. Roose (1987), Patterns of genotypic diversity in clonal plant species, *Am. J. Bot.*, *74*, 123–131.
- Epstein, H. E., M. D. Walker, and A. M. Starfield (2000), A transient, nutrient-based model of arctic plant community response to climatic warming, *Ecol. Appl.*, *10*, 824–841.
- Fetcher, N., and G. R. Shaver (1990), Environmental sensitivity of ecotypes as potential influence on primary productivity, *Am. Nat.*, *136*, 126–131.
- Forbis, T. A. (2003), Seedling demography in an alpine ecosystem, *Am. J. Bot.*, *90*, 1197–1206.
- Franks, S. J., S. Sim, and A. E. Weis (2007), Rapid evolution of flowering time by an annual plant in response to a climate fluctuation, *Proc. Natl. Acad. Sci. U. S. A.*, *104*, 1278–1282.
- Frenzel, B. (1992), *Atlas of Paleoclimates and Paleoenvironments of the Northern Hemisphere*, Gustav Fisher Verlag, Stuttgart.
- Gabrielsen, T. M., and C. Brochmann (1998), Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers, *Mol. Ecol.*, *7*, 1701–1708.
- Giribet, G., and W. C. Wheeler (1999), On gaps, *Mol. Phylogenet. Evol.*, *13*, 132–143.
- Gough, L. (2006), Neighbor effects on germination, survival, and growth in two arctic tundra plant communities, *Ecography*, *29*, 44–56.
- Grundt, H. H., S. Kjølner, L. Borgen, L. Rieseberg, and C. Brochmann (2006), High biological species diversity in the arctic flora, *Proc. Natl. Acad. Sci. U. S. A.*, *103*, 972–975.
- Hamilton, M. B., E. L. Pincus, A. Di Fiore, and R. C. Fleischer (1999), Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites, *Biotechnology*, *27*, 500–507.
- Hardy, O. J., and X. Vekemans (2002), SPAGEDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels, *Mol. Ecol. Notes*, *2*, 618–620.
- Hodkinson, I. D., S. J. Coulson, and N. R. Webb (2003), Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard, *J. Ecol.*, *91*, 651–663.
- Holsinger, K. E. (2000), Reproductive systems and evolution in vascular plants, *Proc. Natl. Acad. Sci. U. S. A.*, *97*, 7037–7042.
- Hultén, E. (1937), *Outline of the History of Arctic and Boreal Biota During the Quaternary Period*, Cramer, New York.
- Hultén, E. (1968), *Flora of Alaska and Neighboring Territories*, Stanford Univ. Press, Stanford.
- Jones, M. H., C. Bay, and U. Nordenhall (1997), Effects of experimental warming on arctic willows (*Salix* spp.): a comparison of responses from the Canadian High Arctic, Alaskan Arctic, and Swedish Subarctic, *Glob. Change Biol.*, *3*, 55–60.
- Jones, M. H., S. E. Macdonald, and G. H. R. Henry (1999), Sex- and habitat-specific response of a high arctic willow, *Salix arctica*, to experimental climate change, *Oikos*, *87*, 129–138.
- Jump, A. S., and J. Penuelas (2005), Running to stand still: adaptation and the response of plants to rapid climate change, *Ecol. Lett.*, *8*, 1010–1020.
- Linhart, Y. B., and J. L. Gehring (2003), Genetic variability and its ecological implications in the clonal plant *Carex scopolorum* Holm. in Colorado tundra, *Arct. Antarct. Alp. Res.*, *35*, 429–433.
- Lloyd, A. H. (2005), Ecological histories from Alaskan tree lines provide insight into future change, *Ecology*, *86*, 1687–1695.
- Marchand, F. L., I. Nijs, H. J. de Boeck, F. Kockelbergh, and S. Mertens (2004), Increased turnover but little change in the carbon balance of High-Arctic tundra exposed to whole growing season warming, *Arct. Antarct. Alp. Res.*, *36*, 298–307.
- Mars, R. A., R. A. Hufbauer, S. M. Bogdanowicz, and R. Sforza (2006), Nine polymorphic microsatellite markers in *Centaurea stoebe* L. [subspecies *C.s. stoebe* and *C.s. micranthos* (S. G. Gmelin ex Gugler) Hayek] and *C. diffusa* Lam. (Asteraceae), *Mol. Ecol. Notes*, *6*, 897–899.
- McGraw, J. B., and J. Antonovics (1983), Experimental ecology of *Dryas octopetala* ecotypes. I. ecotypic differentiation and life-cycle stages of selection, *J. Ecol.*, *71*, 879–897.
- McGuire, A. D., F. S. Chapin, J. E. Walsh, and C. Wirth (2006), Integrated regional changes in Arctic climate feedbacks: implications for the global climate system, *Annu. Rev. Ecol. Syst.*, *31*, 61–91.
- Molau, U., and MøP. Igaard (1996), *ITEX manual*, Danish Polar Cent, Copenhagen.
- Mooney, H. A., and W. D. Billings (1961), Comparative physiological ecology of arctic and alpine populations of *Oxyria digyna*, *Ecol. Monogr.*, *31*, 1–29.
- Murray, D. F. (1995), *Arctic and Alpine Biodiversity: Patterns, Causes, and Ecosystem Consequences*, edited by F. S. Chapin and C. Körner, pp. 21–32, Springer, Heidelberg, Germany.
- Murray, D. F. (1997), Systematics of the ITEX species, *Glob. Change Biol.*, *3*, 10–19.
- Norberg, J., D. P. Swaney, J. Dushoff, J. Lin, R. G. Casagrandi, and S. A. Levin (2001), Phenotypic diversity and ecosystem functioning in changing environments: A theoretical framework, *Proc. Natl. Acad. Sci.*, *98*, 11,376–11,381.
- O'Leary, M. H. (1993), Biochemical basis of carbon isotope fractionation, in *Stable Isotopes and Plant Carbon-Water Relations*, edited by J. R. Ehleringer, A. E. Hall, and G. D. Farquhar, pp. 19–28, Academic, San Diego, Calif.
- Parnesan, C. (2006), Ecological and evolutionary responses to recent climate change, *Annu. Rev. Ecol. Syst.*, *37*, 637–669.
- Peterson, B., J. McClelland, R. Curry, R. Holmes, J. E. Walsh, and K. Aagard (2006), Trajectory shifts in the Arctic and subarctic freshwater cycle, *Science*, *313*, 1061–1066.
- Petit, R. J., et al. (2003), Glacial refugia: hotspots but not melting pots of genetic diversity, *Science*, *300*, 1563–1565.
- Petit, R. J., A. El Mousadik, and O. Pons (1998), Identifying populations for conservation on the basis of genetic markers, *Conserv. Biol.*, *12*, 844–855.
- Philipp, M., and H. R. Siegismund (2003), What can morphology and isozymes tell us about the history of the *Dryas integrifolia*-*octopetala* complex?, *Mol. Ecol.*, *12*, 2231–2242.
- Raven, J. A. (1992), The physiology of *Salix*, *Proc. R. Soc. Edinburgh*, *98B*, 49–62.
- Rehfeldt, G. E., C. C. Ying, D. L. Spittlehouse, and D. A. Hamilton (1999), Genetic responses to climate in *Pinus contorta*: niche breadth, climate change, and reforestation, *Ecol. Monogr.*, *69*, 375–407.
- Robinson, C. H., P. A. Wookey, J. A. Lee, T. V. Callaghan, and M. C. Press (1998), Plant community responses to simulated environmental change at a high arctic polar semi-desert, *Ecology*, *79*, 856–866.
- Serreze, M. C., M. M. Holland, and J. Stroeve (2007), Perspectives on the Arctic's shrinking sea-ice cover, *Science*, *315*, 1533–1536.
- Shaver, G. R., L. E. Street, E. B. Rastetter, M. T. Van Wijk, and M. Williams (2007), Functional convergence in regulation of net CO₂

- flux in heterogenous tundra landscapes in Alaska and Sweden, *J. Ecol.*, *95*, 802–817.
- Simmons, M. P., and H. Ochoterena (2000), Gaps as characters in sequence-based phylogenetic analyses, *Syst. Biol.*, *50*, 454–462.
- Simmons, M. P., H. Ochoterena, and T. G. Carr (2001), Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses, *Syst. Biol.*, *50*, 454–462.
- Skrede, I., P. B. Eidesen, R. P. Portela, and C. Brochmann (2006), Refugia, differentiation and postglacial migration in arctic-alpine Eurasia, exemplified by the mountain avens (*Dryas octopetala* L.), *Mol. Ecol.*, *15*, 1827–1840.
- Steltzer, H., and J. M. Welker (2006), Modeling the effect of vegetation properties on the NDVI-LAI relationship, *Ecology*, *87*, 2765–2772.
- Street, L. E., G. R. Shaver, M. Williams, and M. T. Van Wijk (2007), What is the relationship between changes in canopy leaf area and changes in CO₂ flux in Arctic ecosystems?, *J. Ecol.*, *95*, 139–150.
- Sturm, M., C. Racine, and K. Tape (2001), Increasing shrub abundance in the Arctic, *Nature*, *411*, 546–547.
- Sturm, M., J. Schimel, G. Michaelson, J. M. Welker, S. F. Oberbauer, G. E. Liston, J. Fahnestock, and V. E. Romanovsky (2005), Winter biological processes could help convert arctic tundra to shrubland, *Bioscience*, *55*, 17–25.
- Suda, Y., and G. W. Argus (1969), Chromosome numbers of some North American Arctic and Boreal *Salix*, *Can. J. Bot.*, *47*, 859–862.
- Sullivan, P. F., and J. M. Welker (2007), Variation in leaf physiology of *Salix arctica* within and across ecosystems in the High Arctic: test of dual isotope ($\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$) conceptual model, *Oecologia*, *151*, 372–386.
- Sullivan, P. F., S. J. T. Arens, R. A. Chimner, and J. M. Welker (2008), Temperature and microtopography interact to control carbon cycling in a high arctic fen, *Ecosystems (N. Y., Print)*, *11*(1), doi:10.1007/s10021-007-9107-y.
- Tape, K., M. Sturm, and C. H. Racine (2006), The evidence for shrub expansion in Northern Alaska and the Pan-Arctic, *Glob. Change Biol.*, *12*, 686–702.
- Teeri, J. A. (1973), Polar desert adaptations of a high arctic plant species, *Science*, *179*, 496–497.
- Templeton, A. R., K. A. Crandall, and C. F. Sing (1992), A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. 3. Cladogram estimation, *Genetics*, *132*, 619–633.
- Tremblay, N. O., and D. J. Schoen (1999), Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization, *Mol. Ecol.*, *8*, 1187–1198.
- Vourlitis, G. L., W. C. Oechel, and S. J. Hastings (1993), A system for measuring in situ CO₂ and CH₄ flux in unmanaged ecosystems: an arctic example, *Funct. Ecol.*, *7*, 369–379.
- Wager, H. G. (1938), Growth and survival of plants in the arctic, *J. Ecol.*, *26*, 390–410.
- Walker, M. D., et al. (2006), Plant community responses to experimental warming across the tundra biome, *Proc. Natl. Acad. Sci. U. S. A.*, *103*, 1342–1346.
- Welker, J. M., U. Molau, A. N. Parsons, C. H. Robinson, and P. A. Wookey (1997), Responses of *Dryas octopetala* to ITEX environmental manipulations: a synthesis with circumpolar comparisons, *Glob. Change Biol.*, *3*, 61–73.
- Welker, J. M., J. T. Fahnestock, and G. H. R. Henry (2004), CO₂ exchange in three Canadian High Arctic ecosystems: response to long-term experimental warming, *Glob. Change Biol.*, *10*, 1981–1995.
- Williams, M., and E. B. Rastetter (1999), Vegetation characteristics and primary productivity along an arctic transect: implications for scaling-up, *J. Ecol.*, *87*, 885–898.
- Wilson, J. W. (1964), Annual growth of *Salix arctica* in the High Arctic, *Ann. Bot. (Lond.)*, *28*, 71–72.
- Zhuang, Q., J. M. Melillo, M. C. Sarofim, D. W. Kicklighter, A. D. McGuire, B. S. Felzer, A. Sokolov, R. G. Prinn, P. A. Steudler, and S. Hu (2006), CO₂ and CH₄ exchanges between land ecosystems and the atmosphere in northern high latitudes over the 21st century, *Geophys. Res. Lett.*, *33*, L17403, doi:10.1029/2006GL026972.

M. Casalis, Les Millets, 03290 Dom Pierre sur Besbre, France.

R. Chimner, Ecosystem Science Center, School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA.

R. A. Hufbauer, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, USA.

H. Steltzer, Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523-1499, USA. (steltzer@nrel.colostate.edu)

P. F. Sullivan and J. M. Welker, Environment and Natural Resources Institute, University of Alaska, 707 A St., Anchorage, AK 99501, USA.