The role of polyploidy in the systematics and evolution of arctic alkali grass (*Puccinellia*) in North America

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For Mom and Dad

and Allan

Abstract

In many plant groups, the number of polyploid species increases as one travels further north from the tropics to the Arctic, but little is known about whether any of the arctic species, diploid or polyploid, have origins in the Arctic. I use flow cytometry, AFLP data, sequences of nuclear ribosomal DNA and chloroplast DNA, restriction site analysis, pollen fertility determinations, field morphology, and common garden experiments to investigate the taxonomic limits of diploid putative parental species and to taxonomically delimit and investigate the origins of three polyploid species of *Puccinellia* (Poaceae) that are widespread in the North American Arctic. Six arctic diploid species are genetically and/or morphologically distinct, forming two lineages, while the two temperate North American diploid species form a third lineage. I synonymize three species into *P. arctica*, and describe a new diploid species, *P. banksiensis*, which is found only in the western North American Arctic. The three polyploid species, tetraploid *P. bruggemannii*, hexaploid *P. angustata*, and primarily octoploid *P. andersonii* are genetically distinct. They show allopolyploid origins, having AFLP bands and sequence mutations of two arctic diploids and the arctic triploid/tetraploid *P. phryganodes*. *Puccinellia bruggemannii* has a different nuclear ribosomal DNA history than *P. angustata* and *P. andersonii*, interpreted as resulting from different paths of concerted evolution, but all three share a similar chloroplast history. About 10% of hexaploid specimens had hybrid AFLP patterns and accompanying morphological intermediacy. Evidence from unique AFLP bands, nrDNA sequence differences, and current geographic distributions suggests that introgression accounts for these hybrid patterns. In common garden experiments, half of the characters had significantly different values between field and common garden specimens, but these "plastic" characters changed depending on the species pair analyzed and between experiments. Moreover, several characters were significantly different between species, but these characters were also different in each of the two experiments. Given this variation, I pooled the field and common garden data results and determined the best key characters to distinguish among the polyploids using discriminant analysis of species pairs.

Abrégé

Il existe une augmentation d'especes polyploïdes dans plusieurs groupes de plantes situe dans les tropiques jusqu'au nord de l'arctique, cependant peu est connu des localités de leurs origines. Pour étudier les limites taxonomiques des espèces diploïdes et pour taxonomiquement délimiter et étudier les origines des trois espèces polyploïdes de *Puccinellia* (Poaceae), qui sont répandues dans l'arctique américain du nord, j'ai employé les méthodes de la cytométrie en flux, des données d'AFLP, des séquençages de l'ADN, de l'analyse des sites de restriction, et des expériences de jardinage. Six espèces diploïdes arctiques sont génétiquement et/ou morphologiquement distinctes, formant deux lignées, alors que les deux espèces diploïdes des américaines du nord tempérées forment une troisième lignée. Je mets en synonymie trois espèces dans *P. arctica*, tout en décrivant une nouvelle espèce diploïde, *P. banksiensis*, qui n'est trouvé que dans l'arctique américain du nord occidental. Les trois espèces polyploïdes : la *P. bruggemannii* (tétraploïde), la *P. angustata* (hexaploïde), et la *P. andersonii* (octoploïde), sont génétiquement distinctes. Elles montrent des origines d'allopolyploïd, ayant des bandes d'AFLP et des mutations de séquençages de deux diploids arctiques et de la triploïde/tetraploïde *P. phryganodes* arctique. L'histoire ribosomal nucléaire de l'ADN de *P. bruggemannii* est différente de celui de la *P. angustata* et de la *P. andersonii*, mais chacune sont semblable en ce qui concerne les chloroplastes. Environ 10% des spécimens hexaploïdes avait des modèles hybrides d'AFLP qui était accompagné par des caractères morphologiques intermédiaires. Dans des expériences communes de jardin, la moitié des caractères avaient des valeurs statistiquements différentes lors que les spécimens de champ et les spécimens communs de jardin ont été comparés, mais ces caractères "plastique" ont changé selon la paire d'espèces analysée et entre les expériences. De plus, plusieurs caractères entre les espèces étaient statistiquement différents,mais ces caractères étaient différents dans chacune des expériences. Étant donné que cette variation existe, j'ai déterminé les meilleurs caractères pour distinguer parmi les polyploïdes lors de l'utilisation de l'analyse discriminante des

paires d'espèces ce qui a été accompli avec les données de champ et les données communes de jardin.

Abstract – Inuktitut

ᑭᓱᓕᕆᒻᒪᖔᑕ

ᐅᓄᕐᑐᑦ ᐱᕈᕐᑐᑦ, ᖃᑦᓯᑲᓪᓚᑦ ᐱᕈᕐᑐᑦ ᐱᕈᕐᐸᓪᓕᐊᔪᑎᖏᑦ ᑕᒪᒃᑮᓯᒪᔪᑦ ᐊᒥᓱᕈᕐᐸᓪᓕᐊᓯᒪᒻᒪᑦ ᐅᐊᓐᓇᒧᐊᖅᐸᓪᓕᐊᓪᓗᓂ ᐅᑭᐅᖅᑕᖅᑐᒧᑦ, ᑭᓯᐊᓂᓕ ᖃᐅᔨᒪᔭᐅᑦᓯᐊᓚᓛᓐᖏᒻᒪᑕ ᐅᑭᐅᑕᖅᑑᑉ ᐱᕈᕐᑐᕐᖁᑎᖏᑦ ᓇᑲᓐᖑᕐᓂᑰᒻᒪᖔᑕ ᐅᑭᐅᑕᖅᑐᒥᒃ ᖃᓪᓗᓈᓂᓪᓘᓐᓃᑦ. ᐊᑐᖅᐸᒃᑲᒪ ᐃᒪᕈᔭᖏᓐᓂᒃ (cytometry), AFLP ᑐᓴᖓᒃᓴᑦ, ᐃᔭᕈᐸᐅᔭᖏᓪᓗ ᐱᕈᕐᑐᑦ ᖃᐅᔨᓴᕐᑕᐅᓪᓗᑎᒃ (DNA)ᖑᓂᕋᕐᑕᐅᔭᑦ ᐊᔾᔨᒌᖏᔾᔭᑎᖏᑦ ᐊᒻᒪ ᐃᒪᕈᔭᖏᑦ ᐊᒥᓱᕈᕐᐸᓪᓕᐊᔪᑎᖏᑦ, ᓇᒦᓐᓇᑯᓗᒃ ᐱᕈᕐᒪᖔᑕ, ᐳᔭᕈᔭᖏᑦ ᑎᒃᑕᐅᕙᒃᑐᑦ ᐱᕈᒃᑲᓐᓂᕐᐸᒃᑐᑦ, ᓄᓇᖓᑕ ᑕᐃᔫᓯᖏᑦ, ᐊᒻᒪ ᐱᑕᖃᕋᔭᒃᑐᑦ ᐃᓪᓗᐃᑦ ᖃᓂᒋᔫᓂ ᐱᕈᕐᓯᐊᖑᕙᒃᑐᑦ ᓇᒦᓐᓇᖅ ᐱᕈᕈᓐᓇᕐᒪᖔᑕ ᓇᑭᓐᖓᕐᓂᖏᑦ ᒪᓕᓪᓗᒋᑦ ᑕᐃᔭᐅᔭᑦ *Puccinellia* (Poaceae) ᐱᑕᖃᕋᔭᒃᑐᑳᓘᔭᑦ ᐊᒥᐊᓕᑲᐃᑦ ᐅᐊᓐᓇᖓᓂ. ᐱᖓᓲᔭᕐᑐᖅ ᐅᑭᐅᖅᑕᖅᑐᒥ ᐊᔾᔨᒌᐸᓗᑦ ᐱᕈᕈᓯᖏᑦ ᐃᒪᕐᑯᔭᖏᑎᒍᑦ ᐊᒻᒪ/ᓴᓇᒪᓂᖏᑎᒍᑦ ᐊᔾᔨᒌᖏᒻᒪᑕ, ᒪᕐᕉᒃ ᓯᕗᓕᖏᑦ, ᒪᕐᕉᓪᓗ ᐊᒥᐊᓕᑲᑦ ᐅᐊᓐᓇᖓᓂ ᐱᖓᔭᒋᔭᐅᔭᒥᒃ ᓯᕗᓕᖅᑐᑎᒃ. ᐱᖓᓱᐃᓕᖓᔭᓂᒃ ᓇᓗᓇᐃᕐᓯᓪᓗᖓ ᐅᑯᓂᖓ *P. arctica*, ᐊᒻᒪ ᓄᑖᖅ ᓇᓗᓇᐃᕐᑐᒍ ᓯᕗᓕᖓᓂᒃ, *P. banksiensis*, ᓇᓂᔭᐅᔭᓐᓇᕐᑐᑦ ᑭᓯᐊᓂ ᐊᒥᐊᓕᑲᑦ ᐅᑭᐅᖅᑕᖅᑐᐊᑕ ᐅᐊᓐᓇᖓᓂ ᓇᓂᔭᒃᓴᐅᔭᑦ. ᐱᖓᓱᑦ ᓯᕗᓕᓖᑦ ᐊᔾᔨᒌᖏᑦᑐᓂᒃ, tetraploid *P. bruggemannii*, hexaploid *P. angustata*, ᐊᒻᒪ ᓯᕗᓪᓕᒥᓂᑦ octoploid *P. andersonii* ᐊᔾᔨᒌᖏᑦᑑᒻᒪᑕ. Allopolyploid-ᒥᓐᖓᕐᓂᑯᔭᕐᑐᑦ, ᐃᒪᕈᔭᖏᑎᒍᑦ ᐊᔾᔨᐅᖏᔾᔭᑎᖏᑦ AFLP ᐊᒻᒪ ᓴᓇᒪᓂᖏᑦ ᒪᕐᕈᐃᓕᖓᔭᓐᓃᖓᕐᑑᑉ diploids ᐅᑭᐅᑕᖅᑐᒥᒃ ᐊᒻᒪ ᐅᑭᐅᖅᑕᖅᑑᑉ ᐱᕈᕐᑐᖏᓐᓂᒃ triploid/tetraploid *P. phryganodes*. *Puccinellia bruggemannii* ᐊᔾᔨᒥᓐᖏᑕᖏᓐᓂᒃ ᓴᓇᒪᓂᖃᕐᒥᔭᑦ ᐃᔭᕈᐸᐅᔭᖏᑎᒍᑦ (nuclear ribosomal DNA) ᐃᒻᒪᓂᓴᑐᖃᐅᓂᕐᓴᑦ ᐅᑯᓂᖓ *P. angustata* ᐊᒻᒪ *P. andersonii*, ᑐᑭᓯᓇᕐᑐᑎᒃ ᐱᕈᕐᐸᓪᓕᐊᔭᒥᓂᐅᒋᐊᖏᑦ ᐊᔾᔨᒌᖏᑦᓯᐊᕐᑐᓂᒃ ᐱᕙᓪᓕᐊᓂᖁᓪᓗᑎᒃ, ᑭᓯᐊᓂᓕ ᐱᖓᓱᐃᓕᖓᔭᑦ ᐱᕈᕐᑐᑦ ᐊᔾᔨᒌᐸᓗᓐᓂᒃ ᓯᕗᓕᖅᑐᑦ ᐃᒻᒪᖃ 10% of hexaploid ᐱᕈᕐᑐᑦ ᐊᔾᔨᒌᐸᓘᓪᓗᑎᒃ hybrid AFLP ᓴᓇᒪᓂᖏᑦ ᐊᒻᒪ ᐊᓯᔾᔨᐸᓪᓕᐊᓕᕐᓂᒥᓂᖏᑦ (morphological intermediacy). ᐊᔾᔨᐅᖏᑦᑐᑦ ᓴᖅᑭᕐᑐᑦ AFLP bandsᓂᒃ ᑕᖏᓖᑦ, nrDNA ᐃᒪᕈᔭᖏᑎᒍᑦ ᐊᔾᔨᒌᖏᔾᔭᑎᖏᑦ, ᐊᒻᒪ ᒫᓐᓴ ᓄᓇᓐᖑᐊᒃᑯᑦ ᓯᐊᒃᓯᒪᓂᖏᑦ ᐃᓱᒪᓇᕐᑑᖅ ᑲᑎᐸᓘᓐᓂᑰᒥᐊᖏᑦ. ᐱᕈᕐᓲᕕᓕᓐᓂᓕ ᖃᐅᔨᓴᕐᑐᑎᒃ, ᓇᑉᐸᖏᑦ ᐊᔾᔨᒌᖏᓪᓚᓇᓚᐅᕐᑐᑦ ᐊᑐᓂ ᐊᒻᒪ ᐱᕈᕐᓯᐊᖑᓕᔭᒃᑐᑦ ᐃᓪᓗᐃᑦ ᓴᓂᐊᓐᓂ, ᑭᓯᐊᓂᓕ ᐊᓯᔾᔨᑲᑦᑕᒃᑐᑎᒃ ᒪᓕᒃᑐᒋᑦ ᐊᑕᐅᑦᓯᒥ ᑲᑎᓐᖓᔭᑦ ᐱᕈᕐᑐᑦ ᖃᐅᔨᓴᕐᑕᐅᕙᓪᓕᐊᔭᓪᓗ. ᓱᓕᒃᑲᓐᓂᖅ, ᐱᕈᕈᓱᖏᑦ ᐊᔾᔨᒌᖏᑦᑐᕈᓗᔭᐅᓪᓗᑎᒃ ᐱᕈᕐᑐᑦ, ᑭᓯᐊᓂᓕ ᐃᒥᒃᑯᑦ ᒪᕐᕉᐃᓕᖓᔭᑦ ᖃᐅᔨᓴᕐᑕᐅᒻᒪᑕ ᐊᔾᔨᒌᓗᐅᓐᖏᑦᑐᑦ ᐱᕈᕈᓯᖏᑦ. ᑖᒃᑯᐊ ᐊᔾᔨᒌᖏᓐᓂᖏᑦ ᐃᓱᒪᒋᓪᓗᒋᑦ, ᖃᐅᔨᓴᓚᐅᕋᒪ ᓄᓇᒥ ᐊᒻᒪ ᐃᓗᐃᑦ ᖃᓂᒋᔫᓂ ᐱᕈᕐᓯᐊᖑᓕᔭᒃᑐᓂᒃ ᖃᐅᔨᓚᐅᕐᑕᒃᑲ ᓇᓗᓇᐃᕐᖅᑐᒥᓪᓗᒍ ᐱᐅᓯᓇᓲᖏᑦ ᐱᕈᕐᑐᑦ ᖃᐅᔨᓴᕐᑕᒪ ᐊᔾᔨᒌᖏᒍᑎᖏᓐᓂᒃ ᑲᑎᓐᖓᓪᓗᑎᒃ ᑐᑭᓯᓇᓱᐊᕐᑐᖓ.

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Contributions of Authors

The main part of this thesis consists of four manuscripts. My co-authors were Dr. Marcia Waterway, McGill University, and Dr. Lynn Gillespie, Canadian Museum of Nature. Dr. Waterway was my thesis supervisor, and in that role provided the equipment that I needed for extractions, amplifications, and AFLP analyses and many consumables for these techniques. Dr. Waterway contributed helpful advice and suggestions for some of the alternative procedures and analyses, most significantly suggesting the non-standard technique of using restriction digests of ribosomal DNA regions to potentially identify parental species of the polyploids based on their 'signatures' remaining in the ribosomal repeat region. She was available for many discussions on the results and provided a great number of indispensable criticisms and comments on these manuscripts, including the figures and tables, throughout the writing process. Dr. Gillespie was a member of my thesis advisory committee. She was expedition leader during half of my first field trip, as well as leader of three previous trips during which I had the opportunity to develop my interest in *Puccinellia*. Dr. Gillespie provided DNA for the *Phippsia* and *Catabrosa* outgroup specimens, was always available to discuss arctic botany and the results of my work, and also gave helpful constructive criticism on the chapters as they were developing.

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List of Abbreviations

Abbreviations given below are those commonly used in the text. Other abbreviations that are specific to only one table or figure are defined in the table or figure captions. Herbarium codes are defined in Holmgren et al. (1990).

ACD Acid citrate dextrose solution — anticoagulant solution used for the preservation of fresh red blood cells (Galbraith et al. 1997).

AFLP Amplified fragment length polymorphism — a procedure for detecting polymorphisms in DNA. AFLP analysis involves digesting DNA with restriction enzymes and then selectively amplifying a subset of the fragments by PCR. Mutations are detected by comparing the presence and absence of fragments (Vos et al. 1995).

CNWG Catalogue of new world grasses — a list, with synonymy, of all of the grass species in the New World (Soreng et al. 2003).

cv Coefficient of variation — cv = (standard deviation / mean) X 100%, measuring the variability in the data.

cpDNA Chloroplast DNA

nrDNA Nuclear ribosomal DNA

ETS1 External transcribed spacer region of DNA — two regions of DNA that lie within the IGS region; ETS1 is upstream of the 18S subunit.

GISH Genomic *in situ* hybridization

herb Herbarium

IGS Intergenic spacer region of rRNA gene, situated between the 26S and 18S subunits.

ITS Internal transcribed spacer region of DNA — is transcribed into a region of RNA between subunits of the ribosome: ITS-1 lies between the 18S gene and the 5.8S gene and ITS-2 is located between the 5.8S gene and the 26S gene.

NWT Northwest Territories

PAF Panarctic flora project — a collective of plant names for the circumarctic, compiled by a team of Arctic plant experts (Elven et al. 2003).

PCR Polymerase chain reaction – a procedure for building DNA chains one nucleotide at a time, facilitated by a DNA polymerase (e.g. Taq1) and specified by primers.

PCSP Polar Continental Shelf Project — a department of the Earth Sciences sector of Natural Resources Canada. PCSP co-ordinates support for research in isolated areas of the Canadian Arctic.

rpl16 — intron in ribosomal protein L16

rpoB-trnC — intergenic spacer region in the chloroplast genome between the gene that is transcribed for DNA-directed RNA polymerase subunit beta and the gene that is transcribed for transfer RNA coding for amino acid cysteine (GCA).

Rus Russia

s.l. *sensu lato*

s.s. *sensu stricto*

sp. species

syn. synonym

trnD-T — intergenic spacer region in the chloroplast genome between the genes that are transcribed for the transfer RNAs coding for amino acids aspartate (GUC) and threonine (GGU), respectively.

trnH-psbA — intergenic spacer region in the chloroplast genome between the gene that is transcribed for the transfer RNA coding for the amino acid histidine (GUG) and the gene coding for the photosystem II reaction centre D1 protein.

trnL-F — intron in the gene *trnL* in the chloroplast genome, which is transcribed for the transfer RNA that codes for a leucine (UAG), plus the intergenic spacer region between the genes coding for the transfer RNAs coding for leucine (UAG) and phenylalanine (GAA), respectively.

trnT-L — intergenic spacer region in the chloroplast genome between the genes that are transcribed for transfer RNAs coding for amino acids threonine (UGU) and leucine (UAA), respectively.

1. Introduction

1.1 General overview

Polyploidy has played a major role in plant evolution. It can be an important mechanism in plant speciation, especially when combined with hybridization (Grant 1981; Judd et al. 2001). It has recently been shown that 70% of angiosperms have had polyploid events in their phylogeny (Masterson 1994), accounting, in one estimate, for 2–4% of speciation events in flowering plants (Otto and Whitton 2000). These percentages are even higher if ancient duplications are considered (Adams and Wendal 2005).

Polyploids have more than two basic genomes in their chromosomal composition. They are traditionally divided into autopolyploids and allopolyploids. Autopolyploids have the genomic composition of one parental taxon, i.e., infraspecific polyploidy. Allopolyploids are formed from two or more different taxa, i.e., interspecific polyploidy (Lewis 1980). Duplication of the genome in polyploidy can restore fertility to sterile hybrids and thereby allow the hybrid to reproduce sexually and evolve as a new species (Grant 1981).

In many plant groups the proportion of polyploid taxa increases as one travels further north from the tropics to the Arctic (first observed by Tischler and Hagarup, cited in Stebbins 1950). Stebbins (1950) suggested that polyploidy *per se* must confer physiological or ecological advantages that have allowed successful plant establishment in new post-glacial landscapes and survival in harsh arctic environments. In support of this, Levin (1983) reported several examples of autopolyploid taxa that showed advantages related to modifications from polyploidy (e.g., increased self compatibility, larger cell sizes, more alkaloid production, increased stress tolerance, slower growth, and longer life) which may be advantageous in harsh environments. In more recent work, Stebbins (1985) attributed the success of polyploidy to reproductive stabilization of new hybrids. Indeed, the advantages of polyploidy are often largely attributed to increased heterozygosity from the hybrid origins of allopolyploid taxa (reviewed by Murray 1995; Soltis and Soltis 2000). Soltis and Soltis (2000) further state that higher

levels of heterozygosity are maintained in autopolyploid taxa than their diploid parents through polysomic inheritance which allows independent sorting of chromosomes from different parental genotypes. They give examples of 20 autopolyploid taxa that exhibit polysomic inheritance.

We now know that species at edges of their ranges can have the greatest genetic differences and produce novel hybrids (and allopolyploids) that are well suited to exploit new environments (Bayer 1991). This is relevant to arctic studies because much of the North American Arctic was covered in ice during the last glaciation. Phytogeographical studies by Hultén (1972) suggested one major region of plant refugia during the last glaciation was in the region he named Beringia, in which a land bridge joined Russia with Alaska and the western North American arctic islands. Recent phylogeographical studies (which use molecular evidence) and fossil records are confirming this (Abbott and Brochmann 2003). North American *Dryas integrifolia* (Tremblay and Schoen 1999) (diploid), *Vaccinium uliginosum* subsp. *microphyllum* (diploid) (Alsos et al. 2001), *Saxifraga oppositifolia* (diploid and tetraploid) (Abbott et al. 2000), and *Saxifraga cernua* (many ploidy levels) (Bronken et al. 2001) all have evidence for Beringian refugia during the last glaciation.

These studies do not, however, address the question of whether North American Arctic polyploid species that occupy previously glaciated regions arose in the Arctic from progenitors in Beringian (or other) refugia, or arose further south and migrated to the Arctic. The characteristics of polyploids mentioned above may allow them to survive better in harsh arctic environments than their diploid progenitors. Moreover, because cold treatment can result in increased production of unreduced gametes (Ramsey and Schemske 1998), which are involved in most polyploidization events (DeWet 1980), we may ask: does this result in increased polyploid production in colder regions? This question cannot presently be answered, however, because, as mentioned, it is unknown whether these arctic polyploid taxa originated under cold arctic conditions or spread there from other regions. Indeed, the historical distribution and origin of most arctic polyploid taxa is unknown (Otto and Whitton 2000). Abbott and Brochmann

(2003) cited only three arctic polyploid groups for which the parental origins have been documented (*Cerastium arcticum* Lange and C. *nigrescens* (H.C. Watson) Edmonston ex H.C. Watson, *Saxifraga svalbardensis* D.O. Ovstedal and several species of *Draba*, all studied in Svalbard and adjacent Europe). Three published studies have recently addressed geographic distribution of progenitors for polyploid plant species in the North American Arctic: two that found the origins of North American polyploids from other polyploids (*Dupontia fisheri* R.Br., Brysting et al. 2004; *Saxifraga rivularis* L., Jørgensen et al. 2006), and one that showed a North American (Alaskan) diploid was a likely parent of North American tetraploid and hexaploid *Silene* species (Popp and Oxelman 2007).

It is now believed that most polyploids are of multiple or recurrent origins (Soltis and Soltis 1999; 2000), contributing even more genetic diversity to the plant genomes. Soltis and Soltis (1999) stated that "Polyploid species can maintain high levels of segregating genetic variation through the incorporation of genetic diversity from multiple populations of their diploid progenitors" (p. 351). A prevalence of multiple-origin polyploid taxa in the Arctic, and subsequent mixing during re-contact among them, is suggested as the major contributing factor to explain taxonomic uncertainty of polyploid complexes in these regions (Soltis and Soltis 1999).

Multiple polyploid origins have explained taxonomic confusion among putative taxa in two frequently cited taxonomically complex arctic plant genera that have been studied in detail in Europe (*Draba* (Brassicaceae) and *Saxifraga* (Saxifragaceae), Brochmann et al. 1992, 1998, respectively). Moreover, at least the 16-ploid "species" *Draba corymbosa* R.Br. ex DC was proven to be polyphyletic, which means that it has evolved from different sets of diploid progenitors. The authors found that it may be impossible to establish a taxonomic treatment that correctly reflects the evolutionary history of this taxon. The extent of multiple origins of most polyploid plant species, however, is unknown (Soltis and Soltis 1999; 2000).

Polyploids may become apomictic, which means fruits form without fertilization. Apomixis can result in variants forming independently evolving

clones that are difficult to group into species as traditionally defined, and which form "microspecies" (Asker and Jerling 1992). This has been found in studies of the complex genus *Antennaria* (e.g., Bayer 1991). On the other hand, with the increased self compatibility attributed to polyploids, inbreeding, with biological isolation, can result in the formation of "sibling biological species" (Grant 1981, p. 67) that are slightly linked by cross-pollination to form species (Grant 1981; Grant 1985), but which may also form variant populations that appear to defy taxonomic classification.

The impact of polyploidy on morphological character variation was addressed in the *Stellaria longipes* Goldie complex (Macdonald et al. 1988). Among three cytotypes (4x, 6x, 8x) of *S. longipes* and a suspected diploid progenitor *S. longifolia* Muhl. ex. Willd., phenotypic plasticity was found to be highest in the diploid taxon and there was no difference in phenotypic plasticity among the three polyploid cytotypes. Macdonald et al. (1988) therefore showed a negative correlation between genotypic diversity and phenotypic plasticity. In a study of diploid and a synthetic tetraploid *Phlox*, some characters showed slightly decreased phenotypic plasticity in the tetraploid (Garbutt and Bazzaz 1983). Although studies have been done of changes in characters with increased ploidy (e.g. Schranz and Osborn 2000; examples cited by Levin 1983), the range of phenotypic plasticity related to ploidy level has not been frequently studied.

In summary, we currently know that a) the majority of angiosperms have polyploidy in their history, b) in many plant groups the number of polyploids is greater in the Arctic than in more southern regions, c) polyploids may develop inbreeding or apomixis, and d) that probably most polyploid plant species are of multiple or recurrent origin. However, we do not know 1) the geographical origins of North American arctic polyploid plant species, indeed the origins of most polyploid species, 2) the extent of multiple or recurrent origins in most polyploid species, 3) whether multiple origins contribute to taxonomic difficulty in North American arctic polyploid complexes, and 4) the general relative degree of phenotypic variation in polyploid species as compared with their diploid or lower-ploidy progenitors.

1.2 Main objectives

One of my main objectives is to investigate the origins of North American arctic polyploids and the extent of multiple polyploidy in these species, by obtaining empirical evidence from a widespread polyploid genus. The genus *Puccinellia*, described below, is a taxonomically difficult plant group in the North American Arctic that has a moderate number of species that appear to have different ploidy levels. My second main objective is to use the answers to the above unknowns about polyploidy to resolve the systematics for some of the most challenging species in this genus.

1.3 Specific objectives and hypotheses

My specific objectives and hypotheses outlined below aimed to reveal morphological and molecular patterns in *Puccinellia* taxa (Objective 1), identify the extent of multiple genetic origins and morphological variation related to environment that may blur these patterns (Objectives 2 and 3, respectively), and to thereby develop a systematic classification based on the phylogeny of the species.

1.3.1 Objective 1: Delimitation of taxa

My first objective was to determine whether there is a genetic basis for traditional morphological limits in arctic *Puccinellia* by testing the following hypotheses. Hypothesis 1A: If chromosome number is consistent in the specimens and populations examined for each taxon group, then we may conclude that the taxa represent distinct ploidal levels. Hypothesis 1B: In the diploid taxa, if we find no pattern in molecular and morphological data that would correspond to distinct groups, then we may conclude that the putative diploid taxa are conspecific. Hypothesis 1C: In the polyploid taxa, if molecular data and morphological data are congruent and form patterns corresponding to these taxa, then we may conclude that the putative polyploid taxa are distinct species.

Hypothesis 1A would be rejected if the ploidy levels are not consistent within the taxonomic groups found by testing Hypotheses 1A and 1B. Hypotheses 1B and 1C would be rejected in many ways because of the complexity of genetic variation in plants. Several sample patterns of morphological and molecular variation were sought in this study: A) no pattern in morphological or molecular data; B) patterns in morphological and molecular data that are congruent; C) patterns in morphological and molecular data that are incongruent; D) patterns in morphological data but none in molecular data; E) patterns are found in molecular data but none in morphological data.

Incongruence in molecular patterns in polyploid taxa would potentially be accounted for by complex genetic variation owing to hybrid origin, multiple origins, or autopolyploidy. These were tested in Objective 2. Incongruence in morphological patterns in or among putative taxa could be owing to wide potential latitude of morphological expression affected by environmental conditions. This was tested in Objective 3.

1.3.2 Objective 2: Origins of polyploid taxa

a) Arctic or non-arctic origins. This objective was to determine whether the polyploid taxa in the Canadian Arctic arose from species in the North American Arctic (NAA) and nearby arctic areas (including Greenland and a species from nearby Russia) or non-arctic North America. Each polyploid taxon was tested in turn. Hypothesis 2A: If a polyploid taxon shares sets of diagnostic molecular markers with one or more of the lower ploidy NAA taxa (or non-NAA parental taxa), then we may conclude that this polyploid taxon arose from NAA parental taxa (or non-NAA parental taxa). This hypothesis was to be rejected if no clear association of markers was found in polyploids with taxa of lower ploidal level. This may imply an ancient origin for these polyploids.

b) Mode of speciation of polyploid taxa. This objective was to determine whether the polyploid taxa in the Canadian Arctic were of autopolyploid or allopolyploid origin, and whether their origin was polyphyletic. The putative taxa *P. bruggemannii* and *P. andersonii* are distributed over North America and

Greenland, and *P. angustata* is distributed in North America, Greenland, Russia, and Scandinavia (Fig. 2.1). Therefore, a large part of the range of these three polyploids could be examined in North American collections. Each polyploid taxon was tested separately.

Hypothesis 2B: If we observe, in a polyploid taxon, diagnostic markers from more than one taxon of lower ploidy level, we may conclude that the polyploid taxon arose from allopolyploid origins. Otherwise, it would be probable that the taxon is of autopolyploid origin. Hypothesis 2C: If we observe, in a polyploid taxon, diagnostic markers from different sets of parental taxa, we may conclude that the polyploid taxon has polyphyletic origins. Otherwise, the taxon would be considered to be of monophyletic origin. Hypothesis 2D: If we observe, in a polyploid taxon, markers from different populations of parental taxa then we may conclude that the polyploid taxon has multiple origins. Otherwise, the taxon would be considered to be of single origin.

1.3.3 Objective 3: Morphological variation related to environment

This objective was to determine the degree of divergence or convergence of morphological traits and characters in putative taxa taken from their original sites and grown under common garden conditions. Hypothesis 3: In the greenhouse, if we see a stable significant difference between taxa in a morphological trait when grown under common garden conditions, we may conclude that this trait distinguishes between genotypes (putative taxa) in the experimental environment.

2. Literature Review

2.1 The genus *Puccinellia*

Puccinellia is a grass with a worldwide arctic and temperate distribution that grows in salty or alkaline environments (Porsild 1964, Edgar 1996). Sixty-six percent of the taxa that have had chromosome counts to date are polyploid (Table 2.1). This genus has a long history of taxonomic uncertainty. Fernald & Weatherby (1916, p. 1) called it "even to agrostologists, one of the most perplexing groups of grasses…," Polunin (1940, p. 67) said that: "the characters are variable… [*P. angustata*] is a most unsavoury aggregate... ," and Davis (1993, p. 202) called it: "one of the more controversial genera in the grass family in terms of species delimitation."

Whereas over 300 species have been described worldwide, 30 to 80 species have been accepted over the span of the last 20 years (Gould and Shaw 1983, Davis 1983b, Watson and Dallwitz 1999) (Table 2.1). In the checklist of plants being compiled by the Panarctic Flora Committee (Elven et al. 2003), the number of *Puccinellia* species in the circumpolar Arctic is 20–30 and the number in the North American Arctic is 13–19, depending on the final synonymy adopted.

At the infrageneric level, most species belong to the Section *Puccinellia* (Tzvelev 1976, Elven et al. 2003). Section *Pseudocolpodium* contains two species in North America (*P. vahliana* (Liebm.) Scribn. & Merr., *P. wrightii* (Scribn. & Merr.) Tzvelev in Tolm.) that are distinguished by having relatively longer glumes, and contains some species that were originally included in the genus *Colpodium*. Section *Paralochloa* contains one North American species, the stoloniferous species *P. phryganodes* (Trin.) Scribn. & Merr.

Species of *Puccinellia* figure prominently in the ecological literature and many of these studies have been in North America. *Puccinellia phryganodes* is an easily recognized, stoloniferous species found abundantly on sand flats near the coast. It is widely studied in part because of its value as food for geese (it is also called "goosegrass") (Jefferies and Rockwell 2002 plus many other references). Other species that have figured in ecological studies are more controversial

taxonomically. In their studies of arctic plant life history and ecophysiology, Bell and Bliss (1980) and Grulke (1983) studied *P. vaginata* (Lange) Fernald and Weath., which has not otherwise been recorded from the islands on which they worked and voucher specimens were not cited for verification. McKendrick (2001) found that the species *P. borealis* Swallen was extremely useful for revegetation of disturbed pipeline areas in Alaska. This boreal species has been synonymized with the temperate species complex *P. nuttalliana* (Schult.) Hitchc. because key characters have not yet been found to satisfactorily distinguish them (Davis and Consaul 2007).

Several *Puccinellia* species have been reported as rare in Canada and the Canadian Arctic (Argus and Pryer 1990). Five of these are arctic species (*P. agrostidea* T.J. Sørensen, *P. arctica* (Hooker) Fernald & Weath., *P. andersonii* Swallen*, P. bruggemannii* T.J. Sørensen*, P. poacea* T.J. Sørensen). The first two are globally imperiled; *P. poacea* is critically imperiled (Argus and Pryer 1990) and *P. bruggemannii* and *P. poacea* are narrow, high arctic endemics (McJannet et al. 1993). We may find that these rare *Puccinellia* species are more common than current herbarium collections reveal by increased surveying.

2.2 Background systematic studies

Very few systematic studies have been done for *Puccinellia*, although many regional floristic treatments exist. Sørensen (1953) studied morphology and leaf anatomy in Greenland *Puccinellia*. He provided keys to species from Greenland and the eastern North American Arctic using several new terms to describe characters. He described and named several new, mostly rare, taxa from Greenland (Sørensen 1953) and North America (Sørensen 1953, 1955). Several of these taxa have been synonymized in recent treatments (Elven et al. 2003; Davis and Consaul 2007).

Davis (1983a, 1983b, 1993, Davis and Manos 1991) studied the temperate *P. nuttalliana* complex in North America. They examined phenotypic plasticity, leaf anatomy, and isozyme data. Several species were distinguished based on

isozyme profiles, including six "isozyme species" of *P. nuttalliana,* most of which have not yet been characterized morphologically.

Jefferies and Gottlieb (1983) studied populations of one arctic species (*P. phryganodes*) using isozyme data. They found high genetic variation within the populations that suggested that this species had some sexual reproduction, although all their specimens had triploid chromosome counts.

Consaul and Gillespie (2001) carried out a morphological study of Canadian Arctic island species to resolve the definitions of some of the key characters that had been used for the arctic taxa and did ordinations of morphological data for the putative taxa. They found (2001 - Fig. 9) that most species represented the ends of morphological continua rather than forming well-defined entities.

2.3 Chromosome information

North American *Puccinellia* appears to have a lower proportion of polyploids in the Arctic than in temperate regions, contrary to the general pattern in many plant groups of having a higher number of polyploids with increasing latitude (Table 2.1). In temperate North America, 9 of 11 species are reported as polyploid (82%), whereas in the North American Arctic, only 9 of 14 species are reported as polyploid (64%). This may be a result of the higher number of species counted, but the proportion of polyploid species is also relatively low in the Russian Arctic, where 10 of 18 species counted (55%) are polyploid. This may be because the Arctic has the most favorable habitats for *Puccinellia.* For other plant groups that have a higher number of polyploids in southern regions, Lewis (1980) suggested that the areas with the higher number of polyploids are more stressful for that plant group. The lowest number of *Puccinellia* polyploids of all is found in the Beringian region, where half of the taxa are diploid. This suggests that Beringia may have been a refugium for the diploid taxa during the last glaciation.

The chromosome counts to date for the North American Arctic taxa of *Puccinellia* are given in bold in Table 2.2. This table shows that *P. angustata* (R.Br.) E.L. Rand & Redfield has 12 chromosome counts, *P. vahliana* has eight,

and the rest of the species have only one to four counts each. Most of the taxa have consistent ploidy levels according to these counts, except that *P. pumila* (Macoun ex Vasey) Hitchc. has a hexaploid count and an octoploid count, a subspecies of *P. phryganodes* has a diploid count and a tetraploid count, and *P. vaginata* has a hexaploid count and four octoploid counts. The latter species was not included in the present study because I chose one example of each putative ploidy level for this study, and *P. andersonii* was more appropriate, being widespread and frequently found with *P. angustata*. Moreover, the collection locations for *P. vaginata* were different from the other taxa in the study, which would have required separate arctic field trips, which was not feasible. More counts were needed for all of the species to determine the level of consistency of chromosome numbers for the rest of the taxa.

2.4 Morphological complexity

Consaul and Gillespie (2001) found in the Canadian Arctic that although two diploid species and the triploid were relatively distinct morphologically, one diploid aggregate (of three putative species) and six polyploid taxa were very difficult to identify based on existing morphological keys and descriptions. This was not unexpected based on observations in the field. *Puccinellia* taxa are almost impossible to assign to species in some locations (L. Gillespie, Canadian Museum of Nature, L. Consaul, personal observations in arctic; J. I. Davis, Cornell University, personal observations in temperate regions). Figure 2.1 shows the circumpolar distribution of the putative species of the Canadian arctic islands and other arctic diploid taxa that are potential parents of the polyploids.

Distinct taxa – Four North American arctic species are quite distinct morphologically from other taxa and from each other.

1) *Puccinellia langeana* is a diploid species that belongs to section *Puccinellia*. It is distinguished by very small lemmas and almost a complete lack of hairs and is found in the southern Arctic (Fig. 2.1A). The typical subspecies is widespread in eastern and central regions. *Puccinellia langeana* subsp. *alaskana*

is found in Alaska (and the Russian Far East) (Fig. 2.1A). It is treated by some authors as possibly being a separate species (*P. alaskana*) and the complex to which these taxa belong requires study at a circumpolar level. The related *P. tenella* is found exclusively in Russia.

2. *Puccinellia vahliana* is a diploid species that belongs to section *Pseudocolpodium* (Tzvelev 1976). In the past it has been put in the separate genus *Colpodium*. It is widely distributed on the arctic islands (Fig. 2.1B).

3. *Puccinellia wrightii* is a diploid species from Alaska and nearby Russia (Fig. 2.1C). It also belongs to section *Pseudocolpodium*, but is distinguishable from *P. vahliana* by having unequal glume lengths and strong venation on the lemmas.

4. *Puccinellia phryganodes* subsp. *neoarctica* (North American subspecies that is reportedly triploid) is widespread over the North American Arctic islands (Fig. 2.1D). This taxon is very easily recognized as it is usually the only stoloniferous grass growing in mats on the damp sand of tidal flats. It belongs to section *Paralochloa* (Tzvelev 1976). *Puccinellia phryganodes* subsp. *phryganodes* is from Alaska and Russia, and has been reported as tetraploid (Table 2.2, Fig. 2.1D).

Problematic taxa: Diploid – The three taxa of the *Puccinellia arctica* aggregate sensu Polunin (1959) (*P. agrostidea* T.J. Sørensen, Fig. 2.1E, *P. arctica*, Fig. 2.1F, and *P. poacea*, Fig. 2.1G) are difficult to distinguish morphologically and were not compared with each other when they were originally described. Morphological characters from the literature separating these putative taxa (plant size, inflorescence branching, lemma texture) are non-discrete, and states are difficult to define. *Puccinellia poacea* is found in the far north; *P. arctica* and *P. agrostidea* have been collected in the southwestern Arctic, but from different sites. Few collections for *P. arctica* and *P. agrostidea* exist, so a true picture of the morphological variation within these putative taxa has not been possible. In a principal components analysis of morphological data of specimens keyed to these three taxa, there was almost complete overlap (Figure

9 in Consaul and Gillespie 2001). The species in this aggregate are characterized by having long anthers.

Puccinellia phryganodes subsp. *geniculata* (V.I.Krecz.) Tzvelev is a putative diploid taxon that has been recorded once in Alaska, twice in non-arctic Russia (Table 2.2; see Fig. 2.1D, marked with *) and is also found eleswhere in non-Arctic Asia. The Alaskan record is a single specimen collected in 1926 and is questionable.

Problematic taxa: Polyploid – *Puccinellia bruggemannii* is a putative tetraploid that is found in the western North American Arctic islands (Fig. 2.1H). Its main key characters are curved tips on the lemmas and an inflorescence less than 4 cm long. To date, *P. bruggemannii* has only been collected from the northern, central and western Canadian arctic islands (and northern Greenland). *Puccinellia hauptiana* (Trin. ex V. Krecz.) Kitig. (Fig. 2.1I) is a widespread tetraploid reported from Alaska and eastern Eurasia. *Puccinellia phryganodes* subsp. *phryganodes* in Alaska has also been reported as tetraploid. No tetraploid species except *P. bruggemannii* have been reported from the eastern North American Arctic or Greenland.

Puccinellia angustata is a putative hexaploid that has a circumpolar distribution and has been the most commonly collected *Puccinellia* species in the North American Arctic (Fig. 2.1J). It is characterized by small (0.6-0.8 mm) anthers (Porsild 1964) and hairy lemmas and paleas.

Puccinellia bruggemannii and *P. angustata* are often found in the same sites and are often difficult to distinguish. Although allowing that *P. bruggemannii* may be mistaken for stunted *P. angustata*, Sørensen (1955) stated that this resemblance is superficial, because *P. bruggemannii* has somewhat incurved lemmas, and lacks stomata on the underside of the leaves, "a rare feature in *Puccinellia* – and one only very rarely known to occur in *P. phryganodes,*" (p. 81). *Puccinellia bruggemannii* and *P. angustata* are separated from other *Puccinellia* species by having short anthers and very hairy lemmas and paleas (Consaul and Gillespie 2001). They were also somewhat separable from each

other based on principal components axes and principal coordinates axes by size characteristics, but formed part of a continuum.

The putative hexaploids *P. borealis* and *P. pumila* are also found in the southern North American Arctic. These are primarily boreal and temperate species that will not be examined in detail in this study. This study focussed on three arctic polyploid species that have similar ranges, therefore similar general ecosystem requirements, to minimize the number of factors affecting phenotypic expression in the field.

The putative octoploids *P. andersonii* (Fig. 2.1K) and *P. vaginata* (Fig. 2.1L) are not always easy to distinguish because the characters (hairs and other trichomes on the lemmas) that should separate them are sometimes intermediate (Consaul and Gillespie 2001). Moreover, there can be particular difficulties in distinguishing these two putative octoploids from other putative taxa growing at the same site when intermediate character states and plant habits are found. In this thesis I will address the more common species *P. andersonii* and compare it with *Puccinellia* species of different ploidy levels. Information obtained from this study can be used to investigate the distinction and origins of *P. vaginata* in a future study.

2.5 Molecular evidence in arctic *Puccinellia*

In their study of arctic *Poa*, Gillespie et al (1997), Gillespie and Boles (2001), and Gillespie and Soreng (2005) also included eight species of *Puccinellia* for comparison. They found one restriction site difference for each of *P. vahliana* and *P. phryganodes* from the six other species of *Puccinellia* in the Canadian Arctic (among which there were no differences).

2.6 Putative hybrid origins

The options for parentage of these putative species are numerous given their geographical distribution. Since multiple polyploidization has been demonstrated as possibly being more common than previously thought in the Arctic (Soltis and Soltis 1999), it would not be surprising to find it in *Puccinellia*.

Alternatively, these species may have originated once (even outside the North Amercian Arctic) and migrated to occupy their current geographical distributions. There are three lines of evidence showing that it is possible that arctic polyploid taxa may not be most closely related to the diploids in the Arctic: 1) Consaul and Gillespie (2001) found that diploid species or species groups are relatively distinct morphologically from each other and the polyploid taxa; 2) the polyploid taxa group together closely in ordination analyses of morphological data (Consaul and Gillespie 2001); and 3) knowledge of geographical distribution is required to distinguish some pairs of arctic and southern taxa in the treatments of North American *Puccinellia* (Davis and Consaul 2007). These polyploid taxa may have arisen in the Arctic from arctic parents, in the arctic from extinct ancestors, or from southern species.

2.7 Breeding systems in *Puccinellia*

Polunin (1959, p. 67) stated that "apomixis is apparently widespread" in *Puccinellia*, but he did not provide evidence for this conclusion. Stebbins (1950, p. 404) also mentioned that *Puccinellia* "contains species that may be apomictic." Apomixis has not been otherwise reported for this genus, however. A pattern where populations of the same putative species appear morphologically different from each other, as in *Puccinellia*, may be explained by apomixis (agamospermy), but it may also be explained by inbreeding. Watson (1990) listed reproductive strategies that have been documented for many genera of grasses. While he did not include *Puccinellia* among genera that have been shown to exhibit agamospermy, he did document that at least some members of this genus have been known to exhibit inbreeding and cleistogamy. Knowledge of the breeding systems is lacking in *Puccinellia*.

2.8 Phenotypic plasticity in *Puccinellia*

Davis (1983a, b) carried out a study of the plasticity of morphological characters in the widespread temperate North American *P. nuttalliana* complex. He determined a set of traits and characters that expressed low phenotypic

plasticity under various levels of salt concentration and water availability. He found many characters in this complex that were taxonomically useful, and that combinations of these characters supported the recognition of five species, which, nevertheless, represented extremes in variation rather than distinct entities. Use of these characters in univariate and multivariate analyses of herbarium specimens in the North American arctic species showed that many of the problematic putative species in the North American Arctic overlapped in the states of these characters (Consaul and Gillespie 2001). The degree of phenotypic variation that may be related to habitat in arctic *Puccinellia* has not previously been studied.

2.9 Rationale for choice of molecular markers

Nuclear ribosomal DNA (nrDNA). Genetic evidence from multiple molecular regions is desirable in order to have a solid basis for taxonomic and evolutionary conclusions (Baldwin et al. 1995). As a source of evidence from the nuclear region, I sequenced the internal transcribed spacer (ITS) region (18S-5.8S-25S) of nuclear ribosomal DNA (nrDNA) to investigate the relationships among the diploid species and test for utility in showing the origins of polyploid taxa. The study of *Lasthenia* (Asteraceae, Chan et al. 2002) showed relationships in ITS sequences among diploid and polyploid species that could be interpreted from an evolutionary viewpoint. Van der Heede et al. (2003) found in *Asplenium* subg. *Ceterach* (Pteridophyta: Aspleniaceae) that by cloning multiple ITS copies, hybrid origin of polyploid species could be determined by the existence of copies found in different parental diploid taxa. Since the ITS region is prone to multi-copy paralogues and concerted evolution, this region would be potentially useful in the study of hybrid or polyploid species (Buckler et al. 1997). Starr et al. (2003) and Beardsley and Olmstead (2002) found increased resolution in the external transcribed spacer (ETS1) region over ITS, but Kelch and Baldwin (2003) found the level of variation was similar between ITS and ETS. I planned to design primers to amplify this region in *Puccinellia*.

Chloroplast DNA (cpDNA). CpDNA data reveals maternal parentage in grasses since it is inherited maternally. The *trnL* intron and the *trnL-trnF* spacer regions have been successfully employed in generic and subgeneric levels in the Poaceae. Hodkinson et al. (2002) showed with plastid *trnL* intron and *trnL-F* spacer sequences that the maternal lineage of *Miscanthus* ×*giganteus* was *M. sacchariflorus*. Brysting et al. (2000) identified the maternal parent of the hybrid *Poa* ×*jemtlandica* using evidence from *trnL* intron and *trnL-F* spacer regions. Incongruence between cpDNA data and nrDNA data can show ancient origins (transferred by the mother in the chloroplast) that may have taken place before subsequent speciation revealed by the nrDNA. This was shown by examining *rbcL* and *trnL* for the Hawaiian mints by Lindqvist and Albert (2002). Moreover, because either species can be the maternal parent in hybrid taxa (therefore contributing the chloroplasts), if different cpDNA patterns are found in a putative taxon, these can lead to the detection of both parents of the polyploid taxon. This has been shown, for example, in *Dryopteris* (Xiang et al. 2000). The *trnL* intron and the *trnL-trnF* spacer have been sequenced in the temperate species *Puccinellia distans* (Gielly and Taberlet 1994) and the *trnL* intron has been sequenced in *P. poacea* (L.J. Gillespie, unpublished data). I chose several regions of chloroplast DNA (cpDNA) from Demesure et al. (1995), Dumolin-Lapegue et al. (1997), and Shaw et al. (2005) that had promising variation levels in the grasses.

Markers sampling across the genome. I used amplified fragment length polymorphism (AFLP) analysis to find markers delimiting diploid taxa and search for origins of the polyploid taxa. This technique has been found to provide reproducible and reliable data in the study of genetic variation among and between closely related taxa (reviewed by Mueller and Wolfenbarger 1999). It has also been useful to detect hybrid origins of species (e.g., *Mentha*, Gobert et al. 2002; *Tsuga*, Pooler et al. 2002; *Cardamine*, Marhold et al. 2002). AFLP analysis has been useful for studying genetic variation within several genera of the grass family (Poaceae) (*Dactylis*, Reeves et al. 1998; *Phyllostachys*, Hodkinson et al.

2000; cereals research, Ridout and Donini 1999). Using AFLP, Hodkinson et al. (2002) showed that the allopolyploid grass *Miscanthus ×sinensis* was equidistant from each of its two putative progenitors and found markers that allowed for corrections to identifications that were confirmed by chromosome counts. This technique involves using polymerase chain reaction (PCR) and chosen primers to amplify small, specific regions of DNA from a digest of the total DNA (Vos et al. 1995). One then compares the polymorphisms found in the banding patterns on gels among individuals.

The following four chapters address the above objectives. These objectives and the hypotheses test taxonomic delimitation, origin of polyploids, and environmental effects on morphology for North American arctic *Puccinellia*. Chapter 3 investigates the taxonomic limits of the diploid taxa by testing Hypotheses 1A and 1B to examine molecular variation using AFLP data and Hypothesis 3 to test morphological variation. Chapter 4 describes a new species discovered in Chapter 3. Chapter 5 investigates the taxonomic limits of the polyploid taxa, testing Hypotheses 1A to determine the consistency of chromosome numbers, 1C, 2A-2D to examine the molecular distinctness and origins of the species using AFLP analyses, and Hypothesis 3 to test the morphological variation. Chapter 6 investigates the evolutionary relationships among the species delimited in Chapters 3 to 5 by testing the hypotheses in Objectives 1 and 2 using nrDNA and cpDNA sequences as well as restriction digest data.

Table 2.1. Number of species of *Puccinellia* described and accepted in the New World and northern parts of Russia and Europe. CNWG = Catalogue of New World Grasses, Davis and Soreng (2003). PAF = Panarctic Flora, Elven et al. (2003). Values in parentheses are the number of known chromosome counts, followed by the number of diploid counts.

Region	Number of taxa described	Number of accepted taxa
World (total) (Davis 1983a, Watson and Dallwitz 1999)	300	30–80
New World (ref. CNWG)	150 (31counts)	38 (21 counts, 8 +? are diploid)
South America (ref. CNWG)		10 (0 counts)
North America (ref. CNWG)		28 (21 counts; 8 are diploid)
		30–40 (31)
Arctic		
Canadian Arctic (ref. PAF)		15 (14 counts, 5 are diploid)
Russian Arctic (ref. PAF)		23 (18 counts, 8 are diploid)
Greenland (ref. PAF)		14 (14 counts, 2 are diploid – possibly a third but the count is doubtful)

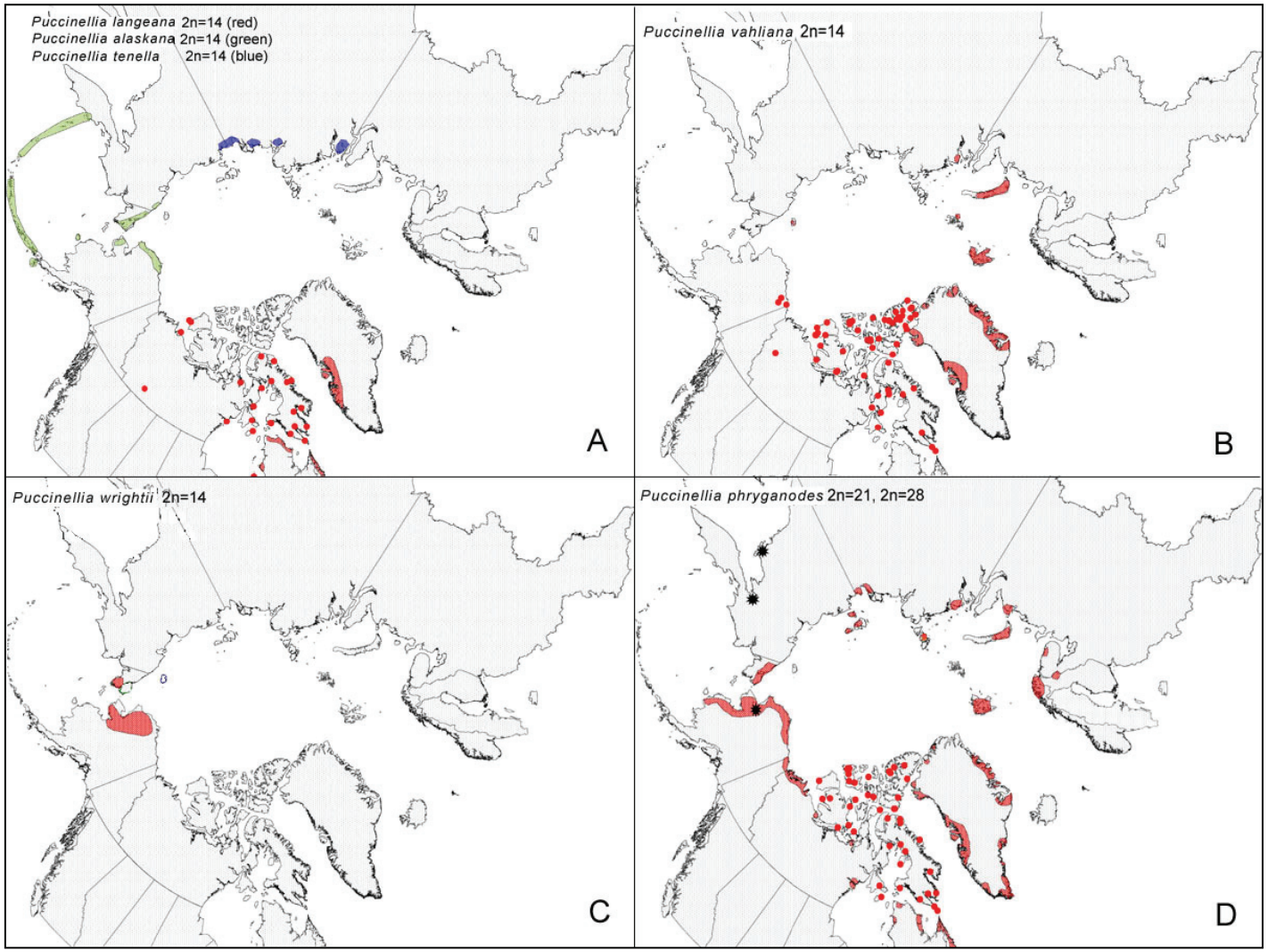
Table 2.2. Chromosome counts for circumarctic and North American temperate species of *Puccinellia* that have been published to date, listed in the Index of Plant Chromosome Numbers, the Cytotaxonomical Atlas of the Arctic Flora (Löve and Löve 1975), or the Panarctic Flora Project (Elven et al. 2003). Subgeneric classification from Tzvelev (1964). Taxa in bold included in this study. Chromosome numbers in parentheses are less common. Ala=Alaska, Can = Canada, Grl = Greenland, Nor = Norway, Rus = Russia, Rfe = Far East Russia, Sib = Siberia. "arctic" = arctic species, "boreal" = boreal species, "temp" = temperate species. (?) = distribution requiring confirmation.

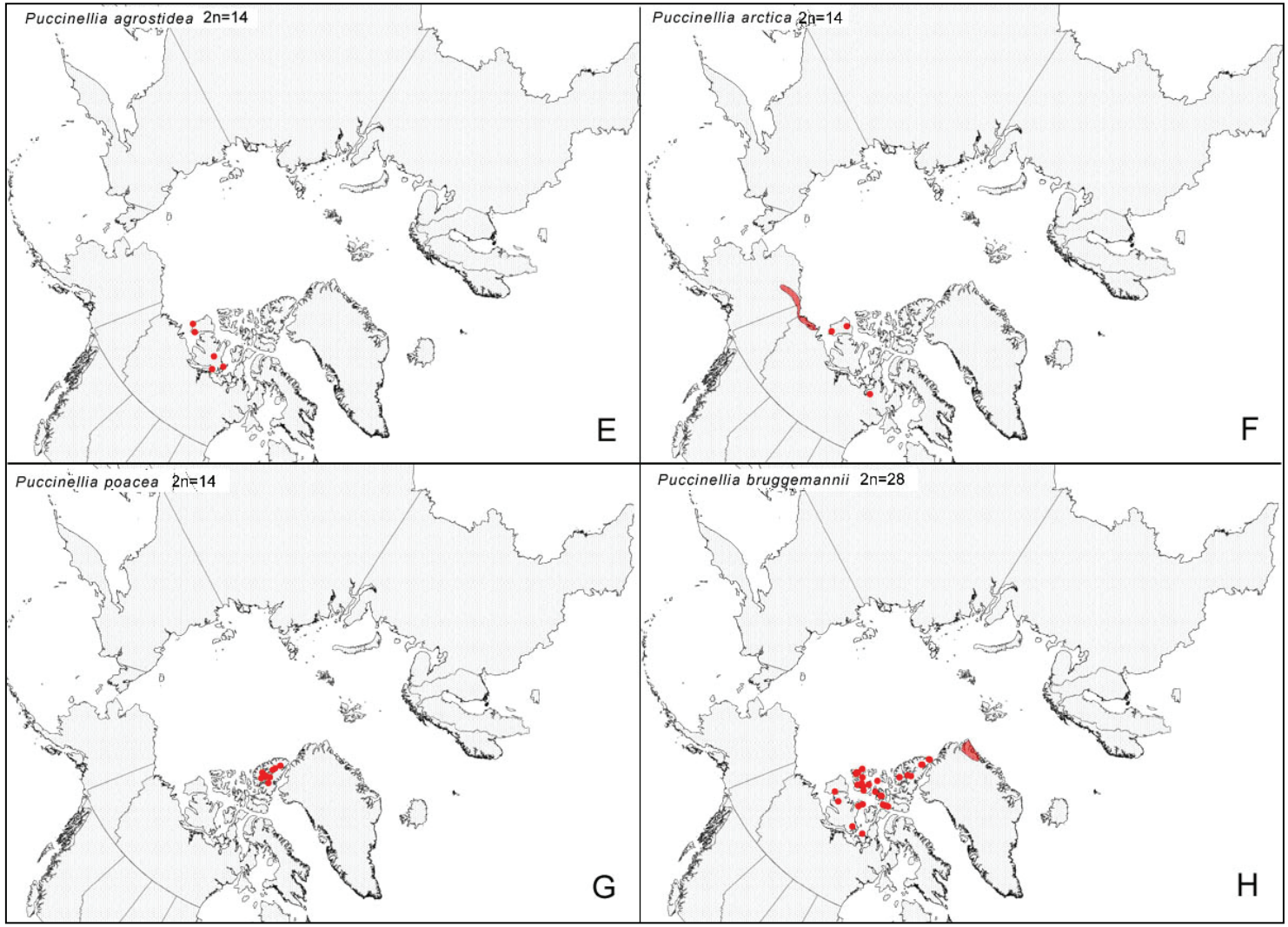
	Locality	Taxon	2*n* =	# of counts
Section *Paralochloa*				
arctic	Rfe Ala	*Puccinellia phryganodes* (Trin.) Scribn. & Merr. subsp. *phryganodes*	(14), 28	1,3
temp, arctic	Rfe (temp) Ala?	*Puccinellia phryganodes* (Trin.) Scribn. & Merr. subsp. *geniculata* (Turcz. ex V. Krecz.) Tzvelev	14	1
arctic	Nor? Can Grl	***Puccinellia phryganodes*** (Trin.) Scribn. & Merr. subsp. ***neoarctica*** (Á. Löve & D. Löve) comb. nov. needed	21	2
Section *Puccinellia*				
arctic	Rus Sib Rfe Ala	*Puccinellia hauptiana* (Trin. ex V. Krecz.) Kitig.	28	2
arctic	Sib Rfe Ala Can	*Puccinellia borealis* Swallen subsp. *borealis*	42	2
arctic	Can Grl	*Puccinellia deschampsioides* T.J. Sørensen	56	2
arctic	Ala Can Grl	***Puccinellia andersonii*** Swallen	56	1
arctic	Nor Rus Sib Rfe Ala? Can Grl	***Puccinellia angustata*** (R. Br.) Rand & Redfield	42	12
arctic	Can Grl	***Puccinellia bruggemannii*** T.J. Sørensen	28	2
arctic	Can	*Puccinellia pumila* (Macoun ex Vasey) Hitchc.	42, 56	2
arctic	Sib Rfe Ala Can Grl	*Puccinellia vaginata* (Lange) Fern. & Weath.	(42?,56)	1,4
arctic/ boreal	Can	*Puccinellia nuttalliana* (Schult.) Hitchc. in Jepson	56	?
arctic/ boreal	Can	*Puccinellia interior* T.J. Sørensen in Hultén	?	

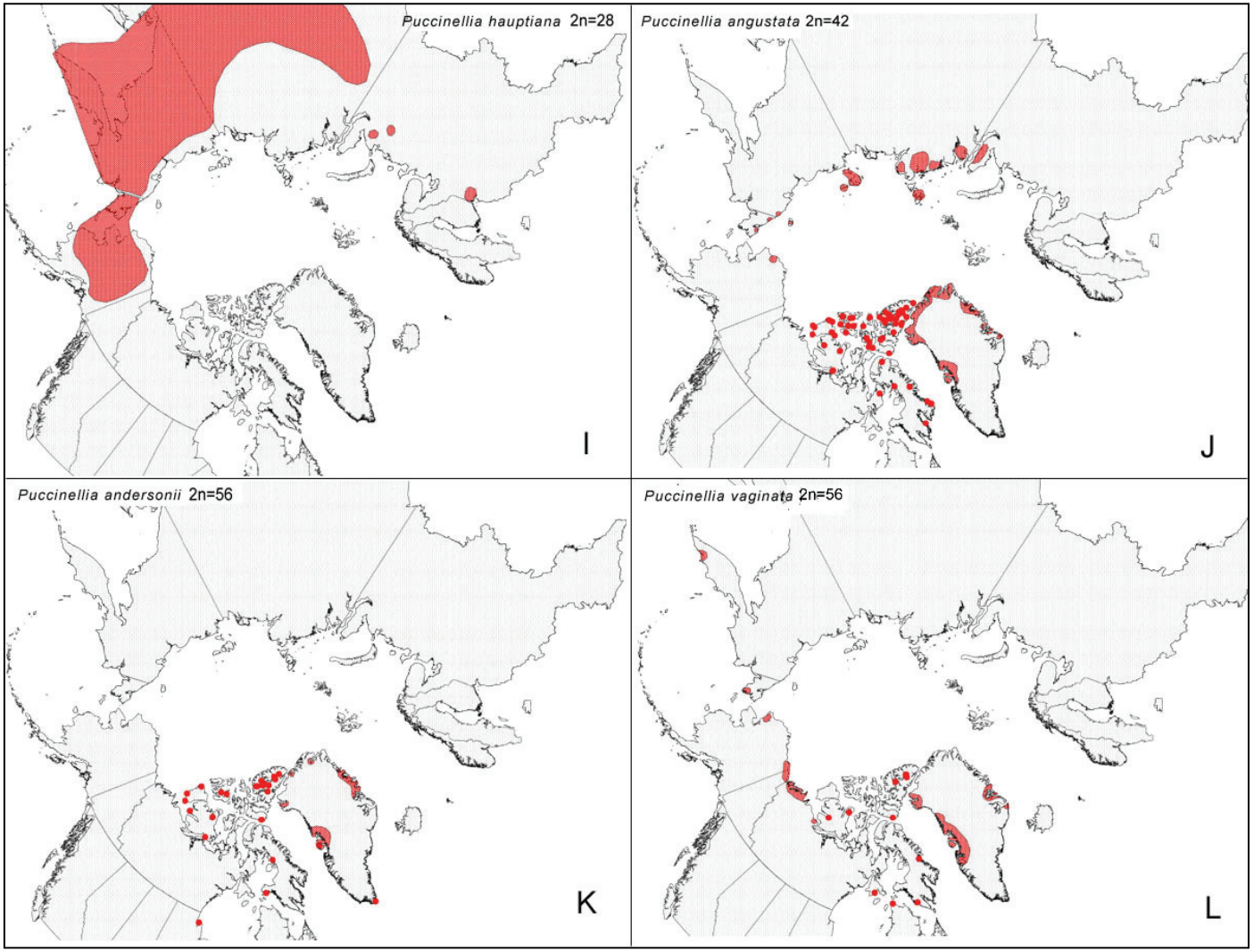
Table 2.2. Continued.

	Locality	Taxon	2*n* =	# of counts
The *Puccinellia arctica* aggregate (*P. agrostidea*, *P. arctica*, *P. poacea*) sensu Polunin (1959)				
arctic	Ala Can	***Puccinellia agrostidea*** T.J. Sørensen	14	2
arctic	Ala Can	***Puccinellia arctica*** (Hook.) Fern. & Weath.	14	2
arctic	Can	***Puccinellia poacea*** T.J. Sørensen	14	2
The *Puccinellia tenella* aggregate (includes *P. alaskana*, *P. langeana*) (currently being debated whether one or several species).				
arctic	Nor? Rfe(?) Ala Can Grl	***Puccinellia langeana*** (Berlin) T.J. Sørensen	14	1
arctic	Rfe Ala	***Puccinellia alaskana*** Scribn. & Merr.	14	1
Section *Pseudocolpodium* Tzvelev				
arctic	Rfe Ala	***Puccinellia wrightii*** (Scribn. & Merr.) Tzvelev in Tolm.	14	4
arctic	Nor Rus Can Grl	***Puccinellia vahliana*** (Liebm.) Scribn. & Merr.	14	8
Non-arctic but North American				
temp	USA (Calif.)	***Puccinellia parishii*** Hitchc.	14	1?
temp	USA	***Puccinellia lemmonii*** (Vasey) Scribn.	14	?
temp	Can	*Puccinellia lucida* Fern. & Weath.	56	**?**
temp	USA	*Puccinellia fasciculata* (Torr.) Bickn.	28	1
temp	USA	*Puccinellia rupestris* (With.) Fern. & Weath.	42	1
temp	USA	*Puccinellia simplex* Scribn.	?	
temp	USA	*Puccinellia howellii* J.I. Davis	?	
temp	Ala	*Puccinellia nutkaensis* (J.S. Presl) Fernald & Weath.	56	?

Figure 2.1. Circumpolar distribution of North American arctic *Puccinellia* species from Aiken et al. (2003), Bubnova (1990), Probatova (1985), Porsild and Cody (1980), Hultén (1968), and Tzvelev (1964). Distributions are shown in red (grey when not in colour) except in A which includes three colours. A. red = *P. langeana* (Canada and Greenland), blue = *P. tenella* (Russia, excluding Far East), and green = *P. alaskana* (Alaska and Far East Siberia). B. *P. vahliana*. C. *P. wrightii*. D. *P. phryganodes*; * = *P. phryganodes* subsp. *geniculata*. E. *P. agrostidea*. F. *P. arctica*. G. *P. poacea*. H. *P. bruggemannii*. I. *P. hauptiana*. J. *P. angustata*. K. *P. andersonii*. L. *P. vaginata*.


Puccinellia langeana 2n=14 (red)
Puccinellia alaskana 2n=14 (green)
Puccinellia tenella 2n=14 (blue)
A
Puccinellia vahliana 2n=14
B
Puccinellia wrightii 2n=14
C
Puccinellia phryganodes 2n=21, 2n=28
D


Puccinellia agrostidea 2n=14
E
Puccinellia arctica 2n=14
F
Puccinellia poacea 2n=14
G
Puccinellia bruggemannii 2n=28
H


Puccinellia hauptiana 2n=28
I
Puccinellia angustata 2n=42
J
Puccinellia andersonii 2n=56
K
Puccinellia vaginata 2n=56
L

3. Systematics of North American arctic diploid *Puccinellia* (Poaceae): Morphology, DNA content, and AFLP markers[1]

Laurie L. Consaul, Lynn J. Gillespie, and Marcia J. Waterway

3.1 Abstract

Alkali grasses (*Puccinellia*) are temperate and arctic grasses of coastal and alkaline habitats, with ploidy levels that range from diploid to octoploid. This paper investigates the species limits of diploid alkali grasses in the North American Arctic. We used flow cytometry to confirm that four to seven of the 13-19 initially recognized taxa in the North American Arctic are diploid. Multivariate analysis of both morphological and amplified fragment length polymorphism (AFLP) data were congruent in resolving five or six diploid species: 1) *P. arctica* of northern and western distribution, including *P. agrostidea* and *P. poacea*; 2) a new diploid species from Banks Island, N.W.T., Canada; 3) *P. tenella* subsp. *langeana*; 4) *P. alaskana*, which had been previously treated as a subspecies of *P. tenella*; and 5) circumpolar (except coastal Beringia) *P. vahliana*, from which 6) the coastal Beringian endemic *P. wrightii* is distinguishable only on size and for which subspecies status may be more appropriate. In common garden experiments, eleven of 21 quantitative morphological characters varied significantly between field and common garden, showing phenotypic plasticity that explains much of the difficulty in identification. We present a map showing

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known geographic ranges of diploid *Puccinellia* species in the North American Arctic.

3.2 Introduction

Alkali grasses (*Puccinellia* Parl., Poaceae, Pooideae, Poeae) are cespitose or stoloniferous pioneering grasses of coastal and interior saline and alkaline habitats in temperate and arctic regions worldwide. In the checklist of plants for the Panarctic Flora Project (Elven et al. 2003), *Puccinellia* is one of many genera with conflicting taxonomic treatments between North America and the Old World. Over half of the species are polyploids. One would expect to find taxonomic difficulties in allopolyploids due to an incorporation of genetic diversity from multiple sources (Soltis and Soltis 1999). Many of the systematic problems in *Puccinellia*, however, are in the diploid species. Of over 300 species of *Puccinellia* that have been described worldwide, 30 to 80 species have been accepted in recent treatments (Gould and Shaw 1983; Davis 1983a; Watson and Dallwitz 1999). Of these, 13 to 17 are in the North American Arctic (Elven et al. 2003) and four to seven of them are diploid, depending on the taxonomy adopted. The seven putative diploid species listed in Table 3.1 with their historical taxonomic treatments are the subject of this paper.

There are several reasons why species delimitation in *Puccinellia* is difficult. As in many plant groups with reduced inflorescences and flowers, there are few characters to use in this grass genus. The species are, with few exceptions, cespitose, with narrow glabrous or slightly scabrous leaves, several florets per spikelet, and often at least some hair on the florets. Combinations of characters such as bract shapes, ratios of bract lengths, and trichome characters (Davis 1983a) are needed to distinguish species. Phenotypic plasticity of these characters can cause problems in determining taxonomic limits. Davis (1983a) studied the plasticity of morphological characters in the polyploid *P. nuttalliana* (Schult.) Hitchc. complex to select the appropriate structural characters for taxonomic purposes. He determined a set of 27 characters that expressed low phenotypic plasticity and significant genetic variation under various levels of salt

concentration and water availability. Although more phenotypic plasticity might be expected in allopolyploids than in diploids due to their mixed genetic constitutions, examples have been published that show diploids are affected by phenotypic plasticity as much or more than polyploids. Macdonald et al. (1988) found lower phenotypic plasticity in the polyploid *Stellaria longipes* Goldie than in its diploid progenitor *S. longifolia* Muhl. ex Willd. and Garbutt and Bazzaz (1983) found slightly decreased phenotypic plasticity in a synthetic tetraploid *Phlox* compared to its parental diploid.

Molecular work on *Puccinellia* has been limited to cpDNA restriction site analysis. Choo et al. (1994) examined 16 temperate species of the genus (including six isozyme variants of *P. nuttalliana* (Schult.) Hitchc. and the two temperate diploid species *P. lemmonii* (Vasey) Scribn. and *P. parishii* Hitchc.) in their study of variation in chloroplast DNA restriction sites. Their resolution was very low, so molecular techniques that provide higher resolution at the species level were sought for examining the species limits in the current study. Amplified fragment length polymorphism (AFLP) analysis has been useful for studying genetic variation and species limits in several genera of grasses (Poaceae) (*Dactylis*, Reeves et al. 1998; cereals research, Ridout and Donini 1999; *Phyllostachys*, Hodkinson et al. 2000; *Bromus*, Peterson et al. 2002). AFLPs provide reproducible and reliable data in the study of genetic variation among and between closely related taxa (Jones et al. 1997; Mueller and Wolfenbarger 1999; McGregor et al. 2000) and congruence with assays using restriction fragment length polymorphisms (RFLP) and microsatellites or simple sequence repeats (SSR) (Powell et al. 1996).

Diploid, triploid, tetraploid, hexaploid, and octoploid *Puccinellia* have been reported in the North American Arctic. Our ongoing study of possible hybrid origins and relationships among the polyploid species requires a better understanding of the diploids. Some of the diploid *Puccinellia* species have been placed in synonymy at one time or another, raising the question whether all seven North American arctic diploid species are distinct or whether some should be considered conspecific. They were coded as “accepted,” through “tentatively

accepted," to "accepted/excluded" in Davis and Soreng (2003). These taxonomic uncertainties in the diploid species are considered here. Our objectives are to determine: a) whether distinct groups of diploid *Puccinellia* are supported by both morphological and AFLP patterns; b) which morphological differences are retained when plants are grown in a common garden experiment; and c) which structural characters are most useful to discriminate among taxa.

3.2.1 Taxonomic history

We summarize the taxonomic history of the North American Arctic diploid members of *Puccinellia* in Table 3.1. Three main groups of arctic taxa are recognized currently: section *Puccinellia,* section *Pseudocolpodium* Tzvelev (Tzvelev 1976), and the *P. arctica* aggregate (Polunin 1959), which has not yet been placed in a formal section. For simplicity in describing our experimental work, we refer to each of the North American taxa by the names they have been given at the species level, as given in the first column of Table 3.1.

Species of section *Puccinellia* (Tzvelev 1976) are distinguished from other *Puccinellia* by being smooth and glabrous or nearly so, with smaller spikelets and smooth or crenulate glume and lemma margins. The three species in this group considered here are *P. langeana*, *P. alaskana*, and *P. tenella* s.s., the latter species from Russia only but we compare it with the other two because of its close association in the taxonomic literature. These three taxa have previously been treated by Sørensen (1953) as subspecies of *P. langeana*: *P. langeana* subsp. *langeana*, *P. langeana* subsp. *alaskana*, and *P. langeana* subsp. *asiatica*. *Puccinellia alaskana* has most recently been synonymized under the polyploid *P. pumila* (Davis and Soreng 2003). These taxa are distributed in the Low Arctic (south of 74°N in North America and Greenland).

Puccinellia vahliana and *P. wrightii* were previously included in a separate genus, *Colpodium* Trin., but are now in *P.* section *Pseudocolpodium* (Table 3.1), distinguished by having glumes that are relatively even in size, with the first glume usually over ½ the length of the first lemma, anthers usually over 1.0 mm long, and thick, crinkled roots. *Puccinellia vahliana* is circumpolar

except in the coastal Beringian region, where *P. wrightii* is found instead (Tzvelev 1964, 1976).

In the *P. arctica* aggregate, *P. agrostidea* and *P. poacea* were considered as synonyms of *P. arctica* in Davis and Soreng (2003), based on the draft treatment in Flora of North America (Davis and Consaul 2007), in turn from preliminary evidence in Consaul and Gillespie (2001), but this synonymy has not yet been confirmed by detailed morphological or molecular study. Long anthers (> 1.2 mm) distinguish these species from other *Puccinellia*. *Puccinellia arctica* and *P. agrostidea* have been collected in the southwestern Arctic, but from different sites, and *P. poacea* is found farther north on Ellesmere and Axel Heiberg Islands. These three taxa were not compared directly with each other when they were originally described. Although the type specimens are quite different in stature and habit appears different in the field, the characters of the spikelets themselves are very similar. Plant size, inflorescence branching, and lemma texture have been used to separate these taxa, but there is overlap between taxa, and character states are difficult to define (Consaul and Gillespie 2001). It has not been possible to assess the morphological variation within and among these three putative taxa prior to this study because few collections for *P. arctica* and *P. agrostidea* existed.

3.3 Materials and Methods

3.3.1 Plant sampling

We sampled *Puccinellia* populations in the Canadian Arctic during the summers of 2003 and 2004 as leaves dried on silica gel, herbarium vouchers, and living plants. We also obtained dried leaf samples for DNA analysis from other researchers collecting in Alaska, Yukon, and other regions in the Canadian Arctic. Samples included in the analyses for this paper are given in Table 3.2. The collections made for this study allowed us to examine in detail the relationships among the taxa of the *P. arctica* aggregate and their relationship to the other widespread North American diploid species, *P. langeana* and *P. vahliana*. We obtained smaller samples of *P. alaskana* from Alaska and *P. wrightii* from Russia

and Alaska, as well as *P. lemmonii* and *P. parishii* from the southwestern United States to include all diploid taxa in North America. We also included specimens of *P. tenella* s.s. (sensu Tzvelev 1964) of Russia, for comparison with *P. langeana*. We coded two sets of specimens that could not be be identified using current keys as *P.* sp.1 'Banks Island' and *P.* sp.2 'octoploid'. *Puccinellia* sp.1 'Banks Island' is a diploid and studied in this paper. *Puccinellia* sp.2 is a polyploid included in the common garden experiments (described below) that will be discussed in a complementary paper on the polyploid species. Specimens collected for this study, other herbarium specimens, and additional literature records for Greenland published by Sørensen (1953) were mapped using ArcView Version 3.2 (ESRI Canada, Montreal, Quebec).

3.3.2 DNA content and ploidy determination

We examined meiotic chromosomes in pollen mother cells of inflorescences collected from vigorously growing greenhouse plants representing different putative diploid species (Table 3.2). We fixed young inflorescences in Carnoy's fixative (3:1:1 100% ethanol: acetic acid: chloroform), stored them in 70% ethanol at -20°C, stained them in Snow's stain (Snow 1963) following the procedure of Radford et al. (1974), dissected and squashed anthers, and photographed chromosomes at Metaphase I or diakinesis using a Nikon F1200 camera.

To determine nuclear DNA content, we used flow cytometry on fresh leaves from 122 putative diploid plants (Table 3.2), which had been collected live in the field and subsequently grown in a greenhouse. Fifty mg of fresh leaves were chopped with a razor blade and cells were stained using the procedure of Arumuganathan and Earle (1991) modified by using 1 mg/mL rather than 5 mg/mL propidium iodide (PI), incubating overnight at 4°C, and giving a second filtration through a 40μm filter. As an internal standard, 0.7 μL of fresh red blood cells from chicken, suspended in acid citrate dextrose (ACD) by the method of Galbraith et al. (1997), and diluted 1:50 with extraction buffer minus PI, were added to the 200 μL of resuspended plant cells. This suspension was diluted to 1

mL with PBS buffer (0.02M sodium phosphate buffer + 0.15M sodium chloride, pH 7.4) and fluorescence was read in a BD FACS Aria flow cytometer (BD Biosciences, San Jose, CA). Fluorescence was read for 10,000 cells, and the mean fluorescence and coefficient of variation (*cv*) recorded. We did ten replicate runs on a single specimen of *P. arctica* from the Yukon with a diploid chromosome count to obtain an average diploid DNA amount. Over half of our readings had a *cv* < 5%; many plants not growing as well as they would in their native arctic environment had a *cv* = 5−10%. Plants that are less healthy give fluorescence readings with higher *cv*'s (Arumuganathan and Earle 1991).

3.3.3 DNA extraction

We extracted DNA from silica-dried material or leaves from herbarium specimens, either manually using a modified CTAB method (Doyle and Doyle 1987) or with an automated system. In the second method, 10 mg of dried leaves were frozen in liquid nitrogen for 60 seconds, ground for 2 minutes in an AutoGen AutoGrinder 48 (Holliston, MA), incubated in 500μL 2% CTAB buffer (as in the manual extraction) with 0.5μL RNase A at 65°C for 30 minutes, and extracted in the AutoGen 850 automated DNA isolation system. We followed the manufacturer's protocol Version 4 for plants, adapted by using reagents modified from the protocol of Dellaporta et al. (1983): 5M Potassium acetate with acetic acid (60 mL of 5M potassium acetate, 11.5 mL of glacial acetic acid, 28.5 mL of ddH_20); 3 % SDS; 100% chloroform; 100% isopropanol; 70% ethanol; and resuspended in 100 μL TE buffer.

3.3.4 Amplified fragment length polymorphism (AFLP) analysis

We analyzed a total of 145 specimens of 11 taxa (Table 3.2). We did restriction, ligation and preamplification following the procedure of Vos et al. (1995) using recipes of Wolf et al. (2004), modified to use a 25 μL volume in the preamplification procedure. We used the following restriction enzymes, adapters, and primers: *MseI* (New England Biolabs, Ipswich, Massachusetts), *EcoRI* (Invitrogen, Burlington, Ontario), MseI adapter, EcoRI adapter, and preselective

primers EcoRI+A and MseI+C (Sigma Genosys, Oakville, Ontario). We did selective amplifications on 2 µL of diluted preamplification product using the selective amplification kit from LI-COR Biosciences (Lincoln, Nebraska), following the manufacturer's instructions, reducing the primer volumes to 0.5µL of EcoRI+ 3 labeled primer (Sigma Genosys, Oakville, Ontario) and 1 µL of MseI+ 3 primer per reaction. We ran the products on a LI-COR 4300 DNA analyzer and scored fragments on the gels using SAGA version 3.2 (LI-COR Inc.) corrected by visual evaluation. After testing 40 primer pairs, we chose the selective primer pairs E+AAG / M+CTT, E+ACG / M+CAA, and E+ACC / M+CTC, which gave the clearest, most easily read bands. Bands that were not consistent among initial replicated test samples were excluded. We analyzed the presence/absence matrix scored on each band for each sample using principal coordinates analysis in MVSP (Kovach Computing Services, Anglesey, Wales, UK, Version 3.13).

3.3.5 Morphology

For the common garden (CG) experiments, we included both diploid and polyploid species, but only report results for the diploid species in this paper. We tried to simulate field conditions in the experiments. We conducted preliminary survival trials in soils of 1:1 sand:loam, and either 1:1:1 or 1:1:2 sand:loam:baked clay. The plants grew well in all three soil combinations, but the pH for the sand:loam was easier to adjust to pH 8.0 than soil with baked clay, which retains more cations, therefore resisting change to pH with lime. We therefore chose to use a combination of 1:1 sand:loam, with pH adjusted to 7.8–8.0. The plants were collected from natural populations in the field by stratified random sampling across a moisture gradient measured as distance from the nearest body of water. We planted them in separate 10 cm pots, vernalized the plants in a growth chamber for 8 weeks at 6°C at 8 hours low light and 16 hours dark, watered to saturation once per week, and transferred them to a computer-controlled greenhouse set for temperature and humidity averaged for NWT and Nunavut locations in July based on Environment Canada climate data (18˚C day/ 5˚C night,

relative humidity 65%). Day length was set to 18 hours and plants were watered with 100mL water every other day to avoid drought stress. We performed two separate CG experiments, and analyzed them separately, because we had collections from two different years (2003 and 2004). Pots for CG#1 were arranged in a complete randomized block design and CG#2 in a complete randomized design.

Our sample of 244 plants for the morphological analysis comprised the 91 plants that flowered in the common garden experiments, the 91 associated field vouchers, and 62 extra field specimens (Table 3.2). Fewer plants were measured than planted in the original common garden setup because some plants (including one entire taxon, *P. vahliana*) did not flower during the experiments. We measured the characters (Table 3.3) used by Consaul and Gillespie (2001).

We output descriptive statistics for each character as boxplots of the medians and quartiles (McGill et al. 1978) separately for field and common garden data, analyzed the difference between the field and common garden specimens with a post hoc Tukey HSD (honestly significant difference) analysis (Sokal and Rohlf 1995), and performed a Kruskal-Wallis test on the qualitative characters testing for differences between field and common garden specimens and also analyzing variation of the qualititative characters within the common garden experiments, using Systat, Version 11 (San Jose, CA).

The Generalized Linear Model procedure (PROC GENMOD) in SAS (1999, Version 9, SAS Institute Inc., Cary, NC) was used with the gamma error distribution and logarithmic link, appropriate for analysis of length values (Lefkovitch 1993), on the 21 quantitative morphological variables in CG#1 to assess differential responses among populations. In CG#1 the factors were three blocks, seven taxa within blocks, and 16 populations within species (populations collected in 2003, number of populations in brackets). We tested four putative diploid taxa: *P. agrostidea* (2)*, P. arctica* (2)*, P. poacea* (2)*, P.* sp.1 'Banks Island' (2), and three polyploid taxa (not analyzed in this paper): *P. bruggemannii* T.J. Sørensen (3)*, P. angustata* (R. Br.) E.L. Rand and Redfield (3), and *P. andersonii* Swallen (2), with 12 replicates per population. In CG#2 the factors

were six taxa and nine populations (populations collected in 2004, except where noted); we included two diploid taxa (*P. langeana* (1) and *P. vahliana* (1)) and four polyploid taxa (*P. bruggemannii* (3)*, P. angustata* (2)*, P. andersonii* (2003) (1)*,* and *P.* sp.2 'octoploid' (2003) (1)), with 12 replicates per population. In CG#1 we used non-orthogonal planned contrasts with Dunn-Sidak adjustment of significance level (Sokal and Rohlf 1995) to compare populations of *P. arctica* versus *P. poacea, P. agrostidea* versus *P. poacea, P. arctica* versus *P. agrostidea, P.* sp.1 'Banks Island' versus the *P. arctica* aggregate (*P. agrostidea* + *P. arctica* + *P. poacea*), *P. bruggemannii* versus *P. angustata*, and *P. angustata* versus *P. andersonii*. The contrasts involving *P. bruggemannii*, *P. angustata*, and *P. andersonii*, as well as the generalized linear model analyses and planned contrasts for CG#2 will be reported in a following paper on the polyploid species. In the present paper, CG#2 results were used only to compare the common garden versus field values.

We performed principal coordinates analyses in MVSP (Multi-Variate Statistical Package, Kovach Computing Services, Wales, UK) using all 40 non-ratio characters with the Gower Similarity Coefficient (Gower 1971) for mixed data.

Discriminant analysis on the quantitative characters in SAS (PROC CANDISC and PROC DISCRIM) was used defining the groups on geographical criteria and then on AFLP groupings, to determine whether these groups could be distinguished using only morphological data. PROC STEPDISC was used to determine which morphological characters best discriminated among the resulting groups.

3.4 Results

3.4.1 Ploidy and DNA content

We obtained diploid chromosome counts of $n = 7$ for *P. agrostidea*, *P. arctica*, *P.* sp.1 'Banks Island'*, P. poacea*, and *P. vahliana* (Figure 3.1, Table 3.4). In flow cytometry, a diploid *P. arctica* specimen from the Yukon that we assigned as the standard had 3.11 pg/2C DNA per nucleus, when averaged over

10 separate runs. The DNA amounts for putative species comprising the *P. arctica* complex (*P. agrostidea*, *P. arctica*, and *P. poacea*) were not statistically different. *Puccinellia langeana* had approximately 0.97 times the amount of DNA per nucleus as the *P. arctica* standard, but this was not statistically different from *P. arctica*. *Puccinellia vahliana* had approximately 1.2 times the DNA per nucleus of the *P. arctica* standard and two populations of *P.* sp.1 'Banks Island' had 0.92 times the diploid amount of DNA per nucleus, both of these statistically different from the *P. arctica* standard.

3.4.2 AFLP analysis

A total of 80 bands were clear and consistent for the three primer pairs. Sixteen of these were monomorphic and were not scored. Thirty-nine bands were found only in certain putative taxa or combinations of putative taxa but no bands were found exclusively with a frequency (*f*) of 1.00 in a taxon. Two bands were unique to a single taxon (*P.* sp.1 'Banks Island') ($f = 0.6 – 0.95$); three bands to the *P. arctica* aggregate (*P. arctica* + *P. agrostidea* + *P. poacea*) ($f = 0.77–0.98$); four bands to *P. vahliana* + *P. wrightii* ($f = 0.73–0.94$); seven bands to *P. arctica* + *P.* sp.1 'Banks Island' ($f = 0.65–1.00$); four bands to *P. langeana* + *P. tenella* ($f = 0.17–0.96$); three bands to *P. langeana* + *P. vahliana* + *P. wrightii* ($f = 0.75–1.00$); three bands to *P. alaskana* + *P. langeana* + *P. vahliana* + *P. wrightii* ($f = 0.25–1.00$); two bands to *P. langeana* + *P. vahliana* + *P. wrightii* +*P.* sp.1 'Banks Island' ($f = 0.17–1.00$); two bands to *P. langeana* + *P. arctica* ($f = 0.04–0.58$); six bands to *P. arctica* + *P. vahliana* ($f = 0.12–0.98$); three bands to all but *P. langeana* ($f = 0.17–1.00$). The remaining 25 bands appeared with moderate to low frequency in several taxa. We found the following number of bands per sample in each putative taxon: *P. agrostidea*, 25.3 ± 0.9; *P. arctica*, 26.3 ± 1.1; *P. poacea*, 24.8 ± 0.8; *P.* sp.1 'Banks Island', 19.7 ± 1.0; *P. langeana*, 18.2 ± 1.0; *P. alaskana*, 18.5 ± 1.0; *P. vahliana*, 23.8 ± 1.0; *P. wrightii*, 22.5 ± 1.0. The single specimens of *P. lemmonii* and *P. parishii* had six and four unique bands, respectively, and shared three bands unique to this pair. These bands

distinguished them from the arctic species, but because no population comparisons could be made, we did not include them in further analyses.

In the principal coordinates analysis of AFLP data (Fig. 3.2), specimens clearly separate in three dimensions into five groups. Specimens of the *P. arctica* complex form a single tight group (group 1) while specimens of *P.* sp.1 'Banks Island' are clustered together in a group distinct from all others (group 2). The three specimens of *P. tenella* group with *P. langeana* (group 3), and the specimens of *P. alaskana* form a distinct group that is between *P. langeana* and *P. vahliana* (group 4). The three specimens of *P. wrightii* are included in the *P. vahliana* group (group 5).

3.4.3 Morphology

When we analyzed all diploid, arctic *Puccinellia* taxa for which we had both field and common garden (CG) plants, 12 out of 24 quantitative morphological characters were significantly different between field and CG (noted as "All: $p \leq X$" above the graphs in Fig. 3.3a and 3.3b; Table 3.3), including all six characters related to habit and inflorescence shape and 6 of 18 spikelet characters. When we analyzed each taxon independently, three inflorescence characters were significantly different between field and common garden for all taxa ($p < 0.05$), whereas the rest were insignificant for some of the taxa. Anther length was stable between field and CG for species with short anthers, but anthers were significantly smaller in size in the CG for all three taxa that had anthers over 1.2 mm long (*P. arctica*, *P. agrostidea*, *P. poacea*). Seven out of 18 qualitative characters were significantly different ($p \leq 0.05$) between field and common garden plants (Table 3.3).

The ranges for most characters overlapped among all taxa (Fig. 3.3). The exceptions were: lemma length, palea length, and extent of hair on palea and lemma (less in *P. langeana* and greater in *P. vahliana* than in most specimens of other species), and anther length (smaller in *P.* sp.1 'Banks Island' and *P. langeana* than in other species). The width to length ratio of the second glume is

much smaller in *P. vahliana* than in other species, demonstrating that the shape of the second glume is different in *P. vahliana* than in the other species analyzed.

In the generalized linear model for the first common garden experiment, all quantitative characters varied significantly among all populations (Appendix C). The Dunn-Sidak adjusted alpha equals 0.009. Planned contrasts showed that two of the 24 characters (second glume length, lemma length) differed significantly ($p < 0.008$) between *P. arctica* and *P. poacea,* three (height, inflorescence length, and lemma hair extent) were significantly ($p < 0.007$) different between *P. arctica* and *P. agrostidea*, and four characters (height, inflorescence length, primary branch length, and panicle internode length) differed significantly ($p < 0.004$) between *P. agrostidea* and *P. poacea*. The ranges of each of these characters, however, overlapped considerably (Fig. 3.3). When testing *P.* sp.1 'Banks Island' versus the three species in the *P. arctica* aggregate, there were 14 significantly different characters (height, inflorescence length, primary branch length, panicle internode length, spikelet length, second glume length, second glume width, rachilla length, lemma length, lemma hair extent, palea length, palea hair extent, anther length, and ratio of palea width/length). Kruskal-Wallis tests on the qualitative characters showed that all but four of the qualitative characters (ligule apex shape, ligule hairy or glabrous, first glume apex shape, and glumes opposite) contributed significantly to the variation in the matrix (Kruskal-Wallis Test Statistic (KW) < 9.00, $p > 0.05$). The qualitative characters with very high KW values (KW > 110, $p < 0.001$), indicating greatest contributions to the variation in the dataset, were: second glume shape, lemma distal margins smooth or scabrous, lemma hair, first glume shape, branches and pedicels smooth or scabrous.

Figure 3.4a shows the results of a principal coordinates analysis of the field specimens using all 40 qualitative and quantitative non-ratio characters. Out of the seven original North American arctic taxa plus one Russian taxon and a putative new taxon, this ordination resolves five groups: 1) *P. arctica*, *P. agrostidea*, *P. poacea*; 2) *P.* sp.1 'Banks Island', 3) *P. langeana, P. tenella*; 4) *P. alaskana*; 5) *P. vahliana*, *P. wrightii*. For the ordination of CG specimens we

lacked specimens of groups 4 and 5 as outlined in the methods, but the remainder of the specimens clearly resolved into the same groups 1-3 (graph not shown). When we analyzed field and common garden specimens together, however, in an ordination using all 40 characters, groups 1 and 2 overlapped (Fig. 3.4b). In an ordination including only specimens from six taxa, two groups clearly resolved (Fig. 3.4c). When we analyzed the same specimens using only the nine stable characters from Table 3.1, groups 1, 2, and 3 were separated (Fig. 3.4d).

In discriminant analysis, when the quantitative data were analyzed with groups defined using AFLP groupings, the analysis discriminated the same five groups (not shown) as the morphological ordination (Fig. 3.3a), with no specimens misclassified. When the groups were defined based on nine geographically distinguished putative groups (seven original North American species, one Russian species, and the new taxon from Banks Island), only the same five groups could be distinguished (Fig. 3.5a) – the same groups distinguished based on the AFLP (Fig. 3.2) and morphological (Fig. 3.4a) ordinations. The characters that were significant in the discrimination ($p \leq 0.05$) were 1, 6, 8, 21, 22, 28, 29, 34, 36, 39, 40, 41, and 44 (characters defined in Table 3.3). We took the groups that were not clearly resolved in Fig. 3.5a (groups 2, 3, and 4) and analyzed them separately, defining the groups as *P. alaskana*, *P. langeana*, *P. tenella*, and *P.* sp.1 'Banks Island', to see if the Russian taxon *P. tenella* would separate, but still only the same three groups resolved (groups 2, 3, and 4). The significant characters in this discrimination were 15, 25, 34, 36, 38, 40, 41, 42, and 44 (Table 3.3). Figure 5c shows that the separate population groups of the *P. arctica* aggregate were not distinct when groups were defined on geography (*P. agrostidea* and *P. arctica* from different locations on Banks Island and *P. poacea* from Ellesmere and Axel Heiberg Islands). Significant characters in this discrimination were 1, 10, 25, and 40 (Table 3.3). Nor was resolution of groups attained when specimens within the *P. arctica* aggregate were placed in two groups (Banks Island versus Ellesmere Island); 16% of Banks Island and 21% of Ellesmere Island specimens were misclassified (graph not shown). Figure 3.5d shows that when *P. vahliana* and *P. wrightii* were defined as separate groups, the

discriminant analysis did separate these two taxa, with significant characters 1, 8, 36, 42 (Table 3.3).

3.4.4 Geographic distribution

Distributions of the six species determined to be distinct in this study (see Discussion) are shown in Figure 3.6. *Puccinellia arctica* is distributed in the southwestern Arctic on the islands and neighboring continental Northwest Territories and Nunavut (syn. *P. agrostidea*, *P. arctica s.s.*), and disjunct in the north on Ellesmere and Axel Heiberg Islands (syn. *P. poacea*). The new species *P.* sp.1 'Banks Island', is currently known from three locations on southern Banks Island and one site in Alaska. *Puccinellia tenella* subsp. *langeana* occurs in the southern arctic islands, on the North American continent and in Greenland; *P. tenella* subsp. *tenella* is only found in Russia and is not shown on this map. *Puccinellia alaskana* is found in far western Alaska out to the Aleutian Islands. *Puccinellia vahliana* is distributed all over the arctic islands and on the continent as far west as eastern Alaska while *P. wrightii* has a Beringian distribution in coastal far western Alaska and neighboring Russia.

3.5 Discussion

Our results showed that *P. arctica*, *P. agrostidea,* and *P. poacea* had similar AFLP patterns, had a similar amount of DNA in the nucleus, and grouped together in morphological analyses. Polunin (1959) treated these taxa as the *P. arctica* aggregate, while Davis and Soreng (2003) treated them as a single species based on our preliminary morphological results (Consaul and Gillespie 2001). Our results here from a broader sampling and including genetic analysis confirm this treatment.

Puccinellia wrightii has always been considered a separate species, but in this study it is only separated from *P. vahliana* by the size of its culms and branches. The small AFLP sample analyzed here suggests that *P. wrightii* may be conspecific with *P. vahliana*. Consaul et al. (2005) documented specimens that were intermediate between *P. vahliana* and *P. wrightii*, which further suggests a continuum of morphological entities with *P. vahliana* at one end and *P. wrightii* at

the other. *Puccinellia vahliana* is circumpolar except in coastal Beringia where *P. wrightii* is found instead. These results and this geographical distribution pattern suggest that subspecies treatment of these two species may be appropriate. More extensive sampling of *P. wrightii* and other members of the complex is needed before any taxonomic changes are made.

Our results suggest that *P. tenella* and *P. langeana* should be treated as conspecific. Although we only scored three specimens of *P. tenella* for the AFLP analyses, *P. tenella* and *P. langeana* formed a single distinct group in both the morphological and AFLP analyses. We maintain subspecies status for these two allopatric entities until more extensive sampling of *P. tenella* can be done in Russia for molecular and morphological analyses.

When *P. langeana* and *P. tenella* are treated as subspecies, there is controversy about the correct species name. Sørensen (1953) argued that the correct name was *P. langeana.* He contended that the drawing in Lange's 1882 protologue of *P. tenella* did not agree with the description and was not similar to *P. langeana*, but was more similar to the hexaploid *P. coarctata* Fernald & Weath., which grows in eastern Canada, Iceland, Greenland, Norway, and Russia. He, therefore, neotypified and amended the description of *P. tenella* (*P. tenella* (Lange) nov. emend T. J. Sørensen, 1953, p. 80) to have characteristics closer to *P. coarctata.* For specimens of Russian "*P. tenella*" that were glabrous and therefore similar to *P. langeana*, he published a new name, *P. langeana* subsp. *asiatica.* In contrast to Sørensen's treatment, Tzvelev (1964) argued that Lange's original drawing and a fragment of a specimen cited in the protologue of *P. tenella* did agree with the original description of *P. tenella,* were, in fact, similar to *P. langeana*, and therefore *P. tenella* would be the correct name by priority. Davis and Soreng (2003) noted that the treatment of *P. langeana* as a subspecies of *P. tenella* rested on accepting the arguments about the type material made by Tzvelev. We compared the protologues in each of the original descriptions. The protologues indicate that *P. tenella* ("caespitoso-pulvinata…ramis laevibus…spiculis glabris", with the lemma "obtusa…leviter erosa") and *P. langeana* ("dense caespitosa…paniculis et spiculis glabris", with the lemma

"obtusis…laceratis") are cespitose and glabrous species, with obtuse lemmas that are erose or lacerate. Sørensen's concern is understandable because the drawings of the lemma apex in the *P. tenella* protologue are ambiguous. The apex is described as slightly erose in the diagnosis, but the apices are shown as very erose to serrate in the drawing. Sørensen (1953) interpreted this margin as scabrous and thus aligned the taxon with a scabrous species (*P. coarctata*) that also had scabrous pedicels. There is a problem here, however, because, as quoted above, the original *P. tenella* description and drawing had distinctly glabrous pedicels. We interpret the drawing as depicting an erose or serrate margin, not a scabrous margin. The name *P. tenella* then takes priority over *P. langeana* because the former was described two years earlier. We, therefore, treat *P. langeana* as *P. tenella* subsp. *langeana*, in agreement with Tzvelev (1964, 1976).

In this study, the specimens from Bogoslov Island in the Aleutians and other herbarium specimens that had been identified as *P. alaskana* were distinct. In AFLP analysis they were distinct from *P. tenella,* closer to but separate from *P. vahliana* (Fig. 3.2), while in the morphological analysis, they were arranged in a loose group between *P. tenella* and *P. vahliana* (Figs. 3.3 and 3.4). Further study with more samples from several areas over the range of *P. alaskana* is recommended to investigate these results. This study did not include polyploids; therefore the comparison of *P. alaskana* with *P. pumila* (Vasey) Hitchc., a polyploid with which *P. alaskana* has recently been synonymized (Table 3.2), was not made. Until these species are compared we do not treat the diploid as a subspecies of the polyploid; if *P. pumila* is found to be an autopolyploid of *P. alaskana*, synonymizing these taxa may be appropriate.

Our results revealed a new taxon (here *P.* sp.1 'Banks Island') that is diploid and different from all other diploid species in North America based on DNA content, morphology, and AFLP data. We found three main populations on Banks Island and Alaska. This taxon resembles very closely the tetraploid *P. hauptiana* (Trin. ex V.I. Krecz.) Kitag. of Russia (Tzvelev 1964, 1976) and far western Alaska (Hultén 1968), and the circumpolar hexaploid *P. angustata*. The new species from Banks Island differs from the former by having hairier paleas,

denser inflorescences and from the latter by having much smaller spikelet and floret characters. This new species is also sympatric with the octoploid *P. andersonii*. Because comparisons should also be made with these polyploids, the new species will be described in a separate paper.

Each of the morphological analyses was useful for different aspects of determining the most informative characters that distinguish species. The Kruskal-Wallis (KW) test revealed which qualitative characters distinguished the main groups: scabrosity of primary branches and pedicels; scabrosity of lemma apex margins, scabrosity of the first and second glume apices, palea hair. The common garden experiment showed that most of the quantitative characters overlap among species and that many characters were plastic between field and common garden conditions. Inflorescence and habit characters showed more differences between field and common garden than those related to the spikelet. This is in agreement with previous reports that characters controlled by long periods of meristematic activity have high amounts of plasticity while those related to flowers have little (Bradshaw 1965, Marshall and Jain 1968, Wilken 1977, Lacey 1986, Macdonald et al. 1988). Although all 40 characters can be used in an ordination to get relatively good separation, the values may shift if a population has been collected in a particularly good habitat or in a particularly good year. Greater reliance should therefore be put on characters that did not vary between treatments. It is perhaps not surprising that the first four quantitative characters chosen in stepwise discriminant analysis of all taxa (extent of lemma hair, anther length, lemma length, and extent of palea hair) were also stable between field and common garden, making them useful key characters for distinguishing species.

With our classification of the diploid species in the North American Arctic, six of 14–18 (33–43%) of the species currently recognized in the North American Arctic are diploid. This proportion of diploid species is higher than for temperate North American *Puccinellia* species (2/11 = 18%), in contrast to the general trend of decrease in proportion of diploid species in a genus as one moves farther north (Tischler and Hagarup in Stebbins 1950, Otto and Whitton 2000).

Lewis (1980) listed a few species that have diploid cytotypes in arctic regions and polyploid cytotypes further south (*Campanula rotundifolia* L., *Chamerion angustifolium* (L.) Holub, *C. latifolium* (L.) Holub, *Vaccinium uliginosum* L.), and suggested that these species are under less stress in the arctic regions. The largest concentration of diploid *Puccinellia* species is in the Beringian region, where it appears that these genotypes are well adapted.

Hultén (1972) postulated that a Canadian High Arctic refugium existed during the Wisconsin glaciation (reviewed by Abbott and Brochmann 2003). Geological findings by England (1999) have also suggested that nunataks (small unglaciated mountain tops) existed on eastern Ellesmere Island during the last glaciation. With this geological evidence, several studies have sought evidence for plant or animal refugia associated with these nunataks, but no conclusive evidence has been found in studies of *Dryas integrifolia* (Tremblay and Schoen 1999), *Potentilla* sect. *Niveae* (Eriksen and Töpel 2006), and *Daphnia pulex* (Weider and Hobæk 2003). Similarly, the diploid *Puccinellia* species studied here do not have distinct genetic markers in the north (Ellesmere Island) and provide no conclusive botanical evidence for a High Arctic refugium.

The reclassification of diploid North American *Puccinellia* affects the number of species that have been reported rare in Canada (Argus and Pryer 1990), the Canadian Arctic (McJannet et al. 1993) and the Northwest Territories (McJannet et al. 1995). Of the three diploid species reported as rare in the Arctic (*P. agrostidea*, *P. arctica*, *P. poacea*) the first two were reported as globally imperiled and *P. poacea* as critically imperiled (Argus and Pryer 1990) and a narrow, high arctic endemic (McJannet et al. 1993). *Puccinellia arctica* s.l. occurs only in two disjunct geographical regions 1) Banks Island and the nearby coast of Northwest Territories, and 2) central Ellesmere and Axel Heiberg Islands, now Nunavut. It is rare according to the ranking criteria of The Nature Conservancy (1988) at level S2 in NWT (having 14 known occurrences) and at level S3 in Nunavut (having 45 occurrences), at level N3 in Canada (with 59 known occurrences), and at G3 worldwide. The new species *P.* sp.1 ‘Banks Island’ has only three known occurrences in the Northwest Territories and one in Alaska,

which means the Nature Conservancy rankings are S1, N1, and G1 (critically imperiled).

3.6 Key to diploid *Puccinellia* species in the North American Arctic

1. Pedicels smooth; lemma apical margins smooth or crenulate
   2. Lemmas 3–5 mm long; keels of palea scabrous distally, frequently with curly hairs on the lower portion; anthers 1.0–2.0 mm long
      3. Culms 5–15 cm; panicles 2–4 cm, branches erect to slightly ascending ……..…....*P. vahliana*
      3. Culms 15–40 cm; panicles 5–8 cm, branches usually ascending to horizontal ……...*P. wrightii*
   2. Lemmas 2–3 mm long; keels of palea glabrous or slightly ciliate; anthers 0.5–0.9 mm long
      4. Lemmas 2–2.5 mm, apices obtuse; glume and lemma margins smooth to crenulate; keels of the palea glabrous……..... *P. tenella*
      4. Lemmas 2.5–3.0 mm, apices acute; glume and lemma margins smooth but not crenulate; keels of palea slightly ciliate……. .*P. alaskana*

1. Pedicels scabrous, sometimes slightly so; lemma apical margins scabrous, sometimes with only a few scabrules.
   5. Anthers (0.9)1.2–2.2 mm long; lemmas 2.1–3.8 mm long; paleas lacking curly hair in proximal half ..……*P. arctica*
   5. Anthers 0.5–0.8 mm long; lemmas 1.7–2.3(–2.7) mm long; paleas with curly hair in proximal half ..……..…….*P.* sp.1 ‘Banks Island’

Table 3.1. History of taxonomic treatments for diploid *Puccinellia* in the North American Arctic. n/a = not applicable.

Species	Sørensen 1953 (Greenland), 1955 (western Canadian Arctic Islands)	Hultén 1968 (Alaska)	Porsild 1964 (Canadian Arctic Islands)	Polunin 1959 (Canadian Arctic)	Tzvelev 1964 (Russia)	Tzvelev 1976 (Russia)	Davis and Soreng 2003 (North America)	Davis and Consaul 2007 (North America)
Puccinellia arctica (Hooker) Fernald & Weath.	*P. arctica*	*P. arctica*	n/a	*P. arctica*	*P. arctica*	n/a	*P. arctica*	*P. arctica*
Puccinellia agrostidea T.J. Sørensen	*P. agrostidea*	*P. agrostidea (*as *P. agrostoidea)*	*P. agrostidea*	not accepted, included in *P. arctica* agg.	n/a	n/a	= *P. arctica*	= *P. arctica*
Puccinellia poacea T.J. Sørensen	*P. poacea*	n/a	*P. poacea*	not accepted, included in *P. arctica* agg.	n/a	n/a	=*P. arctica*	= *P. arctica*
Puccinellia langeana (Berlin) T.J. Sørensen	*P. langeana* subsp. *langeana*	*P. langeana* subsp. *langeana*	*P. langeana*	*P. langeana*	*P. tenella* (Lange) Holmb. ex Porsild subsp. *langeana* (Berlin) Tzvelev	*P. tenella* subsp. *langeana*	*P. langeana*	*P. tenella* subsp. *langeana*
Puccinellia alaskana Scribn. & Merr.	*P. langeana* subsp. *alaskana* (Scribn. & Merr.) Tzvelev	*P. langeana* subsp. *alaskana*	n/a	not accepted, included in *P. langeana* agg.	*P. alaskana*	*P. tenella* subsp. *alascana* (Scribn. & Merr.) Tzvelev	= *P. pumila* (Vasey) Hitchc.	= *P. pumila*

Table 3.1 Continued.

Species	Sørensen 1953 (Greenland), 1955 (western Canadian Arctic Islands)	Hultén 1968 (Alaska)	Porsild 1964 (Canadian Arctic Islands)	Polunin 1959 (Canadian Arctic)	Tzvelev 1964 (Russia)	Tzvelev 1976 (Russia)	Davis and Soreng 2003 (North America)	Davis and Consaul 2007 (North America)
Puccinellia vahliana (Liebm.) Scribn. & Merr.	*Colpodium vahlianum* (Liebm.) Nevski	*Colpodium vahlianum*	*Colpodium vahlianum*	*Colpodium vahlianum*	*P. vahliana*	*P. vahliana*	*P. vahliana*	*P. vahliana*
Puccinellia wrightii (Scribn. & Merr.) Tzvelev	n/a	*Colpodium wrightii* Scribn. & Merr.	n/a	*Colpodium wrightii*	*P. wrightii*	*P. wrightii*	*P. wrightii*	*P. wrightii*

Table 3.2. Collections studied for diploid *Puccinellia* species. Morph: number of field specimens, number of common garden specimens measured for morphology (when only one number given, field specimens unless noted as herbarium specimens); Flow: number of specimens analyzed in flow cytometry (specimen with asterisk, chromosome count only); AFLP: number of specimens analyzed for AFLPs. Vouchers at MTMG unless otherwise specified. Samples obtained from herbarium material only are denoted by (herb.).

Taxon	Collection data for populations	Morph	Flow	AFLP
P. agrostidea	Canada, NWT, Banks I, DeSalis Bay, *LLC 2813, LJG, and H. Bickerton*.	10, 10	6	5
	Canada, NWT, Banks I, DeSalis Bay, *LLC 2823, LJG, and H. Bickerton* (chromosome voucher).	10, 10	5	6
	Canada, NWT, Banks I, Thomson River Valley, *S.G. Aiken 99-210* (chromosome voucher).		1	
P. arctica	Canada, NWT, Banks I, Masik R. *LLC 2805, LJG, and H. Bickerton* (chromosome voucher).	5, 11	10	5
	Canada, NWT, Banks I, Masik R. *LLC 2808, LJG, and H. Bickerton*.	11, 12	7	5
	Canada, Yukon, Herschel I, *W.J. Cody 36154* (chromosome voucher).	1	1	1
P. poacea	Canada, Nunavut, Ellesmere I, Eureka. *LLC 2864 and K. Faubert*.	10, 10	6	10
	Canada, Nunavut, Ellesmere I, Tanquary Camp, *LLC 2140 & LJG* (chromosome voucher)		1*	
	Canada, Nunavut, Ellesmere I, Whitsunday Bay, *LLC 2894 and K. Faubert*.		1	
	Canada, Nunavut, Axel Heiberg I, Buchanan Lake, *LLC 2898 and K. Faubert.*	10, 7	8	5
P. sp.1 'Banks Island'	Canada, NWT, Banks I, Masik R. *LLC 2810, LJG, and H. Bickerton* (chromosome voucher).	12, 12	12	6
	Canada, NWT, Banks I, De Salis Bay, *LLC 2814, LJG, and H. Bickerton*.	2		2
	Canada, NWT, Banks I, Egg River, *LLC 2842, LJG and H. Bickerton.*	12, 12	11	11
P. langeana	Canada, NWT, Banks I, Duck Hawk Bluff, *LLC 2856, LJG and H. Bickerton*.	2		2
	Canada, Nunavut, Baffin I, Tarr Inlet, *LLC 2907 and K. Faubert.*	5		5
	Canada, Nunavut, Baffin I, Tarr Inlet, *LLC 3164, 3170 and A. Archambault.*	10, 7	18	10
	Canada, Nunavut, Bylot I, *Tremblay 04.*			3

Table 3.2. Continued.

Taxon	Collection data for populations	Morph	Flow	AFLP
P. alaskana	USA, Alaska, Bogoslof Island, Aleutian Islands, *R. Meehan #BOGO03*.	5		5
	USA, Alaska, Little Diomede Island 14-20 Aug 1926, *A.E. and R.T. Porsild 1644,* (herb).	1		
	USA, Alaska, Kiska quad., Rat Islands, *M. Dick 83* (ALA) (herb).	1		
	USA, Alaska, Kiska quad., Rat Islands, *M. Dick 84*. (ALA) (herb).			
	USA, Alaska, Umnak quad., Bogoslof Island, *B.F. Friedman 80-147*. (ALA) (herb).			2
P. tenella	Russia, Arctic Jakutia. *Petrovsky 8.IX.1955* (herb.).		1	1
	Russia, Sahka Republic (Yakutia), Polyarka, *Solstad & Elven 04/1156* (ALA) (herb.).		1	1
	Chutotka Peninsula, Lavrentiya, 29 Aug 1971, *N.A. Sekretarova et al. s.n.* (ALA) (herb.).		1	
P. vahliana	Canada, NWT, Banks I, Masik R. *LLC 2806, LJG, and H. Bickerton*		5	1 5
	Canada, NWT, Banks I, De Salis Bay *LLC 2822, LJG, and H. Bickerton*			1 5
	Canada, NWT, Banks I, Nelson Head *LLC 2831, LJG, and H. Bickerton*.		5	5
	Canada, NWT, Banks I, Durham Heights *LLC 2838, LJG, and H. Bickerton* (chromosome voucher).			1 5
	Canada, Nunavut, Axel Heiberg I, *LLC 2884 and K. Faubert.*		5	5
	Canada, Nunavut, Beechey I, *LLC 3074 and A. Archambault*.		5	9 7
	Canada, Nunavut, Bathurst I, *LLC 3079 and A. Archambault*.		5	2 2
	Canada, Nunavut, Somerset I, Cunningham Inlet, *LLC 3082 and A. Archambault*.		2	2 2
	Canada, Nunavut, Somerset I, Aston Bay *LLC 3084 and A. Archambault*.	5		2 5
	Canada, Nunavut, Cornwallis I, Resolute, *LLC 3102 and A. Archambault*.	5		14 5
	Canada, NWT, Prince Patrick I, Mould Bay, *LLC 3149 and A. Archambault*.			1 5
	Canada, Nunavut, Baffin I, Nanisivik, *LLC 3171 and A. Archambault*.			2 3
	Canada, NWT, Banks Island, Egg River, 24 July 1971, *K.L. MacInnes s.n*.	1		
P. wrightii	Canada, Alaska, *R. Elven and H. Solstad 09*.			3
	Russia: Chukotka Peninsula, Chaplin Cape, *R. Satpuisau s.n.* (ALA) (herb.).	1		1
	Chukotka Pen., Cape Chaplino, *V. Gavreluk* (ALA) (herb.).	1		
	Chukotka Pen., Lavrentiya, *N.A. Sekretarova et al.* (ALA) (herb.).	1		
	Chukotka Pen., Uelen, *N.A. Sekretarova et al.* (ALA) (herb.).	1		
	USA: Alaska, Cape Prince of Wales, *T. Kelso 82-230* (ALA) (herb.).	1		
P. lemmonii	USA, California. *J. I. Davis 425* (BH, MTMG)			1
P. parishii	USA, New Mexico. *J.I. Davis and P.S. Manos 568* (BH, MTMG)			1
TOTAL		153, 91	122	145

Table 3.3. Morphological characters analyzed in this study. Significant differences (Signif.) found by Tukey HSD analyses (quantitative data) and Kruskal-Wallis tests (qualitative data) between plants from field and common-garden experiments are indicated following each character (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = not significant).

Character with units and character states	Signif.
1. Height (cm)	***
2. Ligule shape: linear = 1; lanceolate = 2; ovate-oblong = 3; transversely oblong = 4	ns
3. Ligule, apex shape: acuminate = 1; acute = 2; obtuse = 3; truncate = 4	ns
4. Ligule apex: entire = 1; erose = 2; lacerate = 3; cleft = 4	***
5. Ligule: hairy = 1; glabrous = 2	ns
6. Ligule length (mm)	**
7. Inflorescence branch number at lowest node	ns
8. Inflorescence length (cm)	***
9. Primary branches and pedicels: smooth = 1; scabrous = 2	ns
10. Primary branch length (cm) (at lowest node)	***
11. Primary branch, distance on branch to first spikelet (cm)	***
12. Panicle basal internode length (cm)	***
13. Inflorescence branches, maximum divergence: erect = 1; ascending = 2; horizontal = 3; descending = 4	**
14. Pedicel width 1 (just below first glume, mm)	***
15. Pedicel width 2 (0.5 mm below first glume, mm)	ns
16. Spikelet length (mm)	**
17. Florets per spikelet number	*
18. First glume shape: oblong = 1; lanceolate = 2; ovate = 3; elliptic = 4. obovate = 5	ns
19. First glume apex margin: glabrous = 1; sparse tiny trichomes < 25 μm = 2; moderately abundant, tiny trichomes < 25 μm (most) = 3; abundant trichomes many reaching > 25 μm long = 4	ns

Table 3.3. Continued.

Character with units and character states	Signif
20. First glume apex shape: with caudate tip = 1; acuminate = 2; acute or obtuse = 3; truncate = 4	***
21. First glume length (mm)	**
22. First glume width (mm)	**
23. Second glume shape (see character 18)	***
24. Second glume apex margin (see character 19)	ns
25. Second glume length (mm)	ns
26. Second glume width (mm)	***
27. Glume postion: "opposite" = 1; 0.1–0.2 mm apart = 2; < 0.3 mm apart = 3	**
28. Rachilla length between 1st and 2nd lemma (mm)	ns
29. Rachilla width (μm)	ns
30. Callus: glabrous = 1; hairy = 2	ns
31. Lemma apex shape: acute = 1; obtuse = 2; truncate = 3	*
32. Lemma distal margins: entire = 1; slightly irregularly serrate or erose = 2; irregularly serrate or erose = 3	ns
33. Lemma apex margin (see character 19)	**
34. First lemma length (mm)	ns
35. Lemma: glabrous = 1; sparsely scaberulous = 2; hairy = 3	ns
36. Extent of hair on lemma from base (mm)	ns
37. Palea veins: glabrous = 1; scabrous or with short, straight hairs = 2; scabrous or hairy at the top, curly-hairy in the lower part = 3	ns
38. Palea length (mm)	ns
39. Palea width (mm)	*
40. Extent of hair on palea from apex (mm)	ns
41. Anther length (mm)	ns
42. First glume width/length	ns
43. Second glume width/length	ns
44. Palea width/length	***

Table 3.4. DNA content and chromosome numbers for diploid *Puccinellia* species and populations. N = number of individuals analyzed for flow cytometry from "p" number of populations; DNA (mean pg/2C) = mean of N plants ± 2 × standard error; Prop = proportion relative to standard diploid value. Specific populations for which we counted chromosomes are listed in a separate line in the table; mean DNA content given for the population; DNA value of the specific plant counted given in round brackets. n/a = not applicable.

Species or population	N(p) =	DNA (mean pg/2C)	Prop	*x* =
P. agrostidea	13 (3)	3.08 ± 0.09	0.99	
P. agrostidea 2823	5	3.16 ± 0.14 (3.14)	1.02	7
P. arctica	25 (4)	3.19 ± 0.08	1.02	
P. arctica 2805	10	3.16 ± 0.08 (3.15)	1.02	7
P. poacea	15 (3)	3.10 ± 0.05	0.99	
P. poacea 2140	0	n/a	n/a	7
P. tenella	26 (4)	3.02 ± 0.06	0.97	
P. vahliana	44 (15)	3.74 ± 0.12	1.20	
P. vahliana 2838	4	3.57 ± 0.16 (3.55)	1.15	7
P. sp.1 'Banks Island'	23 (2)	2.87 ± 0.06	0.92	
P. sp.1 'Banks Island' 2810	12	2.86 (2.86)	0.91	7

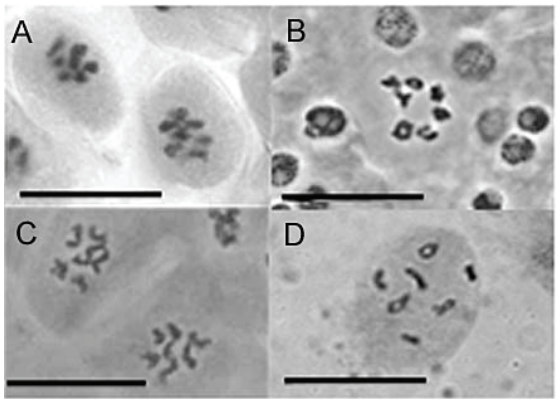
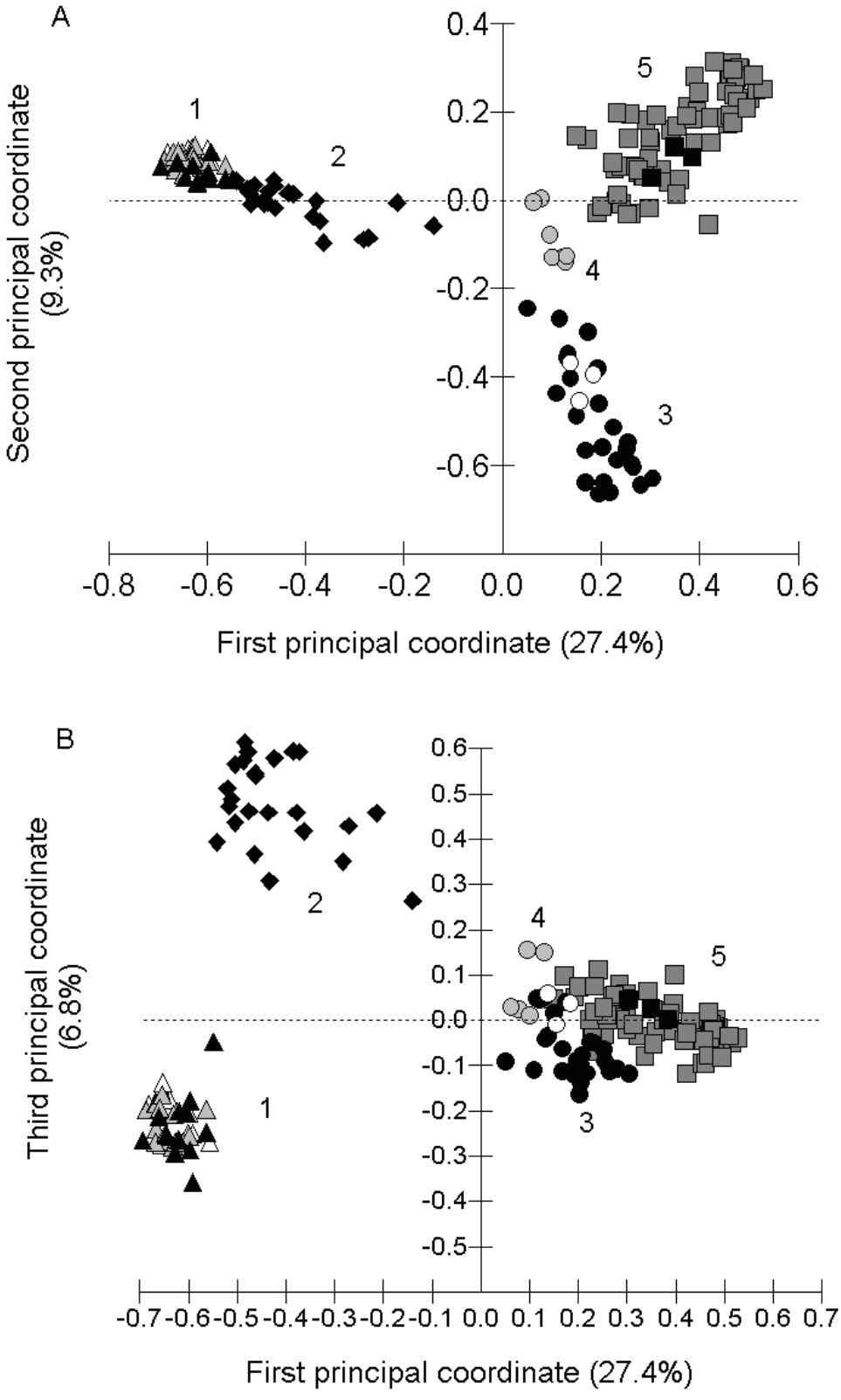


Figure 3.1. Pollen mother cells of diploid arctic *Puccinellia.* A. *P. agrostidea LLC, LJG, and H. Bickerton 2823-13*, metaphase. B. *P. arctica 2805-14*, diakinesis. C. *P.* sp.1 'Banks Island' *2810-5*, metaphase. D. *P. vahliana 2838-5*, diakinesis. Scale bar = 10μm.

Figure 3.2. Principal coordinates analysis of AFLP data using three primer pairs. A. Principal coordinate axes 1 and 2. B. Principal coordinate axes 1 and 3. □ = *P. vahliana*; ■ = *P. wrightii*; ● = *P. langeana*; ○= *P. tenella*; ○ = *P. alaskana*; ◆ = *P.* sp.1 'Banks Island'; ▲ = *P. arctica*; △ = *P. agrostidea*; △ = *P. poacea*. Numbers 1–5 refer to groups discussed in text.


A
Second principal coordinate (9.3%)
First principal coordinate (27.4%)
1
2
3
4
5
B
Third principal coordinate (6.8%)
First principal coordinate (27.4%)
1
2
3
4
5

A

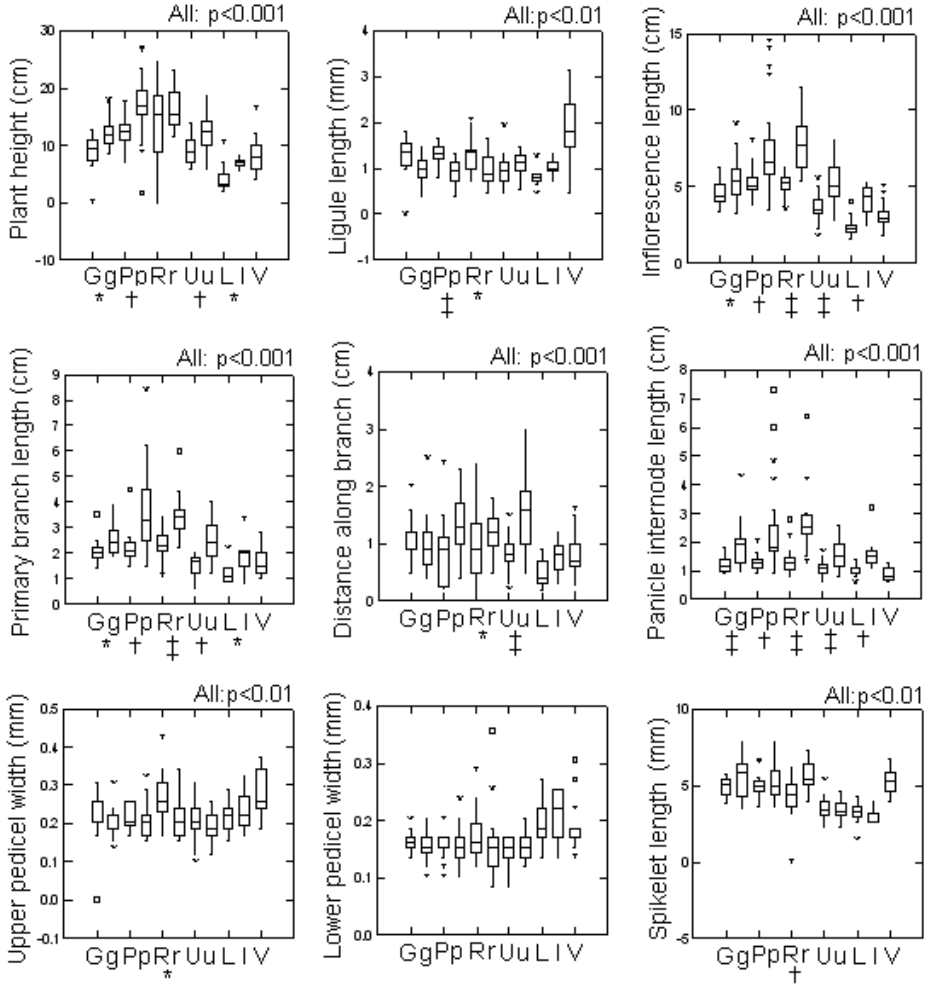


Figure 3.3. Standard box-and-whisker plots of the quantitative characters. Plots show median with upper and lower quartiles (boxes), 1.5× interquartile range (whiskers), and outliers (stars). Letters (upper case = field specimens, lower case = common garden specimens): G = *P. agrostidea*, P = *P. poacea*, R = *P. arctica*, U = *P.* sp.1 'Banks Island', L = *P. langeana*, V = *P. vahliana*. "All = p<X" indicates when significant differences were found between field and common garden specimens when all taxa except *P. vahliana* were included in the analysis. Symbols below letters indicate significant differences for individual taxa.* = $p < 0.05$, † = $p < 0.01$, ‡ = $p < 0.001$. A. Characters of habit, ligule, inflorescence and spikelet size. B. Characters of the florets and rachillas.

B

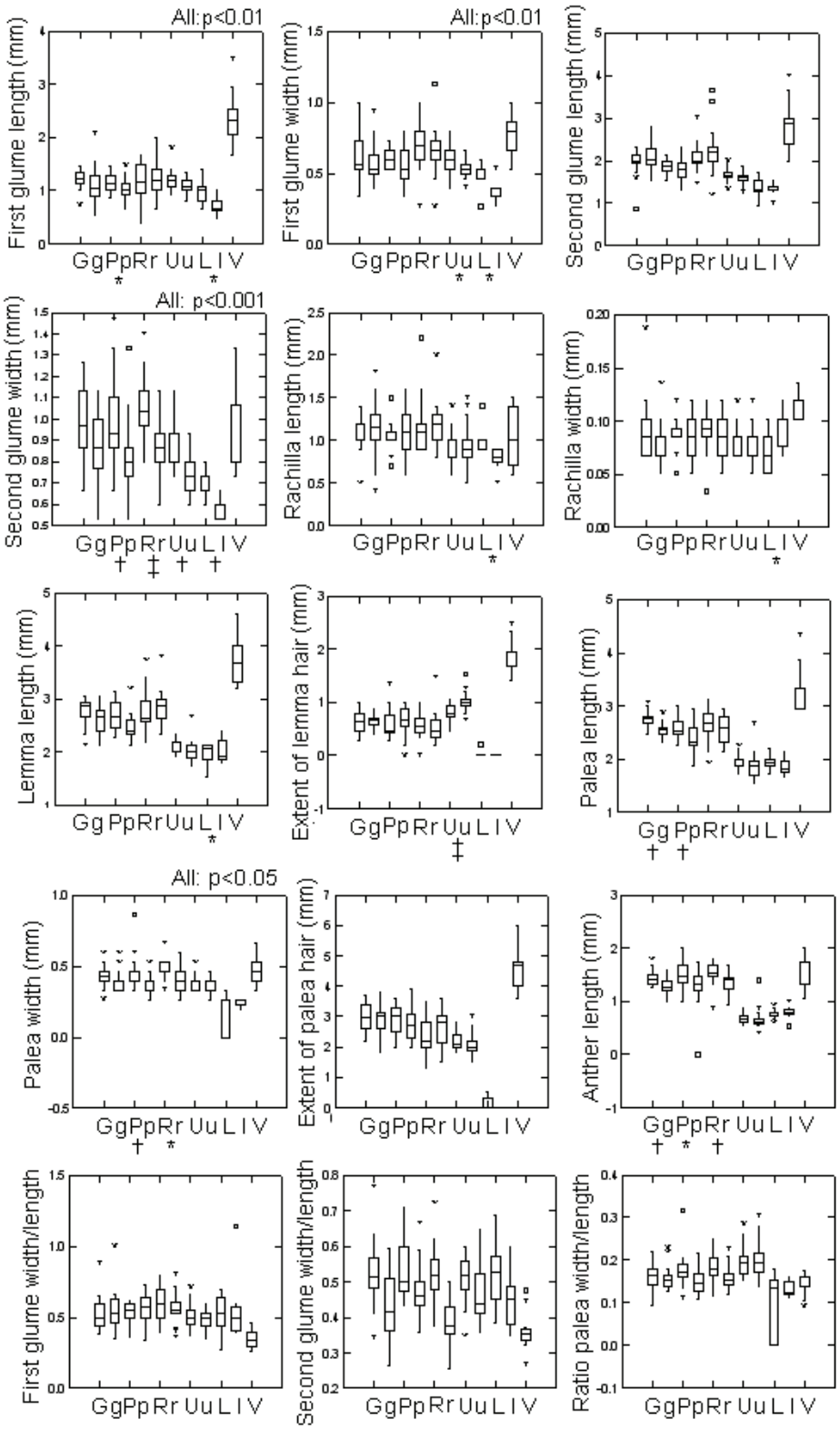

All:p<0.01
First glume length (mm)
All:p<0.01
First glume width (mm)
Second glume length (mm)
All: p<0.001
Second glume width (mm)
Rachilla length (mm)
Rachilla width (mm)
Lemma length (mm)
Extent of lemma hair (mm)
Palea length (mm)
All: p<0.05
Palea width (mm)
Extent of palea hair (mm)
Anther length (mm)
First glume width/length
Second glume width/length
Ratio palea width/length
Gg Pp Rr Uu L I V

Figure 3.4. Principal coordinates analysis of morphological data: A. field specimens of arctic diploid *Puccinellia* species using all 40 non-ratio characters. B. field specimens for all taxa plus common garden specimens for the *P. arctica* aggregate and *P.*sp.1 'Banks Island'using all 40 non-ratio characters. C. as B but without groups 4 & 5. D. as C but using only the nine "stable" characters that did not differ between field and common garden. ▢ = *P. vahliana*; ■ = *P. wrightii*; ● = *P. langeana*; ○= *P. tenella*; ◯ = *P. alaskana*; ◆ = *P.* sp.1 'Banks Island'; ▲ = *P. arctica*; △ = *P. agrostidea*; △ = *P. poacea*. Numbers 1–5 refer to groups discussed in text.

Fig. 3.4

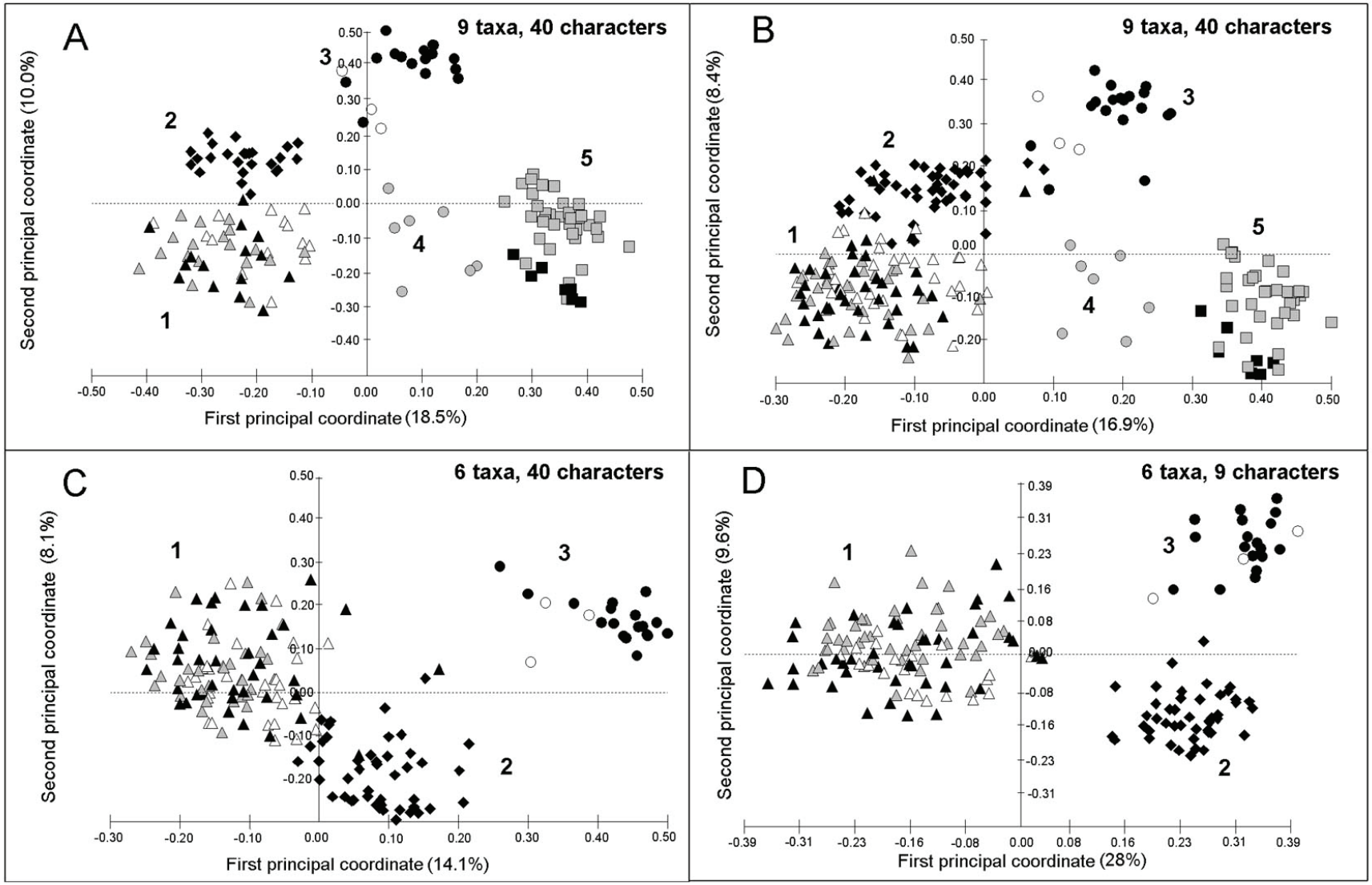
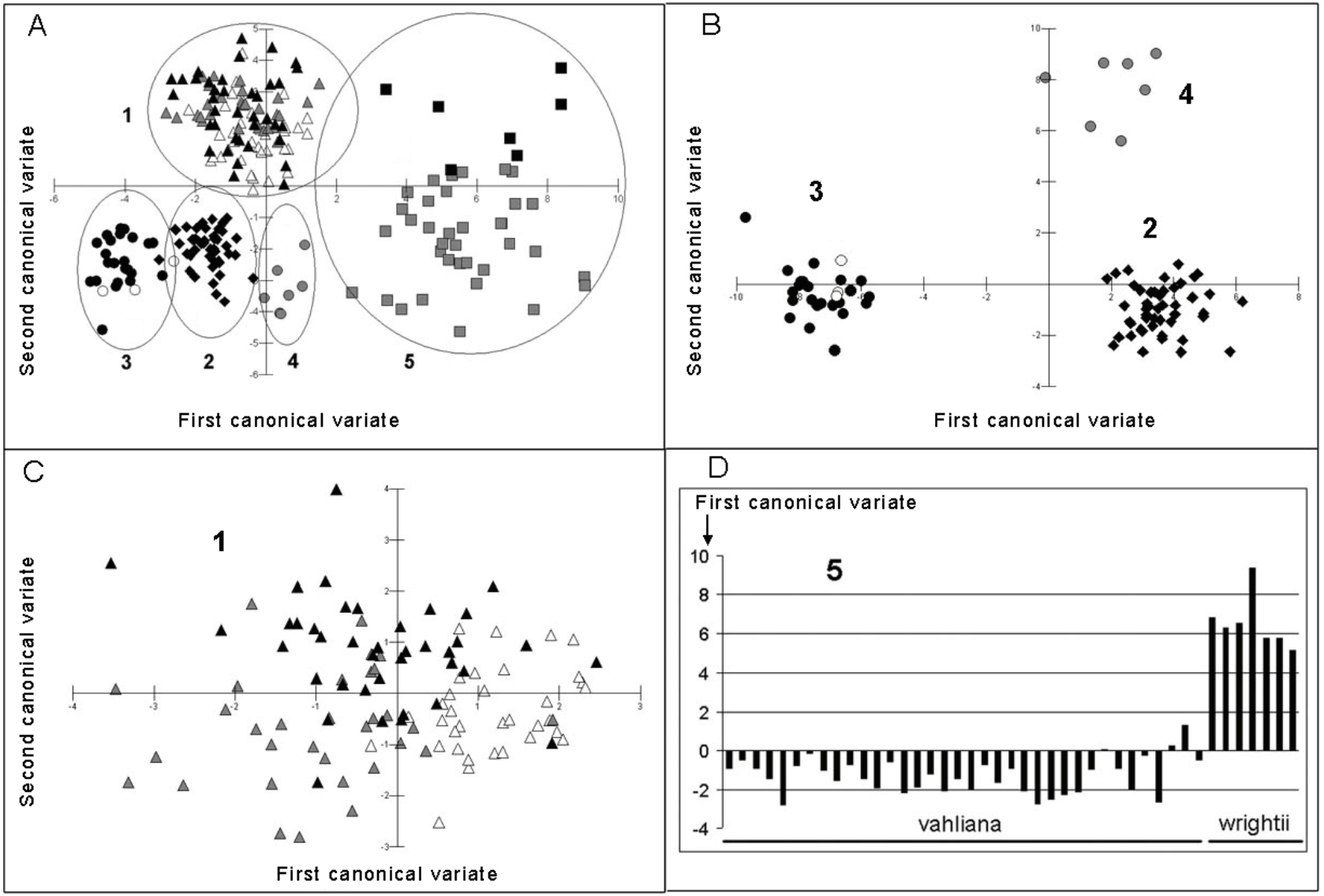

A
9 taxa, 40 characters
Second principal coordinate (10.0%)
First principal coordinate (18.5%)
1
2
3
4
5
B
9 taxa, 40 characters
Second principal coordinate (8.4%)
First principal coordinate (16.9%)
1
2
3
4
5
C
6 taxa, 40 characters
Second principal coordinate (8.1%)
First principal coordinate (14.1%)
1
2
3
D
6 taxa, 9 characters
Second principal coordinate (9.6%)
First principal coordinate (28%)
1
2
3

Figure 3.5. Canonical discriminant analyses on quantitative morphological characters measured from field and common garden specimens. Ellipses clarify the geographically defined groupings used in the analysis. Groups defined on geography. A. All taxa included. B. Only specimens of *P. langeana, P. tenella*, *P. alaskana*, and *P.* sp.1 'Banks Island'. C. *Puccinellia arctica* aggregate only. D. *Puccinellia vahliana* and *P. wrightii* only. □ = *P. vahliana*; ■ = *P. wrightii*; ● = *P. langeana*; ○= *P. tenella*; ◌ = *P. alaskana*; ◆= *P.* sp.1 'Banks Island'; ▲= *P. arctica*; △ = *P. agrostidea*; ◬ = *P. poacea*. Numbers 1–5 refer to groups discussed in text.

Fig. 3.5


A
Second canonical variate
First canonical variate
1
2
3
4
5
B
Second canonical variate
First canonical variate
2
3
4
C
Second canonical variate
First canonical variate
1
D
First canonical variate
5
vahliana
wrightii

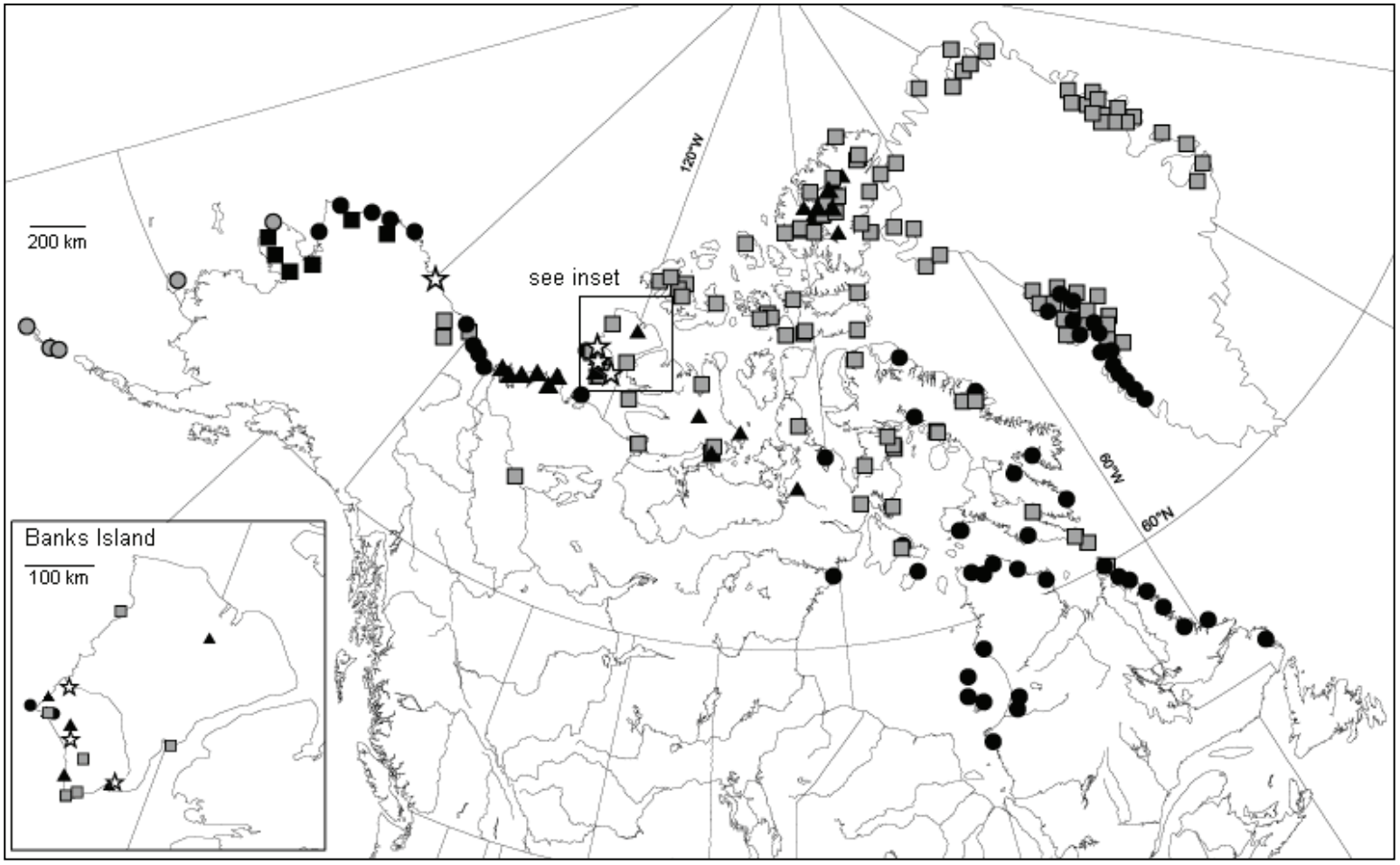


Figure 3.6. Geographic distribution of diploid species of *Puccinellia* in the North American Arctic. The inset shows Banks Island in more detail. ▲= *P. arctica*; ▩ = *P. vahliana*; ■ = *P. wrightii*; ● = *P. langeana*; ○ = *P. alaskana*; ☆ = *P.* sp.1 'Banks Island'.

3.8 Connecting Text

In Chapter 3 the taxonomic limits of North American diploid *Puccinellia* species were modified and clarified based on AFLP patterns, flow cytometry, and morphological data. The data supported five or six diploid species, including a new diploid species from Banks Island, N.W.T., Canada. This species also closely resembles two polyploid species in the North American Arctic. I describe the new taxon in Chapter 4, comparing it with the diploids and polyploids it resembles.

Chapter 4 has been accepted for publication in *Novon* pending minor revision. Laurie L. Consaul, Plant Science, McGill University, Ste Anne de Bellevue, QC H9X 3V9; Lynn J. Gillespie, Research Division, Canadian Museum of Nature, Ottawa, ON K2P 6P4, and Marcia J. Waterway, Plant Science, McGill University, Ste Anne de Bellevue, QC H9X 3V9. A new species of Alkaligrass (*Puccinellia*, Poaceae) from the western North American Arctic.

4. A new species of Alkali grass (*Puccinellia*, Poaceae) from the western North American Arctic

Laurie L. Consaul, Lynn J. Gillespie, and Marcia J. Waterway

4.1 Abstract

A new arctic species of alkali grass, *Puccinellia banksiensis* Consaul from Banks Island, Northwest Territories, Canada and the northern coast of Alaska, U.S.A. is described and illustrated. This diploid species is most similar to the Russian and Alaskan tetraploid *P. hauptiana* Trinius ex V.I. Kreczetowicz, from which it differs by having dense panicles with erect branches and palea keels with curly hairs in the proximal half, to the Siberian species *P. neglecta* ((Tzvelev) Bubnova, which is larger with less dense inflorescences and wider leaves, and to the circumpolar hexaploid *P. angustata* (R. Brown) Rand and Redfield, from which it differs by having smaller spikelets and florets. It differs from other *Puccinellia* species on Banks Island and the northern Alaska coast by having lemmas less than 2.7 mm long with scabrous apical margins and anthers less than 0.9 mm long. Although morphology suggests a very close relationship with *P. angustata, P. hauptiana*, and *P. neglecta*, genetic evidence indicates it is a distinct species. It is local, rare, and grows in low elevation *Dryas*-dominated tundra beside coastal lakes.

4.2 Introduction

During an expedition to Banks Island, Northwest Territories, Canada in 2003, we collected specimens that differed from other arctic *Puccinellia* species in Aiken et al. (2003) and Tzvelev (1964, 1976) by having more erect panicle branches and smaller spikelets. These plants were growing within several kilometers of the coast, near the shores of inland freshwater lakes. The new specimens were not morphologically similar to any of the other *Puccinellia* species growing in the vicinity: *P. arctica* (Hooker) Fernald & Weatherby, *P.*

vahliana (Liebmann) Scribner & Merrill, *P. angustata* (R. Brown) Rand and Redfield, and *P. andersonii* Swallen.

A single specimen in the National Herbarium of Canada matched our new collections. This specimen, with one small culm, had been collected in 1971 in an ecological survey by Kaye MacInnes and John Lambert (Carleton University) of an area on Banks Island near Egg River, Northwest Territories, Canada. It resembled a tiny depauperate *P. angustata* and had been annotated as such by S.G. Aiken in 1989 and by LLC in 2000. Subsequently, our attention was brought to a similar specimen at ALA from Prudhoe Bay labeled as "*P.* sp." by R. Elven and H. Grundt (*s.n.*) in 1998, and annotated as *P. angustata* by A. Batten in 2001. Examination of the additional, more complete specimens that we collected in 2003 suggested that these annotations were incorrect.

Chromosome counts (Consaul et al., Chapter 3) revealed that the Banks Island specimens of the new species are diploid, not hexaploid like plants of *P. angustata* (Löve and Löve 1975; Consaul, unpublished data). We included the diploid specimens of the new species from Banks Island in an investigation of the taxonomic limits of all diploid *Puccinellia* species in the North American Arctic using morphological measurements from field vouchers and common garden experiment vouchers, amplified fragment length polymorphism (AFLP) data, and DNA content from flow cytometry (Consaul et al., Chapter 3). From this investigation we concluded that five or six diploid taxa should be recognized in the Canadian Arctic: *P. arctica* (including *P. agrostidea* Sørensen and *P. poacea* Sørensen), *P. tenella* (Lange) Holmberg ex Porsild subsp. *langeana* (Berlin) Tzvelev, *P. alaskana* Scribner & Merrill, *P. vahliana*, *P. wrightii* (Scribner & Merrill) Tzvelev, and a new species represented by our Banks Island specimens. This new species is distinct from the other diploid species by having much smaller spikelets and florets, a significantly lower amount of DNA per nucleus, and a distinct pattern of AFLP markers (Consaul et al, Chapter 3). Its morphological characteristics suggest it belongs in the section *Puccinellia* (Tzvelev 1976; Ovchinnikova 1989), series *Orientales* Ovchinnikova (Ovchinnikova 1989) being similar to *P. hauptiana* Trinius ex V. I. Kreczetowicz and *P. neglecta* (Tzvelev)

Bubnova of this group (Bubnova 1990). In this paper we describe the new species, compare it to sympatric *Puccinellia* species, and compare it to circumpolar *P. angustata*, Beringian *P. hauptiana*, and Siberian *P. neglecta*, which closely resemble it.

Puccinellia banksiensis Consaul, sp. nov. TYPE: Canada. Northwest Territories: Banks Island, S shore of unnamed lake 2.5 km S of Masik River, 71° 35.638'N, 123° 08.937'W, 19 July 2003, *L. L. Consaul 2810, L. J. Gillespie & H. Bickerton* (holotype, CAN; isotypes, ALA, MO, MTMG, O). Figure 4.1.

Herba perennis, caespitosa, non stolonifera; caules floriferi 5–20 cm alti, erecti vel decumbentes. Paniculae 2–8 cm longae; rami erecti; pedicelli scabrelli. Flosculi 2–4(–5); gluma prima 0.8–1.8 mm longa, gluma secunda 1.3–2 mm longa; lemma 1.7–2.3 (–2.7) mm longum, in dimidio inferiore moderate villosum; carinae paleae ad apicem ciliatae, in dimidio inferiore pilis crispis praeditae. *Puccinellia hauptianae* Trinius ex V.I. Kreczetowicz affinis, sed paniculis densis carinis palearum in dimidio inferiore villosis differt. A *Puccinellia angustata* (R. Brown) Rand and Redfield lemmate et palea breviore differt. A *Puccinellia arctica* (Hooker) Fernald & Weatherby lemmate 1.7– 2.3 (–2.7) mm longo, palea 1.5–2.3 mm longa, carinis paleae in dimidio inferiore villosis, antheris 0.4–0.8 mm longis differt.

Perennial herb, cespitose, lacking stolons; flowering culms 5–20 cm tall, erect or decumbent. Ligules membranaceous, ovate-oblong or transversely oblong, 0.5–2.0 mm; basal leaf blades usually involute, rarely flat, 4–8 × 0.7–1.2 mm when flattened; flag leaf 0.5–1.2 cm, arising above the midpoint of the culm. Panicles 2–8 cm, lowest primary branch 0.6–4.0 cm with spikelets beginning 0.3–3.0 cm from proximal end; lowest panicle internode 0.5–2.6 cm; branches usually erect, occasionally slightly ascending; pedicels scabrous, 0.1–0.3 mm wide. Spikelets 2.2–5.0 mm, with 2–4(5) florets; glumes unequal; first glume

ovate, 0.8–1.8 × 0.4–0.8 mm; second glume ovate, occasionally elliptic, 1.3–2 × 0.5–1.2 mm, apical margin minutely scabrous; lemmas greenish, frequently tinged with purple, often with a yellowish band near the margin, 1.7–2.3 (–2.7) × 0.8–1.0 mm, apex acute or obtuse, occasionally truncate, apical margin minutely scabrous, abaxial surface moderately hairy to 1.5 mm from the base; callus with a ring of hairs 0.1–0.2 mm long; paleas 1.5–2.7 mm long, 0.3–0.6 mm wide, keels with short straight hairs distally and curly hairs proximally almost to the base; rachilla between first and second lemmas 0.5–1.5 mm long, 0.5–0.12 mm wide; anthers 3, 0.4–0.8 mm long. $2n = 14$.

Etymology. This species is named after the type locality, Banks Island, Northwest Territories, Canada.

Distribution and habitat. The new species is known only from three localities in southern Banks Island, Northwest Territories, Canada, and one locality in Alaska, U.S.A. Habitat on Banks Island is in arctic tundra 20–30 m above sea level. *Puccinellia banksiensis* is found infrequently, in localized populations of up to about 100 individuals, in frost-heaved, turfy tundra, with *Dryas integrifolia* Vahl, *Cassiope tetragona* (L.) D. Don, *Astragalus alpinus* L., and *Arnica angustifolia* Vahl. This species grows in a habitat that is more densely vegetated than that of the other *Puccinellia* species found nearby. *Puccinellia banksiensis* should be considered critically imperiled (ranks S1, N1, G1) according to Nature Conservancy rankings (The Nature Conservancy 1988). This species should be sought in other Low Arctic *Dryas*-dominated tundra habitats on Victoria Island, on the adjacent Canadian mainland, and in the Beringian region.

Affinities. Puccinellia banksiensis resembles two species more closely than any others in North America. The first is the tetraploid taxon *P. hauptiana* from far eastern Russia (Tzvelev, 1964, 1976) and far western Alaska (Bowden, 1961). Davis and Consaul (2007) note that the status of *P. hauptiana* as a subspecies should be examined more closely, as it is tetraploid and likely native in North America whereas *P. distans* is introduced, differs from the other subspecies by wider leaves and more hairy palea, and may be entirely hexaploid. Given these differences, particularly the difference in ploidy level, we are using the species

designation in this paper. Morphologically, *P. banksiensis* differs from *P. hauptiana* chiefly by having panicle branches that are almost always erect and palea keels that are spinulose in the distal portion with curly hairs in the proximal half near the base. The tetraploid, on the other hand, has ascending or horizontal branches and palea keels that lack curly hairs in the proximal half (Tzvelev 1964). We compared the amplified fragment length polymorphism (AFLP) patterns of *P. banksiensis* and *P. hauptiana* using the protocols cited in Consaul et al. (Chapter 3) and the paratypes of *P. banksiensis* along with five specimens of *P. hauptiana* listed below. Figure 4.2 illustrates the clear distinction between these two taxa based on AFLP markers.

The second species that *P. banksiensis* closely resembles is the circumpolar hexaploid *P. angustata*. It differs from *P. angustata* by having a denser tuft of basal leaves, and by smaller spikelets, glumes, and florets, the lemmas of *P. banksiensis* being less than 2.5 mm long. *Puccinellia banksiensis* grows in closest geographical proximity to *P. angustata* and the diploid *P. arctica*. It differs from *P. arctica* by its narrow and contracted inflorescence, having much smaller anthers and curly hairs on the lower half of the palea keels. Other *Puccinellia* species growing on southern Banks Island and the northern Alaskan coast are the diploids *P. tenella* subsp. *langeana, P. alaskana*, and *P. vahliana*, and the polyploid *P. andersonii*. The key below gives the main differences among these taxa.

DNA sequence comparisons of arctic *Puccinellia* for internal and external transcribed spacer regions of the nuclear ribosomal genes (ITS and ETS1f) and the chloroplast *rpoB-trnC* intergenic spacer show that *P. banksiensis* forms its own clade (Consaul et al., Chapter 6). Therefore, despite similarities in morphology, genetic evidence suggests that diploid *P. banksiensis* is a distinct species that was not involved in the formation of the tetraploid, *P. hauptiana.*

4.3 Key to *Puccinellia* species on Banks Island, Canada, the southwestern North American Arctic, and closely related species in Siberia

1a. Pedicels smooth; lemma apical margins smooth or crenulate.

2a. Lemmas 3–5 mm long; palea keels scabrous distally, frequently with curly hairs on the lower portion; anthers 1.0–2.0 mm long.

3a. Culms 5–15 cm; panicles 2–4 cm long, branches erect to slightly ascending……..……...……..…….. *P. vahliana*

3b. Culms 15–40 cm; panicles 5–8 cm long, branches usually ascending to horizontal..……..…..…….*P. wrightii*

2b. Lemmas 2–3 mm long; palea keels glabrous or slightly ciliate; anthers 0.5–0.9 mm long.

4a. Lemmas 2–2.5 mm long, apices obtuse; glume and lemma margins smooth to crenulate; palea keels glabrous..…*P. tenella* subsp. *langeana*

4b. Lemmas 2.5–3.0 mm long, apices acute, glume and lemma margins smooth but not crenulate; palea keels slightly ciliate……*P. alaskana*

1b. Pedicels slightly to densely scabrous; lemma apical margins scabrous, sometimes with only a few scabrules.

5a. Lemmas (2.1–) 2.3–5.2 mm long; anthers 0.8–2.2 mm long.

6a. Anthers (0.9)1.2–2.2 mm long…..…….…..……*P. arctica*

6b. Anthers 0.7–1.2 mm long.

7a. Palea keels glabrous in proximal half; lemma apical margin irregularly serrate or moderately to strongly erose……*P. andersonii*

7b. Palea keels hairy in proximal half; lemma apical margin entire to slightly erose……....*P. angustata*

5b. Lemmas 1.5–2.3 (2.7) mm; anthers 0.3--0.8 mm.

8a. Leaves 3–7 mm wide; palea keels shortly ciliate in proximal half, anthers 0.8-1.0 mm…….*P. distans*

8b. Leaves <3 mm wide; palea keels spinulose in upper half; at least some lemmas with curly hair in proximal half or glabrous but not shortly ciliate in lower half; anthers <0.8 mm.

9a. Palea keels smooth in proximal half; lemmas 1.5–1.8(1.9) mm……..…….…..….. *P. hauptiana*

9b. Palea keels usually hairy in proximal half; lemmas >1.7 mm.

10a. Plants 50–80 cm tall; panicles 15–25 mm, diffuse; branches lax……..……. *P. borealis*

10b. Plants <50 cm tall; panicles <10 cm, diffuse or dense, branches erect or lax.

11a Plants 20–25 cm tall; leaves 1.5–2 mm wide; panicles 7–10 cm, dense, branches erect to lax; lemmas 2.3–2.5 (3.0) mm……...…….*P. neglecta*

11b Plants 5–20 cm tall, leaves 0.7–1.2 mm wide; panicles 2–8 cm, slender, branches usually erect, lemmas 1.7--2.3 mm……....*P. banksiensis*

Paratypes. CANADA. **Northwest Territories, Banks Island**: Vicinity of Egg River near junction with Big River, 72°25.65'N, 124°24.39'W, 28 July 2003, *L. L. Consaul 2842, L. J. Gillespie & H. Bickerton* (CAN, MTMG); DeSalis Bay, 1--2 km west of Windrum Lagoon, 71° 28.186'N, 121° 53.051'W, 21 July 2003, *L. L. Consaul 2814, L. J. Gillespie & H. Bickerton* (CAN, MTMG); Egg River, Area #16, 24 July 1971, *K. L. MacInnes s.n.* (CAN). U.S.A. **Alaska**: Arctic coast, Prudhoe Bay, east dock. 70°18-19'N, 148° 18'W, 5 August 1998*, R. Elven & H. H. Grundt s.n.* (ALA).

Puccinellia hauptiana -- Specimens analyzed for AFLP patterns. U.S.A. **Alaska:** Mile 73.8 from Valdez on Richardson Hwy., July 17, 1947, *Dutilly, Lepage and O'Neill 21,314* (CAN); Palmer, on ballast of railroad, June 17, 1947, *Dutilly, Lepage and O'Neill 20,115* (CAN). RUSSIA. Leningrad, July 1, 1919, *Litwinow s.n.*; Ekmus, July 7, 1929, *Kogelnukov 109* (CAN).

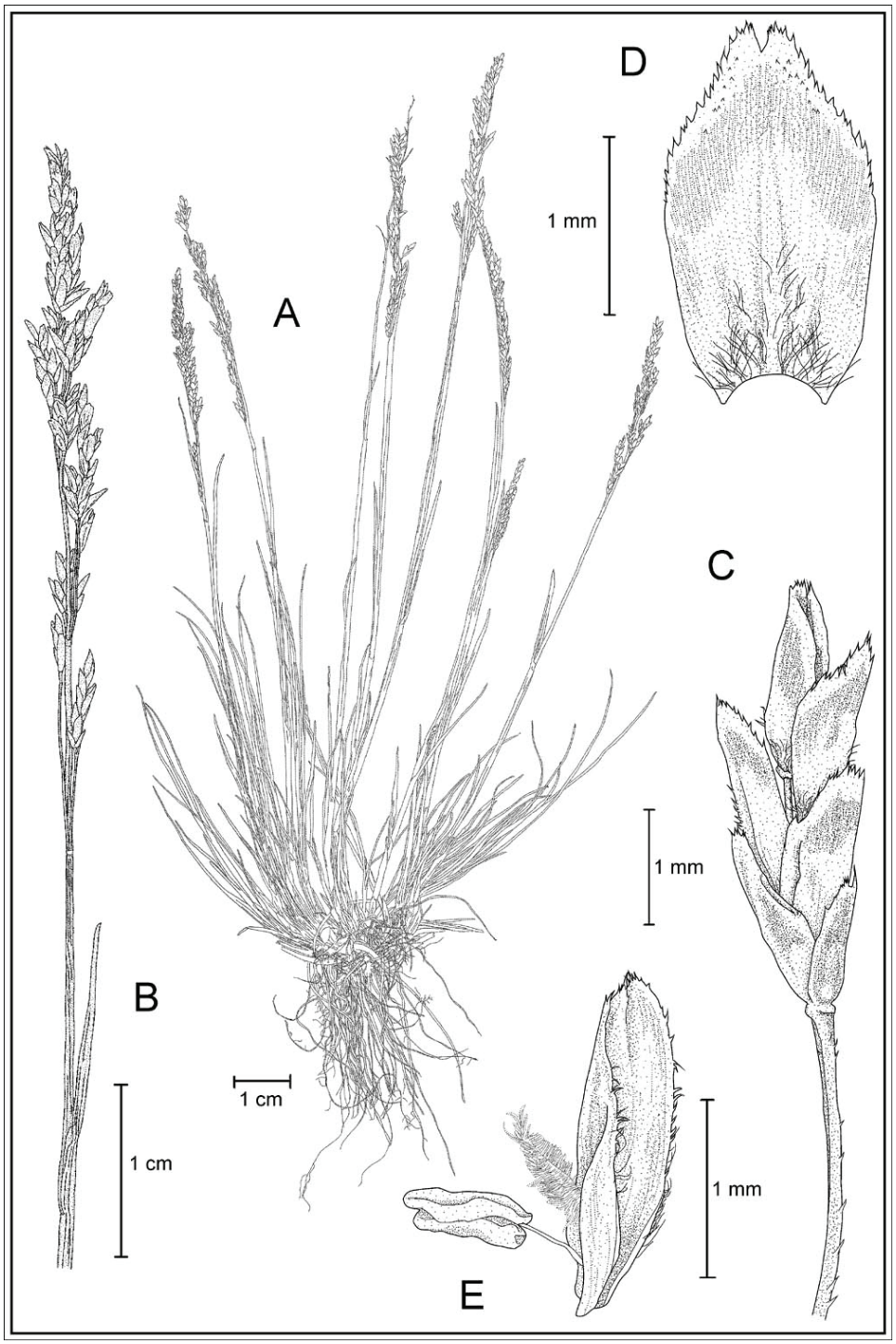


Figure 4.1. *Puccinellia banksiensis* Consaul – A. Plant habit. B. Single culm. C. Single spikelet and subtending pedicel. D. Lemma, abaxial view. E. Palea, oblique abaxial view showing curly hairs on the two keels, stigma attached to top of ovary, and one of three anthers. Drawn from the holotype (*L. L. Consaul 2810, L. J. Gillespie & H. Bickerton*, CAN).

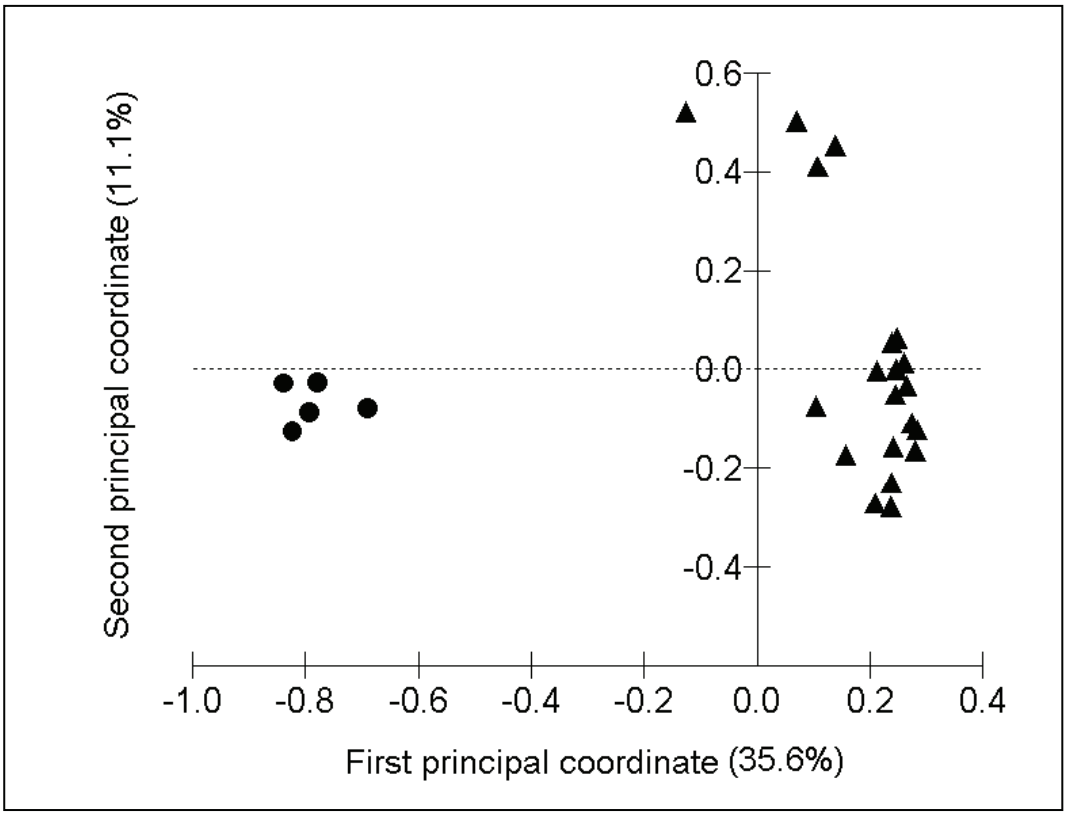


Figure 4.2. Principal coordinates ordination of AFLP presence/absence data. Triangles show *P. banksiensis* specimens and circles show *P. hauptiana* specimens.

4.4 Connecting Text

In Chapters 3 and 4 the delimitation of North American arctic diploid species was clarified and a new diploid species was described. These species are putative parents for polyploid species. In Chapter 5, I use the same techniques as in Chapters 3 and 4 to investigate the taxonomic delimitation of three polyploid species. I also seek evidence for their parentage and whether they are allopolyploid or autopolyploid.

Chapter 5 has been submitted as a manuscript to *Canadian Journal of Botany* for publication. Laurie L. Consaul, Plant Science, McGill University, Ste Anne de Bellevue, QC H9X 3V9; Lynn J. Gillespie, Research Division, Canadian Museum of Nature, Ottawa, ON K2P 6P4, and Marcia J. Waterway, Plant Science, McGill University, Ste Anne de Bellevue, QC H9X 3V9. Systematics of three North American polyploid arctic alkaligrasses (*Puccinellia*, Poaceae): Morphology, ploidy, and AFLP markers.

5. Systematics of three North American polyploid arctic alkali grasses (*Puccinellia*, Poaceae): Morphology, ploidy, and AFLP markers

Laurie L. Consaul, Lynn J. Gillespie, and Marcia J. Waterway

5.1 Abstract

We used flow cytometry, AFLP data, and macromorphology from field and common garden specimens to delimit three polyploid species of *Puccinellia* from the North American Arctic and identify parental taxa. Tetraploid *P. bruggemannii*, hexaploid *P. angustata*, and octoploid *P. andersonii* were generally separable on ploidy and AFLP pattern and showed allopolyploid origin. They had AFLP bands of at least two arctic diploid species and the triploid/tetraploid *P. phryganodes*. Approximately 10% of hexaploid individuals had AFLP patterns that were intermediate between *P. angustata* and *P. bruggemannii* or *P. angustata* and *P. andersonii*. These individuals occupied intermediate positions in the morphological ordinations. Multiple polyploidy or introgression could account for these hybrid patterns, but geographic distributions support introgression more strongly. In common garden experiments, characters that were significantly different between species and those differing significantly between field and common garden experiments changed depending on the species pair analyzed and between the two common garden experiments. Given this variation, we pooled the field and common garden data and determined important key characters by discriminant analysis. The amended distribution of *P. bruggemannii* is primarily restricted to the central Canadian Arctic. We found the first evidence for tetaploid Canadian *P. phryganodes* using flow cytometry.

5.2 Introduction

Polyploidy is a fundamental mechanism for plant speciation. Autopolyploid species, formed by the duplication of a single parental genome, can have a greater capability for genetic modification than their diploid progenitors

because they have more copies of the same genes, which can then undergo modification without loss of the original function. When combined with hybridization, polyploidy can restore fertility to otherwise sterile hybrid plants giving them the potential to develop into new evolutionarily distinct allopolyploid species (Grant 1981). Allopolyploids can incorporate high levels of genetic diversity, combining genomes from multiple parental sources. They may also have multiple or recurrent origins, which can be particularly frequent in the Arctic (Soltis and Soltis 1999). Studies reviewed in Brochmann et al. (2004) have shown that autopolyploidy is frequently recurrent in arctic species, while allopolyploidy can result in variable outcomes: e.g., species of single origin, multiple origins, cryptic species, or different species with the same parents. Three papers have investigated the origins of North American polyploids. Brysting et al. (2004) found that autopolyploids of *Dupontia fisheri* formed recurrently from lower polyploids; Jørgensen et al. (2006) proposed that the tetraploid *Saxifraga rivularis* had a single origin from the diploid *S. bracteata* and *S. hyperborea* lineages, and Popp et al. (2005) and Popp and Oxelman (2007) elucidated parental genomes and the mode of speciation for three circumpolar *Silene* species that occur in arctic North America. Here we examine another genus that may be undergoing polyploid speciation, possibly recurrently, to gain further insight into the origin and evolution of North American polyploid species.

In this paper we examine three closely related arctic polyploid species of *Puccinellia,* one of the largest genera of arctic grasses (Gould and Shaw 1983). *Puccinellia* species colonize harsh alkaline arctic environments and are widely distributed in arctic and temperate areas worldwide. More than half of the *Puccinellia* species whose chromosomes have been counted are polyploids. Seven to 13 of the 13-19 accepted species in the North American Arctic are polyploid (Elven et al. 2003). In any one area in the Arctic there are approximately equal numbers of diploids and polyploids (Elven et al. 2003), but the polyploid species have broader distributions (Davis and Consaul 2007). The three species studied here are common in the North American High Arctic. They are sympatric in range and form a possible chromosome series as putative tetraploid, hexaploid, and

octoploid species, respectively: *P. bruggemannii* T.J. Sørensen, *P. angustata* (R. Br.) E.L. Rand and Redfield, and *P. andersonii* Swallen.

These three polyploid species were originally described at the species level and have neither been synonymized under other names nor reduced to subspecific status. Chromosome counts suggest that *P. bruggemannii* is tetraploid (2 counts, Bowden 1961; Hedberg 1967), *P. angustata* is hexaploid (Löve & Löve (1975) report 12 counts), and *P. andersonii* is octoploid (1 count, Holmen 1952). A survey across many populations is needed to determine whether chromosome number is constant within each species. Flow cytometry is a good method to assess ploidy levels in large numbers of plants when calibrated with chromosome counts (Arumuganathan and Earle 1991).

Despite their stable status as species, these taxa are often difficult to impossible to distinguish from one another. As in many plant groups with reduced floral parts, there are few morphological characters to use in this grass genus. All three species are caespitose, with narrow glabrous or slightly scabrous leaves and several florets per spikelet, often with at least some hair on the florets. These species can be identified by a combination of characters that include bract shapes, ratios of bract lengths, and trichome characters (Davis 1983). Genetic data are needed to obtain evidence for evolutionary relationships of the species and thereby clarify their taxonomic limits. With the clarification of species limits based on genetic data, we can then search more closely for diagnostic morphological characters among the taxa.

Phenotypic plasticity of morphological characters can cause problems in determining taxonomic limits in *Puccinellia* (Davis 1983). Davis (1983) determined a set of 27 characters that expressed low phenotypic plasticity and significant genetic variation under various levels of salt concentration and water availability in temperate and boreal species of the polyploid *P. nuttalliana* (Schult.) Hitchc. complex. Consaul et al. (Chapter 3) found 21 morphological characters were significantly different between conditions in the field and common garden experiments for diploid arctic species, but plasticity in characters has not been studied to date in arctic polyploid *Puccinellia*. Common garden

experiments have been used to distinguish between phenotypic plasticity and underlying genetic differentiation in characters (e.g., Sultan 2000, Stone and Drummond 2006).

The origins of *P andersonii*, *P. angustata,* and *P. bruggemannii* are unknown. They may be allopolyploids or autopolyploids and they may share parental species. AFLP analysis has been useful for studying genetic variation within several genera of the grass family (Poaceae) (*Dactylis*, Reeves et al. 1998; *Phyllostachys*, Hodkinson et al. 2000; cereals research, Ridout and Donini 1999). Having found AFLP bands that diagnose the diploid *Puccinellia* species (Consaul et al., Chapter 3), in the present paper we assay for these same bands in the three polyploid species. If we find matching bands, we consider this to be evidence that the diploid species may be a parent of the polyploid species. Caution must be taken when interpreting comigrating bands as homologous, however (Koopman 2005), since nonhomologous comigrating fragments have been found even within the same individuals (e.g., Hansen et al. 1999; Mechanda et al 2004). However, Mechanda et al. (2004) also showed that level of homology decreases rapidly with increased taxonomic distance, so very closely related species of *Puccinellia* should have a high level of homology in comigrating bands.

Puccinellia phryganodes (Trin.) Scribn. & Merr. is a stoloniferous species, generally sterile in North America. Of the subspecific taxa in North America, *Puccinellia phryganodes* subsp. *phryganodes* is reportedly tetraploid, ranging from Russia east to western Alaska. Plants that are considered to belong to the race '*neoarctica*' (from *Phippsia neoarctica* Á. Löve & D. Löve, not yet validly published in *Puccinellia*) are from Canada and Greenland and have thus far all been counted as triploid (Elven et al. 2003). Jefferies and Gottlieb (1983) found unexpectedly high variation in isozyme patterns of *P. phryganodes 'neoarctica'* in Canada and suggested that the occasional production of viable gametes may occur. If this is so, *P. phryganodes* could be involved in the formation of higher polyploid species.

Our main objectives are to a) determine whether *P. bruggemannii*, *P. angustata*, and *P. andersonii* are cytologically, morphologically and genetically

distinct; b) assay markers that can detect potential parental taxa and evaluate whether these species are allopolyploids or autopolyploids; c) determine how environmental factors affect morphology; and d) find the best morphological characters for identifying these polyploid species.

5.3 Materials and Methods

5.3.1 Plant sampling

We sampled *Puccinellia* populations in the Canadian Arctic, collecting leaves dried on silica gel, herbarium vouchers, and living plants. Locations of the populations included in the analyses for this paper and the voucher information are given in Table 5.1. We sampled most intensively the putative polyploid series consisting of the tetraploid *P. bruggemannii*, the hexaploid *P. angustata*, and the octoploid *P. andersonii*. We coded one population originally collected as *P. andersonii* as *P.* sp.2, because it appeared quite different in morphology. We included five specimens of *P. pumila* (Macoun ex Vasey) Hitchc. (octoploid), since we suspected *P.* sp.2 may be related to this taxon. We added seven populations of *P. phryganodes* because it is reported as a triploid hybrid species (Löve and Löve 1975) and may be involved in the evolution of the polyploids. Collection locations and voucher information for the diploid specimens that we compare with the polyploid specimens are given in Consaul et al. (Chapter 3).

Because the field season spanned the growing season of the plants and some growing seasons start later or are cooler than others, some of the plants had to be collected before flowering commenced. We were able to identify these plants by their previous year's inflorescences and perform all of the analyses except measurements on field plants.

5.3.2 DNA content and ploidy determination

We examined meiotic chromosomes in pollen mother cells from florets of the specimens cited as chromosome vouchers in Table 5.1, using the technique described in Consaul et al. (Chapter 3), to verify the ploidy level for each of the

species and calibrate flow cytometry values. We used flow cytometry on fresh leaf tissue from 241 of the putative polyploid plants that had been collected live in the field and grown in the greenhouse (Table 5.1) to determine nuclear DNA content and compared the DNA content with a standard diploid specimen as described in Consaul et al. (Chapter 3). For some of the flow cytometry runs we included two or more specimens in a single mix to confirm any differences in ploidy levels directly.

5.3.3 Amplified fragment length polymorphism (AFLP) analysis

We performed AFLP analyses as described in Consaul et al. (Chapter 3) using the selective primer pairs E+AAG / M+CTT, E+ACG / M+CAA, and E+ACC / M+CTC. AFLP banding patterns of 203 polyploid specimens (Table 5.1) were compared with the 145 diploid plants previously analyzed (Table 3.2). We examined the data in four ways. 1) Principal Coordinates Analysis (PCoA) performed with the Gower Similarity Coefficient calculated from a presence/absence data matrix of AFLP bands using MVSP (Multi-Variate Statistical Package, Version 3.1, Kovach Computing Services, Wales, UK) shows the relationships among individuals without bias based on *a priori g*rouping. 2) Analysis of Molecular Variance (AMOVA) calculated using WINAMOVA (Version 1.55; Excoffier et al. 1992) with the same AFLP data matrix allowed us to examine the partitioning of variance within populations, among populations within taxa, among taxa, and among other groups that were revealed to be of interest during the analysis. 3) Band frequencies in each population and each species were calculated and depicted in graphs to show which bands were shared between diploid and polyploid populations. 4) Cluster analysis of the band frequency data was performed in MVSP by Unweighted Pair Group Method with Arithmetic mean (UPGMA) using Manhattan distances, which measure the absolute distance between populations rather than an average, to examine phenetic clustering.

5.3.4 Morphology

We measured structural characters (Table 5.2) on 264 polyploid plants comprising 134 plants from the common garden experiments (described below), 123 associated field vouchers, and 7 extra field vouchers from additional populations (Table 5.1). We had a different number of field vouchers than common garden vouchers because a) some field plants lacked the current year's inflorescences (see *Plant sampling*), and b) some plants did not flower in the common garden experiment. We did not measure specimens of *P. phryganodes* because this species produced flowers only extremely rarely in the field and infrequently in the greenhouse. For analyses comparing diploid to polyploid species, we also included the 244 diploid plants cited in Consaul et al. (Chapter 3).

We output descriptive statistics for each character as boxplots of the medians and quartiles (McGill et al. 1978) separately for field and common garden data, analyzed the difference between the field and common garden specimens with a post hoc Tukey HSD (honestly significant difference) analysis (Sokal and Rohlf 1995), and performed a Kruskal-Wallis test on the qualitative characters testing for differences between field and common garden specimens and also analyzing variation of the qualititative characters within the common garden experiments, using Systat, Version 11 (San Jose, CA).

We carried out two common garden experiments (CG), one in 2003 and one in 2004. The CG experiments included both diploid species and polyploid species and used plants collected as described in Consaul et al. (Chapter 3). The Generalized Linear Model procedure (PROC GENMOD) in SAS (1999, Version 9, SAS Institute Inc., Cary, NC) was used with the gamma error distribution and logarithmic link, appropriate for analysis of length values (Lefkovitch 1993), using the model given in Table 5.3. A nested analysis of populations within taxa as well as analyses of the factors taxon, population, and their interaction, was performed on each of the 21 quantitative morphological variables independently to assess differential responses among populations. We also used non-orthogonal planned contrasts (CONTRAST statement, SAS) with a Dunn-Sidak adjustment

(Sokal and Rohlf 1995) (Table 5.3) for CG#1, to be able to compare all three taxa in the *P. arctica* complex (= *P. arctica*, *P. agrostidea*, and *P. poacea*) with each other. In CG#2 we did not need to overlap the comparisons, so orthogonal planned contrasts were calculated (Table 5.3).

We performed PCoA with the Gower Similarity Coefficient (Gower 1971) for mixed data in MVSP using all 40 non-ratio characters. We then separated the data into taxon pairs and did separate analyses on the pairs: *P. bruggemannii* versus *P. angustata* (4X versus 6X), *P. angustata* versus *P. andersonii* (6X versus 8X), and *P. bruggemannii* versus *P. andersonii* (4X versus 8X). This was done based on our preliminary findings of overlap when all three of the species were included in the ordination. The morphological characters separating each of the pairs of putative taxa may be different, which can cause overlap among taxa when all three taxa are included (as found in Consaul and Gillespie 2001), resulting in a lack of separation of the species.

Discriminant analysis of the quantitative characters in SAS (PROC CANDISC and PROC DISCRIM) was used with groups defined on ploidy groupings, to determine whether these groups could be distinguished using only morphological characters. PROC STEPDISC was used to determine which morphological characters best discriminated among the resulting groups.

We tested the viability of pollen in selected specimens by staining anthers overnight in 0.1% cotton blue in lactophenol and examining the pollen grains to determine whether they were stained and healthy or empty and malformed indicating sterility of the pollen (Darlington and La Cour 1960). We tested potential hybrid specimens and a random sample of remaining specimens (*2851-01,04,13,18; 2853-16; 2864-17; 2865-03,05,13,14,16,17,18; 2866-01,19; 2874-19; 3072-2,7,11,18; 3150-01,02; 3151-03*)

5.3.5 Distribution

Specimens from the collections listed here and herbarium specimens from ALA, CAN, and DAO were annotated after this study was completed, and

mapped based on the new identities using ArcView Version 3.2 (ESRI Canada, Montreal, Quebec).

5.4 Results

5.4.1 Ploidy and DNA content

We obtained chromosome counts of $n = 14$ for specimens that had been collected as *P. bruggemannii*, $n = 21$ for specimens that had been collected as *P. angustata* and some populations that had been collected as *P. bruggemannii*, and $n = 28$ for *P. pumila* (Fig. 5.1A, B, C, respectively; Table 5.4). We used *P. pumila* to show the octoploid chromosome count because we had difficulty getting clear counts for *P. andersonii*. Figure 5.1D and Table 5.4 show the main groupings of ploidy level among the studied specimens, along with the chromosome counts. We found five main groupings corresponding with triploid, tetraploid, hexaploid, possible heptaploid, and octoploid levels, including 14 tetraploid readings for *P. phryganodes*. Diploid specimens are only shown for comparison with the triploid values. The amount of DNA in the triploid specimens was slightly over 1.5 times that of the diploid specimens, the DNA amount in the tetraploid specimens was approximately two times that of the standard diploid, but the amounts of DNA in the hexaploids and octoploids were often less than three and four times that in the diploid specimens, respectively.

5.4.2 AFLP analysis

Eighty-four bands were clear and consistent for the three primer pairs and 68 of these were polymorphic. Bands were usually not present at a frequency (f) of 1.0 in an entire taxon or even in an entire population (Figs. 5.2, 5.3). We have coded the diploid and triploid taxa to make referring to the putative parental genotypes easier (R = *P. arctica*, T = *P. tenella* (Lange) Holmb., U = *P. banksiensis* Consaul (Consaul et al., Chapter 4), A = *P. alaskana* Scribn. & Merr., V = *P. vahliana* (Liebm.) Scribn. & Merr., H = bands from *P. phryganodes* or a progenitor species of *P. phryganodes* not yet included in this study.

Figure 5.2 shows frequencies of all bands scored in each population of the

diploid and polyploid taxa. The bands are ordered to demonstrate differences among the diploids and allow direct comparison of these differential banding patterns in the polyploids. The diploid species analyzed in Consaul et al. (Chapter 3) are separated by several groups of band differences and *P. phryganodes* contains bands of every diploid species in addition to three bands that are not found in the diploids. The other polyploid taxa (*P. bruggemannii*, *P. angustata*, and *P. andersonii*, *P.* sp.2 and *P. pumila*) in this study contain some but not all of the bands from each diploid taxon and *P. phryganodes*.

Figure 5.3 is organized to compare the patterns among the polyploids *P. bruggemannii*, *P. angustata*, and *P. andersonii*. Presence/absence data is shown for individuals of only these three taxa to reveal variation patterns within populations. The last column on the bottom right of Fig. 5.3 shows the diploid or triploid genome(s) that could be found in the higher polyploids. From the band similarities shown in Fig. 5.3 we sorted the individuals into groups, which also formed clusters in the UPGMA phenogram of Fig. 5.4. The groups are B, classic tetraploid *P. bruggemannii*; N, classic hexaploid *P. angustata*; D, classic octoploid *P. andersonii*; B6, hexaploids with some *P. bruggemannii* bands; D6, hexaploids with some *P. andersonii* bands; and D7, putative heptaploid *P. andersonii*. When a population was split into two different sets based on differences in chromosome number, we refer to each of these groups as "subpopulations" to refer to the fact that they were part of an original population but are being considered as separate taxa.

B: *P. bruggemannii*

This group of tetraploid plants has four characteristic bands shown (in grey) at the top of the graph in Fig. 5.3. The top two are restricted to typical tetraploid *P. bruggemannii*, not being found in any diploids or *P. phryganodes*. The next two bands are also characteristic of *P. bruggemannii* and are also diagnostic of the diploid *P. vahliana,* with the fourth band also quite common in *P. tenella*. All of the tetraploid *P. bruggemannii* specimens, including tetraploid subpopulations from the two mixed 4X/6X populations (*3072* and *3144*, noted by

*) clustered together in the UPGMA phenogram (Fig. 5.4) and formed part of a cluster of polyploid taxa that is joined closely to a cluster composed only of diploid species *P. arctica* and *P. banksiensis*.

N: *P. angustata*

Typical *P. angustata* populations are characterized by their lack of AFLP bands that are characteristic of *P. bruggemannii* and *P. andersonii* (i.e., by the lack of dark grey bands and light grey bands outlined in black in Fig. 5.3). These hexaploid individuals clustered together the UPGMA tree (Fig. 5.4). In the two populations (*3072* and *3144*, mentioned under *P. bruggemannii*) that had hexaploid and tetraploid individuals, the hexaploid subpopulations grouped with *P. angustata* (shown by * in Fig. 5.4). Five populations had both hexaploid and octoploid individuals (*2851*, *2856*, *2865*, *2877*, and *2878*). The hexaploid subpopulations had variable alignment. The hexaploids in *2877* had typical *P. angustata* AFLP patterns, and clustered in *P. angustata* in the cluster analysis (Fig. 5.4). The remaining four hexaploid subpopulations fell under D6 because they also had *P. andersonii* bands, and are discussed in that section.

D: *P. andersonii*

Of the five populations that had both hexaploid and octoploid individuals, group D is comprised of the octoploid subpopulations. The bottom set of three bands (light grey outlined in black in Fig. 5.3) are generally characteristic of *P. andersonii*, but the first two can also be found in *P. arctica*, *P. banksiensis*, *P. vahliana*, *P. tenella*, *P. phryganodes* and one instance in *P. bruggemannii.* The bottom band is otherwise found only in some populations of *P. phryganodes*. The octoploid *P. andersonii* grouped together in the UPGMA (Fig. 5.4). The four hexaploid subpopulations (D6) also have some "*P. andersonii*" bands and are discussed in that section.

B6: Hexaploids with some *P. bruggemannii* bands

This group comprised several individuals originally collected as *P.*

bruggemannii based on field morphology, but which were later determined by flow cytometry to be hexaploids (therefore coded as B6). This group had some AFLP bands characteristic of *P. angustata* and two characteristic of *P. bruggemannii*, but lacked the top two bands in Fig. 5.3, which are diagnostic for *P. bruggemannii*. Most of the specimens in this group were restricted to three populations on Cornwallis Island (all plants in *LLC et al. 3140, 3150, 3151*), but two plants were from two populations on Banks Island (*2807-1*, *2849-1*) and two from one population on Ellesmere Island (*2866-7, 8*) also had this "hybrid" AFLP pattern. In the cluster analysis, B6 forms a discrete group that links most closely with *P. angustata* and the other hexaploids from mixed populations.

D6: Hexaploids with some *P. andersonii* bands

The plants in these subpopulations were hexaploid plants that had a hybrid AFLP pattern and were from four mixed 6X/8X populations (*2851, 2856, 2865, 2878*). Individuals in these subpopulations possessed bands found only in *P. andersonii* plus bands (CTC0189, CTC0186, CTT0084, CTT0187) more characteristic of *P. angustata* and found only sporadically in *P. andersonii*. Two of these subpopulations, (*2851, 2878*) clustered with *P. angustata*, and two (*2856, 2865*) clustered with *P. andersonii*.

D7: Putative heptaploid *P. andersonii*

These plants had flow cytometry values suggesting they might be heptaploids. They match *P. andersonii* in morphology and AFLP banding pattern so we labeled them D7. They were not plotted as separate "populations" in the UPGMA because they were single individuals with banding patterns matching those of their respective *P. andersonii* populations in Fig. 5.3, but they are examined as individuals in the PCoAs below.

5.4.3 AFLP Ordinations and AMOVAs

The polyploid specimens (grey and black symbols) formed a group distinct from the diploid species (white symbols) and the triploid/tetraploid *P.*

phryganodes specimens (symbols + and ×) in the PCoA of the presence/absence AFLP data (Fig. 5.5A). Specimens of *P.* sp.2 (inverse black triangles) were placed directly between *P. arctica* and *P. vahliana* instead of being offset like the rest of the polyploids.

Puccinellia bruggemannii (B), *P. angustata* (N), and *P. andersonii* (D) formed groups with little overlap when the diploids and *P. phryganodes* were removed from the analysis and the polyploids were coded as defined above (B, N, D, B6, D6, D7) (Fig. 5.5B). *Puccinellia* sp. 2 specimens grouped with *P. andersonii*. Specimens from B6 were found in the *P. angustata* (6X) cloud of points on the edge closest to *P. bruggemannii* (4X), with two specimens placed very close to the *P. bruggemannii* group. Some of the D6 specimens grouped with *P. angustata* and some with *P. andersonii.* The D7 specimens grouped with *P. andersonii* samples.

An AMOVA of the diploid specimens showed that most variation was among taxa (Table 5.5). AMOVA of the polyploid taxon groups B, N, and D showed that the variation among taxa was significant but that the variation within populations was higher than variation among taxa. The AMOVA of the polyploid groups B, N, and B6 showed a similar pattern. AMOVA among each of the polyploid taxa showed that variation within the populations is greater than among the populations.

5.4.4 Morphology

Field versus common garden analyses

Kruskal-Wallis tests of the qualitative data revealed that 10 of 18 qualitative characters were significantly different between plants grown in the field versus in the common garden (Characters 2, 3, 4, 7, 9, 13, 24, 31, 32, and 33 in Table 5.2), including 6 of 8 qualitative characters related to habit, inflorescence shape, and spikelet, and 4 of 12 floret characters. T-tests of the quantitative morphological data showed that 15 of 24 quantitative morphological characters were significantly different between field and common garden (Table 5.6, Fig. 5.6). This included all 6 characters related to habit and inflorescence (Fig. 5.6A)

and 9 of 15 floret characters (Fig. 5.6B). When we considered the taxa separately, three of these characters were significantly different between field and common garden for all taxa ($p<0.05$) (Fig. 5.6A), whereas the rest were significant for at least one taxon. Anther length (Fig. 5.6B) was stable between field and common garden for species with the shortest anthers (*P. bruggemannii* and *P.* sp.2), but anthers were significantly smaller in size in the common garden for the two taxa with larger anthers in the field (*P. angustata* and *P. andersonii*).

Common garden experiments

The results of the generalized linear model analyses for the diploid species are given in Consaul et al. (Chapter 3). For the polyploid species, the generalized linear model analysis of CG#1 showed that all characters were significantly different ($p \leq 0.05$) among taxa. For the nested test of populations within taxa, all of the interactions were significant except for ligule length, pedicel width 2, rachilla length and width, and anther length; in CG#2 no characters except rachilla length, anther length, and ratio of second glume length/width were significant for populations within taxa, but they were significant among taxa for all characters except ligule length, panicle internode distance, pedicel width 1, pedicel width 2 and rachilla width. For the population factor only, in CG#1 all characters had significant differences ($p \leq 0.05$) among all three taxa over the entire model and in CG#2, 13 characters were significantly different among all three taxa over the entire model (Table 5.6). The planned contrasts between *P. bruggemannii* and *P. angustata* for the populations of CG#1 showed three characters were significantly different and for CG#2 showed 13 were significant. Only height was significantly different in both experiments. Three characters differed significantly between *P. andersonii* and *P. angustata* (compared only in CG#1). *Puccinellia* sp. 2 differed significantly from *P. andersonii* for eight characters.

Ordinations – Morphology

The PCoA ordination using morphological characters, coding the specimens based on ploidy level (Fig. 5.7A), showed that the polyploid specimens

(black + grey symbols) form a loose group surrounded by the diploid groups (white symbols). Fig. 5.7B shows overlap among the polyploid groups defined based on ploidy levels in an ordination of polyploid field specimens only. The type specimens of *P. bruggemannii* (B), *P. angustata* (N), and *P. andersonii* (D) grouped in the tetraploid, hexaploid, and octoploid groups of specimens, respectively. Similar overlap was also found in the common garden specimens and deleting the plastic characters (characters which were significantly different between field and common garden) did not increase the resolution (graphs not shown). A canonical discriminant analysis showed that the three groups did not separate cleanly when grouped by ploidy (Fig. 5.7C) and a similar result was obtained when the specimens were grouped by AFLP pattern (not shown).

5.4.5 Ordinations – AFLP and morphology

Figures 5.8–5.10 show the PCoA ordinations of the main taxon groups (B, N, D) in pairs for AFLP and morphological data. These also show where the "uncharacteristic" AFLP groups (B6, D6, D7) fall. Group B6 is included in the *P. bruggemannii* / *P. angustata* comparison (Fig. 5.8) and D6 is included in the *P. angustata* / *P. andersonii* comparison (Fig. 5.10).

P. bruggemannii and *P. angustata.* Fig. 5.8A shows that these two species form non-overlapping groups based on AFLP patterns and that individuals coded as B6 were unambiguously included in *P. angustata*. A discriminant analysis was able to distinguish almost all of the *P. bruggemannii* (B) and *P. angustata* (N) specimens, with only two misclassifications (Table 5.7, *3072-23*, *2853-16*) despite the overlap between the groups seen in the PCoA based on field morphology (Fig. 5.8B). In this PCoA, the B6 specimens are found in a diffuse group at the border of the *P. bruggemannii* and *P. angustata* distributions. In the PCoA of the common garden experiment (Fig. 5.8C) the *P. angustata* specimens that overlapped with *P. bruggemannii* in the upper right quadrat were from small populations in harsh sites on Prince Patrick Island (*LLC 3144, 3154*). B6 specimens were not included in the common garden experiments. Only 2 plants were misclassified in a discriminant analysis of the common garden plants (Fig.

5.8C, Table 5.7); *P. angustata 3144-08* was misclassified as *P. bruggemannii* and *P. bruggemannii 3072-18* was misclassified as *P. angustata*.

P. bruggemannii and *P. andersonii*. Figure 5.9A shows that the AFLP patterns clearly distinguish between *P. bruggemannii* and *P. andersonii* while Fig. 5.9B illustrates limited overlap between these taxa when the analysis is based on field morphology. Discriminant analysis of the field specimens using morphological data correctly classified all specimens of *P. bruggemannii*, and misclassified only two specimens of *P. andersonii as P. bruggemannii* (Table 5.7). Overlap was decreased but not eliminated when only the specimens from the common garden were analyzed (Fig. 5.9C). Interestingly, the individuals that showed overlap in the ordination of common garden specimens were not the same as those in the ordination of field specimens. In the discriminant analysis of common garden specimens, all specimens were correctly classified.

P. angustata and *P. andersonii.* Figure 5.10A shows *P. angustata* and *P. andersonii* (grey circles and black triangles) are non-overlapping based on AFLP patterns, although the two groups are almost contiguous at the bottom of the graph. Individuals of P.sp 2 (inverted black triangles) were grouped with the *P. andersonii* individuals, three being very close to the *P. angustata* specimens in the bottom right quadrat. Intermediate individuals (D6, diamonds) fell between these two groups. Plants belonging to D7 group (light grey triangles) group with *P. andersonii* (D). Individuals of D6 are spread throughout the plot, concentrated in the centre portion. In a similar pattern, plants of *P. angustata* and *P. andersonii* separate in the ordination based on field morphology, with four of the D6 specimens between them (Fig. 5.10B). The difference is that one specimen each of *P. angustata* and *P. andersonii* were completely misclassified based on field morphology. On the other hand, separation is cleaner in the ordination of common garden specimens (Fig. 5.10C), although two different hexaploid specimens were misclassified as *P. andersonii* by discriminant analysis, with the D6 specimens sorting into either *P. angustata* or *P. andersonii*.

Table 5.8 is a list of the specimens that were misplaced or misclassified in the above analyses. The specimens under “A: Morphological misclassification”

are those with consistency between ploidy and AFLPs, but not morphology. The specimens under "B: Inconsistent chromosome numbers" had consistency between AFLP pattern and morphology, but "wrong" chromosome numbers. The specimens under "C: Inconsistent AFLP patterns had consistency between ploidy and morphology, but the "wrong" AFLP pattern.

The best characters for distinguishing *P. bruggemannii*, *P. angustata*, and *P. andersonii* are listed in Table 5.9. These characters all discriminated among at least two pairs of taxa and included the single most important character for discrimination between each pair according to STEPDISC (Table 5.6). The means are calculated from both field and common garden specimens. There was no overlap of the 95% confidence intervals for the means except for rachilla length between *P. angustata* and *P. andersonii* and second glume length between *P. bruggemannii* and *P. andersonii*.

5.4.6 Pollen viability

All plants tested for pollen viability had full, stained, and normally shaped pollen grains except specimens *2865-05* and *2865-14*, which had collapsed and empty pollen. These individuals belong to group D6 and are among the problematic specimens in Table 5.8. Dehisced anthers that still contained a few normal pollen grains were frequently still present in florets that were forming caryopses, often intertwined among the stigmas, implying potential selfing.

5.4.7 Geographic distribution

Distribution of specimens based on updated identifications as determined by the results of the analyses described above is shown in Figure 5.11. The range of *P. bruggemannii* originally extended also to Ellesmere and Axel Heiberg Islands in the north and to Banks Island in the south (Davis and Consaul 2007, Thesis Appendix A), but is now restricted to a more central arctic distribution.

5.5 Discussion

5.5.1 Distinct species

We found groups that are consistent in ploidy level, AFLP pattern, and morphology among the *Puccinellia* populations collected in this study, but these groups did not always match the original identifications. The polyploid plants separated into tetraploid, hexaploid, and octoploid groups, which were usually associated with characteristic AFLP patterns. On the other hand, there was also a set of plants that did not fall into the consistent groups either because of uncharacteristic morphology, inconsistent ploidy-AFLP associations, or intermediate AFLP patterns.

We could name the three taxa as earlier presumed and as subsequently coded (Fig. 5.3), since the holotype specimens of *P. bruggemannii*, *P. angustata*, and *P. andersonii* in the ordination of field specimens using morphological characters (Fig 5.7B) fell within the tetraploid, hexaploid and octoploid groups of the ordination, respectively. Putative heptaploid specimens grouped with the *P. andersonii* or *P. angustata* groups, respectively, with agreement between AFLP and morphological data.

5.5.2 Allopolyploid origins

Puccinellia bruggemannii, *P. angustata*, and *P. andersonii* all appear to have allopolyploid origins, with at least two of the same species involved as parental species for all three. *Puccinellia arctica* and *P. vahliana* are probable parents, based on AFLP band similarities. AFLP bands of *P. phryganodes*, some also present in the diploid species, were also present in all three polyploid species. Shared AFLP bands between putative parental species and hybrid derivatives have been shown in other plant groups (e.g., *Mentha* – Gobert et al. 2002, *Tsuga* – Pooler et al. 2002, *Cardamine* – Marhold et al. 2002). Hodkinson et al. (2002) showed with AFLPs that the allopolyploid grass *Miscanthus ×sinensis* was equidistant from each of its two putative progenitors and found markers that allowed for corrections to identifications that were confirmed by chromosome counts. The polyploid species in the present study are in an intermediate position

similar to that found by Jørgensen et al. (2006) for *Saxifraga rivularis* L., suggesting that they originated from a combination of the diploid species, but they have also accumulated a few mutations such that they share some AFLP bands that are not present in any of the diploid or triploid species in North America. Although another species from Eurasia may be involved in their origin, the low number of unique bands in the polyploids (six, three of these found in *P. phryganodes*) (Fig. 5.3) suggests that the three species *P. arctica*, *P. vahliana*, and either *P. phryganodes* or a parent of *P. phryganodes* that we have not yet tested, were the only parents involved. To account for the unique bands, it is possible that the putative parental species were ancestors of the extant diploids instead of the extant species themselves.

Puccinellia bruggemannii

This species is tetraploid so would be expected to have two different parental genomes if it is an allopolyploid. AFLP evidence supports most strongly *P. arctica* and *P. vahliana* as parents. Although observations during field work revealed that this species is always found with *P. vahliana*, suggesting autopolyploid origin, bands characteristic of *P. arctica* and *P. vahliana* (and not found in each other) are found in *P. bruggemannii*.

After critical identification of specimens based on the new set of distinguishing characters in Table 5.9, *P. bruggemannii* has a much more restricted range than reported in Porsild (1964), Consaul and Gillespie (2001) or Davis and Consaul (2007). We have now identified many of the specimens originally identified as *P. bruggemannii* in this study and in the herbarium at CAN as *P. angustata*. In the field work for this study *P. bruggemannii* was frequently found with *P. vahliana* or on drier upland areas nearby, and additional localities may be found by looking specifically in such habitats.

Contemporary recurrent polyploidy is not likely in *P. bruggemannii*, because one of the putative parents, *P. arctica*, is distributed only in the southwestern and the extreme northern Arctic (Consaul et al., Chapter 3), and is not extant in the central arctic islands where *P. bruggemannii* is now primarily

distributed (Fig. 5.11). However, AFLP evidence did not answer whether *P. bruggemannii* has formed once or several times in the past.

Puccinellia angustata

Puccinellia angustata has a number of bands characteristic of *P. arctica*, *P. vahliana*, and *P. phryganodes*. We considered *P. bruggemannii* as one parent because bands of both of the proposed parents of *P. bruggemannii* are in *P. angustata*. Evidence contradicting this is found, however, in that the four most characteristic bands of *P. bruggemannii* are not found in typical hexaploid *P. angustata*. Although we cannot determine what the order of hybridization is for the species from only the AFLP data, evidence suggests that both *P. arctica* and *P. vahliana* are involved, but without direct formation of *P. angustata* from *P. bruggemannii*. A number of bands found in *P. phryganodes* were also found in P. angustata which may show that it was involved in the origin of this hexaploid.

Group B6 is a hexaploid group that has two of the four main *P. bruggemannii* bands also found in *P. vahliana* as well as all bands characteristic of *P. angustata*. Accordingly, in ordinations they usually grouped with *P. angustata* but were frequently found approaching the *P. bruggemannii* group. B6 could be formed by *P. bruggemannii* hybridizing with one of the diploid species, which would indicate a different origin for these *P. angustata* if we call these B6 by that name. Alternatively, B6 plants may have formed by *P. angustata* plants undergoing introgression with *P. vahliana*, since the "*P. bruggemannii*" bands in B6 are also found in *P. vahliana*. This would imply that the original formation of *P. angustata* was followed by the loss of some of the original *P. vahliana* bands. These B6 plants are mostly from populations that are in the range of *P. bruggemannii*, but two of the plants are from Banks Island and two from Ellesmere Island, in areas where *P. vahliana* is common but *P. bruggemannii* is absent or very scarce making the introgression scenario more likely.

Puccinellia andersonii

This species is primarily octoploid. From the evidence presented here, one hypothesis for the origin of this species is that it forms from *P. angustata* hybridizing with a diploid progenitor of *P. phryganodes* with duplication of the genome to make a fertile polyploid. Three bands that are characteristic of *P. andersonii* plants are abundant in *P. phryganodes*. This formation could be single or multiple; we have no evidence to distinguish between these hypotheses.

Six putative heptaploid individuals aligned with *P. andersonii* (thus we coded them D7) based on AFLP patterns and morphology. The problem with these specimens was determining their ploidy level with confidence. Flow cytometry analyses were successful in giving us generally interpretable data with few exceptions. The ploidy distinctions between diploid and triploid, triploid and tetraploid, and between tetraploid and hexaploid, were clear. However, when the specimens had ploidy counts that were near the dividing line between hexaploid and octoploid, just above 10 pg DNA/2C, we could not conclude with certainty whether the specimens were hexaploid or octoploid. Talent and Dickinson (2005) also found that tetraploid specimens in the Rosaceae subfamily Maloideae could be distinguished, but that the line between pentaploid and hexaploid specimens was blurred. Without successful chromosome counts we do not yet know whether the apparently heptaploid specimens actually have a heptaploid chromosome number, but otherwise they fit within *P. andersonii* and do not pose a problem.

None of the *P. andersonii* populations were completely octoploid. As mentioned, the flow cytometry values should be checked for these hexaploid *P. andersonii* (D6) individuals by obtaining chromosome counts.

5.5.3 Hybridization and Introgression

Two main hybrid molecular patterns occurred in this data set: the B6 pattern and the D6 pattern. We discussed above that B6 plants could be formed by *P. bruggemannii* hybridizing with one of the diploid species or by *P. angustata* plants undergoing introgression with *P. vahliana*. The D6 plants do not fall under any of the above taxa (B, N, or D), being hexaploids that have characteristic *P.*

angustata bands and characteristic *P. andersonii* bands, indicating a hybrid AFLP pattern. The AFLP banding pattern is consistent with a formation of D6 by introgressive hybridization of *P. angustata* with *P. phryganodes*, or *P. angustata* with *P. andersonii*, sharing genetic information but no increase in ploidy (as found in *Elymus* by Mason-Gamer et al. 2005). Additional information obtained by examining DNA sequences may provide a further explanation for these two hybrid patterns.

Plants that have a high rate of selfing would be likely to experience low levels of introgression or hybridization. Some polyploid plants have been reported as having lower self-incompatiblity, and therefore higher rates of autogamy (Levin 1983). In examining the polyploids in this study, plants with developing caryopses often had dehisced anthers wrapped up in the stigmas. Therefore, autogamy is likely in these plants. However, variation in AFLP bands was found to be higher within populations than among populations in AMOVA. This is more characteristic of outcrossing plants (Crawford 1983). Together this evidence suggests that both outcrossing and selfing occurs in these plants. If introgression or hybridization followed by polyploidy occurs, the genetic changes are likely to be characteristic of a whole population due to selfing, which is consistent with the patterns in these *Puccinellia* populations.

5.5.4 Effect of environment on morphology

Changes in environment caused significant changes in half of the quantitative morphological characters in this study. Almost all of the characters relating to inflorescence, ligule, and habit showed significant differences, whereas only half of the floret characters differed between field and common garden conditions. This is consistent with previous findings that characters controlled by long periods of meristematic activity have high amounts of plasticity while those related to flowers have little (Bradshaw 1965, Marshall and Jain 1968, Wilken 1977, Lacey 1986, Macdonald et al. 1988). The characters that were plastic in this study, however, were not identical for each taxon. Gottlieb (1977) found that the patterns of plastic responses between species of closely related *Stephanomeria* did

not vary, whereas Marshall et al. (1986) found that the patterns of responses for two species of *Sesbania* that were from different sections in the genus did differ (Marshall et al. 1986). In the case of *Puccinellia*, the species are very closely related and thus might be expected to have similar plastic responses, but different combinations of parental species have been involved in the speciation of these three polyploids so it would not be surprising that the set of plastic characters differs among them. One other complication occurs because variation in common garden experiments can be due to maternal factors and not genetic influence (Sultan 2000). This variation in plastic characters among taxa means we will not place our first emphasis of the "best characters" on their degree of plasticity.

Greater overlap was found among the polyploid species (this paper) than among the diploid species (Consaul et al. Chapter 3) in ordinations of morphological characters, but was not attributable to higher plasticity. Polyploid and diploid species had similar numbers of characters that did not differ significantly between the field and the common garden experiments: 20 in the polyploids studied here and 21 in the diploids in Consaul et al. (Chapter 3). Schlichting and Levin (1984) hypothesized that diploids, having low diversity within their own genomes, may express higher phenotypic plasticity to adapt because their ability to tolerate a large range of conditions is limited. Marshall and Jain (1968) showed this to be the case in the hexaploid *Avena fatua* which had higher isozyme variation and lower phenotypic plasticity than the diploid *A. barbata*. Garbutt and Bazzaz 1983 (*Phlox*) and Macdonald et al. 1988 (*Stellaria*) found that polyploid species tend to have lower phenotypic plasticity than their diploid progenitors. These findings are consistent with our observations that we did not have more characters differing between field and common garden in the polyploid species.

The morphology of several specimens of all three taxa did not correspond to the ploidy level and AFLP patterns typical of that ploidy level (Table 5.8). The hexaploid specimens that were misclassified as *P. andersonii* were less hairy than normal; therefore they classified with the less hairy taxon *P. andersonii*. Hair characters have been shown to be susceptible to environmental conditions to a

certain extent (Ramesar-Fortner et al. 1995, *Festuca*), but hair extent on the lemma and palea is not usually susceptible to the environment in *Puccinellia*. The characters of hair on lemma and palea did not differ significantly for any taxon between field and common garden in this study (Fig. 5.6) and Davis (1983) found these to be among the most genetically stable and taxonomically important characters. The four hexaploid and octoploid specimens that were misclassified as *P. bruggemannii* were smaller than normal, therefore classifying with the smaller taxon. These may be hybrids, but pollen was fertile for these individuals. They may have aberrant morphological measurements owing to an extreme microenvironmental effect.

5.5.5 Best morphological characters for identifying the species

The most important characters for discriminating among *P. bruggemannii*, *P. angustata*, and *P. andersonii* are rachilla length, which has not previously been used as a diagnostic character in this group, width and length of the second glume, extent of lemma and palea hair, and anther length (Table 5.9), each of which distinguished among pairs of species in the common garden experiments except second glume length. The character means in Table 5.9 were calculated using both the field and common garden plants; thus they distinguish the species over the entire morphological range encountered during fieldwork. The values in the key are calculated using standard deviations from the mean to define the ranges. One should use the inflorescence length and lemma shape as a guide and then verify with the lengths stipulated in the other characters.

5.5.6 Key to three polyploid *Puccinellia* species in the North American Arctic

1. Inflorescence often less than 4 cm long; lemmas with rounded back and often with an incurved tip; rachilla between first and second lemma 0.8–1.3 mm long; second glume 1.7–2.3 mm long, its length/width ratio 0.5–0.8…..*P. bruggemannii*

1. Inflorescences often more than 4 cm long; lemma tip not incurved; rachilla between first and second lemma 1–1.7 mm long; second glume 1.9–3.0 mm long, its length/width ratio 0.3–0.6 …….....……..…2

2. Hair on lemma extending to 0.7–1.5 mm from base; palea usually with hairs on basal half, hairs extending 1.8–2.8 mm from palea apex; inflorescence branches usually scabrous ……...*P. angustata*

2. Hair on lemma extending to 0.1–1.0 mm from base; palea never with hairs on basal half, hairs extending to 1.6–1.9 mm from palea apex; inflorescence branches usually smooth……...…*P. andersonii*

5.6 Conclusions

Despite the observation that the environment affected morphology in half of the characters measured here, most plants in this study can be classified into *P. bruggemannii*, *P. angustata*, and *P. andersonii*. Morphological variation on its own accounted for only some of the problematic specimens that we encountered. The other problems were aligned with hybrid genetic patterns. Over half of the D6 plants had morphological misclassifications, suggesting that genetic intermediacy or hybridity is one of the causes of identification difficulties in *Puccinellia*. Although this may be caused by recurrent polyploidy or introgression, current geographic distribution patterns of the potential parents support the latter more strongly. Analyses of DNA regions that can provide information on homologous sites, such as sequencing of nuclear ribosomal DNA regions to reveal multiple copies and chloroplast DNA regions to reveal the maternal inheritance of these polyploids will be useful to support the proposed parental contributions of these polyploids as *P. arctica*, *P. vahliana*, and *P. phryganodes*.

Table 5.1. Locality data for collections of polyploid *Puccinellia* species studied in this paper, with numbers of individuals per population studied for morphology (Mor), flow cytometry (Flow), and AFLP analysis (AFLP). Orig id = identification made in the field at beginning of the study, given if different from final identification: *and = P. andersonii, ang = P. angustata, bru = P. bruggemannii.* Collectors: *HB = H. Bickerton*, *KF = K. Faubert*, *AA = A. Archambault*. Chromosome vouchers indicated as such. Morphology written as # field specimens, # common garden specimens. Vouchers are at MTMG unless noted otherwise.

Collection data	Mor	Flow	AFLP	Orig. id
P. andersonii				
NWT, Banks I, Duck Hawk Bluff, *LLC 2856b, LJG & HB*	2	3	3	
NWT, Banks I, Worth Point, *LLC 2851b, LJG & HB*	3, 5	4	4	*ang*
Nunavut, Ellesmere I, Eureka, *LLC 2865 & KF*	11,8	16	10	
Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2877a & KF*	9,10	14	5	
Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2878a & KF*	7, 4	8	5	*bru*
P. angustata				
NWT, Banks I, Masik R, *LLC 2807, LJG & HB*		1	1	
NWT, Banks I, Masik R, *LLC 2809, LJG & HB*	1	-	3	
NWT, Banks I, Egg R, coastal plain, *LLC 2849, LJG & HB*		2	2	
NWT, Banks I, Worth Point, *LLC 2851a, LJG & HB*	8, 5	8	5	
NWT, Banks I, Worth Point, *LLC 2853, LJG & HB*	11,12	12	10	*bru*
NWT, Banks I, Duck Hawk Bluff, *LLC 2856c, LJG & HB*	1	2	3	*and*
NWT, Banks I, Sachs Harbour, *LLC 2858* (chromosome voucher)	2	6	5	*bru*
Nunavut, Ellesmere I, Eureka, *LLC 2866 & KF* (chromosome voucher)	9, 8	16	7	
Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2874 & KF*	10,11	14	9	

Table. 5.1. Continued.

Collection data	Mor	Flow	AFLP	Orig. id
Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2877b & KF*	2, 2	2	1	
Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2878b & KF*	5, 4	7	5	*bru*
Nunavut, Ellesmere I, *LLC 2899 & KF*		2	2	
Nunavut, Cornwallis I, Resolute coast, *LLC 3137 & AA*		3		
Nunavut, Cornwallis I, Resolute, *LLC 3141 & AA*		3	3	
NWT, Prince Patrick I, Mould Bay, *LLC 3150 & AA*		5	5	
NWT, Prince Patrick I, Mould Bay, *LLC 3151 & AA*		2	2	
NWT, Prince Patrick I, Mould Bay, *LLC 3144a & AA*	2,12	21	10	*bru*
NWT, Prince Patrick I, Mould Bay, *LLC 3154 & AA* (chromosome voucher)	2,10	17	11	*bru*
P. bruggemannii				
Nunavut, Cornwallis I, Resolute, *LLC 2906 & KF* (chromosome voucher)	11,8	4	9	
Nunavut, Cornwallis I, Resolute coast, *LLC 3063 & AA*	1	1	-	
Nunavut, Cornwallis I, Resolute coast, *LLC 3072 & AA*	8,11	7	7	
Nunavut, Beechey I, *LLC 3075 & AA*	3,6	5	5	
Nunavut, Bathurst I, *LLC 3080 & AA*	6,8	9	10	
Nunavut, Somerset I, Cunningham Inlet, *LLC 3083 & AA*		3	3	
Nunavut, Cornwallis I, Resolute, *LLC 3100 & AA*	5,5	6	4	
NWT, Prince Patrick I, Mould Bay, *LLC 3144b & AA*	1	3	3	

Table. 5.1. Continued.

Collection data	Mor	Flow	AFLP	Orig. id
P. phryganodes				
Nunavut, Axel Heiberg I, Expedition Fiord, *LLC 2321, R.J. Soreng & LJG*			3	
NWT, Banks I, De Salis Bay, *LLC 2818, LJG & HB*			3	
NWT, Banks I, Egg River, shoreline, *LLC 2847, LJG & HB*			3	
Nunavut, Cornwallis I, N of Resolute, *LLC 3123 & AA*		3	3	
Nunavut, Baffin I, Tarr Inlet, *LLC 2908 & KF*		3		
Nunavut, Baffin I, Tarr Inlet, *LLC 3163 & AA*		10	7	
Nunavut, Baffin I, Tarr Inlet, *LLC 3169 & AA*		3	6	
P. sp.2				
NWT, Banks I, Egg River, *LLC 2848, LJG & HB*	10,5	10	10	
P. pumila				
Labrador, Nain, *LLC 2512*			1	
Labrador, Edwards Cove, *LLC 2552*			6	
Labrador, Anaktalak Bay, *LLC 2926*			2	
Labrador, Anaktalak Bay, *LLC 2563* (chromosome voucher)		5	6	
Manitoba, Churchill, *S.G. Aiken 01-342*		1	1	
Total	130,134	241	203	

Table 5.2. Morphological characters analyzed in this study.

Character	Details or character states
1. Height	(cm)
2. Ligule shape:	linear = 1; lanceolate = 2; ovate-oblong = 3; transversely oblong = 4
3. Ligule, apex shape:	acuminate = 1; acute = 2; obtuse = 3; truncate = 4
4. Ligule apex:	entire = 1; erose = 2; lacerate = 3; cleft = 4
5. Ligule:	hairy = 1; glabrous = 2
6. Ligule length	(mm)
7. Inflorescence branch number	at lowest node
8. Inflorescence length	(cm)
9. Primary branches and pedicels:	smooth = 1; scabrous = 2
10. Primary branch length	(cm) (at lowest node)
11. Primary branch, distance on branch to first spikelet	(cm)
12. Panicle basal internode length	(cm)
13. Inflorescence branches, maximum divergence:	erect = 1; ascending = 2; horizontal = 3; descending = 4
14. Pedicel width 1	(just below first glume, mm)
15. Pedicel width 2	(0.5 mm below first glume, mm)
16. Spikelet length	(mm)
17. Florets per spikelet	number
18. First glume shape:	oblong = 1; lanceolate = 2; ovate = 3; elliptic = 4. obovate = 5
19. First glume apex margin:	glabrous = 1; sparse tiny trichomes < 25 μm = 2; moderately abundant, tiny trichomes < 25 μm (most) = 3; abundant trichomes many reaching > 25 μm long = 4
20. First glume apex shape:	with caudate tip = 1; acuminate = 2; acute or obtuse = 3; truncate = 4
21. First glume length	(mm)
22. First glume width	(mm)
23. Second glume shape	(see character 18)
24. Second glume apex margin	(see character 19)
25. Second glume length	(mm)
26. Second glume width	(mm)
27. Glume postion:	"opposite" = 1; 0.1–0.2 mm apart = 2; < 0.3 mm apart = 3
28. Rachilla length	between 1st and 2nd lemma (mm)
29. Rachilla width	(μm)
30. Callus:	glabrous = 1; hairy = 2

Table 5.2. Continued.

Character	Details or character states
31. Lemma apex shape:	acute = 1; obtuse = 2; truncate = 3
32. Lemma distal margins:	entire = 1; slightly irregularly serrate or erose = 2; irregularly serrate or erose = 3
33. Lemma apex margin	(see character 19)
34. First lemma length	(mm)
35. Lemma:	glabrous = 1; sparsely scaberulous = 2; hairy = 3
36. Extent of hair on lemma from base	(mm)
37. Palea veins:	glabrous = 1; scabrous or with short, straight hairs = 2; scabrous or hairy at the top, curly-hairy in the lower part = 3
38. Palea length	(mm)
39. Palea width	(mm)
40. Extent of hair on palea from apex	(mm)
41. Anther length	(mm)
42. First glume width/length	
43. Second glume width/length	
44. Palea width/length	

Table 5.3. Generalized linear model for morphological data from common garden experiments to test population differences.

Factors	df	Populations															
Common garden #1																	
Tax	6	*agr*	*agr*	*arc*	*arc*	*poa*	*poa*	*ban*	*ban*	*bru*	*bru*	*bru*	*ang*	*ang*	*ang*	*and*	*and*
ID		*2813*	*2823*	*2805*	*2805*	*2864*	*2898*	*2810*	*2842*	*2906*	*2853*	*2878*	*2851*	*2866*	*2874*	*2865*	*2877*
Pop	15	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Pop(tax)	90																
Reps		12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Contrast 1	1	1	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0
Contrast 2	1	0	0	1	1	-1	-1	0	0	0	0	0	0	0	0	0	0
Contrast 3	1	-1	-1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Contrast 4	1	1	1	1	1	1	1	-3	-3	0	0	0	0	0	0	0	0
Contrast 5	1	0	0	0	0	0	0	0	0	1	1	1	-1	-1	-1	0	0
Contrast 6	1	0	0	0	0	0	0	0	0	0	0	0	-2	-2	-2	3	3
Post Hoc		0	0	0	0	0	0	0	0	3	0	0	-1	-1	-1	0	0

Common garden #2											
Tax	5	*bru*	*bru*	*bru*	*bru*	*ang*	*ang*	*and*	*sp.2*	*lan*	*vah*
ID		*3072*	*3080*	*3075*	*3100*	*3154*	*3144*	*2878*	*2848*	*3164*	*3102*
Pop	9	1	2	3	4	5	6	7	8	9	10
Pop(tax)	45										
Reps		12	12	12	12	12	12	12	12	12	12
Contrast 1	1	1	1	1	1	-2	-2	0	0	0	0
Contrast 2	1	0	0	0	0	0	0	1	-1	0	0

Note: Tax = Taxon, codes are original identifications: *agr*: *P. agrostidea*; *arc*: *P. arctica*; *poa*: *P. poacea*; *ban*: *P. banksiensis*; *bru*: *P. bruggemannii*; *ang*: *P. angustata; and*: *P. andersonii*. "ID" is the LLC population code found in Table 5.1 (polyploids) or Consaul et al. (Chapter 3). Pop = Population number given in the common garden experiments. Pop(tax) = test of populations nested within taxa. Reps: number of replicates (= pots) per population. Contrast = values given to each of the populations for the individual contrasts in the CONTRAST statement. Post Hoc = tests made after the original analysis, based on revised identifications of some specimens in the polyploid populations.

Table 5.4. DNA content based on flow cytometry and chromosome numbers for populations of polyploid *Puccinellia* spp.

Ploidy level or population	N(p)	DNA (mean pg/2C)	Prop	*n*
Triploid (*P. phryganodes*)	19(6)	5.01 ± 0.12 (4.62–5.56)	1.61	
Tetraploid (*P. phryganodes*)	14(2)	6.31 ± 0.13 (6.31–6.79)	2.02	
Tetraploid (*P. bruggemannii*)	61(8)	6.07 ± 0.09 (5.63–6.78)	1.95	
2906 (*P. bruggemannii*)	6	6.37 ± 0.24 (5.96–6.78)	2.08	14
Hexaploid (*P. angustata*)	160(17)	8.51 ± 0.13 (7.14–10.09)	2.73	
2858 (*P. angustata*)	6	8.74 ± 0.38 (8.13–9.39)	2.84	21,21
2866 (*P. angustata*)	16	8.41 ± 0.29 (7.76–9.39)	2.73	~21
3154 (*P. angustata*, orig. ID : *P. bruggemannii*)	20	8.29 ± 0.19 (7.63–9.56)	2.66	21
cf. Heptaploid (*P. angustata* & *P. andersonii*)	7(4)	10.26 ± 0.17 (10.04–10.52)	3.30	
Octoploid (*P. andersonii*)	44(6)	11.34 ± 0.21 (10.57–12.79)	3.64	
2563 (*P. pumila*)	1	10.65	3.42	28

Note: Population given as *LLC* population number from Table 5.1. N = number of plants analyzed for flow cytometry from "p" number of populations; DNA (mean pg/2C) = mean of N plants ± 2× standard error, range in round brackets, single number given if only a single plant was analyzed. Prop = proportion of standard diploid amount; *n* = haploid chromosome number. Chromosome counts given for particular individuals within the listed populations, but the corresponding DNA content value is the mean for that population.

Table 5.5. AMOVA results for AFLP presence/absence data from diploid and polyploid populations of *Puccinellia*. df = degrees of freedom.

Source of variation	df	Variance Component[a]	p-value[b]
Diploid			
Among taxa	3	10.435 (66.93%)	< 0.001
Among populations within taxa	4	1.553 (09.97%)	< 0.001
Within populations	56	3.600 (23.10%)	< 0.001
Polyploid (Groups B, D, and N)			
Among taxa	2	3.001 (28.80%)	< 0.001
Among populations within taxa	17	1.574 (15.13%)	< 0.001
Within populations	111	5.830 (56.02%)	< 0.001
Polyploid (Groups B, B6, and N)			
Among taxa	2	2.591 (25.95%)	< 0.001
Among populations within taxa	12	1.493 (14.95%)	< 0.001
Within populations	89	5.902 (59.10%)	< 0.001
P. bruggemannii			
Among populations	5	1.957 (23.54%)	< 0.001
Within populations	31	6.356 (76.46%)	0.001
P. angustata			
Among populations	8	1.253 (18.26%)	< 0.001
Within populations	52	5.609 (81.74%)	0.001
P. andersonii			
Among populations	4	1.857 (24.87%)	< 0.001
Within populations	22	5.610 (75.13%)	0.001

Note: [a] Percentage of the total variance is given in parentheses.
[b] Probability of having a more extreme variance component than the observed value by chance alone, computed by nonparametric analyses from 500 random permutations.

Table 5.6. Significance values of morphological characters for common garden experiments.

Table 5.6A. Quantitative morphological characters. Significance values for field versus common garden comparisons (F vs CG) as determined by Tukey-Cramer HSD tests illustrated in Fig. 5.6, (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = not significant). P-values from the generalized linear model results with planned comparisons of morphological data from common garden experiments of *Puccinellia* polyploid specimens, and importance of the characters as determined in the discriminant analysis between pairs and coded in columns Stepwise, CG and Stepwise, F. Codes in the last two columns represent species pair being compared (*bru* = *P. bruggemannii*, *ang* = *P. angustata*, *and* = *P. andersonii*) followed by the order of significance in the analysis given by numbers 1-7.

Quantitative Characters	F vs CG	Pop CG#1	*P. bru.* vs *P. ang.* CG#1	*P. ang.* vs *P. and.* CG#1	Pop CG#2	*P. bru.* vs *P. ang.* CG#2	*P. and.* vs *P.* sp. 2 CG#2	Stepwise, CG	Stepwise, F
1. Height	*	< 0.0001	0.0063	ns	0.0005	0.0302	< 0.0001	*bru* vs *and*: 2	ns
6. Ligule length	*	< 0.0001	ns	ns	0.0025	ns	0.0020	*bru* vs *and*: 3 *bru* vs *ang*: 5	ns
8. Inflorescence length	***	< 0.0001	ns	ns	0.0034	0.0005	0.0246	ns	*ang* vs *and*: 2
10. Branch length	**	0.0018	ns	ns	0.0407	0.0139	ns	ns	ns
11. Branch first spikelet	***	0.0007	ns	ns	ns	ns	ns	ns	ns
12. Basal internode	***	0.0024	ns	ns	0.0056	0.0052	0.0064	ns	ns
14. Pedicel width 1	ns	< 0.0001	ns	ns	ns	ns	ns	*bru* vs *ang*: 3	ns
15. Pedicel width 2	ns	< 0.0001	ns	0.0009	ns	ns	ns	*bru* vs *and*: 7	ns
16. Spikelet length	ns	< 0.0001	ns	ns	< 0.0001	ns	ns	*ang* vs *and*: 2	ns
21. 1st glume length	***	< 0.0001	ns	ns	< 0.0001	ns	ns	*bru* vs *and*: 6	ns
22. 1st glume width	***	< 0.0001	ns	ns	< 0.0001	0.0004	ns	*bru* vs *ang*: 4	*bru* vs *and*: 2
25. 2nd glume length	*	< 0.0001	ns	ns	< 0.0001	ns	ns	*bru* vs *ang*: 1 *ang* vs *and*: 4	*bru* vs *ang*: 1 *bru* vs *and*: 5
26. Second glume width (mm) (A)	**	< 0.0001	ns	ns	< 0.0001	0.0045	ns	*bru* vs *ang*: 2 *bru* vs *and*: 1	*bru* vs *ang*: 2 *bru* vs *and*: 4

Table 5.6A. Continued.

Quantitative Characters	F vs CG	Pop CG#1	*P. bru.* vs *P. ang.* CG#1	*P. ang.* vs *P. and.* CG#1	Pop CG#2	*P. bru.* vs *P. ang.* CG#2	*P. and.* vs *P.* sp. 2 CG#2	Stepwise, CG	Stepwise, F
28. Rachilla length	**	< 0.0001	ns	ns	< 0.0001	0.0246	0.0480	*bru* vs *ang*: 6 *bru* vs *and*: 1	*bru* vs *ang*: 3 *ang* vs *and*: 4
29. Rachilla width	ns	< 0.0001	ns	ns	ns	0.0044	ns	*ang* vs *and*: 5	ns
34. Lemma length	***	< 0.0001	0.0040	ns	< 0.0001	ns	0.0039	ns	*ang* vs *and*: 3
36. Lemma hair extent Extent	ns	< 0.0001	0.0005	0.0036	0.0039	ns	ns	*ang* vs *and*: 3	*bru* vs *ang*: 4 *ang* vs *and*: 1
38. Palea length	**	< 0.0001	ns	ns	< 0.0001	ns	0.0183	ns	ns
39. Palea width	ns	< 0.0001	ns	ns	< 0.0001	0.0031	ns	ns	*bru* vs *and*: 6
40. Palea extent of hair from apex	ns	< 0.0001	ns	0.0006	< 0.0001	ns	ns	*ang* vs *and*: 1 *bru* vs *and*: 5	ns
41. Anther length	***	< 0.0001	ns	ns	< 0.0001	0.0032	0.0025	*ang* vs *and*: 6 *bru* vs *and*: 4	*bru* vs *and*: 3
42. 1st glume W/L	ns	0.0011	ns	ns	ns	0.0190	ns	ns	ns
43. 2nd glume W/L	ns	< 0.0001	ns	ns	< 0.0001	0.0038	ns		
44. Palea W/L	**	< 0.0001	ns	ns	< 0.0001	0.0001	ns	ns	ns

Table 5.6B. Qualitative morphological characters. Significance of F versus CG as determined by Kruskal-Wallis tests and significance in distinguishing among taxa, indicated in columns KW,F and KW, CG.

Qualitative Characters	F vs CG	KW, CG	KW, F	Qualitative Characters	F vs CG	KW, CG	KW, F
2. Ligule shape	***	ns	ns	**20.** 1st glume apex shape	ns	ns	ns
3. Ligule apex shape	*	ns	ns	**23.** 2nd glume shape	ns	***	***
4. Ligule apex entire	**	ns	ns	**24.** 2nd glume trichomes	*	***	***
5. Ligule hair	ns	ns	ns	**27.** Glumes opposite	ns	***	***
7. Branch #	***	ns	ns	**30.** Callus hairy.	ns	ns	ns
9. Branches scabrous	*	ns	ns	**31.** Lemma apex shape	*	**	**
13. Branches divergence	***	*	ns	**32.** Lemma distal margins	**	**	**
17. Floret number	ns	ns	ns	**33.** Lemma apex trichomes	**	***	***
18. 1st glume shape	ns	ns	ns	**35.** Lemma glabrous/hairy	ns	***	***
19. 1st glume apex trichomes	ns	***	***	**37.** Palea keel hairs	ns	***	***

Note: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = not significant. CG#1 = Common garden #1; CG#2 = Common garden #2. Pop = p-value based on the entire model analyzing all populations in the experiment, significance values given if $p \leq 0.05$. ns = non-significant. In individual comparisons of CG#1 contrasts are significant when $p \leq 0.009$ because of Dunn-Sidak adjustment for non-orthogonal comparisons; for CG#2, contrast are orthogonal, therefore they are significant when $p \leq 0.05$.

Table 5.7. Stepwise discriminant analysis results showing number of plants classified in each taxon (Discriminant classification) as a fraction of the total number assigned to the original taxon by ploidy level (Original classification).

Original classification		Discriminant classification		
		bruggemannii	*angustata*	*andersonii*
bruggemannii	– field	23/23	0/23	0/23
	– common garden	19/20	1/20	0/20
angustata	– field	2/33	27/33	4/33
	– common garden	1/40	37/40	2/40
andersonii	– field	2/20	1/20	17/20
	– common garden	0/17	0/17	17/17

Table 5.8. Problematic specimens. A. Specimens misclassified with reference to morphology, but consistent for ploidy and AFLP patterns; B. Specimens with the wrong ploidy level, but consistent for the morphology and AFLP patterns. C. Specimens with the wrong AFLP pattern, given consistency between ploidy and morphology.

Specimen	Group	Morph	Specimen	Group	Morph	Specimen	Group	Morph
A. Morphological misclassification only			***B. Inconsistent chromosome numbers***			***C. Inconsistent AFLP patterns***		
2853-16	N	*D*	**2851-04*	D6	*D,N*	*2851-01*	D6	*N*
2865-03	D	*B*	**2851-13*	D6	*D,N*	*2856-10*	D6	*N*
2874-15	N	*D*	*2856-02*	D6	*D*	*2856-12*	D6	*N*
**2877-06*	D7	*N,D*	**2865-14*	D6	*D,N*	*2878-23*	D6	*N*
3072-18	B	*N*	*2865-05*	D6	*D*	*2856-06*	D	*B*
3072-23	N	*B*	*2878-16*	D6	*D*			
3144-08	N	*B*	**2878-19*	D7	*D,N*			

Note: Specimen codes refer to *LLC et al.* collections (Table 5.1). Group is based on previous AFLP-ploidy determinations (see Fig. 5.3). Morph = classification based on discriminant analysis of morphological data with groups defined on ploidy level. Asterisk (*) = plants positioned in different groups in field and common garden morphological data, both given.

Table 5.9. Characters separating *P. bruggemannii*, *P. angustata*, and *P. andersonii*, chosen as the highest in discriminatory value over all data sets in STEPDISC analysis (see Table 2).

	Rachilla length	Second glume length	Second glume width	Second glume width/length	Extent of lemma hair	Extent of palea hair	Anther length
P. bruggemannii 4X (n=73)	1.05 ± 0.04	1.98 ± 0.08	1.20 ± 0.06	0.62 ± 0.04	0.86 ± 0.07	2.10 ± 0.10	0.80 ± 0.04
P. angustata 6X (n=117)	1.34 ± 0.06	2.44 ± 0.10	1.10 ± 0.02	0.46 ± 0.12	1.10 ± 0.06	2.31 ± 0.08	0.89 ± 0.04
P. andersonii 8X (n=57)	1.37 ± 0.09	1.95 ± 0.14	1.04 ± 0.03	0.46 ± 0.04	0.58 ± 0.10	1.73 ± 0.16	0.95 ± 0.04

Note: Values given are mean ± 2 × standard error, pooling field and common garden specimens.

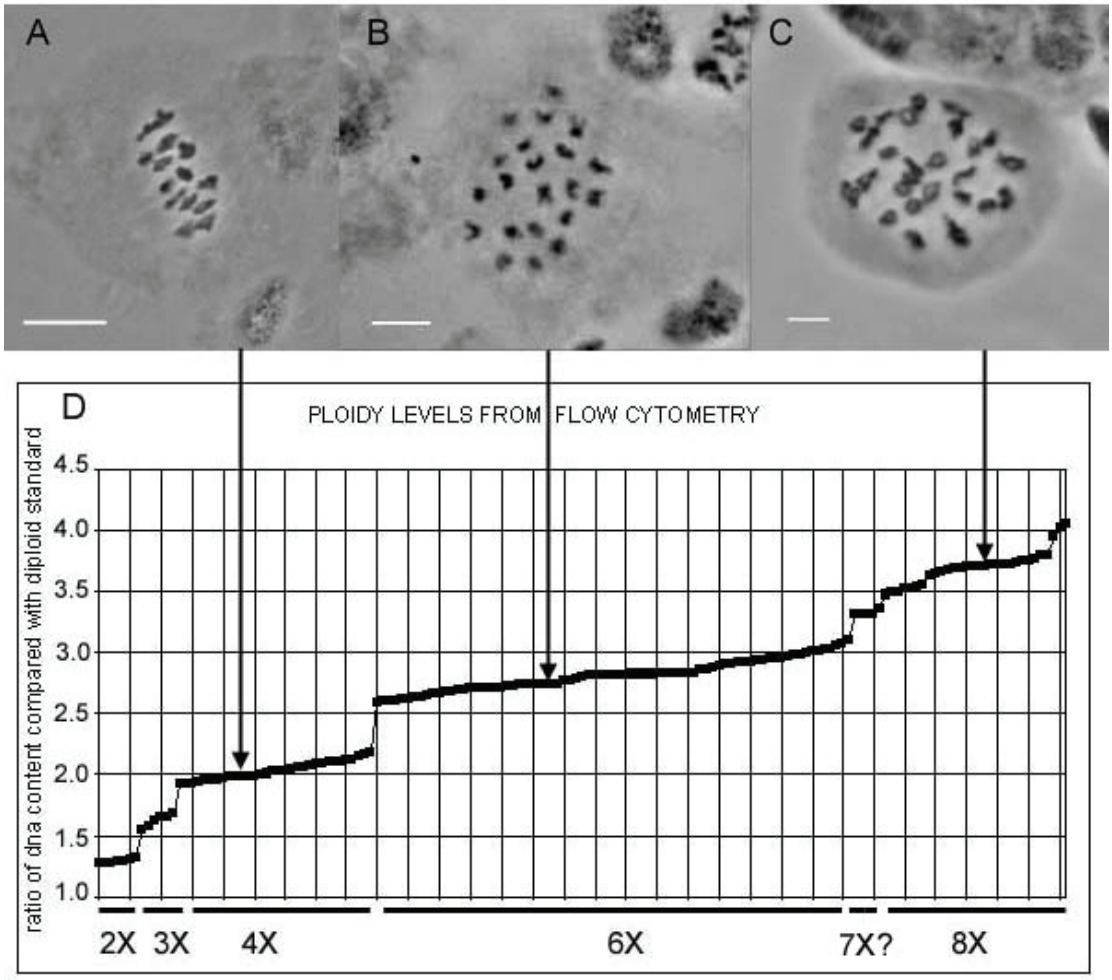


Figure 5.1. Chromosome counts and flow cytometry graph of polyploid *Puccinellia* species. A. *P. bruggemannii,* 4X, *n*=14, *2906-5*, late diakinesis; B. *P. angustata*, 6X, *n*=21, *2858-11,* metaphase; C. *P. pumila*, 8X, *n*=28, *2563-12*, diakinesis. Scale bar = 10 μm. D. Graph showing amounts of DNA (pg/2C) as measured by flow cytometry. Diploid specimens of *P. vahliana* included for comparison only.

Figure 5.2. AFLP band frequencies. Distributional representation of all fragments scored in the diploid and polyploid populations of *Puccinellia*. Columns represent populations and rows correspond to specific AFLP fragments. Black indicates presence in >75% of individuals in a population, dark grey indicates presence in 50–75%, medium grey indicates presence in 25–50%, light grey indicates presence in <25%, and white indicates absence in 100% of individuals in a population. Letters on left indicate regions where bands appear to be characteristic for a single diploid or triploid species; codes given on the X-axis.

Fig. 5.2.

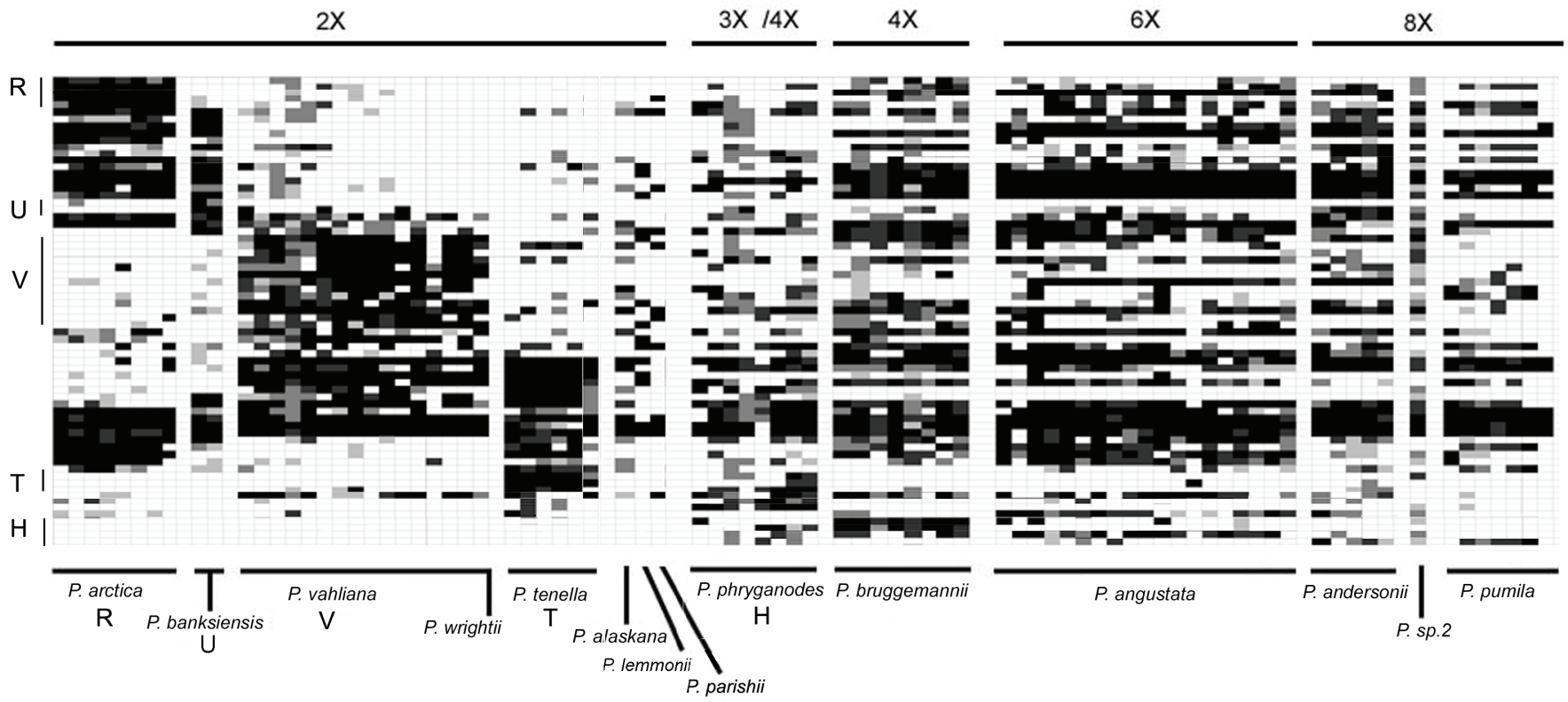

2X
3X /4X
4X
6X
8X
R
U
V
T
H
P. arctica
R
P. banksiensis
U
P. vahliana
V
P. wrightii
P. tenella
T
P. alaskana
P. lemmonii
P. parishii
P. phryganodes
H
P. bruggemannii
P. angustata
P. andersonii
P. sp.2
P. pumila

Figure 5.3. AFLP band presence/absence of fragments in single specimens of polyploid species *P. bruggemannii*, *P. angustata*, and *P. andersonii*. Bands shaded to represent characteristic alignment with typical species: Medium grey – *P. bruggemannii*; black – *P. angustata* (and *P. bruggemannii* or *P. andersonii*); light grey outlined in black – *P. andersonii*; white – no band present. Letters (B, B6, N, D6, D7, D) given for each of the characteristic ploidy-AFLP groups discussed in the text. Diploid code letter or polyploid species aligned with particular bands given at the lower right of the graph. *bru* = *P. bruggemannii*; *ang* = *P. angustata*; *and* = *P. andersonii.*

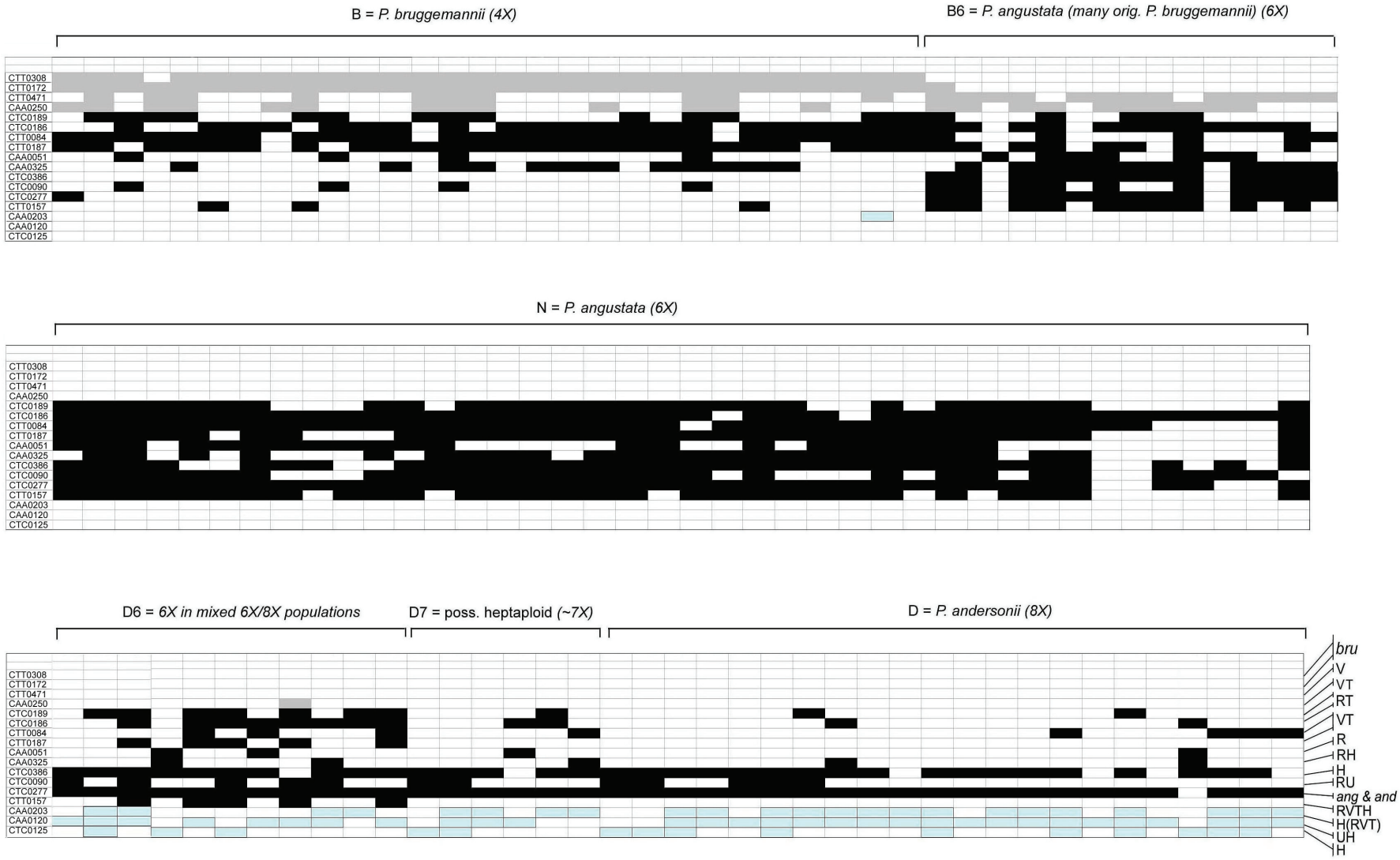

B = P. bruggemannii (4X)
B6 = P. angustata (many orig. P. bruggemannii) (6X)
N = P. angustata (6X)
D6 = 6X in mixed 6X/8X populations
D7 = poss. heptaploid (~7X)
D = P. andersonii (8X)
CTT0308
CTT0172
CTT0471
CAA0250
CTC0189
CTC0186
CTT0084
CTT0187
CAA0051
CAA0325
CTC0386
CTC0090
CTC0277
CTT0157
CAA0203
CAA0120
CTC0125
bru
V
VT
RT
VT
R
RH
H
RU
ang & and
RVTH
H(RVT)
UH
H

Figure 5.4. UPGMA phenogram of AFLP band population frequencies. Manhattan character distance values given on X axis. Species names of *Puccinellia* given to identify clusters. B6 = hexaploid plants with some characteristic *P. bruggemannii* bands, discussed in text. " * " = plants from tetraploid/hexaploid populations (*3072, 3144*). "+" = D6 populations. Arrow points to population *2877*, which was a group of good 6X *P. angustata* plants from an 8X population.

Fig. 5.4

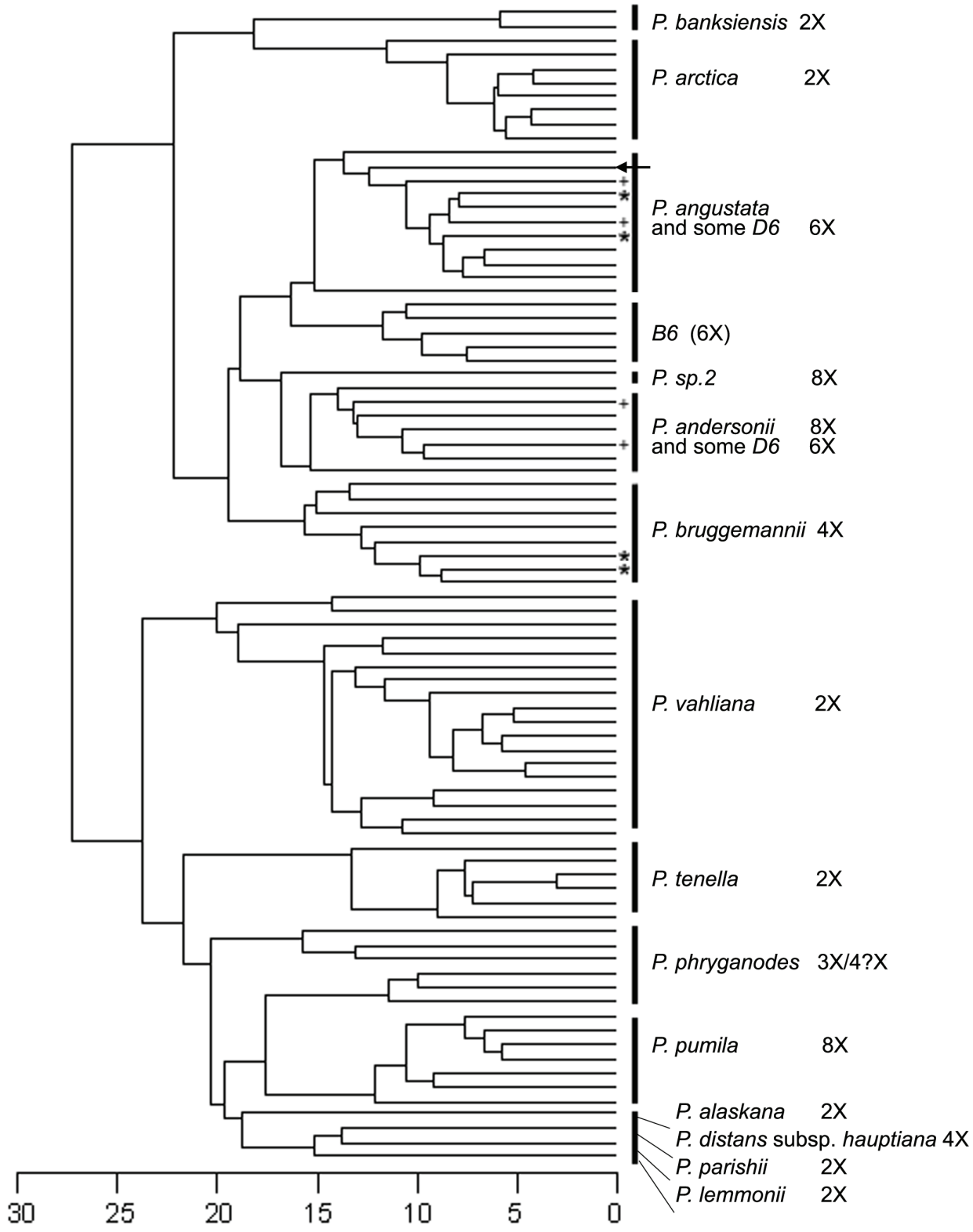
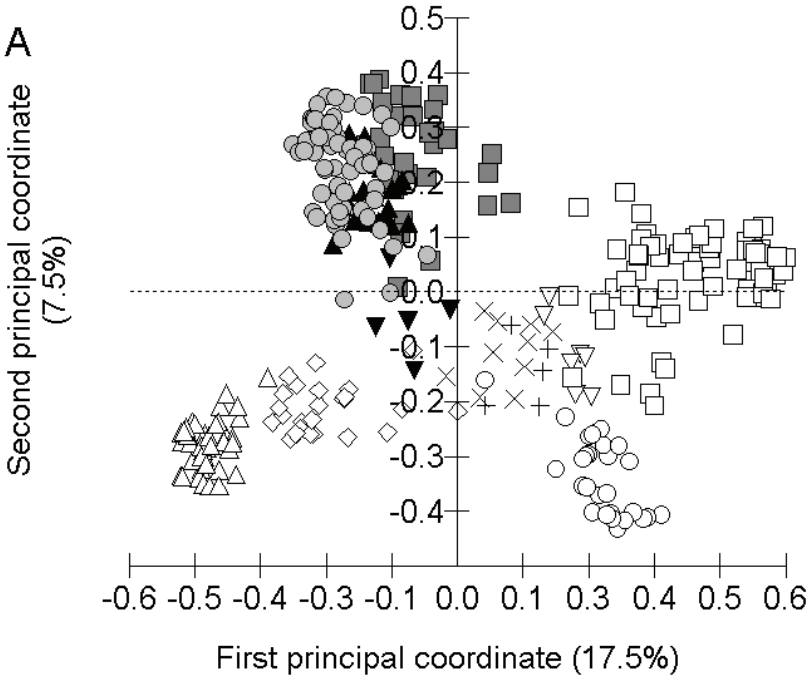

P. banksiensis 2X
P. arctica 2X
P. angustata
and some D6 6X
B6 (6X)
P. sp.2 8X
P. andersonii 8X
and some D6 6X
P. bruggemannii 4X
P. vahliana 2X
P. tenella 2X
P. phryganodes 3X/4?X
P. pumila 8X
P. alaskana 2X
P. distans subsp. hauptiana 4X
P. parishii 2X
P. lemmonii 2X
30
25
20
15
10
5
0

Figure 5.5. PCoA plots from analysis of AFLP presence/absence matrix for three primer pairs. A. Polyploid and diploid specimens. White symbols represent diploid species: △ = *P. arctica*; ◇ = *P. banksiensis;* ○= *P. tenella*; ▽= *P. alaskana*; □ = *P. vahliana* and *P. wrightii*. Crossed symbols represent *P. phryganodes*: × = triploid *P. phryganodes*, + = putative tetraploid *P. phryganodes*. Grey and black symbols represent specimens of polyploid taxa studied in this paper: ■ = tetraploids; ●= hexaploids; ▲ = octoploids; ▼= *P.* sp.2 (octoploid). B. Specimens of putative *P. bruggemannii*, *P. angustata*, and *P. andersonii* only. ■ = B, *P. bruggemannii*; ● = N, *P. angustata*; ▲ = D, *P. andersonii*; ▼ = *P.* sp.2 (octoploid); ⊠ = B6; ⊕ = D6; ▲ = D7.


A
Second principal coordinate (7.5%)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.6 -0.5 -0.4 -0.3 -0.1 0.0 0.1 0.3 0.4 0.5 0.6
First principal coordinate (17.5%)

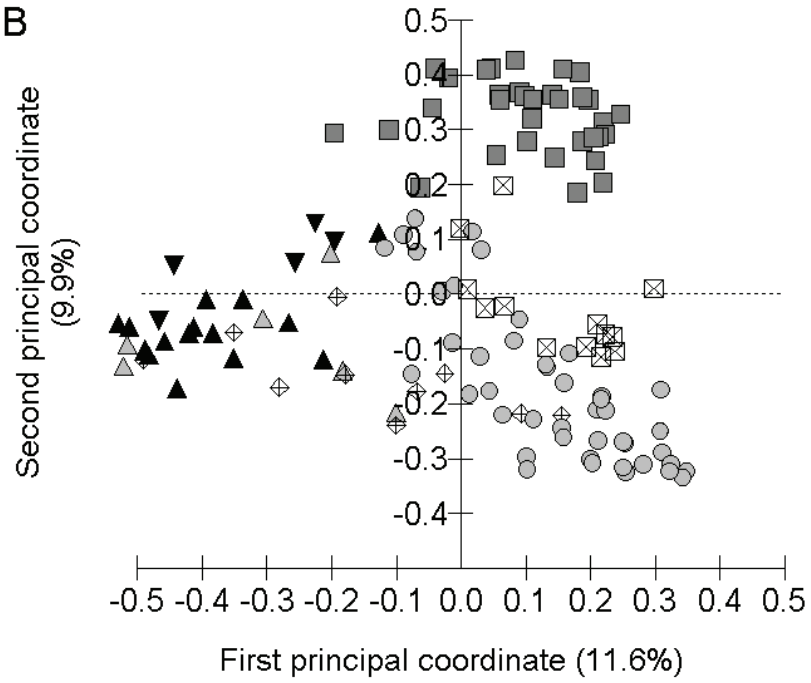

B
Second principal coordinate (9.9%)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5 -0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2 0.3 0.4 0.5
First principal coordinate (11.6%)

Fig. 5.6

A

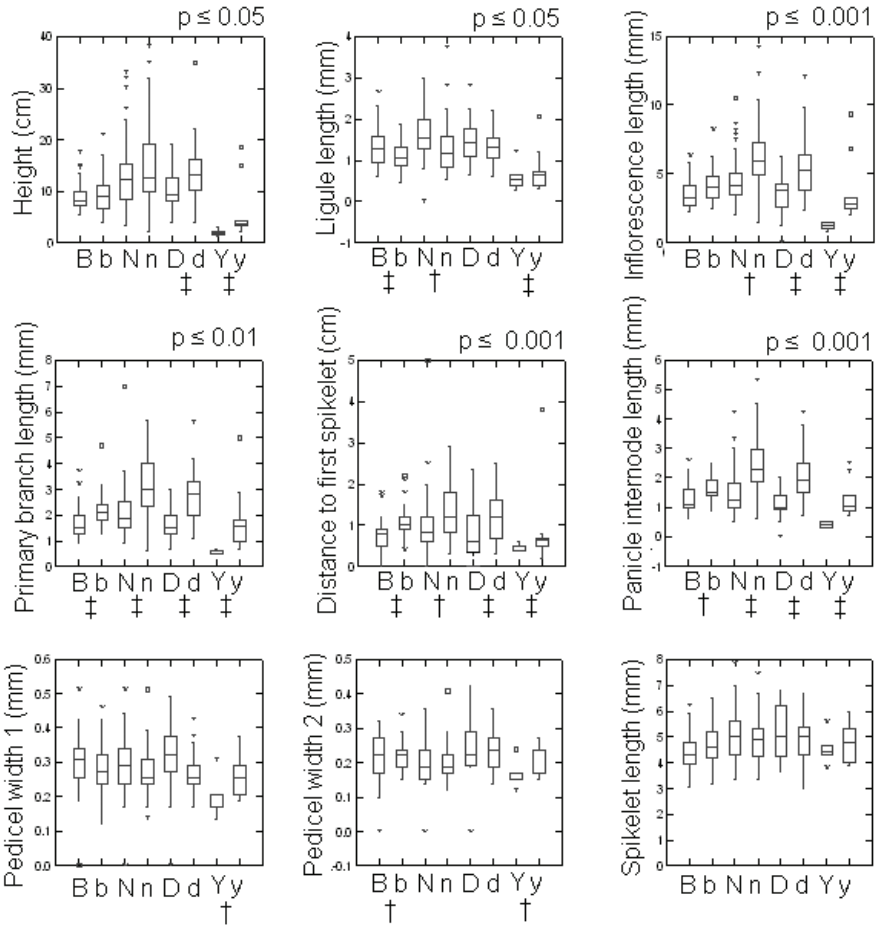


Figure 5.6. Boxplots of medians and quartiles for quantitative morphological characters. Dots outside the whiskers indicate extreme values. Significance of field versus common garden for all taxa given by p ≤ X above each graph. Significance of field versus common garden for each species given below the graphs: * = p ≤ 0.05, †= p ≤ 0.01, ‡ = p ≤ 0.001. B, b = *P. bruggemannii*; N, n = *P. angustata*; D, d = *P. andersonii*, Y, y = *P.* sp.2 (capital letters are field specimens, lower case letters are common garden specimens). A. Characters of inflorescence and spikelets. B. Characters of the florets.

B

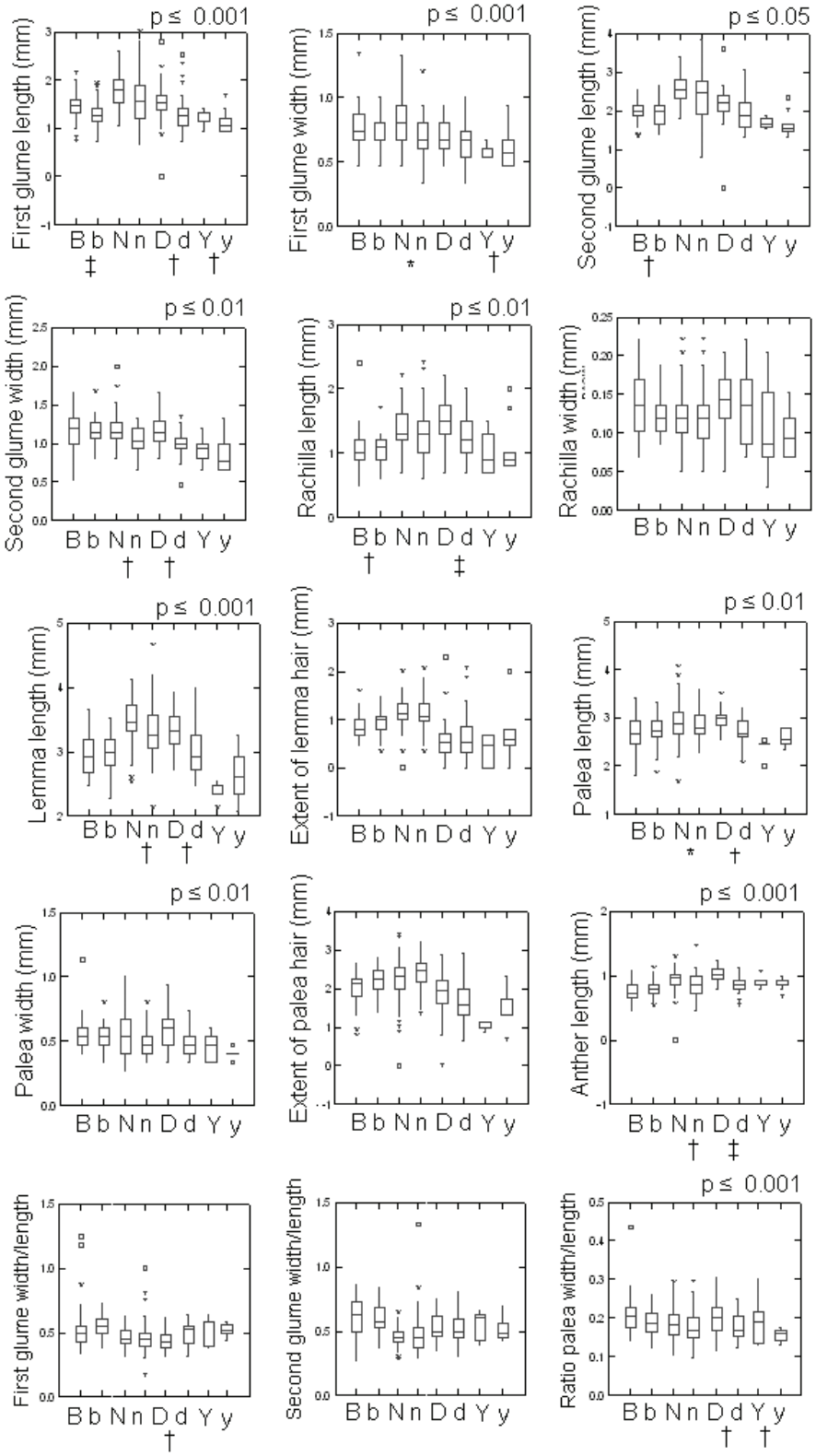
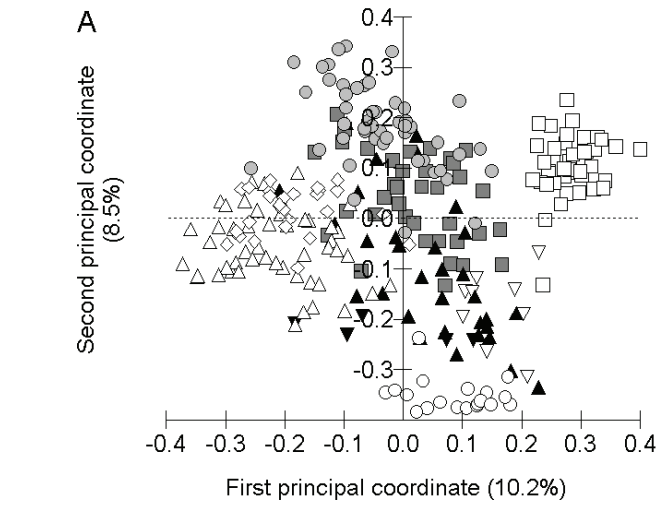
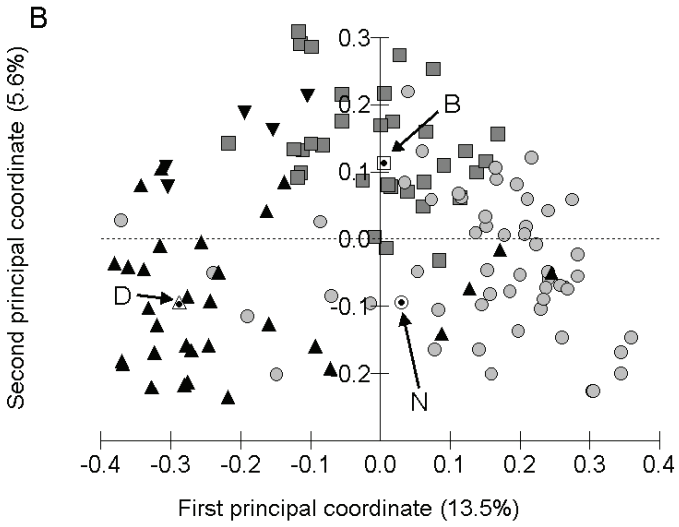

First glume length (mm)
p ≤ 0.001
First glume width (mm)
p ≤ 0.001
Second glume length (mm)
p ≤ 0.05
Second glume width (mm)
p ≤ 0.01
Rachilla length (mm)
p ≤ 0.01
Rachilla width (mm)
Lemma length (mm)
p ≤ 0.001
Extent of lemma hair (mm)
Palea length (mm)
p ≤ 0.01
Palea width (mm)
p ≤ 0.01
Extent of palea hair (mm)
Anther length (mm)
p ≤ 0.001
First glume width/length
Second glume width/length
Ratio palea width/length
p ≤ 0.001
B b N n D d Y y

Figure 5.7. PCoA ordinations of morphological data for *Puccinellia* polyploid individuals. △ = *P. arctica*; ◇ = *P. banksiensis;* ○ = *P. tenella*; ▽ = *P. alaskana*; □ = *P. vahliana* and *P. wrightii*. ■ = B; ● = N; ▲ = D; ▼ = *P.* sp.2 (octoploid); ⊠ = B6; ✣ = D6; ◭ = D7. A. Diploid and polyploid taxa, field specimens. B. Polyploid taxa only, field specimens. Arrows with B, D, and N point to the type specimens of *P. bruggemannii* (⊡), *P. andersonii* (◬), and *P. angustata* (⊙), respectively. C. Canonical discriminant analysis of field and common garden specimens with groups defined based on ploidy level, which are given as 4X, 6X, 8X.


A
Second principal coordinate (8.5%)
First principal coordinate (10.2%)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2 0.3 0.4


B
Second principal coordinate (5.6%)
First principal coordinate (13.5%)
B
D
N
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2 0.3 0.4

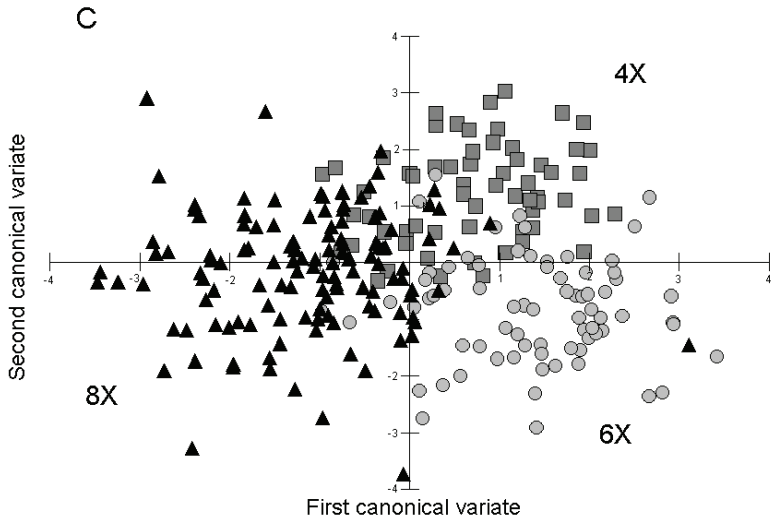

C
Second canonical variate
First canonical variate
4X
8X
6X

Figure 5.8. PCoA ordinations of AFLP and morphological data for *P. bruggemannii* and *P. angustata*. A) AFLP band presence/absence data; ploidy levels given as 4X or 6X. B) Morphology, field specimens. C) Morphology, common garden specimens. ■= *P. bruggemannii*, ○ = *P. angustata*, ☒ = B6.

Fig. 5.8

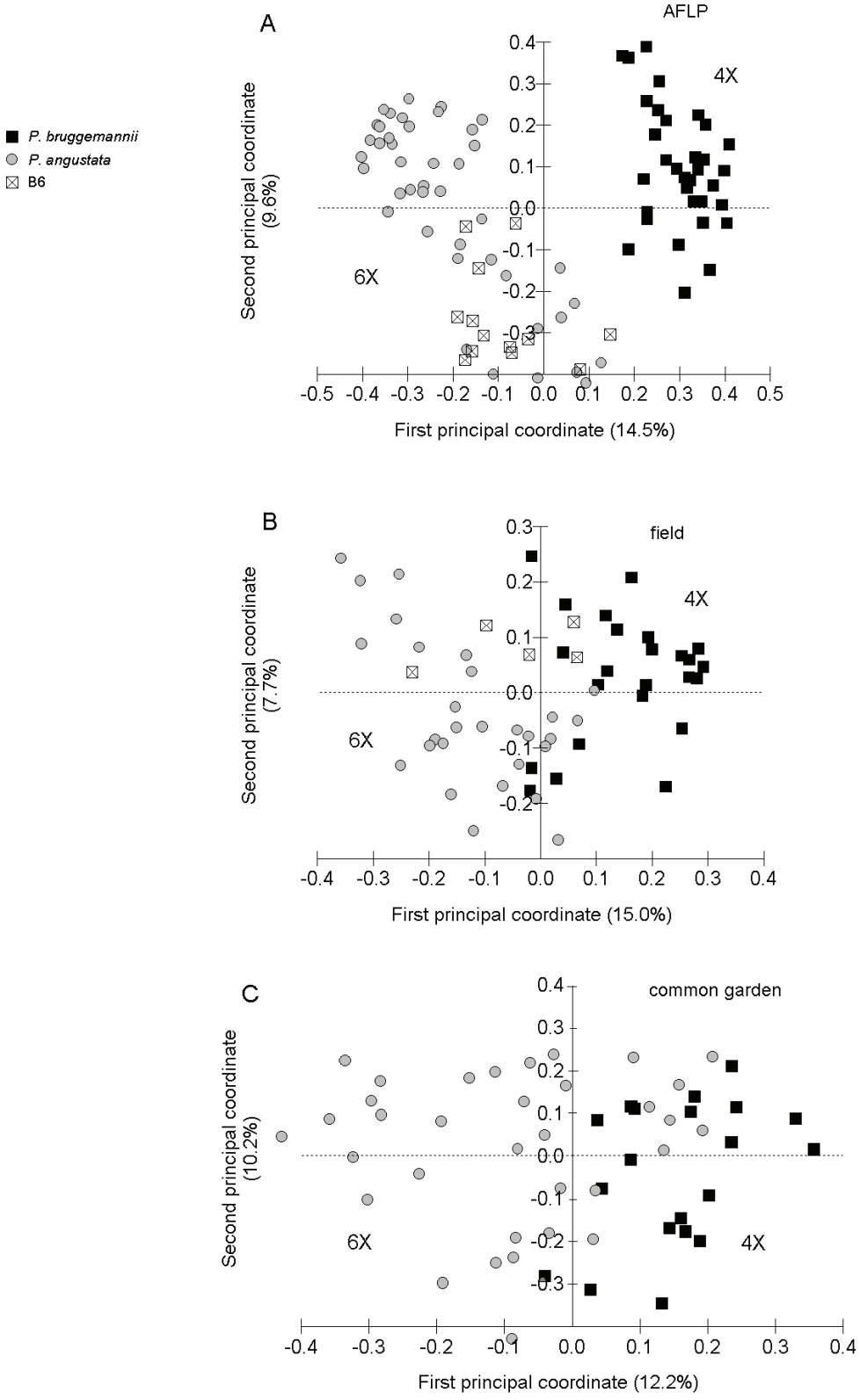

P. bruggemannii
P. angustata
B6
A
AFLP
4X
6X
Second principal coordinate (9.6%)
First principal coordinate (14.5%)
B
field
4X
6X
Second principal coordinate (7.7%)
First principal coordinate (15.0%)
C
common garden
6X
4X
Second principal coordinate (10.2%)
First principal coordinate (12.2%)

Figure 5.9. PCoA ordinations of AFLP and morphological data for *P. bruggemannii* and *P. andersonii*. A) AFLP band presence/absence data; ploidy levels given as 4X or 8X. B) Morphology, field specimens. C) Morphology, common garden specimens. □ = *P. bruggemannii*, ▲ = *P. andersonii*.

Fig. 5.9

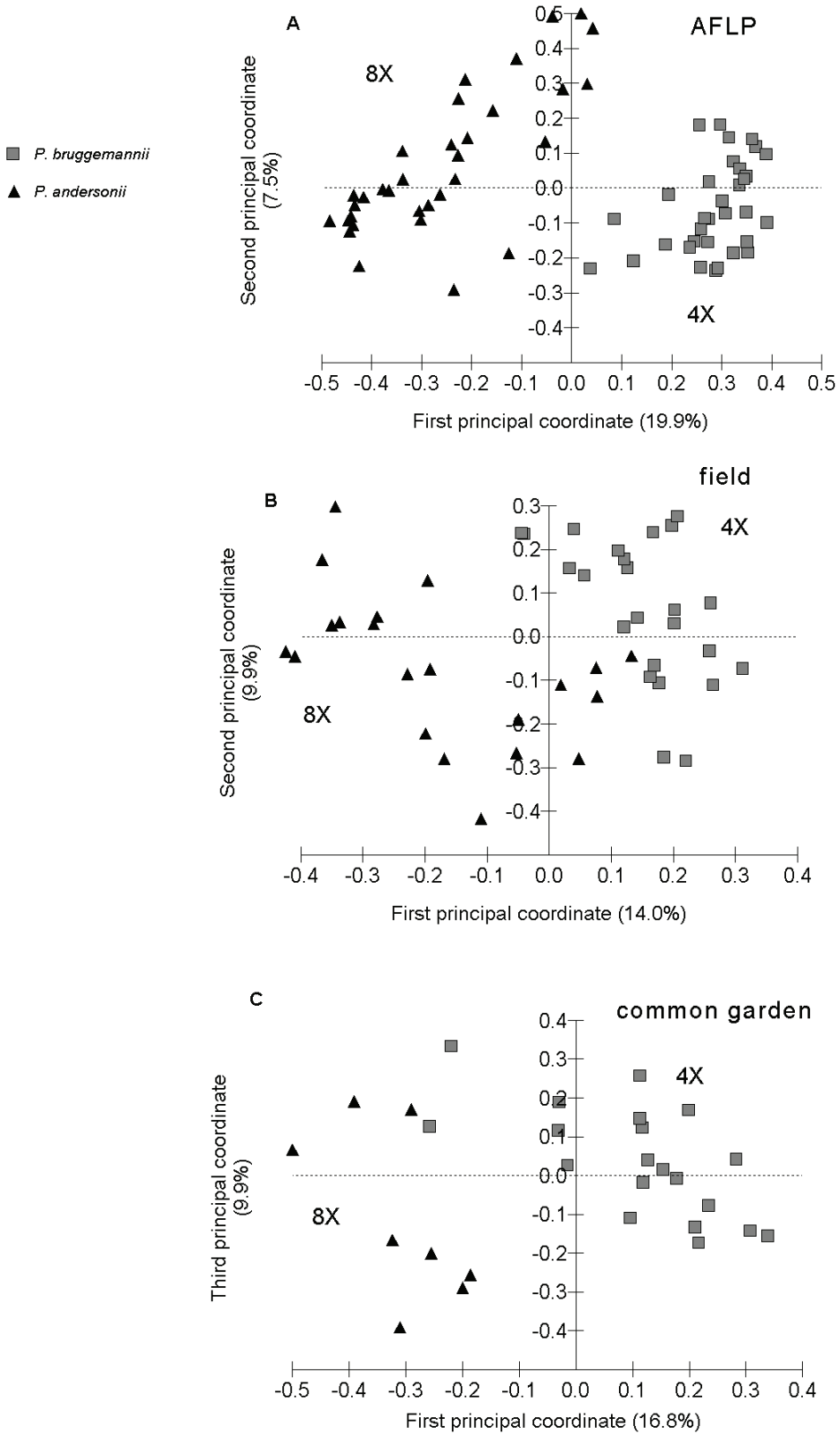
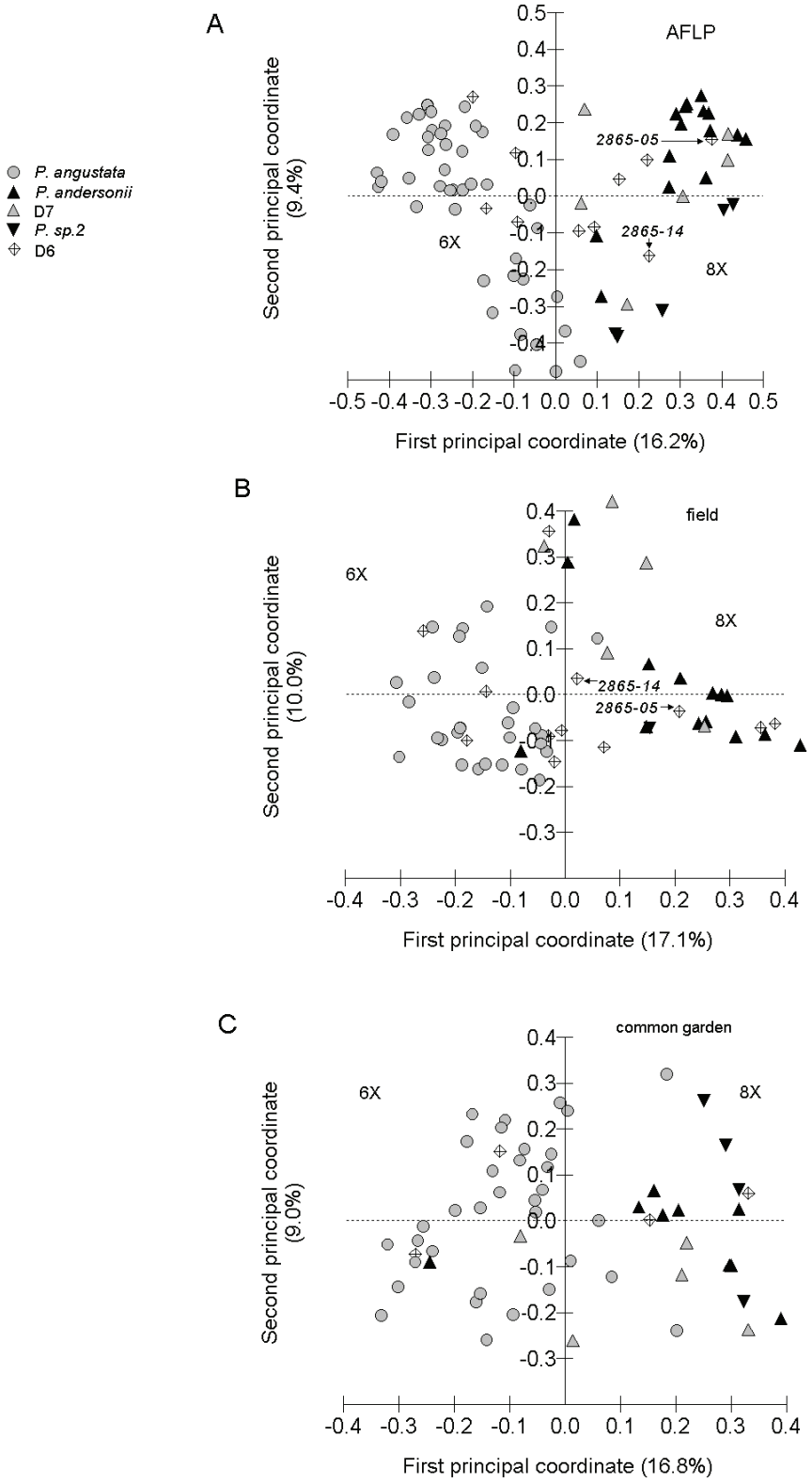

P. bruggemannii
P. andersonii
A
AFLP
8X
4X
Second principal coordinate (7.5%)
First principal coordinate (19.9%)
B
field
4X
8X
Second principal coordinate (9.9%)
First principal coordinate (14.0%)
C
common garden
4X
8X
Third principal coordinate (9.9%)
First principal coordinate (16.8%)

Figure 5.10. PCoA ordinations of AFLP and morphological data for *P. angustata* and *P. andersonii*. A) AFLP presence/absence data, ploidy levels given as 6X or 8X, except that D6 is always hexaploid. B) Morphology, field specimens. C) Morphology, common garden specimens. ○ = *P. angustata*, ▲ = *P. andersonii*, △ = D7, ▼ = *P.* sp. 2, ⊕ = D6.

Fig. 5.10


A
AFLP
P. angustata
P. andersonii
D7
P. sp.2
D6
Second principal coordinate (9.4%)
First principal coordinate (16.2%)
2865-05
2865-14
6X
8X
B
field
Second principal coordinate (10.0%)
First principal coordinate (17.1%)
C
common garden
Second principal coordinate (9.0%)
First principal coordinate (16.8%)

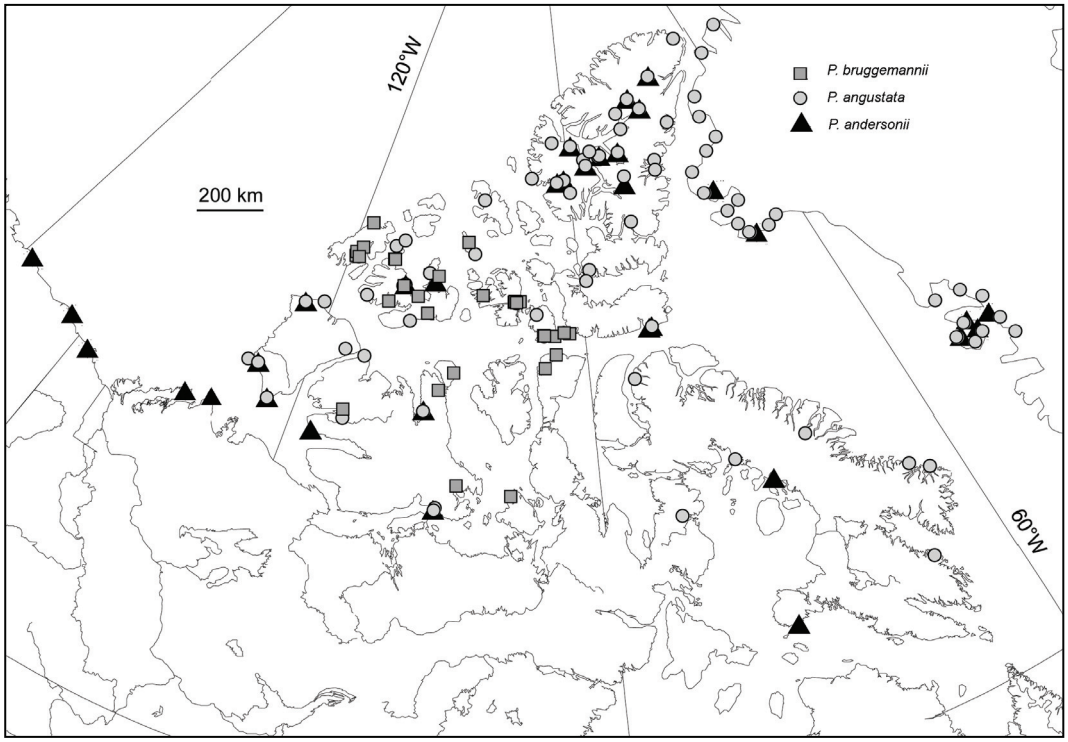


Figure 5.11. Geographic distributions in the North American Arctic of the three polyploid species of *Puccinellia* examined in this study. ■ = *P. bruggemannii*; ○ = *P. angustata*; ▲ = *P. andersonii.*

5.7 Connecting Text

In Chapters 3 to 5, I have investigated the taxonomic limits of six diploid and three polyploid species of *Puccinellia* using flow cytometry, AFLP analysis and morphology of field and common garden plants. Determination of the species limits was a prerequisite to studying the evolutionary relationships among the species and investigating the origins of the polyploids. I address the evolution and origins of three arctic polyploid species in Chapter 6.

Chapter 6 has been prepared as a manuscript to be submitted to *American Journal of Botany*. Laurie L. Consaul, Plant Science, McGill University, Ste Anne de Bellevue, QC H9X 3V9; Lynn J. Gillespie, Research Division, Canadian Museum of Nature, Ottawa, ON K2P 6P4, and Marcia J. Waterway, Plant Science, McGill University, Ste Anne de Bellevue, QC H9X 3V9. Evolution in North American Arctic *Puccinellia* (Poaceae) based on nuclear ribosomal spacer and chloroplast DNA sequences.

6. Evolution in North American Arctic *Puccinellia* (Poaceae) based on nuclear ribosomal spacer and chloroplast DNA sequences

Laurie L. Consaul, Lynn J. Gillespie, and Marcia J. Waterway

6.1 Abstract

Nuclear ribosomal DNA regions ITS and ETS1f plus two cpDNA regions are used to infer the phylogeny of diploid North American *Puccinellia* and, combined with restriction site analysis, the origins and evolution of three arctic polyploid species. Results confirm the proposed taxonomic limits for the diploid and polyploid species based on recent studies using morphology, ploidy, and AFLP markers. Among the diploids, the arctic species *P. arctica* and *P. banksiensis* formed one lineage, the arctic species *P. tenella, P. alaskana*, *P. vahliana*, and *P. wrightii* belonged to a second lineage, and two temperate species*, P. lemmonii* and *P. parishii*, comprised a third distinct lineage. *Puccinellia bruggemannii* (tetraploid), *P. angustata* (hexaploid), and *P. andersonii* (primarily octoploid) are shown to have similar chloroplast histories, belonging to the same major clade as *P. arctica* and *P. banksiensis.* The nrDNA sequences of *P. bruggemannii* are the only ones to match those of the diploids *P. vahliana* and *P. wrightii*. *Puccinellia angustata* and *P. andersonii* have a primary sequence that is most similar to the hybrid species *P. phryganodes*, and a secondary sequence similar to that of *P. arctica*, showing hybrid origins with ITS sequences that have not undergone concerted evolution.

6.2 Introduction

The origin of arctic polyploid species in relation to their putative diploid progenitors has received much attention in European studies. Abbott and Brochmann (2003) and Brochmann et al. (2004) summarized reports on species in

seven genera (*Festuca*, *Poa, Cerastium, Silene, Saxifraga, Potentilla,* and *Vaccinium*) that have shown insights into their evolution, including evidence for recurrent formation of polyploids. Studies on North American arctic polyploid plant species are just beginning. Brysting et al. (2004) showed that higher polyploid races of the polyploid genus *Dupontia fisheri* R.Br. form recurrently at the circumarctic scale. Popp et al. (2005) and Popp and Oxelman (2007), although they did not include any Canadian arctic specimens, elucidated parental genomes and the mode of speciation for three circumpolar *Silene* species that occur in arctic North America. Cold treatment of flowering plants can result in increased production of unreduced gametes (Ramsey and Schemske 1998), which are involved in most polyploidization events (deWet 1980). Therefore, it may be hypothesized that polyploid events are more likely in colder regions, and that polyploid events in the Arctic happen frequently, as discussed by Soltis and Soltis (1999). Here we investigate the origins of an arctic polyploid complex in North America, where almost all of the Arctic was covered by ice sheets during the last glaciation, to determine whether these North American arctic species originated in the Arctic or farther south.

Puccinellia is a genus of pioneering grasses that colonizes coastal and harsh alkaline areas and is one of the largest genera of arctic grasses. About half of the 13-19 recognized arctic species are polyploid (Elven et al. 2003), but their speciation mechanism is unknown. The arctic polyploid species of *Puccinellia* may have formed, or are recurrently forming, from the arctic diploid or lower polyploid species, or from non-arctic species. They may be autopolyploid or allopolyploid. Our recent phenetic studies of diploid species showed that there are five or six diploid species in the North American Arctic and two diploid taxa in temperate North America (Consaul et al. Chapter 5). Given the geographical distribution of the diploid and polyploid *Puccinellia* species (Chapter 5), the options for origin and parentage of the polyploid species are numerous. Our study of three polyploid species, *P. bruggemannii* T.J. Sørensen, *P. angustata* (R.Br.) E.L. Rand & Redfield, and *P. andersonii* Swallen, using amplified fragment length polymorphism (AFLP) evidence suggested that they have distinct ploidy

levels and AFLP patterns and demonstrated that *P. arctica* (Hooker) Fernald & Weath. and *P. vahliana* (Liebm.) Scribn. & Merr. are likely parents for all three, and *P. phryganodes* (Trin.) Scribn. & Merr. is an additional probable parent of *P. angustata* and *P. andersonii* (Chapter 5). The morphological characteristics overlap among the species, but the most problematic specimens to identify have an intermediate or hybrid AFLP pattern.

Phylogenetic work on *Puccinellia* to date has been limited to chloroplast DNA (cpDNA) restriction-site analysis. Choo et al. (1994) examined 14 temperate taxa of the genus (including six isozyme "species" of *P. nuttalliana* (Schult.) Hitchc. and the two temperate diploid species, *P. lemmonii* (Vasey) Scribn. and *P. parishii* Hitchc.), in their cpDNA restriction-site study. They used five restriction endonucleases and presented a cladistic analysis in which all 21 accessions of the 16 taxa formed a monophyletic group. There was very little structure within this group. A polytomy consisted of nine polyploid individuals and two clades. One clade included *P. lemmonii* and *P. parishii* and the second clade comprised ten polyploid accessions, each clade supported by one restriction-site mutation. In their studies of restriction-site variation in arctic *Poa*, Gillespie et al. (1997), Gillespie and Boles (2001), and Gillespie and Soreng (2005) also included 8 species of *Puccinellia* for comparison. In the most complete study, Gillespie and Soreng (2005) found only one restriction-site difference (using 44 chloroplast region-restriction enzyme combinations) for *P. vahliana* and two for *P. phryganodes* from six other species of *Puccinellia* in the Canadian arctic (*P. andersonii*, *P. angustata*, *P. nuttalliana*, *P. arctica*, *P. bruggemannii*, and *P. poacea* T.J. Sørensen), among which there were no differences. They showed that *Phippsia algida* (Sol.) R.Br. was an appropriate outgroup taxon to *Puccinellia*.

The subgeneric classification in *Puccinellia* is incomplete. Tzvelev (1964, p. 237) stated: "*Puccinellia* … can scarcely be divided into infrageneric subdivisions of the rank of subgenera or sections." Tzvelev (1976) later divided the genus into five sections, three of which included North American species as well as Russian species. One North American species *P. phryganodes* is classified in section *Paralochloa* (Krecz.) Bor, while two North American species, *P.*

vahliana and *P. wrightii* (Scribn. & Merr.) Tzvelev, belong to section *Pseudocolpodium* Tzvelev. Section *Puccinellia* includes the rest of the North American Arctic species except *P. arctica*, which is endemic to North America and has not been placed into any of Tzvelev's sections. An investigation of the phylogenetic relationships among the species will test this subgeneric classification and suggest appropriate placement for *P. arctica*.

Our goals are to compare DNA sequences of arctic *Puccinellia* to test: (1) whether groups delimited by sequence data are congruent with groups obtained through studies of morphology, ploidy, and AFLP patterns (Chapters 3 and 5); (2) whether there are consistent nucleotide mutations that are diagnostic for the diploid taxa and can be used to infer both the phylogeny of the diploid species and the parentage and geographical origin of the polyploid taxa; (3) whether there has been recurrent polyploid formation within any of the polyploid species; and (4) whether the current sectional treatment reflects the evolutionary relationships in *Puccinellia.*

6.3 Materials and Methods

6.3.1 Specimen sampling

Puccinellia samples were collected from across the North American Arctic as described in Consaul et al. (Chapter 5). The samples analyzed in this paper are a subset of those analyzed in Consaul et al. (Chapter 5). Table 6.1 gives the voucher information, the number of individuals sequenced, and the number of individuals with restriction enzyme analyses for each population.

Our strategy was to study most intensively the putative polyploid series consisting of the tetraploid *P. bruggemannii*, the hexaploid *P. angustata*, and the octoploid *P. andersonii*. We included five populations of *P. phryganodes* from across its range as a reported triploid that may be involved in the evolution of the polyploids if fertile gametes are occasionally produced. We coded one octoploid population originally collected as *P. andersonii* as *P.* sp.2 because it differed in morphology and molecular characters from other octoploid *P. andersonii* samples (Chapter 5). We included three populations of *P. pumila* (octoploid) because we

suspected *P.* sp.2 may be related to this taxon and sequenced two specimens of *P. hauptiana* (Trinius ex V. I. Kreczetowicz) W. E. Hughes because this tetraploid taxon is very similar in appearance to the diploid *P. banksiensis* and we sought evidence for autopolyploidy.

6.3.2 Primer design

Primers for the 5' end of the ETS1 region for the Poaceae have been developed for tribe Paniceae (Duvall et al. 2003), and tribe Triticeae (Sallares and Brown 2004), but these primers did not amplify *Puccinellia* DNA (tribe Poeae), so we designed new primers for this group. To do so, we amplified a test group of specimens coded IGS in the Appendix (*P. alaskana* Scribn. & Merr., *P. arctica*, *P. banksiensis* Consaul ined., *P. tenella* (Lange) Holmb. ex Porsild, *P. vahliana*, and *Phippsia algida*) for the entire intergenic spacer region (IGS) of nuclear ribosomal DNA (nrDNA), using the primers in the coding regions 26S and 18S (26S-F and 18S-R, Starr et al. 2003). Regions of IGS that were conserved across all sampled *Phippsia* and *Puccinellia* species were used to design a primer (ETS1fP) for part of the ETS1 region (ETS1f) at approximately 817 bp from the 18S-R primer. This ETS1fP was then used with the 18S-R primer and PCR conditions 3 min at 94°C, 25X [1 min at 94°C, 1 min at 54°C, 1 min at 72°C], 7 min at 72°C, for amplification of the ETS1f region.

6.3.3 DNA sequence data

DNA was extracted as described in Consaul et al. (Chapter 5). We analyzed a set of 69 specimens of *Puccinellia,* plus 3 outgroup specimens (two of *Phippsia algida*, one of *Catabrosa aquatica* (L.) P. Beauv.) for two nrDNA regions. We sequenced ten *Puccinellia* specimens (= ten putative taxa) plus *Phippsia algida* as the outgroup for six cpDNA regions. We analyzed the same 69 *Puccinellia* specimens as for the nrDNA analysis, plus 2 outgroup specimens (lacking *Catabrosa*, which has not yet amplified successfully for these regions), for the two most variable of these non-coding cpDNA regions (Table 6.2).

Findings by Choo et al. (1994) and Gillespie and Soreng (2005) suggested that *Phippsia* and *Catabrosa* are appropriate outgroups.

nrDNA—The entire internal transcribed spacer region (ITS1/5.8S/ITS2) was amplified using forward primer N18L18 (Wen and Zimmer 1996) and reverse primer ITS4 (White et al. 1990). We included DMSO in the reaction mix because Buckler et al. (1997) and Álvarez and Wendel (2003) warned against using only ITS sequences in phylogenetic studies because paralogous copies or non-functional pseudogenes may be preferentially amplified if reagents like dimethylsulfoxide (DMSO) or betaine are not included. We amplified a fragment of the ETS1f region, using primers ETS1fP (design described above) and 18S-R (Starr et al. 2003).

cpDNA—We assessed six chloroplast regions by amplifying and sequencing eleven plants, representing the taxa *P. alaskana*, *P. arctica*, *P. banksiensis*, *P. andersonii*, *P. angustata*, *P. bruggemannii*, *P. lemmonii*, *P. parishii*, *P. tenella,* and *P. vahliana*, and the outgroup taxon *Phippsia algida*. We used primers from Taberlet et al. (1991) for *trnL-F* (including *trnL* intron) and *trnT-L*; for *trnH-psbA* we used the primer from Tate and Simpson (2003) for *trnH,* and from Sang et al. (1997) for *psbA*; for *trnD-T* we used the primer of Demesure et al. (1995) for *trnD* and the primer from Sun (2002) for *trnT*; we used primers from Shaw et al. (2005), modified from Ohsako and Ohnishi (2000), for *rpoB-trnC*, and primers from Small et al. (1998) for *rpl16*. After comparing the number of variable and potentially informative sites for each of these regions, we selected *rpoB-trnC* and *rpl16* to sequence for the whole 69-specimen data set.

Amplified PCR products were sequenced in one of two ways: 1) Purification was done with a QIAGEN QIAquick PCR Purification Kit (Qiagen), cycle sequencing performed with the amplification primers, products resuspended in HiDi Formamide (Applied Biosystems, Warrington, UK), and run on an ABI 310 sequencer (Applied Biosystems). For some samples internal primers ITS2 and ITS3 were also used for internal sequencing. 2) Purification, cycle sequencing

using the amplification primers, and sequencing were done by GenomeQuebec (Montreal, QC) on an ABI 3730XL.

6.3.4 Phylogenetic analyses

Sequences were edited and aligned with Vector NTI 6.0 or Sequencher (ver. 4.5) and ClustalX (ver. 1.81), with visual examination and manual editing when necessary. Indels were coded as additional characters using the simple indel coding method of Simmons and Ochoterena (2000).

Phylogenetic analyses of sequence data were performed using PAUP* 4.0b10 (Swofford 2003). The level of incongruence among the data sets was measured with the Incongruence Length Difference (ILD) test (Farris et al. 1994) implemented as the partition homogeneity test in PAUP*, with 100 replicates. Maximum parsimony analysis (MP) was performed with the heuristic search option, 100 random addition-sequence replicates, and tree bisection-reconnection (TBR) branch swapping. All minimal trees were saved during the branch-and-bound search and branches were collapsed (creating polytomies) if maximum branch length was zero. Molecular evolutionary models for maximum likelihood analyses (ML) were determined by the Akaike information criterion in Modeltest (Posada and Crandall 1998), version 3.7. The model TIM + Γ (transitional model with site-specific rate homogeneity estimated by the gamma distribution, Posada 2005) was used for the diploid data set combining both nrDNA and cpDNA sequences. Reliability of clades was assessed in PAUP* using nonparametric bootstrapping with MP and ML (Felsenstein 1985), with 100 bootstrap replicates and 10 random-addition sequences per bootstrap replicate. We analyzed the dataset using Bayesian inference in MrBayes (Version 3.1.2; Huelsenbeck and Ronquist 2001) with the model GTR + Γ (general time reversible model with site-specific rate heterogeneity estimated by the gamma distribution, Yang 1993) for ITS, HKY + G (Hasegawa et al. 1985) for ETS1f, and F81 + I (Felsenstein 1981) for both *rpoB-trnC* and *rpl16*, and the priors set to the defaults in MrBayes. We ran the Markov-chain Monte Carlo simulation for 50,000 to 150,000 generations (until the average standard deviation of split frequencies reached 0.01), sampling

every 100 generations. Consensus trees and posterior probabilities for the branches were calculated after at a minimum of 2500 trees were discarded and a check was made to ensure that the log probability of observing the data was at a plateau.

We constructed a phylogenetic network using SplitsTree (version 4.3, Huson and Bryant 2006). The Jukes-Cantor distance (Jukes and Cantor 1969) was used, because it not only computes the proportion of nucleotide changes for which the samples differ, but it also allows for multiple changes. The network was constructed from the distance matrix by the Neighbor-Net agglomerative method (Bryant and Moulton 2004), which recovers reticulations. Whenever there is conflicting signal, Neighbor-Net produces a box-like section in the graph. To compare the AFLP data (Chapter 5) with the sequences, we constructed a SplitsTree network of the entire AFLP data and a second network of only the 69 *Puccinellia* specimens sequenced in this study, using the uncorrected P-distance (Nei 1987), which computes the proportion of positions at which the samples differ.

6.3.5 Restriction digests

For each of the mutations diagnostic for one of the diploid arctic taxa we found a restriction enzyme that cut once or twice in the entire ITS sequence. We were thus able to test for multiple copies within an individual of ITS sequences originating from different parents in a single direct amplification by subjecting the amplification products to restrictions with each of these enzymes and visualizing the fragments on a 2% agarose gel. These enzymes, *AluI*, *BanII*, *BbvI*, and *Sau96I*, and the locations of the mutations at which they cut are indicated in Fig. 1C. The enzyme *DpnII* was chosen to cut at the mutation that was present only in the polyploids and some individuals of *P. phryganodes*, but not the diploid species (Fig 3A). The ITS amplification products were digested using the five restriction endonuclease enzymes (New England Biolabs, Ipswich, Massachusetts) following the manufacturer's specifications. We were not able to find an enzyme that would cut at the mutations of *P. lemmonii* and *P. parishii*, so

our interpretations of relationships with these taxa are based on sequence data. Four to 10 individuals were sampled per diploid species and 25 to 58 specimens were sampled per polyploid species. The number of individuals sampled per population is indicated by RE = in Table 6.1, with the numbers listed for the 206 individuals in Thesis Appendix H.

6.4 Results

6.4.1 Primer design

The amplifications of the entire IGS region gave single bands at approximately 3000 bp for almost all of the specimens. The bands of *P. tenella* and *P. banksiensis* were longer than those of the other species and also different in length from each other (Figure in Thesis Appendix). From the aligned sequences of the test samples for the reverse strand (sequenced by 18S-R), conserved regions were selected to amplify the area approximately 817 base pairs upstream from the start of the 18S-R fragment (ETS1f), comprising the first 817 base pairs of the ETS1 region. We designed the primer ETS1fP (5'-TTCCAGTCGGACGGATTC-3'). We used the primers ETS1fP and 18S-R for the ETS1f amplifications. We also found conserved regions that would amplify in ETS2. These conserved motifs were repeated several times in the IGS fragment, however, so we did not use primers to amplify ETS2 regions for this study.

6.4.2 Tree congruence

The ILD tests between ITS and ETS1f and between *rpoB-trnC* and *rpl16* for the diploid species had a significance value of p = 1.00, meaning the data sets are congruent. The test among all four data sets gave a significance value of p = 0.99, still non-significant and indicating strong congruence. Therefore, we combined the ITS and ETS data to give a nrDNA tree, combined *rpoB-trnC* and *rpl16* to give a cpDNA tree, and finally combined all four data sets to give a total combined tree which had higher bootstrap values for two of the clades (Fig. 1). For the polyploid species the chloroplast and nuclear regions were incongruent, for the most part because *P. bruggemannii* shifts from one clade in the nuclear

trees to a different clade in the chloroplast trees. Therefore, for the diploid + polyploid data we combined ITS + ETS1f and combined *rpoB-trnC* + *rpl16* but analyzed the nrDNA and cpDNA regions separately.

6.4.3 Diploid taxa

We coded the diploid taxon groups with a single letter in order to refer to them more easily and to be able to refer to the diploid genome composition of the polyploid taxa in later sections. The codes are *P. alaskana* = A, *P. arctica* = R, *P. banksiensis* = U (for "unknown" as it was discovered as a new entity in Consaul et al. Chapter 5), *P. lemmonii* = M, *P. parishii* = S, *P. tenella* = T, *P. vahliana* and *P. wrightii* = V (codes are shown in Fig. 1). Since *P. vahliana* and *P. wrightii* have the same genotype we refer to them together as *P. vahliana* s.l.

nrDNA — Sequence statistics are given in Table 6.2. Sequencing of the ITS region gave sequences of 595 base pairs that aligned without problem. Maximum parsimony (MP) analysis of the ITS region for the diploid individuals resulted in a single most parsimonious tree, with five clades (not shown, but clades are those in the ITS+ETS tree in Fig. 1A). The ITS mutations are coded in Fig. 1A with a capital I under the bars. Sequencing of the ETS1f region gave 818 base pairs that aligned without problem. The same groups were resolved as in the ITS analysis, but the number of variable and potentially informative sites was higher (Table 6.2). ETS mutations are those not coded with I in Fig. 1A.

The nrDNA MP consensus tree of the combined ITS and ETS1f sequences (Fig. 1A) is based on six most parsimonious trees (consistency index (CI) = 0.972). All of the groups formed strongly supported clades except Group R, for which one specimen (*P. arctica 2808-14*) was not resolved within either Group R or Group U but did form part of the strongly supported arctic clade combining those groups. The other major clade contained both arctic and temperate species.

cpDNA— Sequences of the chloroplast regions were 911-915 bp for the *trnL-F* (includes *trnL* intron plus *trnL-F* spacer) region, 630 bp for the *trnH- psbA*

region, 817 bp for the *trnT-L* region, 1110 for the *trnD-T* region, 794-836 bp for *rpl16,* and 1153-1197 bp for the *rpoB-trnC* region. The *trnL-F* region had no variable sites, *trnH-psbA* had 1 variable and potentially informative site, *trnT-L* had 4, *trnD-T* had 6, *rpl16* had 7 variable sites that were all potentially informative, and *rpoB-trnC* had 10 variable sites, 8 being potentially informative. Figure 6.2 shows where each of these mutations is located on a maximum parsimony consensus tree using one sample of each taxon and all six chloroplast region sequences. By considering the number of potentially informative sites and the distribution of these mutations for distinguishing the diploid species we chose *rpl16* and *rpoB-trnC* to analyze the larger data set of 69 diploid and polyploid *Puccinellia* species. The detailed sequence statistics for *rpl16* and *rpoB-trnC* are given in Table 6.2.

The cpDNA MP analyses of *rpoB-trnC* and *rpl16* sequences for all 35 diploid samples resulted in 24 most parsimonious trees and the strict consensus tree shown in Fig. 1B (CI = 0.984). The arctic species (Groups R, U, T, V, and A) formed a clade separate from the southern species (Groups M and S) with moderate to low support (Fig. 1B). Groups R, U, T, and A were each resolved with bootstrap values over 77%. The V genome did not form a clade; although a V-genome clade was present in the Bayesian consensus tree, the posterior probability was only 67%.

Comparing nrDNA and cpDNA sequences— The nrDNA and cpDNA trees were combined into a single MP tree (Fig. 1C). The R+U clade is distinct from the T, A, V, M, and S clades. Combining nrDNA and cpDNA data increased support for the M+S clade (the southern USA species *P. lemmonii* and *P. parishii*), and placed *P. arctica 2808-14* within the R clade. Although the ILD test indicated that the nrDNA and cpDNA data sets were congruent in this analysis of diploids, *P. lemmonii* and *P. parishii* are supported as sister to the T+A+V clade in the nrDNA tree, whereas in the cpDNA data, these southern species are sister to all of the arctic species as a group. The maximum likelihood (ML) tree (Fig.

1D) resulted in the same clades as the MP tree, with high bootstrap values for all of the major clades.

6.4.4 Polyploid taxa

nrDNA— Fig. 6.3A shows the consensus tree of 97951 most parsimonious trees (CI = 0.180) for the 46 unique *Puccinellia* sequences in the dataset, including both diploid and polyploid taxa. Direct comparison of the sequences revealed that *P. andersonii* is distinguished from *P. angustata* by one mutation in ETS1f that it also shares with *P. arctica* and that *P. bruggemannii* has the ITS sequence of *P. vahliana*. *Puccinellia angustata* and *P. andersonii* had at least two different ITS sequences per plant. The primary sequences (angustata and andersonii) were similar to each other but not closely related to any diploid species. The secondary sequence of *P. angustata* (angustata2) was similar to *P. arctica* and *P. banksiensis*, including a four-nucleotide deletion diagnostic of the R+U clade, while the secondary sequence of *P. andersonii* (andersonii2) also had this diagnostic deletion but did not group in the R+U clade (Fig. 6.3A). All five of the *P. phryganodes* plants, from the northern, south-western, and south-eastern Arctic, were consistent in having six single nucleotide sites that had two (or three in one case) subequally strong bases in the electropherogram of the same ITS sequence (see Thesis Appendix F), indicating more than one sequence in the amplification product. These sites were coded as N in the analyses.Table 6.3 shows the alignments these bases had with the other taxa in this study.

The phylogenetic network based on nrDNA data (Fig. 6.4) shows the diploid groups A, M, R, S, U, T, and V are all quite distinct. *Puccinellia bruggemannii* groups with *P. vahliana* (V). The group comprised of *P. angustata* and *P. andersonii* is distinct. The putative hybrid *P. phryganodes* specimens have a variety of different sequences but are essentially located between the diploid species + *P. bruggemannii* and the *P. angustata* / *P. andersonii* group.

cpDNA sequencing— Maximum parsimony analysis of the polyploids and diploids together resulted in 285922 most parsimonious trees for which the

consensus is shown in Fig. 6.3B (CI = 0.215). The consensus tree showed *P. bruggemannii, P. angustata,* and *P. andersonii* are all in the same clade with diploid species having the R and U genomes. This same clade also includes the other two polyploid species we sampled for comparison, *P. pumila* and *P.*sp 2 (Fig. 3B). Individuals of the diploids *P. tenella*, *P. alaskana*, and *P. lemmonii* each form species clades, but these clades are part of a large basal polytomy ("comb") with individuals of the other sampled diploids (*P. vahliana*, *P. parishii*) and triploid *P. phryganodes*. Constructing the MP cpDNA tree using all six chloroplast regions for one representative of each species did not increase the resolution of the main clades (Fig. 6.2).

The phylogenetic network resulted in a two-group structure, with the A, M, S, T, and V diploids and *P. phryganodes* at one end (Fig. 6.5). Both R and U diploid groups and the polyploids are at the other end. *Puccinellia bruggemannii* and *P. angustata* are separate; individual *and2878-16* of the intermediate group D6 (Chapter 5) is at the base of these two groups. *Puccinellia andersonii*, *P. pumila*, and *P.* sp.2 are in a separate branch, with *P. banksiensis*. *Puccinellia arctica* is on a separate branch.

Restriction enzyme analyses **—** Table 6.4 summarizes the presence/absence data from the restriction digest analyses. For ITS amplifications of the diploids, it confirms that *BanII* cuts only *P. arctica* and *P. banksiensis* to distinguish the R and U genomes from the others. *AluI* cuts only *P. banksiensis* to distinguish it (U-genome) from all other species; *BbvI* cuts only *P. tenella* to distinguish it from all other species as the T-genome. *Sau96I* cuts the ITS region in all diploid species except *P. alaskana* once, with the cut referred to as *Sau96I* "R,U,T,&V" in Table 6.4. *Sau96I* also cuts *P. alaskana* ITS once at a different site, to distinguish it from the other species (Fig. 1C); it cuts the ITS region of *P. vahliana* s.l. at both sites, so that it has a double cut indicating the V-genome. *DpnII* does not cut the ITS region in any of the diploid species, but does so in some of the polyploids, including *P. phryganodes*, distinguishing the Z-genome.

In the chloroplast genome (*rpoB-trnC* region), *Sau96I* cuts all but *P. vahliana*, distinguishing it from the rest of the diploid species (Table 6.4).

For ITS in the polyploids, *P. bruggemannii* has only the V-genome pattern. *Puccinellia angustata* and *P. andersonii* have the R-genome and the Z-genome, with most of the fragments in the ITS amplification product from each specimen cutting for the Z-genome. *Puccinellia phryganodes* does not have the R-genome pattern. In our small sample, the triploid *P. phryganodes* individuals do not have the Z-genome and the tetraploid *P. phryganodes* individuals do. *Puccinellia pumila* and most of the specimens (75%) in the population *P.* sp 2 have only the R-genome pattern. The remainder (25%) of the specimens in population *P.* sp. cut with the same pattern as *P. andersonii* (R-genome and Z-genome). For the chloroplast patterns from restriction of the *rpoB-trnC* region, none of the polyploid species have the uncut pattern of the V-genome.

AFLP data— A phylogenetic network constructed for the entire AFLP data set was too complex to interpret. The network constructed for the 69-specimen *Puccinellia* data set of sequenced specimens shows all of the taxa on separate arms of the network (Fig. 6.6). This network has characteristics of both the cpDNA and nrDNA networks. It resembles that of the cpDNA network in having two distinct ends, but a shorter branch connecting them. It resembles the nrDNA network in that *P. bruggemannii* and *P. vahliana* are on the same branch.

6.5 Discussion

6.5.1 Delimited groups

Puccinellia species defined on patterns of morphology, ploidy, and AFLP data in Chapters 3 and 5 are monophyletic in the DNA sequence-based analyses presented in this chapter. The diploid genomes R, U, T, A, V, M, and S correspond to five arctic (*P. arctica*, *P. banksiensis*, *P. tenella*, *P. alaskana*, and *P. vahliana*) and two temperate (*P. lemmonii, P. parishii*) *Puccinellia* species, supported in this paper by nrDNA and cpDNA mutations (Fig. 6.1). As in Consaul et al. (Chapter 3), which focuses on AFLP data, *P. wrightii* is

indistinguishable from *P. vahliana* based on sequence data, but in that chapter we argued that more specimens would need to be analyzed before synonymizing these two species formally. The three higher polyploid species that we addressed in detail, *P. bruggemannii*, *P. angustata*, and *P. anderson*ii, showed variable degrees of support, but were most clearly distinguished in phylogenetic networks. Their origins are not simple, however, and the genetic patterns of each of these polyploids will be discussed below.

Several different sources of DNA data were helpful here because the resolution attained by sequencing the different regions of DNA was variable. The number of mutations was low in ITS sequences, but these mutations distinguished the diploid species reliably, thus being appropriate for doing restriction-site tests on the populations. We found increased resolution in the ETS1f region over ITS. This has also been found, for example, by Starr et al. (2003) and Beardsley and Olmstead (2002), but Kelch and Baldwin (2003) found the level of variation was similar between ITS and ETS. Sequences of the chloroplast regions gave low resolution within *Puccinellia*.

6.5.2 Phylogenetic relationships

Three distinct evolutionary lineages appear to have arisen in the diploid species: one lineage formed by the arctic species *P. arctica* and *P. banksiensis*, the second comprising the arctic species *P. alaskana*, *P. tenella*, *P. vahliana*, and *P. wrightii*, and the third with the southern USA species *P. lemmonii* and *P. parishii*. *Puccinellia lemmonii* and *P. arctica* are very difficult to tell apart based on morphological features and the key of Davis and Consaul (2007) is heavily based on geography for this pair. Their separation in the diploid trees (Fig. 6.1) and the polyploid/diploid MP trees (Fig. 6.3), indicating that these two taxa may have been separate since at or before the speciation of the polyploids, justifies their species status. The R and U groups (*P. arctica* and *P. banksiensis*), which included specimens from both the southwestern and northern Arctic, were found to be basal to all other species in the diploid nrDNA tree (Fig. 6.1). The cpDNA results show, instead, the southern species (M and S) as basal, but support is much

lower for the cpDNA MP tree. We might expect the origin of the arctic diploid species to be in the Beringian region because the largest number of diploid species is found there (Brochmann et al. 2004), but the basal relationships between arctic and temperate *Puccinellia* species remains unsolved based on the sequence data obtained here.

6.5.3 Diploid mutations to track polyploid evolution

Each diploid species had one to 12 unique nrDNA mutations and zero to four cpDNA mutations. In the cpDNA tree, *P. vahliana* s.l. was the only diploid not supported as monophyletic. The results presented here support allopolyploid status for *P. bruggemannii*, *P. angustata*, and *P. andersonii*. Table 6.5 lists the genomes found in each of *P. bruggemannii*, *P. angustata*, and *P. andersonii* based on the sequencing data and the AFLP data from Chapter 5. Table 6.6 summarizes the putative origins of these three taxa based on the evidence listed in Table 6.5.

Puccinellia bruggemannii is a tetraploid species (Chapter 5) and the evidence suggests that the male parent was *P. vahliana* and the female parent was *P. arctica* or an ancestor of *P. arctica* and *P. banksiensis*. We suggest an ancestor of *P. arctica* and *P. banksiensis*, because *P. bruggemannii* has sequences that are sister to, but not exactly identical to, the R and U genomes in cpDNA (Fig. 6.3B). However, the AFLP patterns (Chapter 5) indicated the contemporary U-genome was not a likely parent, by showing that *P. bruggemannii* lacked one of the diagnostic bands of *P. banksiensis*. The phylogenetic networks also supports this; when based on cpDNA data, *P. bruggemannii* specimens group with *P. angustata* on the same main "arm" as both *P. arctica* and *P. banksiensis*. To account for the male parent, *P. bruggemannii* has a sequence that is identical to *P. vahliana* (the V-genome) in the nrDNA (Fig. 3A), with neither secondary sequences visible in the electropherograms nor multiple cutting patterns in the restriction digest gels. This nrDNA pattern of *P. vahliana*, which appears to be maintained unchanged in *P. bruggemannii*, suggests that concerted evolution has homogenized the ITS DNA to such an extent that sequences of only the male parent are now present.

Although multiple branches in the cpDNA network could indicate *P. bruggemannii* has formed more than once, the chances that it is recurrently forming at the present time are extremely low. The phylogenetic network shows specimens of *P. vahliana* and *P. bruggemannii* at the base of the circled branch "V" (Fig. 6.4), implying that speciation of *P. bruggemannii* occurred before subsequent mutations in each of the two species. *Puccinellia bruggemannii* also has chloroplast sequences that form a sister clade to *P. angustata*, which forms a polytomy with *P. arctica* and *P. banksiensis*, but neither *P. arctica* nor *P. banksiensis* is found sympatrically with *P. bruggemannii* in its current known distribution (Chapter 5).

Puccinellia angustata is hexaploid (Chapter 5) and evidence here shows that it is an allopolyploid species. *Puccinellia angustata* has two different ITS sequences. The primary ITS sequence (the Z-genome), was not found in any of the diploid species, and was similar to sequences of all *P. andersonii* and 83% of *P. phryganodes* specimens by having the diagnostic mutation detected by restriction enzyme *DpnII*. The other sequence is similar to that of *P. arctica* and *P. banksiensis,* characterized by the presence of a four nucleotide deletion found in the R+U clade; this sequence was present in low quantity when sequenced directly from a PCR amplification and in restriction digest analyses and thus we refer to it as the secondary sequence. In the MP tree based on cpDNA (Fig. 6.3), *P. angustata* is in the clade sister to *P. bruggemannii*, which forms a polytomy with *P. arctica* and *P. banksiensis*.

Puccinellia angustata has [R or U] + H genomes in the nrDNA (this chapter) and V-genome bands abundant in the AFLP patterns (Chapter 5), but evidence of the V-genome was not found in sequence data. Since these V-genome AFLP bands are abundant, they are likely to result from amplification of DNA in the nuclear rather than the chloroplast genome. This would suggest the possibility that *P. vahliana* was a male parent and that harmonization of the ITS sequence resulted in the loss of the male component, (as found, for example, in Wendel et

al. 1995). This leaves the R/U ancestor copy (instead of the V-genome copy as in *P. bruggemannii*) and thus suggests that *P. angustata* may not have formed simply by *P. bruggemannii* hybridizing with a diploid.

The nrDNA (Z-genome) evidence suggests the R/U ancestor would have hybridized with *P. phryganodes*. The hybridization of a tetraploid with a triploid has been shown to occur rarely, because some triploids can, rarely, form reduced or unreduced gametes that can hybridize with a different diploid or tetraploid to result in a fertile polyploid (Harlan and deWet 1975; Burton and Husband 2001).

Puccinellia andersonii is primarily octoploid with a few hexaploid specimens (Chapter 5) and is very closely related to *P. angustata.* It has the same nrDNA and cpDNA pattern in general as *P. angustata,* but can be distinguished by one ETS1f mutation that it also shares with *P. arctica.* It has the same primary ITS sequence as *P. angustata,* and a secondary sequence, that, although not identical, also had the four-nucleotide deletion diagnostic of the R+U clade. In the MP tree based on cpDNA, *P. andersonii* (including the hexaploid *P. andersonii* specimen *2865-5*) forms an unresolved polytomy basal to *P. angustata* and *P. bruggemannii* (Fig. 6.3B).

One hypothesis for the origin of *P. andersonii* is that it may have evolved from a hybridization of *P. angustata* with *P. phryganodes* (Table 6.6). This would account for the similar composition of *P. angustata* and *P. andersonii* plus the extra bands of *P. andersonii* that are also characteristic of *P. phryganodes* (Fig. 5.3. There may be one other factor distinguishing *P. andersonii* from *P. angustata*, in that *P. andersonii* and *P. banksiensis* were situated on the same arm in the cpDNA network, raising a question as to whether *P. banksiensis* and not *P. arctica* was an original diploid maternal parent of *P. andersonii*. One of the characteristic *P. banksiensis* AFLP bands is found in all populations of *P. andersonii* and the other characteristic band is not found in any *P. andersonii* populations (Chapter 5). Therefore, *P. banksiensis* may be an original parent of *P. andersonii.* On the other hand, an ancestor of both *P. arctica* and *P. banksiensis*

may have been an original parent, which does not refute the proposal that *P. andersonii* evolved from *P. angustata*.

Other arctic polyploid species— To account for the proposed modes of speciation put forth for the evolution of *P. angustata* and *P. andersonii* above, the triploid hybrid species *P. phryganodes* would have to produce some viable haploid gametes on occasion. Although we found tetraploid flow cytometry values for *P. phryganodes* in Chapter 5, these would not normally form the haploid gametes we need here. Triploids can rarely form haploid or diploid gametes (i.e., reduced or unreduced gametes) (Harlan and deWet 1975, Burton and Husband 2001). We also looked for evidence of the diploid progenitor of *P. phryganodes*. One of the original species in the hybrid origin of *P. phryganodes* may have been *P. tenella*, based on evidence from AFLP data (Chapter 5) and sequence data (Fig. 6.5). AFLP bands and sequence mutations characteristic of *P. tenella* are not prevalent, however, in *P. bruggemannii*, *P. angustata*, or *P. andersonii*, so *P. tenella* does not appear to be one of the parents of these polyploids. Another progenitor of the triploid and tetraploid *P. phryganodes* is likely *P. phryganodes* subsp. *geniculata* (V.I.Krecz.) Tzvelev. This subspecies may have been involved in the origin of *P. angustata* and *P. andersonii*, although the likelihood is very low because it is known only from non-arctic Asia (one diploid count in Russia by Sokolovskaya (1968)) and one location in far western Alaska (Tzvelev 1976) (Fig. 2.1).

Puccinellia sp. 2 was examined as a putative distinct species because it was different than the other polyploid species based on AFLP data (Chapter 5). These plants differed in morphology from other *Puccinellia* species in the arctic islands by their small and tightly tufted habit, with smaller florets than other species except *P. banksiensis.* Our results here show that this species has a nrDNA pattern that is different from *P. angustata* and *P. andersonii*, but similar to *P. pumila*. *Puccinellia pumila* grows on the Atlantic and Pacific coasts of North America in temperate regions and boreal regions. It just reaches the Arctic in the East, and Alaska (primarily boreal but a few arctic specimens) in the West (Davis

and Consaul 2007). It has been recorded from the south-eastern Arctic islands (Southampton Island; Iqaluit, Baffin Island), but has not been reported for the western arctic islands. Our molecular data provides evidence that most of the plants in this population are a small form of *P. pumila*, but one was consistent with *P. andersonii* rather than *P. pumila*, making population 2848 a mixed, octoploid population of *P. andersonii* and *P. pumila*.

Puccinellia hauptiana was included for comparison with the new species *P. banksiensis* (Chapter 2), which it very closely resembles. It does not have the deletion characteristic of the R+U clade, which indicates that it is not closely related to *P. banksiensis*. The nrDNA MP tree (Fig. 3A) shows that its nrDNA is similar to that of *P. pumila*, however, and the possibility that it is a tetraploid parent of the octoploid *P. pumila* should be examined in future studies.

Subgeneric classification— The sequence data clearly indicate that *P. arctica* and *P. banksiensis* form a lineage distinct from the other diploid taxa, suggesting that a separate section (here referred to as section "*Arctica*") would be appropriate for these species. The subgeneric classification of the genus is complicated, however, by the fact that the polyploid species have a hybrid origin, with putative parents of the hybrids from different sections. *Puccinellia tenella* is in section *Puccinellia*, *P. vahliana* in section *Pseudocolpodium*, and *P. phryganodes* in section *Paralochloa* (Tzvelev 1976). Tzvelev (1976) also placed the polyploids *P. angustata* and *P. andersonii* in section *Puccinellia*, but *P. bruggemannii* has not been formally placed in a section. Thus, the most likely parental species for *P. bruggemannii* belong to sections *Pseudocolpodium* and "*Arctica*," while the most likely parental species of *P. angustata* and *P. andersonii* belong to sections *Pseudocolpodium*, "*Arctica*", and *Paralochloa.* Given this reticulate pattern across currently recognized sections, Tzvelev's (1964) initial hesitancy to divide the genus into infrageneric subdivisions seems justified. Sørensen's (1953) informal subgeneric classification of North American *Puccinellia* puts the species studied here into the Langeana group (which includes *P. tenella* and *P. alaskana*), the Andersonii group (*P. andersonii*), the Angustata

group (*P. angustata*), the Maritima group (*P. phryganodes*), the Nuttalliana group (*P. arctica*), and into the genus *Colpodium* (*P. vahliana*). This is not a natural grouping either, based on the results here. A solution would be to recognize sections only for the diploid species. Intersectional hybridization has been shown, for example, in *Clarkia* (Ford and Gottlieb 2003), *Costa* (Sytsma and Pippen 1985) and *Potentilla* (Hansen et al. 2000). The polyploid species of *Puccinellia* considered as having intersectional hybrid origins would not be placed into a section.

Conclusion— We were able to confirm species limits and determine phylogenetic patterns among these diploid species by sequencing nrDNA and cpDNA regions. We were able to detect allopolyploid origins of the polyploid species by studying DNA regions that were diagnostic for the diploid species. We obtained insights into the different origins of *Puccinellia* polyploids by using nrDNA and cpDNA regions and by comparing them with AFLP bands generated from total DNA extractions. Constructing trees of the diploid species was informative, but constructing trees including the polyploids resulted in analyses that took days to complete and gave trees with low resolution, possibly because of hybridization or reticulate evolution. Phylogenetic networks showed the hybrid relationships in the polyploid species. A next step may be investigating further the proposed hypotheses for hybrid origin and the putative genome components (Table 6.6) further. Sequencing clones of low copy nrDNA may confirm hypothesized relationships in these species. Low copy nuclear regions have been shown to clarify resolution as well as maintaining multiple copies that can be identified by cloning; for example, the low-copy nuclear gene *waxy* (encoding granule-bound starch synthase I, GBSSI) has been very useful at various taxonomic levels in the Poaceae, most recently helping to reveal the allopolyploid origin of *Eragrostis tef* (Ingram and Doyle 2003) and introns in the second largest subunit of RNA polymerases have elucidated relationships in *Silene* (Oxelman et al. 2004; Popp and Oxelman 2007). In addition, genomic *in situ* hybridization (*Poa*, Brysting et al. 2000), using microsatellite markers, or using other cpDNA

regions found useful in plant systematics and evolution (Shaw et al. 2007) are all promising lines of investigation to resolve these persistent questions.

Table 6.1. Collections of *Puccinellia* studied in this paper, with voucher information for each collection. Number of individuals for each collection sequenced coded as S = "number". Number of individuals analyzed by restriction site digests indicated by R = "number". "IGS" indicates populations sampled for IGS sequence for ETS1 primer design. Samples at MTMG unless stated otherwise.

Diploid species—

P. alaskana. USA. Alaska, Bogoslof Island, Aleutian Islands, *R. Meehan #BOGO01, 02, 03*, S = 2, R = 3, IGS; *B.F. Friedman* 80-147 (ALA), S = 1; Kiska Quad, Rat Islands, Buldir Island, *M. Dick* 84 (ALA).

P. arctica. CANADA. NWT, Banks I, Masik R, *LLC, LJG, and H. Bickerton 2805*, S = 1, R = 1; NWT, Banks I, Masik R, *LLC, LJG, and H. Bickerton 2808*, S = 2, R = 1, IGS; Yukon, Herschel I, *W.J. Cody 36154*, S = 1. NWT, Banks I, De Salis Bay, *LLC, LJG, and H. Bickerton 2813*, (syn. *P. agrostidea*) S = 1, R = 5, IGS; NWT, Banks I, De Salis Bay, *LLC, LJG, and H. Bickerton 2823* (syn. *P. agrostidea*), S = 1, IGS; Nunavut, Ellesmere I, Eureka. *LLC and K. Faubert 2864* (syn. *P. poacea*), S = 2, R = 1; Nunavut, Ellesmere I, Whitsunday Bay. *LLC and K. Faubert 2894* (syn. *P. poacea*), S = 1; Nunavut, Axel Heiberg I, Buchanan Lake, *LLC and K. Faubert 2898* (syn. *P. poacea*), S = 1; Nunavut, Ellesmere I, Tanquary Camp, *LJG and S.G. Aiken 5744* (syn. *P. poacea*), S = 1.

P. banksiensis. CANADA. NWT, Banks I, Masik R. *LLC, LJG, and H. Bickerton 2810*, S = 2, R = 3; NWT, Banks I, Egg River, *LLC, LJG and H. Bickerton 2842*, S = 2, R = 1, IGS.

P. lemmonii. USA. Oregon. *J. I. Davis 425* (BH, MTMG), S = 2.

P. parishii. USA. New Mexico. *J.I. Davis and P.S. Manos 568* (BH, MTMG), S = 1.

P. tenella subsp. ***langeana***. CANADA. Nunavut, Baffin I, Lower Savage I, *LLC, LJG and R.J. Soreng 2352*, S = 1, R = 1; NWT, Banks I, Duck Hawk Bluff, *LLC, LJG and H. Bickerton 2856*, S = 1; Nunavut, Baffin I, Tarr Inlet, *LLC, and K. Faubert 2907,* S = 1, R = 3; Nunavut, Baffin I, Tarr Inlet, *LLC and A. Archambault 3164*, S = 1, R = 3.

P. tenella subsp. ***tenella***. RUSSIA. Arctic Jakutia. *Petrovsky 8.IX.1955*. S = 1.

P. vahliana. CANADA. NWT, Banks I, Masik R, *LLC, LJG and H. Bickerton 2806*, S = 1, R = 1; NWT, Banks I, De Salis Bay, *LLC, LJG and H. Bickerton 2822*, S = 1; NWT, Banks I, Egg River, *LLC, LJG and H. Bickerton 2836*, S = 2, R = 3; Nunavut, Ellesmere I, Eureka, *LLC and K. Faubert 2881*, S =

1, IGS; Nunavut, Axel Heiberg I, Expedition Fiord, *LLC and K. Faubert 2884*, S = 1, R = 1; Nunavut, Cornwallis I, Resolute, *LLC and A. Archambault 3102*, R = 1.

P. wrightii. USA. Alaska, *R. Elven and H. Solstad 09* (O), S = 2, R = 3; Alaska, Cape Prince of Wales, *T. Kelso 82-230* (ALA), S = 1.

Polyploid species—

P. andersonii. CANADA. NWT, Banks I, Worth Point, *LLC 2851b, LJG, and H. Bickerton*, R = 3; NWT, Banks I, Duck Hawk Bluff, *LLC 2856b, LJG and H. Bickerton* S = 1, R = 4; Nunavut, Ellesmere I, Eureka, *LLC 2865 and K. Faubert* S = 2, R = 10; Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2877a and K. Faubert*, S = 1, R = 3; Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2878a and K. Faubert*, S = 1, R = 5.

P. angustata. CANADA. NWT, Banks I, Egg R, coastal plain, *LLC 2849, LJG, and H. Bickerton* R = 1; NWT, Banks I, Worth Point, *LLC 2851a, LJG, and H. Bickerton*, S = 1, R = 3; NWT, Banks I, Worth Point, *LLC 2853, LJG, and H. Bickerton*, S = 1, R = 6; NWT, Banks I, Duck Hawk Bluff, *LLC 2856c, LJG and H. Bickerton* R = 2; NWT, Banks Island, Sachs Harbour, *LLC 2858*, S = 1, R = 2; Nunavut, Ellesmere I, Eureka, *LLC 2866 and K. Faubert* S = 2, R = 10; Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2874 and K. Faubert*, S = 1, R = 8; Nunavut, Nunavut, Axel Heiberg I, Gypsum Hill, *LLC 2877b and K. Faubert*, R = 2; Axel Heiberg I, Gypsum Hill, *LLC 2878b and K. Faubert,* R = 4; Nunavut, Ellesmere I, *LLC 2899 and K. Faubert* R = 1; NWT, Prince Patrick I, Mould Bay, *LLC 3144b and A. Archambault*, S = 1, R = 5; NWT, Prince Patrick I, Mould Bay, *LLC 3150 and A. Archambault*; NWT, Prince Patrick I, Mould Bay, *LLC 3151 and A. Archambault*; NWT, Prince Patrick I, Mould Bay, *LLC 3154 and A. Archambault*, S = 1, R = 7.

P. bruggemannii. CANADA, Nunavut, Cornwallis I, Resolute, *LLC 2906 and K. Faubert* S = 2, R = 4; Nunavut, Cornwallis I, Resolute coast, *LLC 3063 and A. Archambault*, R = 4; Nunavut, Cornwallis I, Resolute coast, *LLC 3072 and A. Archambault*, R = 5; Nunavut, Beechey I, *LLC 3075 and A. Archambault*, R = 4; Nunavut, Bathurst I, *LLC 3080 and A. Archambault,* S = 2, R = 5; Nunavut, Somerset I, Cunningham Inlet, *LLC 3083 and A. Archambault*, R = 10; Nunavut, Cornwallis I, Resolute coast, *LLC 3100 and A. Archambault*, R = 4; NWT, Prince Patrick I, Mould Bay, *LLC 3144b and A. Archambault*, S = 1, R = 3.

P. hauptiana. USA. Alaska, Palmer, *Dutilly, Lepage, and O'Neill 20115*, S = 1; Russia, *Bubnova, 26 June 1986*, R = 1; Russia, *Litwinow 4106b*, R = 1; Russia, *Kogelbreckob 605*, R = 1.

P. phryganodes. CANADA. Nunavut, Axel Heiberg I, Expedition Fiord, *LLC 2321, R.J. Soreng, & LJG*, S = 1; NWT, Banks Island, DeSalis Bay, *LLC 2818, LJG and H. Bickerton*, R = 3; NWT, Banks I, Southwestern coastal plain,

LLC 2847, LJG and H. Bickerton, R = 4; Nunavut, Cornwallis I, north of Resolute, *LLC 3123 and A. Archambault*, S = 1, R = 3; Nunavut, Baffin I, Tarr Inlet, *LLC and K. Faubert 2907*, S = 1; Nunavut, Baffin I, Tarr Inlet, *LLC and A. Archambault 3163*, S = 2, R = 8.

P. pumila. CANADA. Labrador, Mouth of Little Reid Brook, *LLC 2552*, S = 1, R = 1; Labrador, Anaktalak Bay, *LLC 2563*, R = 3; Labrador, Edward's Cove, *LLC 2926*, R = 2.

P. sp.2. CANADA. NWT, Banks I, Southwestern coastal plain, *LLC 2848, LJG and H. Bickerton*, S = 3, R = 8.

Outgroups—

Phippsia algida. CANADA. Nunavut, Cornwallis I, Valley near SE coast, *LJG 6913 and LLC*, S = 1; Nunavut, Baffin I, Nanisivik, *LJG 6253*, S = 1, IGS.

Catabrosa aquatica. CHILE, *R.J. Soreng and N. Soreng 7150* (US), S = 1.

Table 6.2. Summary of DNA sequence characteristics for ITS, ETS1f, *rpoB-trnC*, and *rpl16*, for the ingroup alone and including the outgroup. If the latter values were different, they are given in square brackets following the values for the ingroup.

	ITS1+ 5.8S +ITS2	ETS1f	*rpoB-trnC*	*rpl16*	all
Length range:	591–595	807–809	1153–1197	794–836	
Aligned length:	595	809	1204 [1208]	836	3444 [3448]
G+C content range (%):	60.1–60.2	56.7–57.1	30.4–30.7	31.8–32.6	30.4–60.2
Number of indels:	1	2	6 [10]	6	15 [19]
Number of variable sites:	56	81 [84]	26 [34]	12 [21]	175 [195]
Potentially informative sites:	17	54 [57]	8 [12]	7 [9]	86 [95]
Constant sites:	539	737 [734]	1182 [1178]	855 [846]	3313 [3297]

Table 6.3. Positions in *P. phryganodes* ITS sequence having more than one base represented in a single amplification product, seen as multiple peaks on the chromatogram. R = *P. arctica*, U = *P. banksiensis*, T = *P. tenella*, A = *P. alaskana*, V = *P. vahliana*, S = *P. parishii*, M = *P. lemmonii*, *bru = P. bruggemannii, ang = P. angustata, and = P. andersonii, phry = P. phryganodes*.

Position	Bases (Taxa having each base)
82	G (RUTAVPL *bru*)/ A (*ang and*)
91	G (T) / A (RAVLP *bru, ang, and*)
100	G (TAV *bru*) / T (RU *ang and*)
137	A (TAV *bru*) / T (*ang and*) / G (R U L P)
436	G (RUTAVLP *bru*) / T (*ang and*)
540	C (all except *phry*) / T (only *phry*)

Table 6.4. Frequencies of restriction site presence/absence in 13 arctic *Puccinellia* species for nrDNA (ITS, to detect various genomes) and cpDNA (*rpoB-trnC*, to detect the V genome when no cut). Letters in brackets and in columns "Nuclear genome and Chloroplast genome" refer to the genomes defined in Figs. 6.1, 6.2, and 6.3. Genome letters in parentheses at the top of each column indicate the genomes that are cut by the associated restriction enzyme.

		ITS								*rpoB-trnC*	
Species (n)	Ploidy	*BanII* (R,U)	*Sau96I* (R,U,T,V)	*Sau96I* (V)	*Sau96I* (A)	*AluI* (U)	*BbvI* (T)	*DpnII* (Z)	Nuclear genome	*Sau96I*	Chloroplast genome
P. alaskana (4)	2X	0	0	0	1	0	0	0	A	1	A
P. arctica (10)	2X	1	1	0	0	0	0	0	R	1	R
P. banksiensis (5)	2X	1	1	0	0	1	0	0	U	1	U
P. vahliana (10)	2X	0	1	1	0	0	0	0	V	0	V
P. wrightii (2)	2X	0	1	1	0	0	0	0	V	0	V
P. tenella (7)	2X	0	1	0	0	0	1	0	T	1	T
P. phryganodes (5)	3X	0.2	1	0	0	0	0	0	0	1	not V
P. phryganodes (8)	4X	0	1	0	0	0	0	1	Z	1	not V
P. bruggemannii (41)	4X	0	1	1	0	0	0	0	V	1	not V
P. hauptiana (3)	4X	1	1	0	0	0	0	0	R	1	not V
P. angustata (58)	6X	1	1	0	0	0	0	1	R, Z	1	not V
P. andersonii (25)	8X	1	1	0	0	0	0	1	R, Z	1	not V
P. sp.2 (8)	8X	1	1	0	0	0	0	0.25	R [Z]	1	not V
P. pumila (6)	8X	1	1	0	0	0	0	0	R	1	not V

Table 6.5. Genomes represented in *Puccinellia bruggemannii*, *P. angustata*, and *P. andersonii* from AFLP and sequencing data. R = *P. arctica*, U = *P. banksiensis*, A = *P. alaskana*, T = *P. tenella*, V = *P. vahliana* and *P. wrightii*, H = *P. phryganodes*, *bru* = *P. bruggemannii*. When codes are separated by a comma, there is evidence of both genome in the sequences. In the AFLP columns, "characteristic bands" are those in a species that are almost non-existent in the other polyploid species; when codes are not separated by a comma a single band was found in all of the species listed.

Species	AFLP data for characteristic bands	AFLP data for bands shared with other taxa	nrDNA sequences and restriction enzymes	cpDNA sequences	cpDNA restriction enzymes
P. bruggemannii	*bru*, V, VT	RT, VT, R, RH	V	R, U	not V
P. angustata		RV, VT, R, RH, H, RU, V	Z and R	R, U	not V
P. andersonii	H(RVL), UH, H	H, RU, V	Z and R	R, U	not V

Table 6.6. Flow chart depicting hypothesized origins of polyploid *Puccinellia* species. R = *P. arctica*, U = *P. banksiensis*, V = *P. vahliana*, H = *P. phryganodes*.

DNA Region plus whether male (♂) or female (♀) parent	Hypothesized origins and putative genomic content		
	P. bruggemannii	*P. angustata*	*P. andersonii*
Chloroplast DNA			
♀ *P arctica/banksiensis* ancestor →	*P. arctica/banksiensis* **R/U** or ancestor →	*P. arctica/banksiensis* **R/U** or ancestor →	*P. arctica/banksiensis* **R/U** or ancestor
Nuclear DNA			
ITS+ETS			
♂= (R/U + V, followed by harmonization)	**V** →	**R + Z(H)** →	**R +Z(H) + U?**
AFLP (nuclear genome highly represented)	**R/U + V** →	**R +V+H** →	**R + V + H + HorU**

Figure 6.1. *Puccinellia* diploid species trees. MP = maximum parsimony. ML = maximum likelihood. Bars represent mutations that are consistent within each clade. Black bars = transversions, white bars = transitions, black circles = insertions, white circles = deletions. Bootstrap values >50% are shown above the branches on the trees. Posterior probabilities for branches from Bayesian inference analysis are shown below the branches. Geographical designations: N = northern Arctic, SE = southeastern Arctic, SW = southwestern Arctic, S USA = south-central United States. *Puccinellia* species are indicated by their specific epithets only. The diploid genome codes in A are explained in "Results – Diploid taxa" section: R = *P. arctica*; U = *P. banksiensis*; T = *P. tenella*; V = *P. vahliana* and *P. wrightii*; A = *P. alaskana*; M = *P. lemmonii*; S = *P. parishii*. A. Strict consensus of six most parsimonious trees based on nrDNA regions ITS + ETS1f. Capital I under bars indicates an ITS mutation, the rest are ETS mutations. B. Strict consensus of 24 most parsimonious trees based on cpDNA regions *rpoB-trnC* and *rpl16*. Dashed line shows species included in a clade present in Bayesian tree but not present in strict consensus tree. C. Strict consensus tree of 24 most parsimonious trees based on both nrDNA and cpDNA regions. D. The single best maximum likelihood tree based on both nrDNA and cpDNA regions. Scale bar units are substitutions/site.

Fig. 6.1

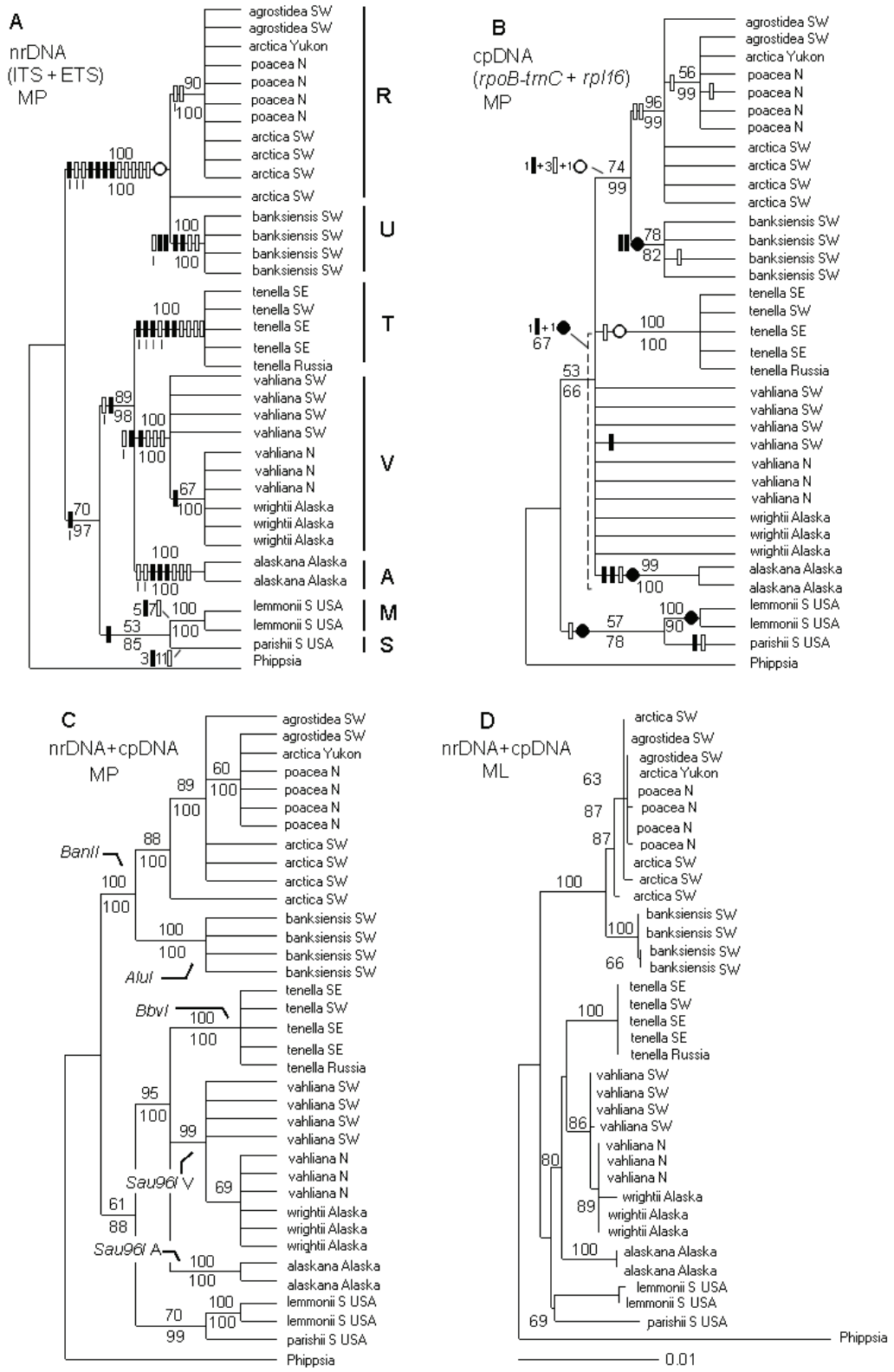

A
nrDNA
(ITS + ETS)
MP
agrostidea SW
agrostidea SW
arctica Yukon
poacea N
poacea N
poacea N
poacea N
arctica SW
arctica SW
arctica SW
arctica SW
banksiensis SW
banksiensis SW
banksiensis SW
banksiensis SW
tenella SE
tenella SW
tenella SE
tenella SE
tenella Russia
vahliana SW
vahliana SW
vahliana SW
vahliana SW
vahliana N
vahliana N
vahliana N
wrightii Alaska
wrightii Alaska
wrightii Alaska
alaskana Alaska
alaskana Alaska
lemmonii S USA
lemmonii S USA
parishii S USA
Phippsia
R
U
T
V
A
M
S
B
cpDNA
(rpoB-trnC + rpl16)
MP
C
nrDNA+cpDNA
MP
BanII
AluI
BbvI
Sau96I V
Sau96I A
D
nrDNA+cpDNA
ML
0.01

Figure 6.2. *Puccinellia* diploid and polyploid taxa. Strict consensus of 11 most parsimonious trees in an MP analysis of six cpDNA regions with a single specimen representing each of the taxa. *Puccinellia* species are indicated by their specific epithets only; "del" = deletions, "ins" = insertions, "ti" = transitions, "tv" = transversions. Each bar represents one mutation. Each DNA region may have more than one mutation (numbers given in parentheses). *Puccinellia lemmonii* and *P. parishii* have several mutations in each region but these regions are omitted from the graph to avoid crowding. Values above the branches are bootstrap values > 50%. The diploid genome codes on the right are explained in "Results – Diploid taxa" section and Fig. 6.1.

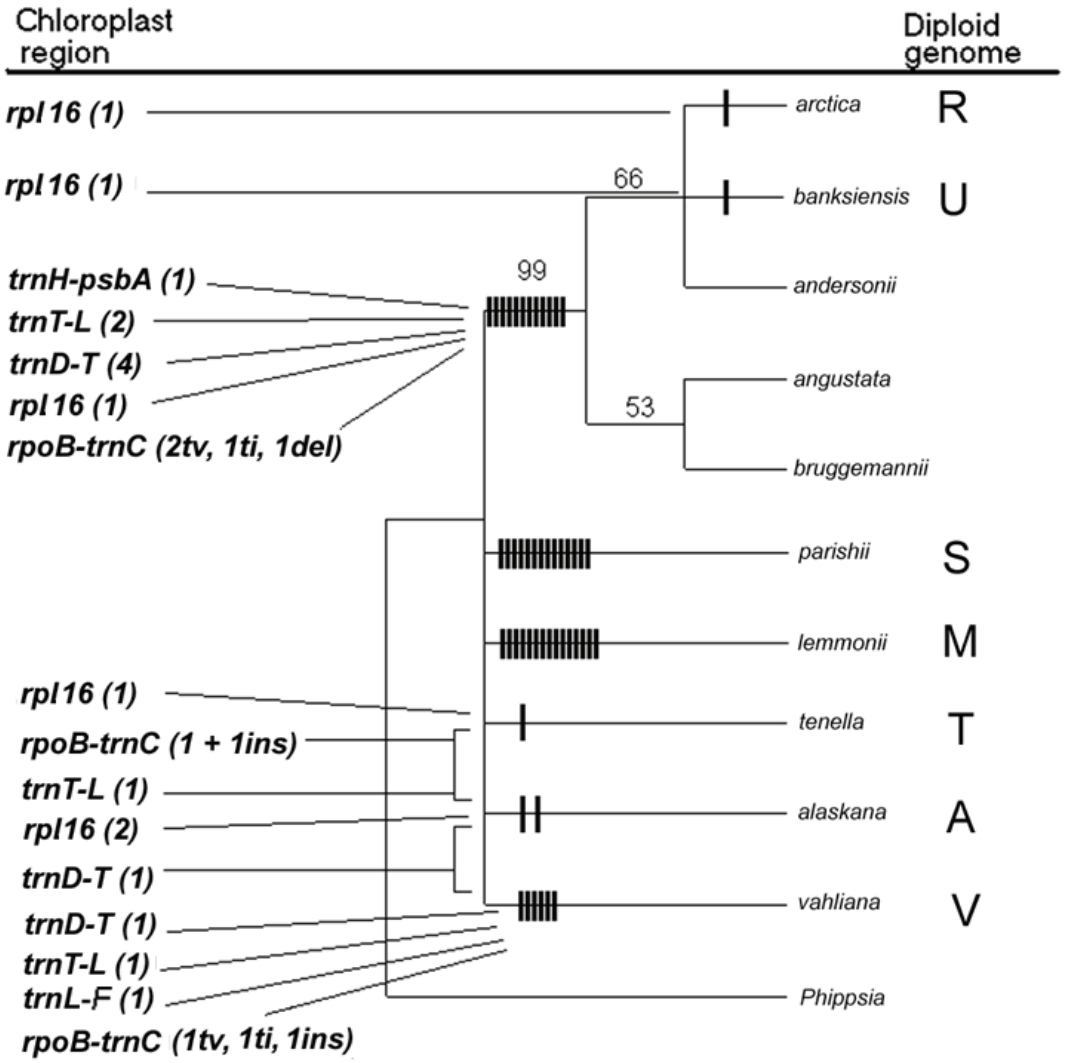

Chloroplast region
Diploid genome
rpl16 (1)
rpl16 (1)
trnH-psbA (1)
trnT-L (2)
trnD-T (4)
rpl16 (1)
rpoB-trnC (2tv, 1ti, 1del)
rpl16 (1)
rpoB-trnC (1 + 1ins)
trnT-L (1)
rpl16 (2)
trnD-T (1)
trnD-T (1)
trnT-L (1)
trnL-F (1)
rpoB-trnC (1tv, 1ti, 1ins)
66
99
53
arctica
banksiensis
andersonii
angustata
bruggemannii
parishii
lemmonii
tenella
alaskana
vahliana
Phippsia
R
U
S
M
T
A
V

Figure 6.3. Strict consensus trees resulting from parsimony analysis of *Puccinellia* diploid and polyploid taxa. *Puccinellia* species are indicated by their specific epithets only. Codes angustata2 and andersonii2 are the secondary sequences (they are not in the cpDNA tree). All evolutionary taxonomic units (ETUs) are unique sequences; number after species name is the number of individuals with a particular sequence. Values above the branches are bootstrap values >50% and values below the branches are Bayesian posterior probabilities. * = hexaploid *P. andersonii*. *Phippsia* consists of two samples of the same species. Line shows the change in position of *P. bruggemannii* between the nrDNA tree and the cpDNA tree. C = central arctic, N = northern arctic, SW = southwestern arctic, (N) = *P. angustata*, (D) = *P. andersonii*, (D6) = group D6 defined in Chapter 5. A. Strict consensus of 97951 most parsimonious trees based on both ITS and ETS1f data (nrDNA). B. Strict consensus of 285922 most parsimonious trees based on both *rpoB-trnC* and *rpl16* data (cpDNA).

Fig. 6.3.

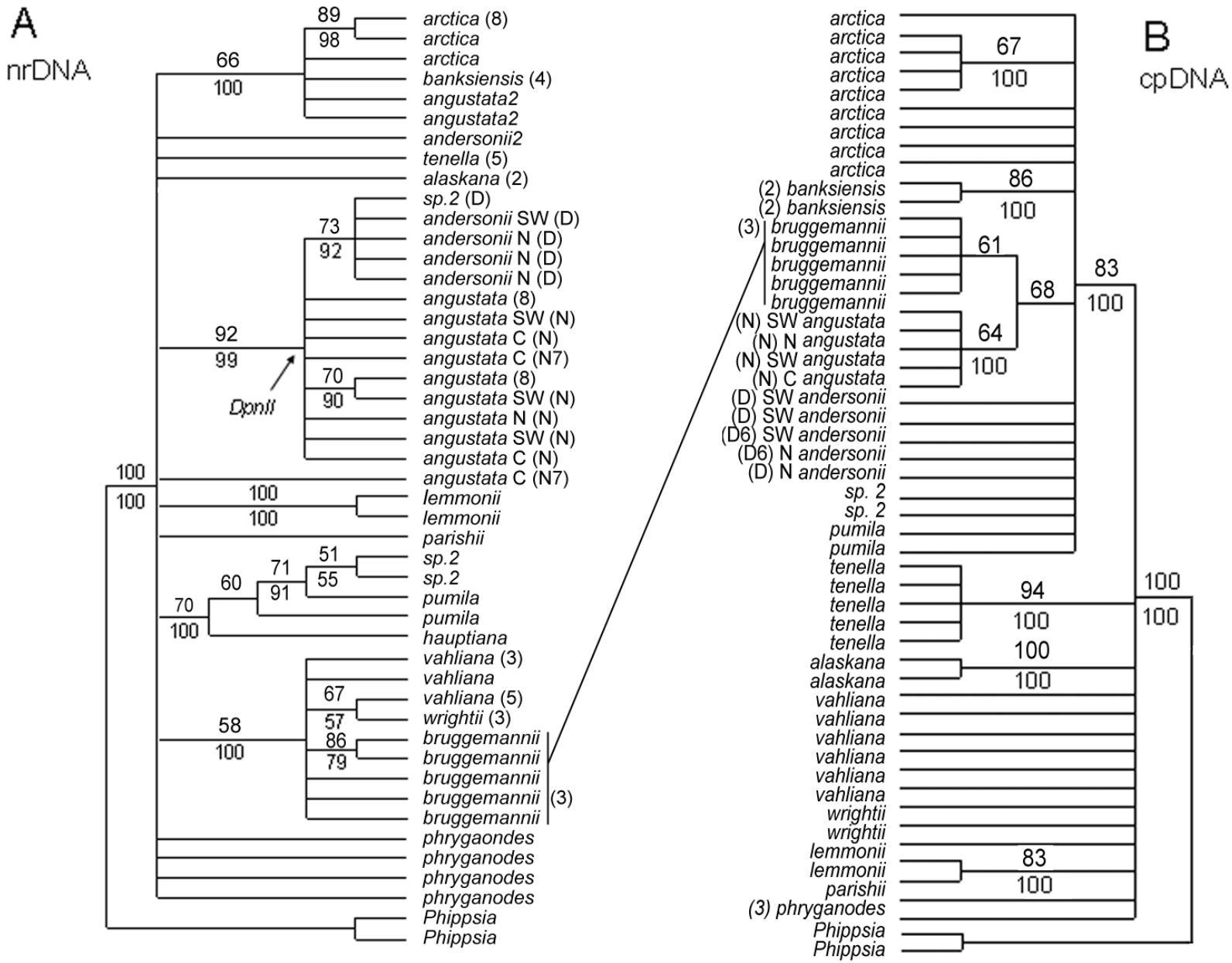

A
nrDNA
arctica (8)
arctica
arctica
banksiensis (4)
angustata2
angustata2
andersonii2
tenella (5)
alaskana (2)
sp.2 (D)
andersonii SW (D)
andersonii N (D)
andersonii N (D)
andersonii N (D)
angustata (8)
angustata SW (N)
angustata C (N)
angustata C (N7)
angustata (8)
angustata SW (N)
angustata N (N)
angustata SW (N)
angustata C (N)
angustata C (N7)
lemmonii
lemmonii
parishii
sp.2
sp.2
pumila
pumila
hauptiana
vahliana (3)
vahliana
vahliana (5)
wrightii (3)
bruggemannii
bruggemannii
bruggemannii
bruggemannii
bruggemannii
(3)
phrygaondes
phryganodes
phryganodes
phryganodes
Phippsia
Phippsia
DpnII
B
cpDNA
arctica
arctica
arctica
arctica
arctica
arctica
arctica
arctica
(2) banksiensis
(2) banksiensis
(3) bruggemannii
bruggemannii
bruggemannii
bruggemannii
bruggemannii
(N) SW angustata
(N) N angustata
(N) SW angustata
(N) C angustata
(D) SW andersonii
(D) SW andersonii
(D6) SW andersonii
(D6) N andersonii
(D) N andersonii
sp. 2
sp. 2
pumila
pumila
tenella
tenella
tenella
tenella
tenella
alaskana
alaskana
vahliana
vahliana
vahliana
vahliana
vahliana
vahliana
wrightii
wrightii
lemmonii
lemmonii
parishii
(3) phryganodes
Phippsia
Phippsia

Figure 6.4. Phylogenetic network of nrDNA sequence data. Circles delimit groups in the network, which are labeled with the species name, ploidy level, and genome code in that order, as appropriate. Genome codes at the branch tips are the same as those in Fig. 6.2 with a three-letter prefix corresponding to the taxon. Scale bar shows a distance of 0.001 substitutions per site.

Fig. 6.4 nrDNA

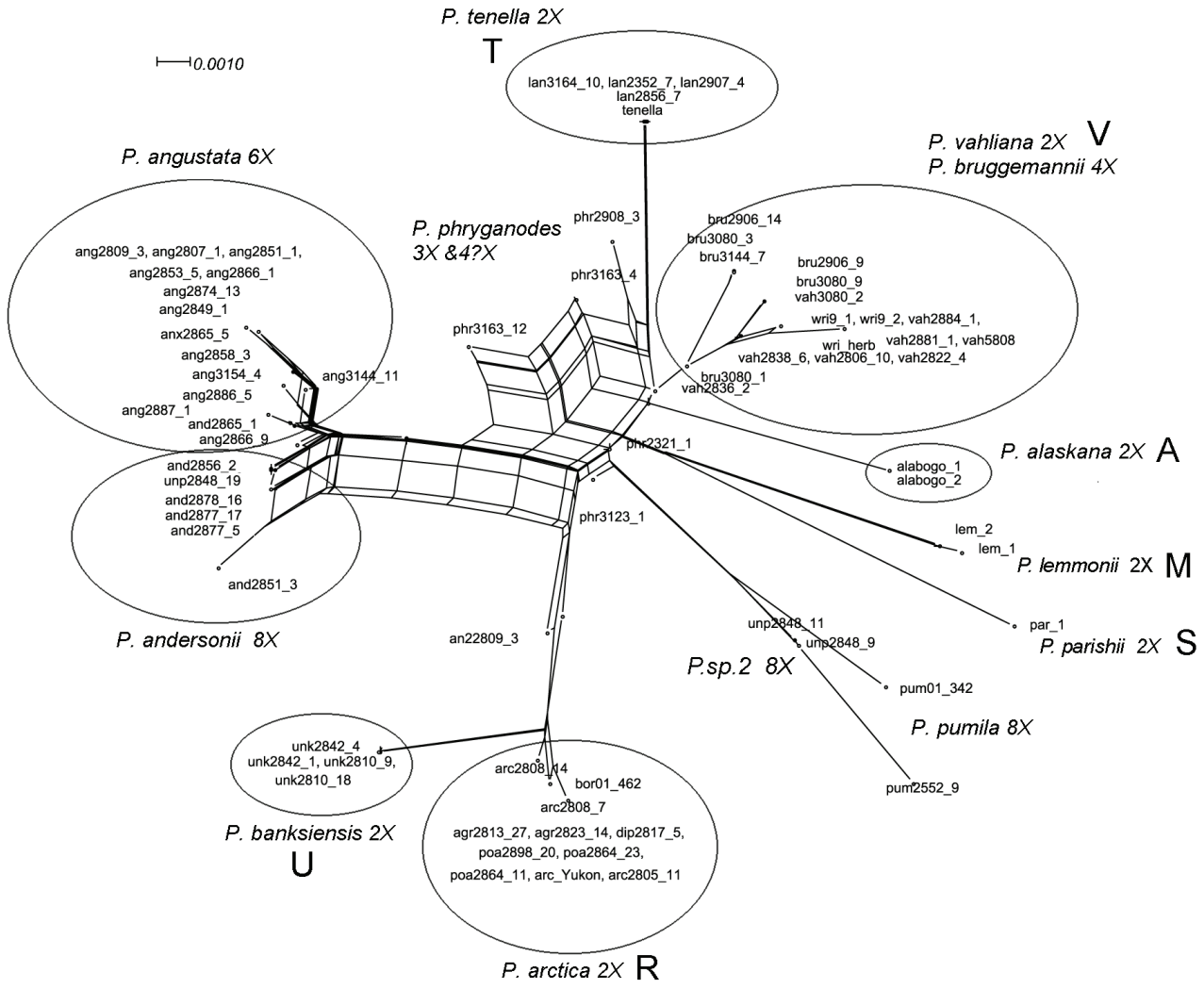

P. tenella 2X
T
0.0010
lan3164_10, lan2352_7, lan2907_4
lan2856_7
tenella
P. vahliana 2X V
P. bruggemannii 4X
P. angustata 6X
P. phryganodes
3X &4?X
phr2908_3
bru2906_14
bru3080_3
bru3144_7
bru2906_9
bru3080_9
vah3080_2
wri9_1, wri9_2, vah2884_1,
wri_herb
vah2881_1, vah5808
vah2838_6, vah2806_10, vah2822_4
bru3080_1
vah2836_2
phr3163_4
phr3163_12
ang2809_3, ang2807_1, ang2851_1,
ang2853_5, ang2866_1
ang2874_13
ang2849_1
anx2865_5
ang2858_3
ang3154_4
ang3144_11
ang2886_5
ang2887_1
and2865_1
ang2866_9
phr2321_1
P. alaskana 2X A
alabogo_1
alabogo_2
and2856_2
unp2848_19
and2878_16
and2877_17
and2877_5
and2851_3
phr3123_1
lem_2
lem_1
P. lemmonii 2X M
par_1
P. parishii 2X S
P. andersonii 8X
an22809_3
unp2848_11
unp2848_9
P.sp.2 8X
pum01_342
P. pumila 8X
pum2552_9
unk2842_4
unk2842_1, unk2810_9,
unk2810_18
P. banksiensis 2X
U
arc2808_14
bor01_462
arc2808_7
agr2813_27, agr2823_14, dip2817_5,
poa2898_20, poa2864_23,
poa2864_11, arc_Yukon, arc2805_11
P. arctica 2X R

Fig. 6.5. Phylogenetic network of cpDNA sequence data. Circles delimit groups in the network, which are labeled with the species name, ploidy level, and genome code in that order, as appropriate. Genome codes at the branch tips are the same as those in Fig. 6.2 with a three-letter prefix corresponding to the taxon. Scale bar shows a distance of 0.001 substitutions per site.

Fig. 6.5. cpDNA

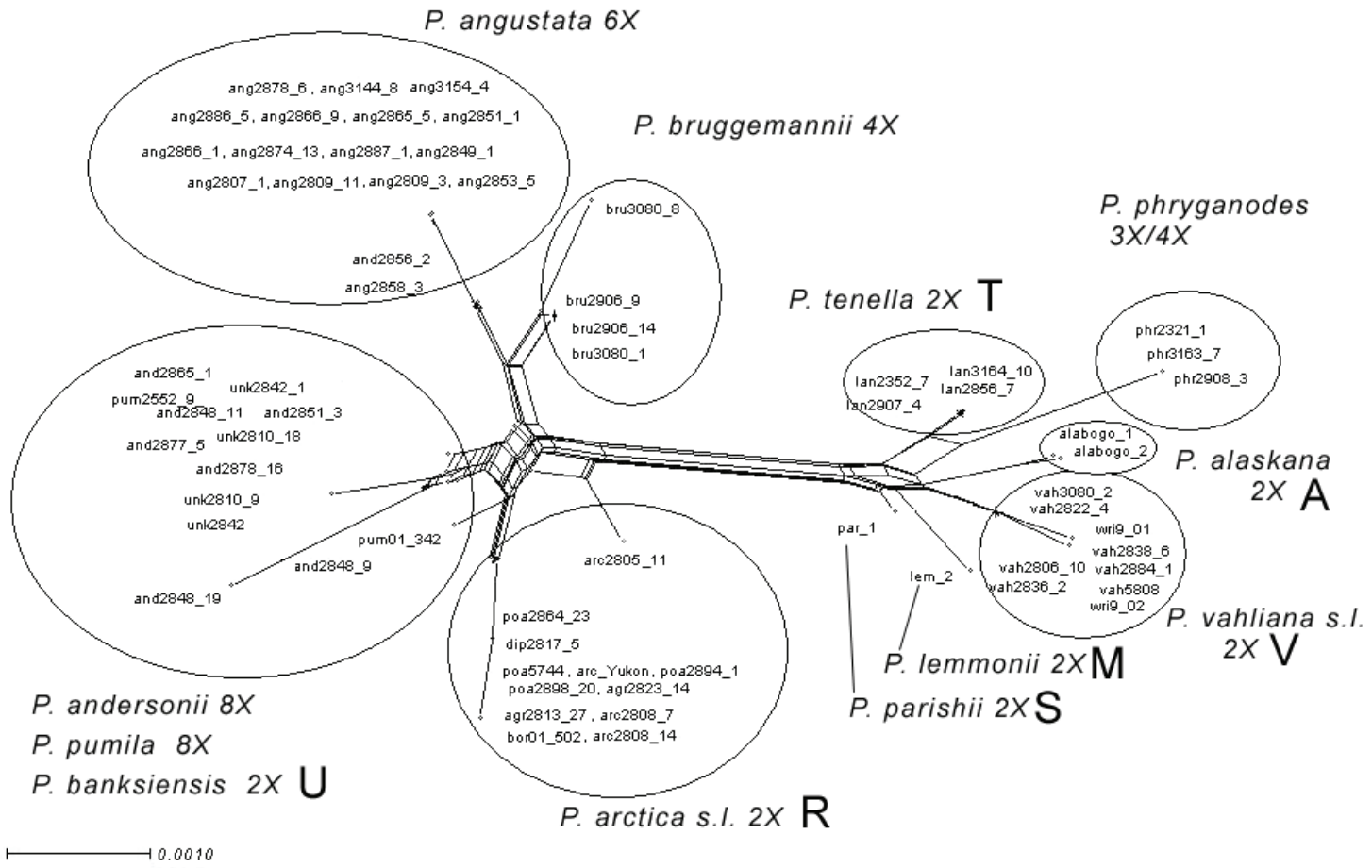
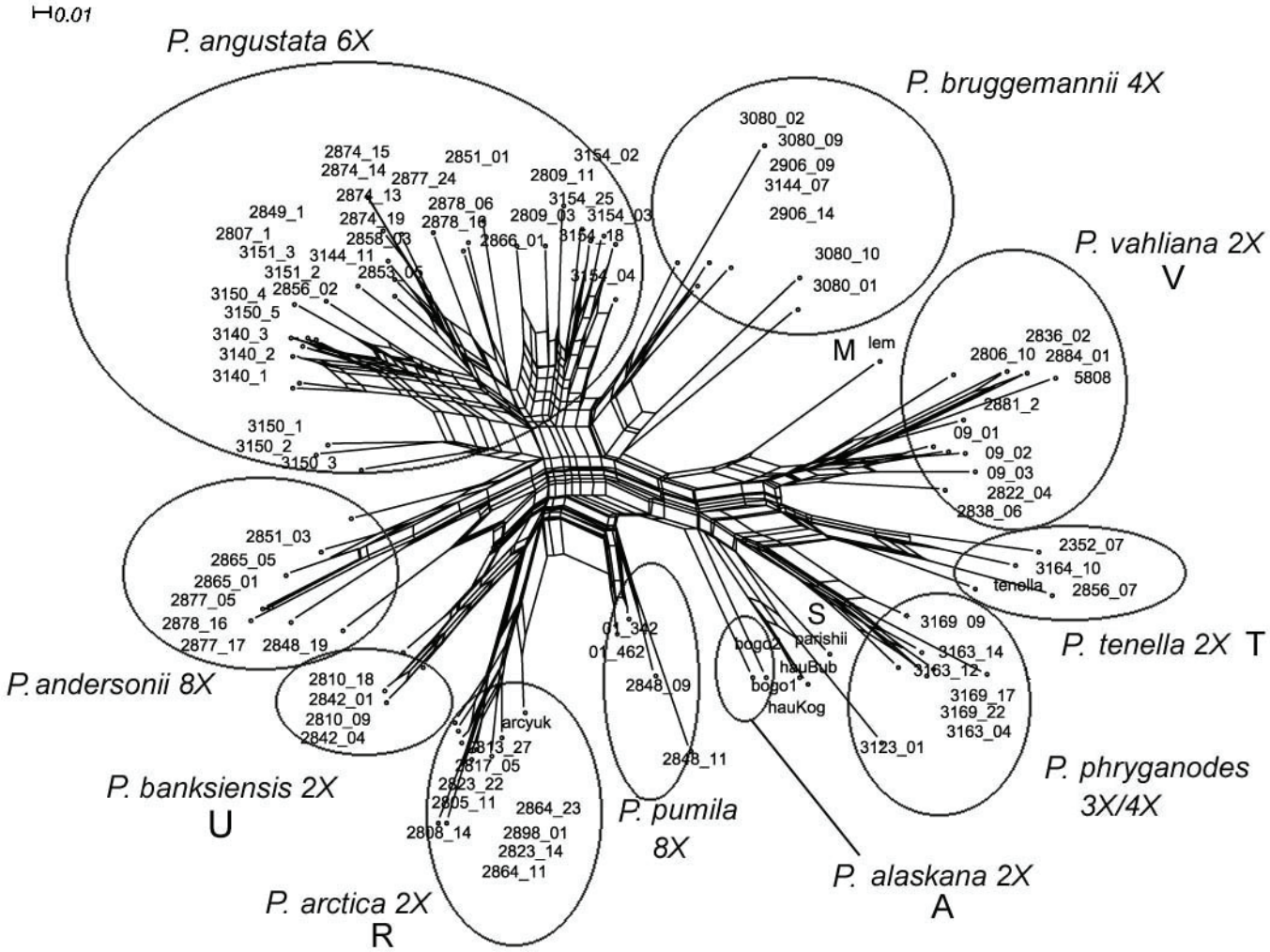

P. angustata 6X
ang2878_6, ang3144_8 ang3154_4
ang2886_5, ang2866_9, ang2865_5, ang2851_1
ang2866_1, ang2874_13, ang2887_1, ang2849_1
ang2807_1, ang2809_11, ang2809_3, ang2853_5
and2856_2
ang2858_3
P. bruggemannii 4X
bru3080_8
bru2906_9
bru2906_14
bru3080_1
P. phryganodes 3X/4X
P. tenella 2X T
lan2352_7
lan3164_10
lan2856_7
lan2907_4
phr2321_1
phr3163_7
phr2908_3
alabogo_1
alabogo_2
P. alaskana 2X A
vah3080_2
vah2822_4
wri9_01
vah2838_6
vah2884_1
vah5808
wri9_02
vah2806_10
vah2836_2
P. vahliana s.l. 2X V
par_1
lem_2
P. lemmonii 2X M
P. parishii 2X S
and2865_1
unk2842_1
pum2552_9
and2848_11
and2851_3
and2877_5
unk2810_18
and2878_16
unk2810_9
unk2842
pum01_342
and2848_9
and2848_19
P. andersonii 8X
P. pumila 8X
P. banksiensis 2X U
arc2805_11
poa2864_23
dip2817_5
poa5744, arc_Yukon, poa2894_1
poa2898_20, agr2823_14
agr2813_27, arc2808_7
bor01_502, arc2808_14
P. arctica s.l. 2X R
0.0010

Fig. 6.6. Phylogenetic network of AFLP data. Circles delimit groups in the network, which are labeled with the species name, ploidy level, and genome code in that order, as appropriate. Species names and ploidy levels are omitted for diploid species *P. lemmonii* (M) and *P. parishii* (S). Genome codes at the branch tips are same as those in Fig. 6.2 with a three-letter prefix corresponding to the taxon. Scale bar shows a distance of 0.01 substitutions per site.

Fig. 6.6. AFLP


0.01
P. angustata 6X
P. bruggemannii 4X
P. vahliana 2X
V
P. tenella 2X T
P. andersonii 8X
P. banksiensis 2X
U
P. arctica 2X
R
P. pumila
8X
P. alaskana 2X
A
P. phryganodes
3X/4X
M lem
S
2874_15
2851_01
3154_02
2874_14
2877_24
2809_11
2874_13
3154_25
2849_1
2878_06
2809_03
3154_03
2874_19
2878_16
2807_1
2858_03
2866_01
3154_18
3151_3
3144_11
3154_04
3151_2
2853_05
3150_4
2856_02
3150_5
3140_3
3140_2
3140_1
3150_1
3150_2
3150_3
3080_02
3080_09
2906_09
3144_07
2906_14
3080_10
3080_01
2836_02
2806_10
2884_01
5808
2881_2
09_01
09_02
09_03
2822_04
2838_06
2352_07
3164_10
tenella
2856_07
3169_09
3163_14
3163_12
3169_17
3169_22
3163_04
3123_01
2851_03
2865_05
2865_01
2877_05
2878_16
2877_17
2848_19
2810_18
2842_01
2810_09
2842_04
01_342
01_462
2848_09
2848_11
bogo2
parishii
hauBub
bogo1
hauKog
arcyuk
2813_27
2817_05
2823_22
2805_11
2864_23
2808_14
2898_01
2823_14
2864_11

7. Summary and Conclusions

The origins of plant species in the North American Arctic are of interest because this area was largely covered with ice until approximately 10,000 years ago (Pielou 1991). No study comparing molecular markers in diploid populations of North American arctic plant species with sympatric polyploid populations had been carried out before the present study. In this research project, I aimed to 1) find the origins of North American arctic polyploids and the extent of multiple polyploidy in arctic North American *Puccinellia*, and 2) clarify systematics in North American arctic members of the genus *Puccinellia*.

In Chapter 3, I proposed changes to the taxonomic treatment of North American diploid *Puccinellia* species. Hypotheses 1A, that taxa represented distinct ploidy levels, was accepted for all of the diploid species. Hypothesis 1B, that the diploid taxa belonged to a single species, was accepted for the group including *P. arctica, P. agrostidea*, and *P. poacea*, in that no AFLP pattern or sequence mutation was found to separate them, but was rejected for the other diploid species, which had distinct molecular and/or morphological patterns. The six diploid species that were distinct based on AFLP patterns and morphological ordinations were: 1) *P. arctica* of northern and western distribution, including *P. agrostidea* and *P. poacea*; 2) a new diploid species from Banks Island, N.W.T., Canada; 3) *P. tenella* subsp. *langeana*, which had been previously treated as a full species *P. langeana*; 4) *P. alaskana*, which had been previously treated as a subspecies of *P. tenella*; and 5) circumpolar (except coastal Beringia) *P. vahliana*, from which 6) the coastal Beringian endemic *P. wrightii* is distinguishable only on size and for which subspecies status may be more appropriate.

In Chapter 4, I described the new species discovered in Chapter 3. This species was closest to *P. arctica* in AFLP patterns, but was distinct morphologically by having extremely small florets. Its distinctiveness was maintained in common garden experiments and was consistent across its range. I

also showed that it was distinct from the tetraploid species, *P. hauptiana*, which it closely resembles.

In Chapter 5, I investigated three widespread polyploid species of arctic alkali grass, *P. bruggemannii*, *P. angustata*, and *P. andersonii*, and sought evidence for their parentage using flow cytometry, morphology, and AFLP data to compare them with the diploid species and the triploid *P. phryganodes*. Hypothesis 1A, that taxa represented distinct ploidy levels, was accepted for all species except *P. andersonii* and *P. phryganodes*, because *P. bruggemannii* was tetraploid and *P. angustata* was hexaploid, but *P. andersonii* had both hexaploid and octoploid and *P. phryganodes* had both triploid and tetraploid flow cytometry readings within the same populations. Tetraploid, hexaploid, and octoploid levels were correlated with AFLP banding patterns for the most part and morphologically associated with type specimens of *P. bruggemannii*, *P. angustata*, and *P. andersonii*, respectively. Thus, Hypothesis 1C, that the polyploid species were distinct, was accepted for the three polyploid taxa, although there were two patterns of intermediate individuals that required further investigation. The three polyploid species each had diagnostic markers from at least two parental species, thus Hypothesis 2B, that the polyploid species were allopolyploids, was accepted. Hypothesis 2A, that the polyploid species arose from North American arctic (NAA) species, was accepted because the polyploids had molecular markers characteristic of NAA *P. arctica*, *P. vahliana*, and *P. phryganodes*, although the possibility remains that an additional Eurasian parent also contributes to the parentage of *P. angustata* and *P. andersonii*. Hypothesis 2C, that the polyploid taxa had polyphyletic origins, was rejected because I did not find evidence that different sets of taxa contributed to each of the polyploid species. I was not able to test Hypothesis 2D, that the polyploid taxa had multiple origins from different populations within the same parental taxa, directly, because there were no consistently different genotypes within the diploid taxa. However, the multiple branching in phylogenetic networks suggests that multiple origins in the past for each of the polyploid species are possible. Multiple polyploidy or introgression may account for the groups of individuals with hybrid AFLP

patterns, which require further study. Common garden (CG) experiments revealed phenotypic plasticity in more than half the characters and overlap in character ranges between species even when significantly different, are most likely a consequence of their allopolyploid origins. Hypothesis 3, that morphological characters distinguished consistently among taxa in the greenhouse was accepted for some characters and rejected for others, because different characters were important in distinguishing between different pairs of taxa.

In Chapter 6, I used nuclear ribosomal DNA regions ITS and ETS1f plus two cpDNA regions to infer the phylogeny of diploid North American *Puccinellia* and, combined with restriction site analysis, the origins and evolution of three arctic polyploid species. The diploid species that we had proposed in Chapter 3 formed monophyletic groups in three lineages. The basal relationship between the temperate North American diploid species and the Arctic species is still unknown, however, since in nrDNA the arctic species *P. arctica* and *P. banksiensis* are basal to all of the other North American diploid species, while in the cpDNA tree, the two temperate diploid species *P. lemmonii* and *P. parishii* are basal to all of the other North American species. *Puccinellia bruggemannii* (tetraploid), *P. angustata* (hexaploid), and *P. andersonii* (primarily octoploid) have similar chloroplast histories, belonging to the same major clade as *P. arctica* and *P. banksiensis.* The nrDNA sequences of *P. bruggemannii* matched only those of the diploid *P. vahliana* s.l. *Puccinellia angustata* and *P. andersonii* have a primary sequence that is most similar to the hybrid species *P. phryganodes*, and a secondary sequence similar to that of *P. arctica*.

The origin of *P. bruggemannii* was possibly from a *P. arctica/P. banksiensis* ancestor as female parent and *P. vahliana* as male parent, with an ITS harmonization to *P. vahliana*; Evidence suggests that *P. angustata* may have had the same first hybridization as *P. bruggemannii*, but with an ITS harmonization towards *P. arctica*, and then a subsequent hybridization with *P. phryganodes* as the male parent and that *P. andersonii* being may have originated with *P. angustata* as the female parent and a subsequent hybridization involving *P. phryganodes* as the male parent. I found evidence in the cpDNA sequences that *P.*

banksiensis may have been involved early in the origins of *P. andersonii*, which requires further testing. Multiple polyploidy was suggested but not confirmed by multiple branching in phylogenetic networks.

The molecular markers used in this study were a powerful combination. AFLP data revealed important information about mutations across the genome. ITS and ETS sequences and restriction digests gave us important information both when multiple copies were retained in the genome and when one parent was lost through concerted evolution. Chloroplast DNA data allowed us to sort out which of the diploid (or triploid) genomes were maternal.

These results lead to other questions about polyploid *Puccinellia* evolution. A study of *P. phryganodes*, which is a common, circumpolar triploid/tetraploid species (perhaps also diploid and hexaploid in some non-North American parts of its range, Elven et al. 2003) has potential to provide further information on the polyploid origins. Studies of chromosome numbers and analysis of the DNA regions studied here from plants across their circumpolar range are needed to discover how many *P. phryganodes* subspecies there are and how they contribute to the other polyploid species in the Arctic. The populations of *P. bruggemannii*, *P. angustata*, and *P. andersonii* having intermediate characteristics require further study to interpret whether or not these plants are formed by multiple polyploidy or introgression resulting from hybridization and backcrossing. The study of low-copy nuclear genes, GISH, microsatellites, and other cpDNA regions, discussed at the end of Chapter 6, show potential for helping to answer these additional questions on polyploidy and arctic *Puccinellia*.

8. Contributions to Science

1. I proposed changes to the taxonomic treatment of North American diploid *Puccinellia* species, which involved providing molecular and morphological support for the distinctness of four species, synonymizing three other species into one, and providing evidence for a new diploid species.
2. I showed that these North American diploid species formed monophyletic groups in three lineages based on sequences of nrDNA regions ITS and ETS1f plus two cpDNA regions, combined with restriction site analysis.
3. I determined seven distinct diploid genotypes in *Puccinellia* that could be used to determine the parental species of the polyploids.
4. I discovered, described, and named a new diploid species (*P. banksiensis*) from the western North American Arctic.
5. I determined that *P. bruggemannii*, *P. angustata*, and *P. andersonii* were allopolyploids, formed from lineages of North American Arctic parental species
6. I proposed hypothesized origins of the polyploids as: *P. bruggemannii* = *P. arctica/P. banksiensis* lineage x *P. vahliana* lineage; *P. angustata* = *P. arctica/P. banksiensis* lineage x *P. vahliana* lineage x *P. phryganodes* lineage; *P. andersonii* = *P. angustata* x *P. phryganodes*.
7. I reported the first tetraploid chromosome numbers for *Puccinellia phryganodes* from Canada.
8. I reidentified plants of *P. bruggemannii* to *P. angustata*, resulting in a new geographic distribution of the former, concentrated in the Central North American Arctic.
9. I designed a primer that amplifies the ETS1f nuclear ribosomal DNA spacer region for members of the *Puccinellinae* of the *Poeae* (Poaceae).
10. I demonstrated that three of the Arctic diploid species reported as Rare in the North American Arctic could not be distinguished using morphological or molecular data and should therefore be synonymized under the name *P.*

arctica, which even in this broader sense still merits Rare status in the Canadian Arctic based on National Conservancy criteria.

11. I prepared new keys that can be incorporated into future revised versions of Flora of the Canadian Arctic Archipelago (Aiken et al. 2003) and The Flora of North America (Davis and Consaul 2007).
12. I showed that for both diploid and polyploid species approximately half of the morphological characters varied significantly between field and common garden, showing phenotypic plasticity that explains some of the difficulty in identification.
13. I determined that high variation within the polyploid species could be explained either by multiple polyploidy or by post-speciation hybridization or introgression, needing further research.

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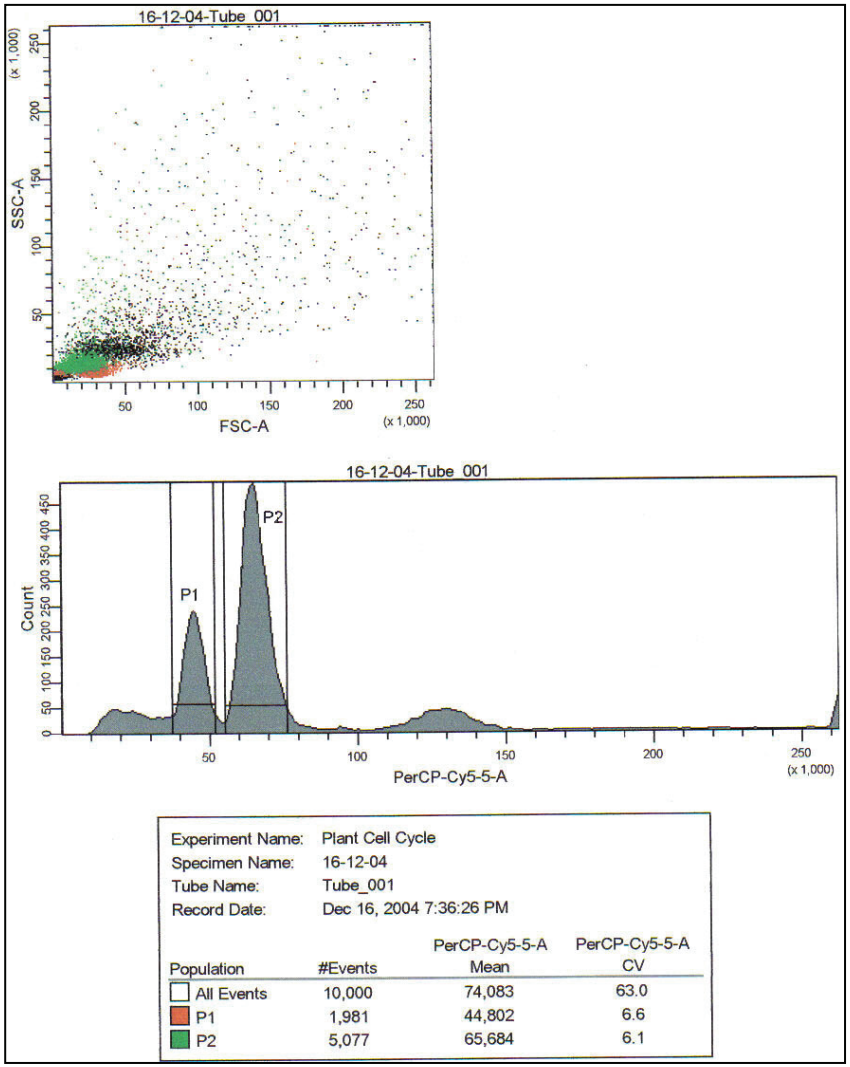
Appendix A. Flow cytometry output graphs

Examples of output from the BD FACS Aria flow cytometer are given in the following figures. Readings of fluorescence were made for each of the samples with an acid citrate dehydrogenase treated blood standard (labelled P1 in each of the graphs). It is imperative to take the readings on the linear scale and not the logarithmic scale in order to calculate the amount of DNA in the nuclei (J. Dolezel personal communication, 2004), although a logarithmic scale makes the peaks look tighter. Therefore, each of the following graphs depicts the fluorescence in linear scale. The coefficients of variation (cvs) are given for each of the peaks.

The upper scatter plot gives the size of each cell recorded by a front scatter detector (FSC-A) and a side scatter detector (SSC-A). The cells of higher ploidy are larger. The lower frequency graph depicts the amount of fluorescence per cell. The PerCP-Cy5-5-A axis (X-axis) is the intensity of fluorochrome from the propidium iodide dye (which stains the chromosomes) detected per nucleus by the laser, indicating the amount of DNA per nucleus. Some of the graphs have two test individuals in the same mix, because because the cvs of some of the samples were high owing to weak plants. Therefore, I needed to confirm differences in ploidy level by combining samples in pairs in the same mix for direct comparison.

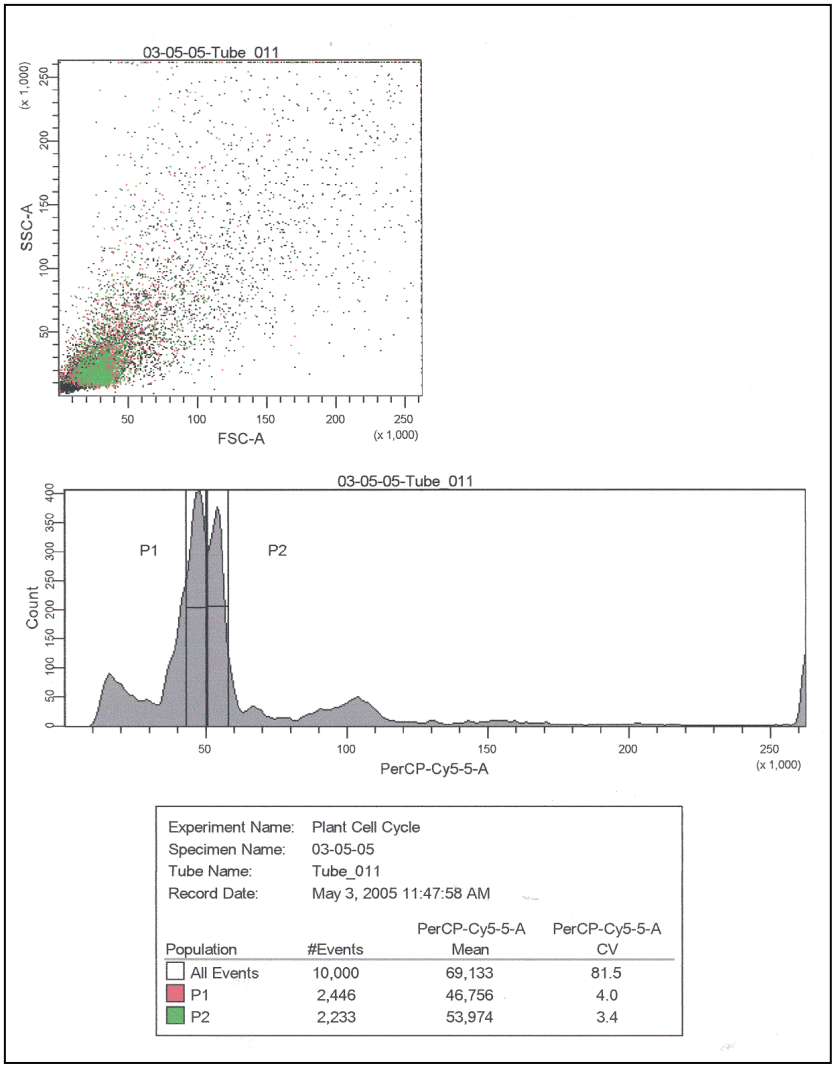
P2 = *Puccinellia arctica* Yukon (2X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	16-12-04
Tube Name:	Tube_001
Record Date:	Dec 16, 2004 7:36:26 PM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	74,083	63.0
P1	1,981	44,802	6.6
P2	5,077	65,684	6.1

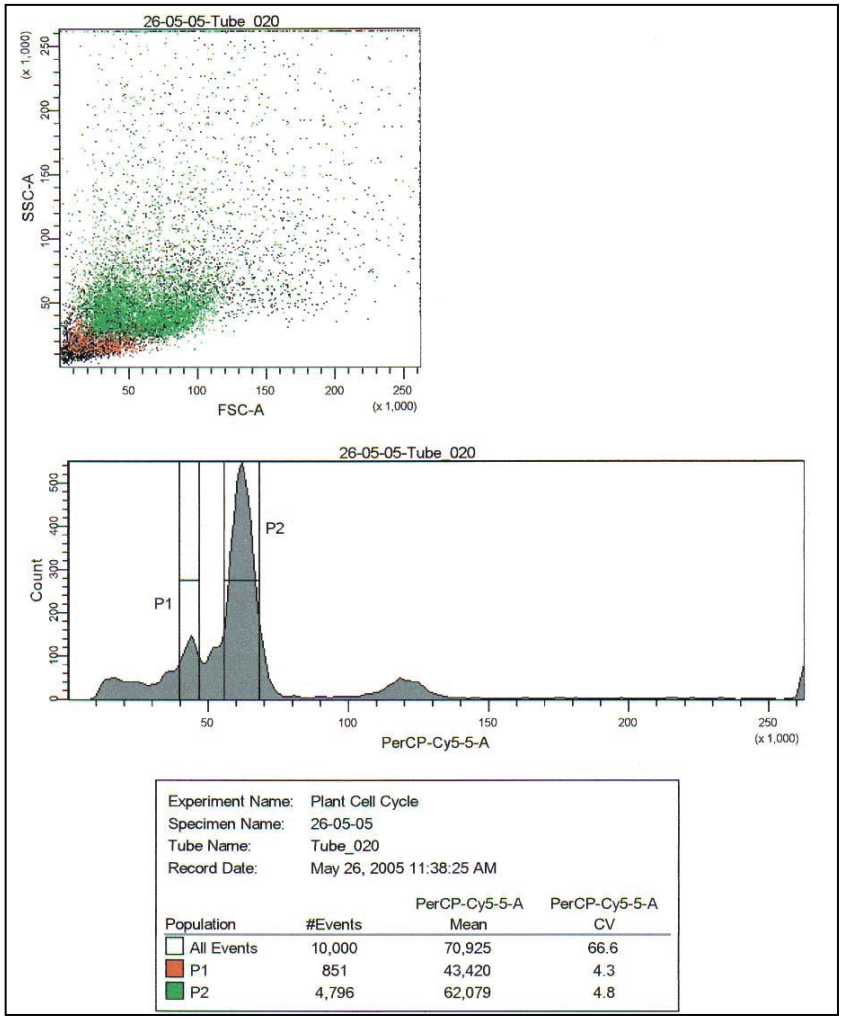
P2 = *P. banksiensis* (2X)



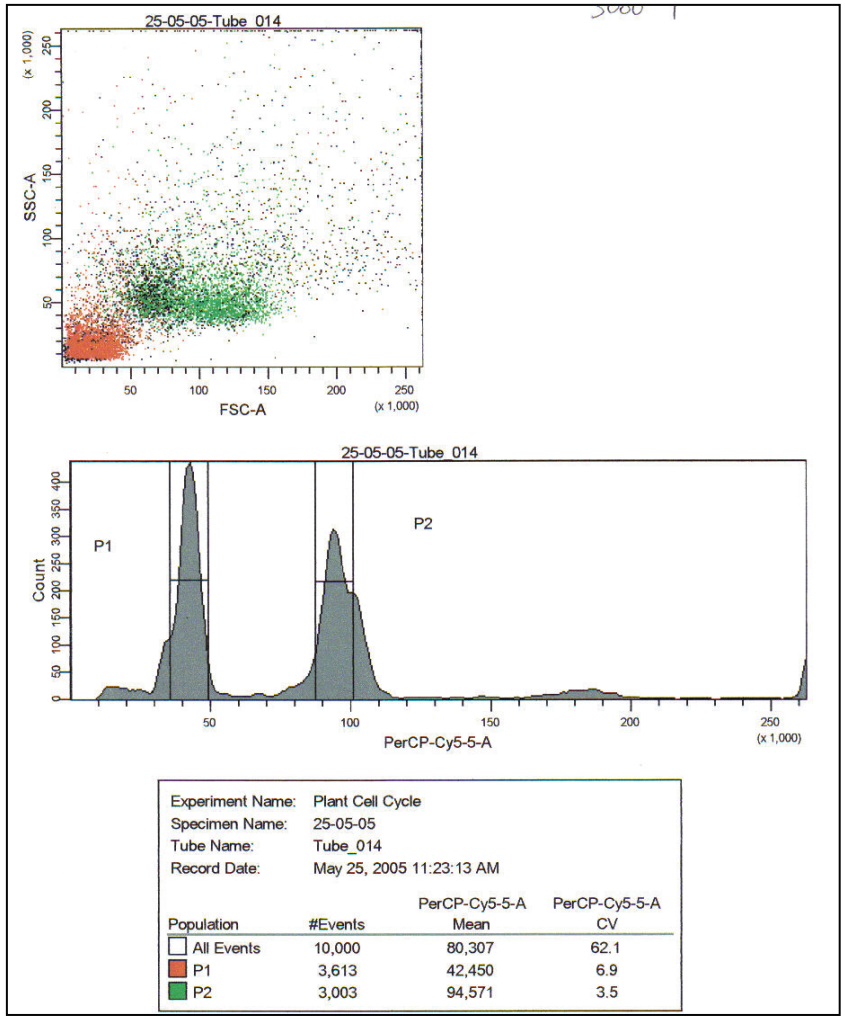
Experiment Name:	Plant Cell Cycle
Specimen Name:	03-05-05
Tube Name:	Tube_011
Record Date:	May 3, 2005 11:47:58 AM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	69,133	81.5
P1	2,446	46,756	4.0
P2	2,233	53,974	3.4

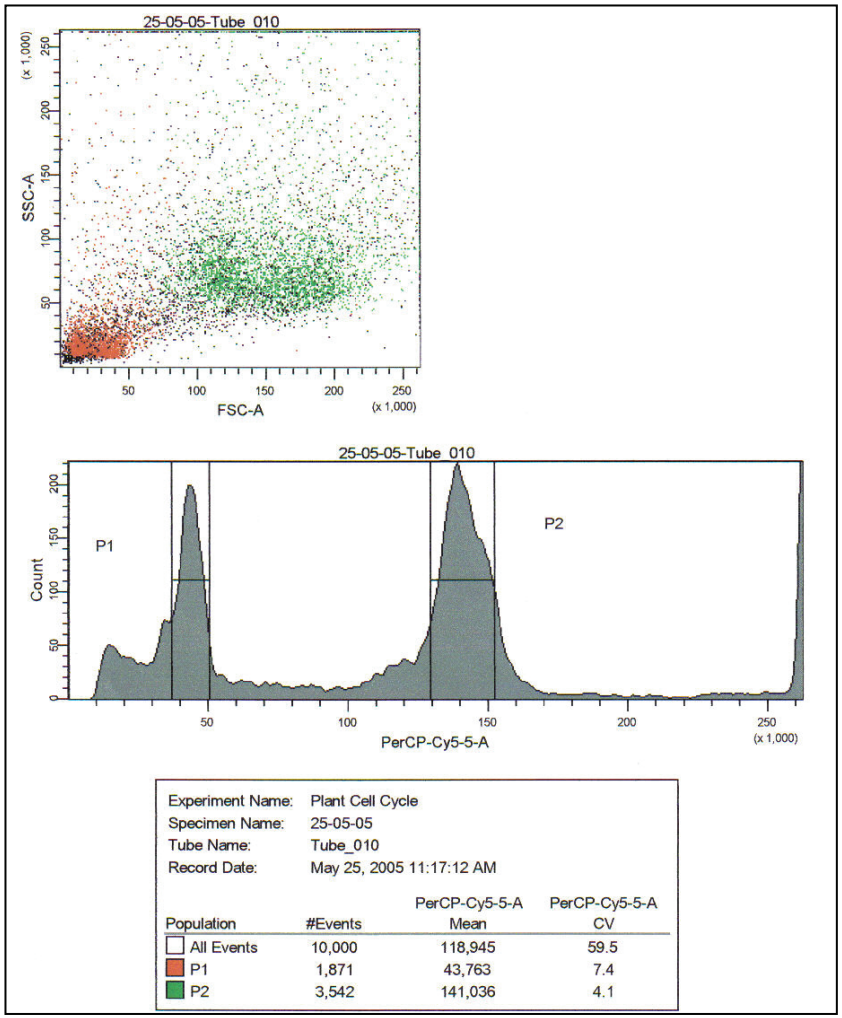
P2 = *P. vahliana* 3078_2 (2X)



P2 = *P. bruggemannii* 3080_9 (4X)



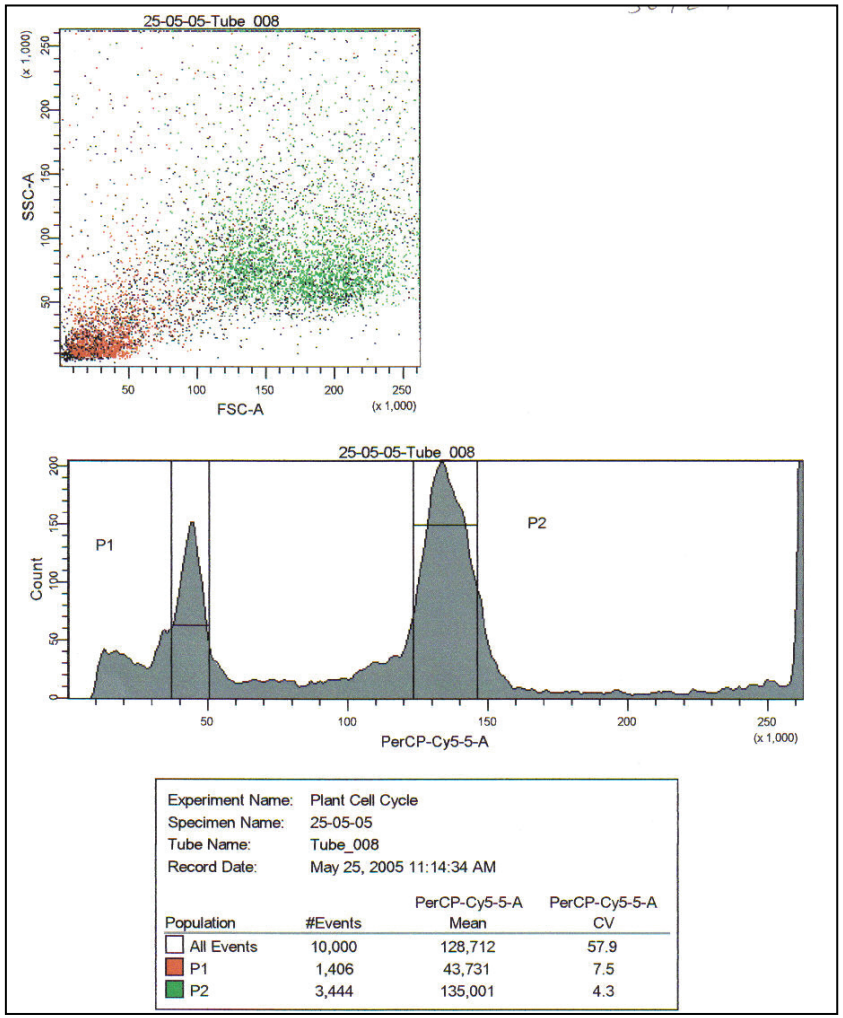
P. angustata 3154_1 (6X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	25-05-05
Tube Name:	Tube_010
Record Date:	May 25, 2005 11:17:12 AM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	118,945	59.5
P1	1,871	43,763	7.4
P2	3,542	141,036	4.1

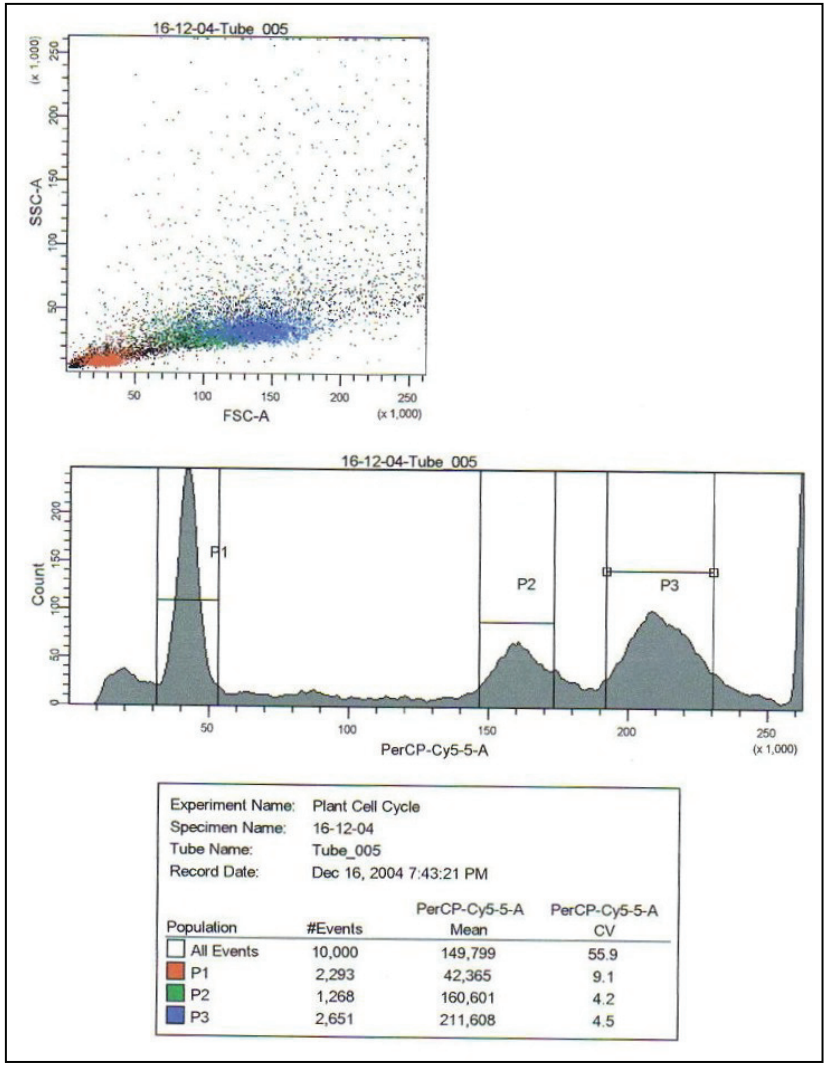
P. angustata 3072_4 (6X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	25-05-05
Tube Name:	Tube_008
Record Date:	May 25, 2005 11:14:34 AM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	128,712	57.9
P1	1,406	43,731	7.5
P2	3,444	135,001	4.3

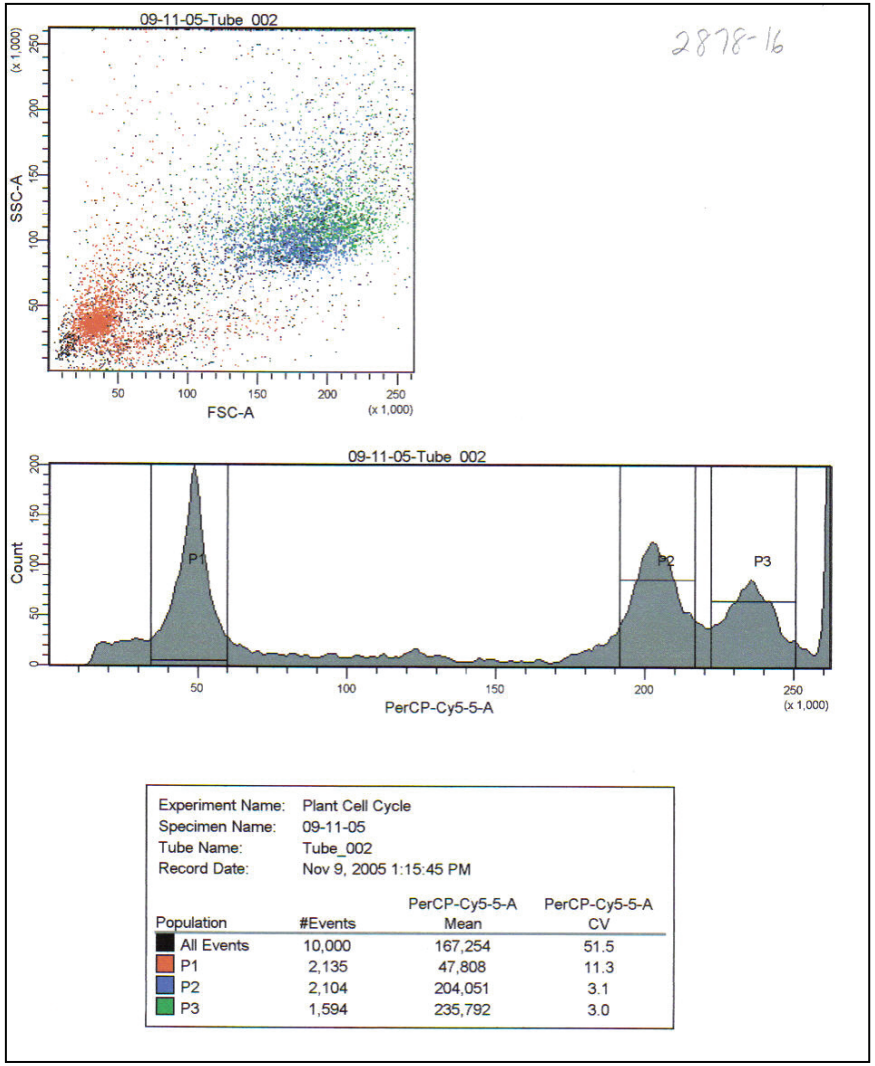
P. angustata P2 = 2866-19 (6X) and
P. andersonii P3 = 2878-14 (8X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	16-12-04
Tube Name:	Tube_005
Record Date:	Dec 16, 2004 7:43:21 PM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	149,799	55.9
P1	2,293	42,365	9.1
P2	1,268	160,601	4.2
P3	2,651	211,608	4.5

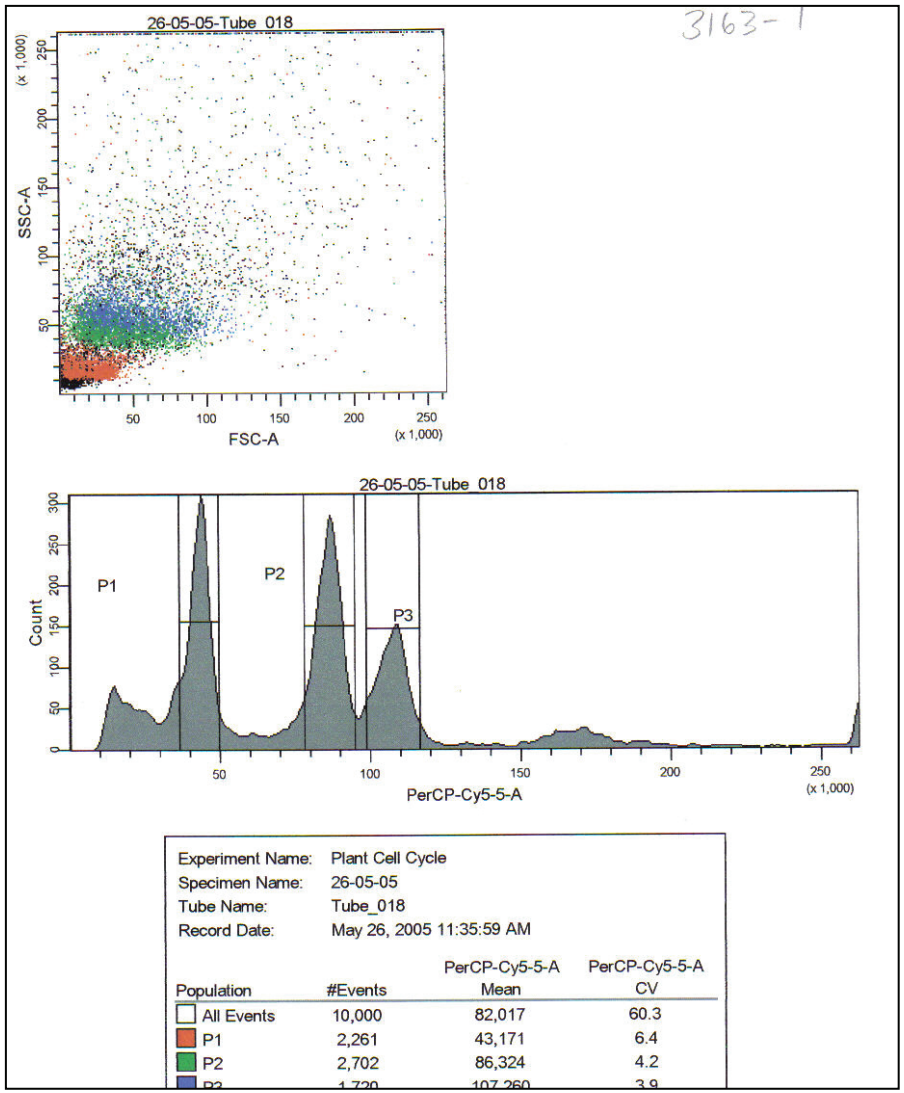
P. andersonii 2856_12 (7X) + 2878_16 (8X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	09-11-05
Tube Name:	Tube_002
Record Date:	Nov 9, 2005 1:15:45 PM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	167,254	51.5
P1	2,135	47,808	11.3
P2	2,104	204,051	3.1
P3	1,594	235,792	3.0

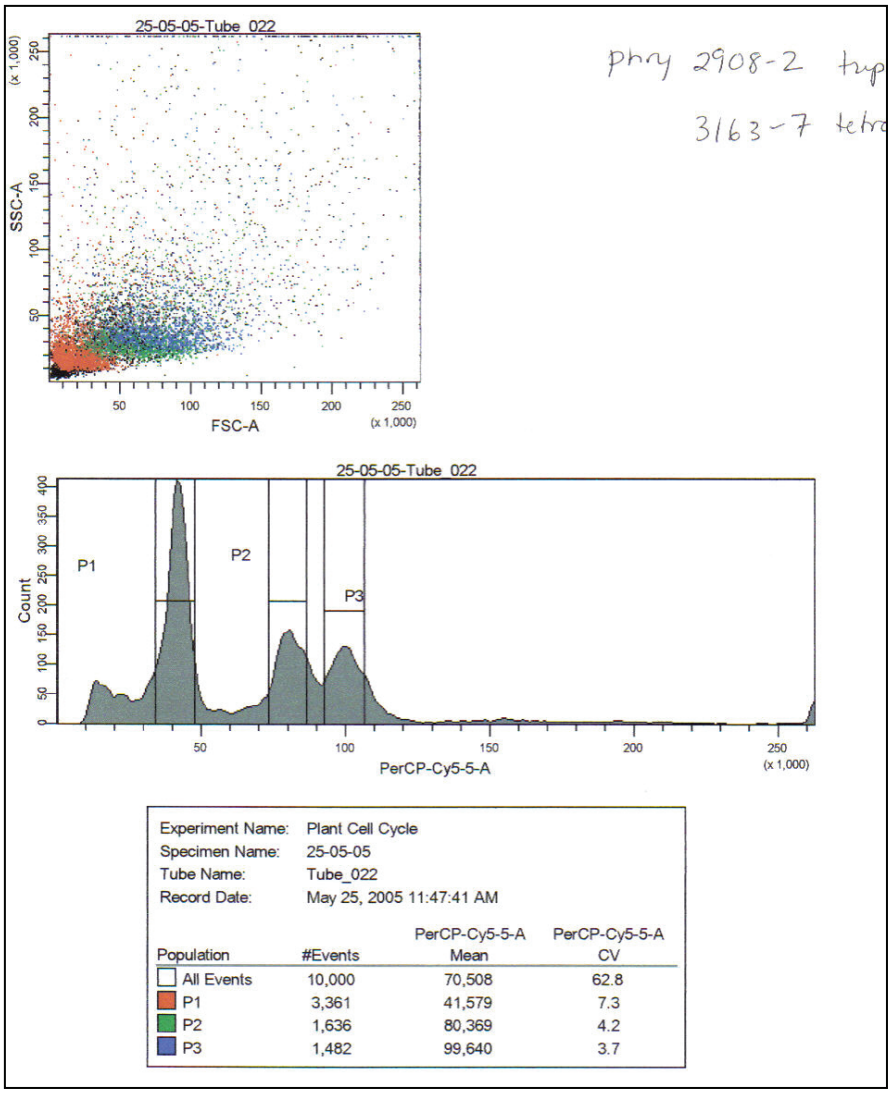
P. phryganodes P2 = 2818_17 (3X) ; P3 = 3163_1 (4X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	26-05-05
Tube Name:	Tube_018
Record Date:	May 26, 2005 11:35:59 AM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	82,017	60.3
P1	2,261	43,171	6.4
P2	2,702	86,324	4.2
P3	1,720	107,260	3.9

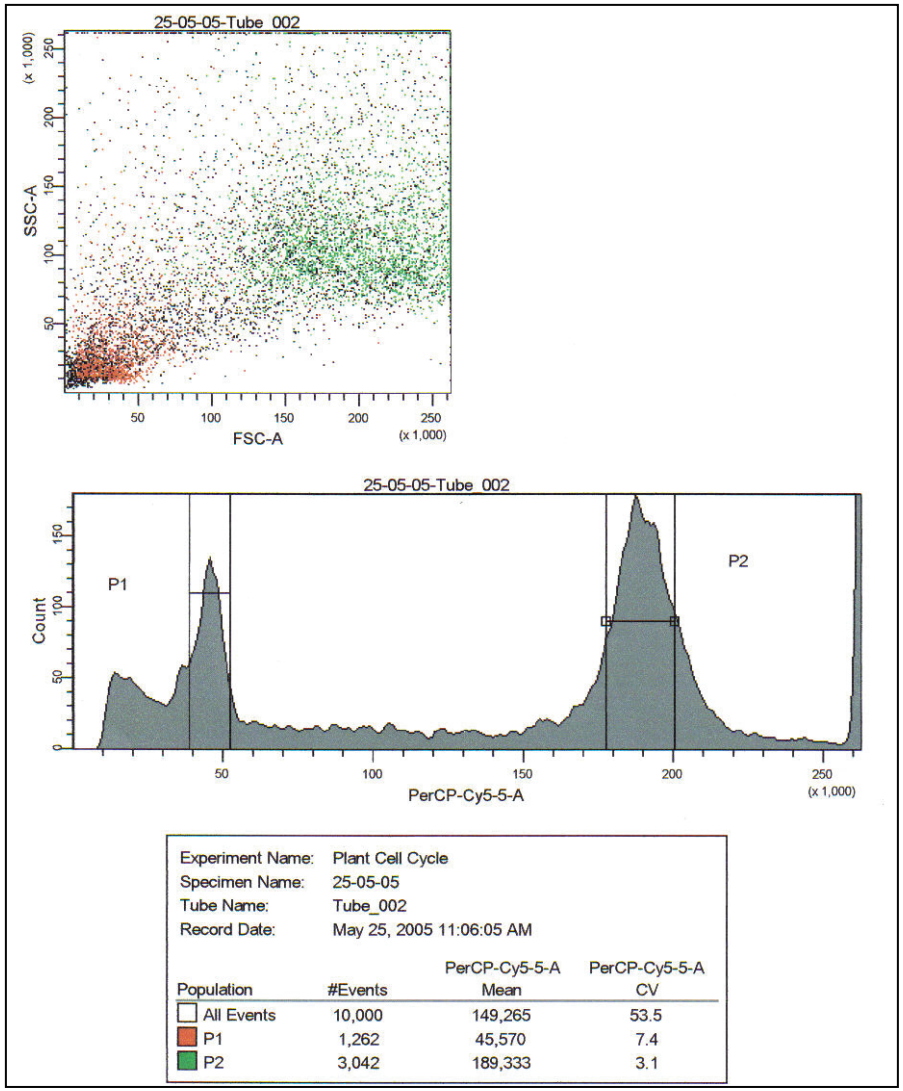
P. phryganodes P2 = 2908_2 (3X); P3 = 3163_7 (4X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	25-05-05
Tube Name:	Tube_022
Record Date:	May 25, 2005 11:47:41 AM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	70,508	62.8
P1	3,361	41,579	7.3
P2	1,636	80,369	4.2
P3	1,482	99,640	3.7

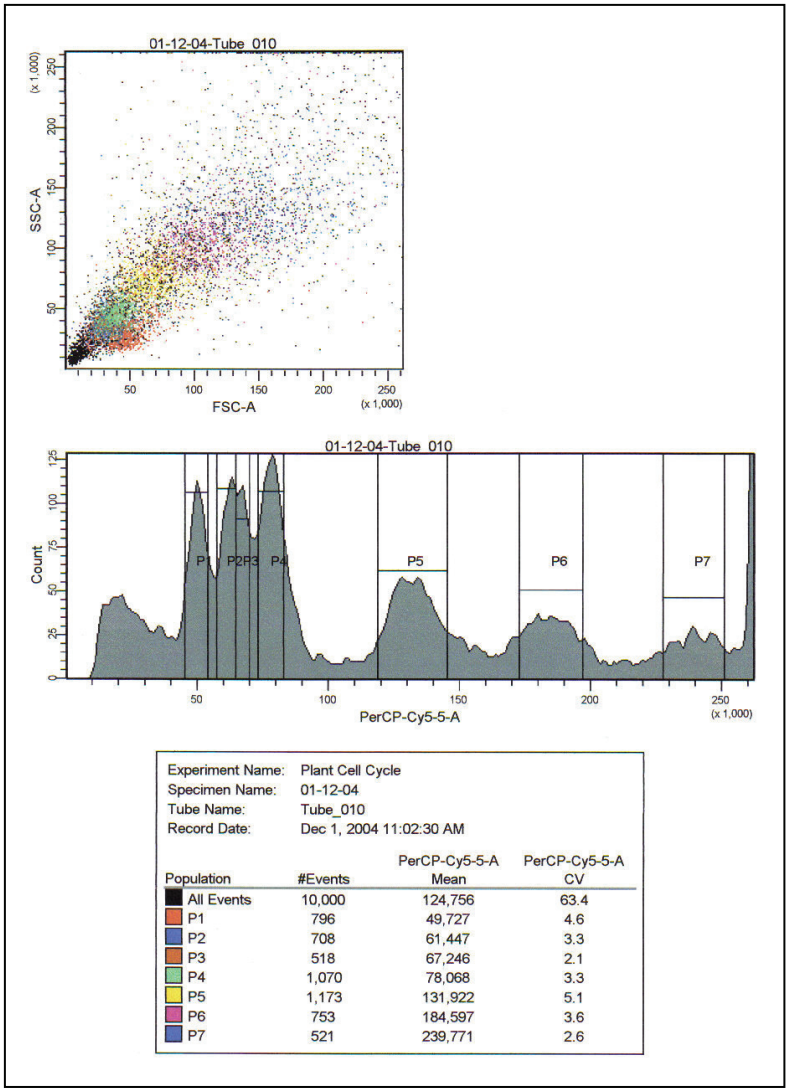
P2 = *P.* sp. 2 2848_8 (8X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	25-05-05
Tube Name:	Tube_002
Record Date:	May 25, 2005 11:06:05 AM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	149,265	53.5
P1	1,262	45,570	7.4
P2	3,042	189,333	3.1

P1 = ACD blood; P2 = *P. banksiensis* 2810_5 (2X); P3 = *P. arctica* (*P. agrostidea*) 2823_13 (2X) ; P4 = *P. vahliana* 3101_2 (2X); P5 = *P. bruggemannii* 3100_1 (4X); P6 = *P. angustata* 2866_19 (6X); P7 = *P. andersonii* 2877_5 (8X)



Experiment Name:	Plant Cell Cycle
Specimen Name:	01-12-04
Tube Name:	Tube_010
Record Date:	Dec 1, 2004 11:02:30 AM

Population	#Events	PerCP-Cy5-5-A Mean	PerCP-Cy5-5-A CV
All Events	10,000	124,756	63.4
P1	796	49,727	4.6
P2	708	61,447	3.3
P3	518	67,246	2.1
P4	1,070	78,068	3.3
P5	1,173	131,922	5.1
P6	753	184,597	3.6
P7	521	239,771	2.6

Appendix B. Flow cytometry table

Flow cytometry values for specimens sampled in this study. Fluor. = fluorescence; crbc = Chicken red blood cells; cv = coefficient of variation; propdip = proportion of diploid DNA amount; pg/2C = amount of DNA calculated per cell. Parcstand = *P. arctica* standard diploid, confirmed with meiotic chromosome counts. Replicate readings made on a different set of leaves on different days except Parcstand1- Parcstand5 were different sets of leaves run on the same day. Parc3 = a second standard calibrated in case of death of first standard. Plants coded with first three letters of their original id, and placed in the table under their new id. D6 individuals underlined and italicized.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
Standard *P. arctica 36154*, from Yukon							
PARCSTAND1	51519	4.8	40806	5.6	0.95	2.94	0.22
PARCSTAND2	49674	4.9	38899	6.3	0.96	2.98	0.24
PARCSTAND3	49310	5.2	37587	6.0	0.98	3.06	0.24
PARCSTAND4	55295	5.4	41336	6.8	1.00	3.12	0.27
PARCSTAND5	53777	6.0	41743	7.1	0.97	3.00	0.28
PARCSTAND	60856	3.9	48077	6.0	0.95	2.95	0.21
PARCSTAND	45781	6.2	35816	8.3	0.96	2.98	0.31
PARCSTAND	57099	6.3	42760	7.3	1.00	3.11	0.30
PARCSTAND	65478	4.1	48079	7.0	1.02	3.17	0.26
PARCSTAND	39353	5.0	28549	7.6	1.03	3.21	0.29
PARCSTAND	65104	4.4	44393	5.3	1.10	3.42	0.24
PARCSTAND	100324	4.0	67680	6.3	1.11	3.45	0.26
mean					1.00	3.11	0.05
P. arctica 3							
PARC3	63718	4.7	51786	6.5	0.92	2.87	0.23
PARC3	68352	4.5	51721	4.1	0.99	3.08	0.19
PARC3	56706	6.0	41869	7.8	1.01	3.16	0.31
PARC3	69663	4.9	48999	4.0	1.07	3.31	0.21
PARC3	60503	5.4	40025	7.7	1.13	3.52	0.33
PARC3	52721	4.6	42051	5.2	0.94	2.93	0.20
PARC3	40897	4.6	30568	6.9	1.00	3.12	0.26
PARC31	59685	5.5	46769	6.6	0.96	2.97	0.26
PARC300MG	56645	3.6	46223	5.9	1.01	3.14	0.20
PARC32	57469	4.9	47251	6.6	0.91	2.83	0.23
PARC325MG	56926	3.9	46816	6.2	1.00	3.11	0.21

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
PARC350MG	56081	4.3	45793	5.9	1.01	3.14	0.21
mean					1.00	3.10	0.06
P. arctica (orig. P. agrostidea)							
AGR2813-07	47428	5.0	38186	6.8	0.99	3.08	0.24
AGR2813-10	44190	5.2	34415	7.6	1.02	3.19	0.28
AGR2813-14	47925	11.1	48550	11.3	1.00	3.12	0.49
AGR2813-17	47051	4.8	38314	6.7	0.98	3.05	0.24
AGR2813-27	59345	8.3	59378	8.5	0.89	2.77	0.37
AGR2823-03	45602	5.1	35869	5.4	1.01	3.15	0.22
AGR2823-13	67657	2.3	50129	6.6	1.01	3.14	0.22
AGR2823-22	50867	10.8	50197	10.8	1.06	3.31	0.48
AGR2823-24	43413	5.3	33215	6.4	1.04	3.24	0.25
AGR99-210	45452	4.9	36914	7.0	0.92	2.87	0.25
AGR99-210	56972	3.8	48499	4.7	0.97	3.01	0.17
mean					1.00	3.08	0.05
P. arctica							
ARC2805-03	45969	4.7	35894	8.2	1.02	3.18	0.28
ARC2805-04	47444	4.7	36185	10.5	1.05	3.25	0.35
ARC2805-06	46759	4.3	36807	8.2	1.01	3.15	0.27
ARC2805-11	57092	7.7	58535	6.9	0.95	2.96	0.31
ARC2805-14	56393	4.4	45818	5.2	1.01	3.15	0.20
ARC2805-14	55925	4.1	44396	5.4	1.04	3.23	0.20
ARC2805-09	63026	7.0	61546	8.8	0.99	3.06	0.36
ARC2808-04	42959	4.5	33811	5.6	1.01	3.15	0.21
ARC2808-08	42637	5.3	31652	7.1	1.07	3.34	0.28
ARC2808-13	61029	7.1	61546	8.8	0.95	2.97	0.35
ARC2808-14	60379	7.1	58535	6.9	1.01	3.13	0.32
ARC2808-20	48315	11.7	50197	10.8	0.93	2.89	0.48
ARC2808-24	55854	4.1	43462	5.6	1.06	3.29	0.21
ARC2805-15	52308	5.3	44175	4.1	0.97	3.03	0.18
ARC2805-15	62548	5.8	48743	5.9	1.06	3.29	0.25
ARC2805-15	65785	4.4	50644	5.6	1.07	3.33	0.22
ARC2817-01	57974	5.1	45374	5.2	1.05	3.27	0.22
ARC2817-08	56180	5.1	45144	5.1	1.02	3.19	0.21
ARC2817-12	57861	5.1	45415	5.3	1.05	3.26	0.22
ARC2817-13	57438	4.8	47941	4.8	0.99	3.07	0.19
ARC2817-17	58434	5.6	42147	8.1	1.14	3.55	0.32
ARC2817-20	57935	5.5	41736	7.9	1.14	3.55	0.31
ARC2817-05	50401	10.4	46738	8.9	1.08	3.35	0.46
ARC2817-06	44919	8.4	46598	7.4	0.97	3.02	0.34
mean					1.03	3.19	0.03
P. arctica (orig. P. poacea)							
POA2864-10	44209	4.4	35289	5.9	1.00	3.11	0.21

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
POA2864-11	48364	10.8	48550	11.3	1.01	3.14	0.48
POA2864-14	56712	4.0	44972	4.8	1.01	3.13	0.18
POA2864-17	57578	5.0	47426	4.9	0.91	2.83	0.20
POA2864-23	58704	7.5	44709	8.0	0.98	3.04	0.45
POA2864-07	48002	10.0	46738	8.9	1.03	3.19	0.43
POA2894-01	59880	6.9	44709	8.0	1.00	3.10	0.44
POA2898-01	48907	4.7	35644	5.9	1.03	3.20	0.24
POA2898-14	54951	4.1	43416	4.9	1.01	3.14	0.19
POA2898-14	48731	8.2	50829	7.5	0.99	3.08	0.33
POA2898-20	63451	6.7	60809	7.3	1.04	3.25	0.32
POA2898-22	53850	4.1	44540	4.9	0.96	3.00	0.18
POA2898-22	62195	5.3	50762	5.9	0.92	2.85	0.23
POA2898-22	44147	4.3	35901	6.1	0.98	3.05	0.21
POA2898-03	47673	4.8	36240	6.0	0.99	3.07	0.24
mean					1.00	3.10	0.03
P. andersonii							
AND2848-09	185196	5.3	40721	9.9	3.41	10.60	1.19
ANG2851-02	192823	3.4	42290	8.6	3.42	10.62	0.98
ANG2851-03	175686	5.1	46196	8.1	3.80	11.83	1.13
ANG2851-18	201936	2.6	44687	7.0	3.39	10.53	0.79
ANG2851-12	180297	3.9	47965	6.5	3.76	11.69	0.89
AND2856-02	226527	4.3	50781	7.8	3.35	10.43	0.93
AND2856-02	183631	3.2	40785	8.5	3.37	10.49	0.95
AND2856-06	170986	6.4	46160	7.6	3.70	11.52	1.14
AND2856-09	181261	3.8	48456	7.1	3.74	11.63	0.94
AND2865-01	184688	4.8	50163	7.1	3.68	11.45	0.98
AND2865-09	193756	6.3	42085	9.3	3.45	10.73	1.20
AND2865-11	180693	4.0	39001	8.6	3.47	10.79	1.02
AND2865-12	199984	4.9	54664	8.9	3.69	11.47	1.16
AND2865-13	205812	3.2	56525	8.6	3.64	11.32	1.04
AND2865-17	183869	5.0	49296	7.5	3.73	11.60	1.05
AND2865-20	183440	5.9	49531	8.3	3.70	11.52	1.17
AND2877-01	173479	2.3	42716	6.6	3.34	10.40	0.66
AND2877-03	172412	5.3	45306	9.0	3.81	11.84	1.24
AND2877-05	224085	3.7	50197	10.8	3.53	10.98	1.55
AND2877-05	240723	4.2	50129	6.6	3.60	11.19	0.88
AND2877-05	187030	5.4	36564	9.2	3.83	11.92	1.27
AND2877-05	208871	5.1	63953	6.7	4.01	12.47	0.83
AND2877-06	189135	4.8	40923	8.4	3.46	10.77	1.04
AND2877-17	203585	4.8	54664	8.9	3.75	11.67	1.17
AND2877-18	195979	4.9	43187	7.6	3.40	10.57	0.96
AND2877-18	189912	4.6	36927	10.1	3.85	11.98	1.33
AND2877-19	122860	5.3	27446	4.5	3.35	10.43	0.73
AND2877-25	142415	5.3	27949	13.1	3.82	11.87	1.68
AND2877-26	134501	4.5	26483	11.5	3.80	11.83	1.46

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
AND2877-27	134757	4.7	26206	15.6	3.85	11.98	1.95
BRU2878-01	154901	5.2	33259	8.5	3.49	10.85	1.08
BRU2878-09	136908	4.7	28899	10.0	3.55	11.04	1.22
BRU2878-09	139551	4.5	28190	10.9	3.71	11.53	1.36
BRU2878-10	183202	5.8	35499	9.8	3.87	12.02	1.37
BRU2878-10	233593	3.7	67401	7.1	3.47	10.78	0.86
BRU2878-14	166292	5.4	44709	8.0	3.72	11.57	1.12
BRU2878-14	213665	4.6	54664	8.9	3.94	12.25	1.22
BRU2878-14	211608	4.5	42365	9.1	3.74	11.64	1.18
BRU2878-15	152329	3.9	32758	8.1	3.48	10.83	0.97
BRU2878-16	180687	3.4	39163	8.2	3.46	10.75	0.95
BRU2878-16	205281	4.6	41606	10.3	3.70	11.50	1.30
BRU2878-16	196660	4.3	48550	11.3	4.11	12.78	1.52
mean					3.65	11.34	0.09
putative heptaploids							
AND2848-15	208672	5.0	48395	6.9	3.23	10.05	0.86
AND2848-05	226140	6.6	52436	8.3	3.23	10.05	1.07
BRU2878-19	179511	3.2	39121	8.0	3.44	10.52	0.92
AND2856-12	157338	4.9	47646	7.5	3.30	10.27	0.92
AND2865-09	154290	5.0	46620	7.7	3.31	10.29	0.94
AND2865-18	201197	3.0	45450	7.5	3.32	10.31	0.83
ANG2851-14	212214	2.6	48999	6.0	3.24	10.09	0.66
					3.28	10.19	0.14
P. angustata							
ANG2419-01	165424	4.6	54664	8.9	3.05	9.49	0.94
ANG2420-01	171942	3.8	59344	12.3	2.79	8.69	1.24
ANG2420-01	168501	4.7	61546	8.8	3.11	9.66	0.83
ANG2807-01	169935	4.5	62736	8.2	2.71	8.42	0.79
ANG2849-01	160810	4.5	59378	8.5	2.71	8.42	0.81
ANG2851-01	173897	4.5	64175	6.4	2.71	8.43	0.66
ANG2851-04	171019	3.5	53646	5.8	2.39	7.43	0.50
ANG2851-04	144272	2.8	42081	6.6	2.57	7.99	0.57
ANG2851-05	166209	2.8	51800	5.6	2.40	7.48	0.47
ANG2851-05	149838	2.8	45023	6.2	2.49	7.75	0.53
ANG2851-08	167422	2.8	52369	5.4	2.40	7.45	0.45
ANG2851-08	142835	3.1	41465	6.9	2.58	8.03	0.61
ANG2851-09	155088	7.1	50197	10.8	2.98	9.26	1.24
ANG2851-13	150129	5.1	54664	8.9	2.75	8.54	0.88
ANG2851-16	170064	2.5	55066	5.5	2.31	7.20	0.43
BRU2853-01	156488	4.1	45691	9.3	2.57	7.98	0.81
BRU2853-02	168285	3.8	53248	8.4	2.37	7.36	0.68
BRU2853-02	152788	2.2	45283	6.7	2.53	7.86	0.55
BRU2853-03	165884	2.9	51618	5.9	2.41	7.49	0.49

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
BRU2853-05	169102	4.8	62687	8.1	2.70	8.39	0.79
BRU2853-06	167275	2.9	52564	6.0	2.38	7.41	0.49
BRU2853-06	147772	2.7	42801	7.0	2.59	8.04	0.60
BRU2853-07	152598	5.2	54664	8.9	2.81	8.75	0.89
BRU2853-08	160478	3.5	46011	6.8	2.61	8.13	0.62
BRU2853-09	168429	5.5	54664	8.9	3.11	9.66	1.00
BRU2853-15	170491	2.5	54200	5.4	2.36	7.33	0.44
BRU2853-15	150655	2.6	44958	6.7	2.51	7.81	0.56
BRU2853-16	164822	2.8	50940	5.4	2.42	7.54	0.46
AND2856-02	189752	4.4	61724	7.9	3.07	9.56	0.86
ANG2856-10	148646	2.4	43664	7.8	2.55	7.93	0.65
ANG2856-10	140620	5.2	50102	6.8	2.81	8.73	0.75
ANG2856-12	186671	4.5	50397	7.8	2.78	8.66	0.78
AND2856-12	185917	1.9	48771	5.7	3.14	9.76	0.53
BRU2858-11	136958	5.1	48507	7.1	2.82	8.78	0.77
BRU2858-12	137478	5.1	48548	7.1	2.83	8.81	0.77
BRU2858-14	163869	5.5	54664	8.9	3.02	9.40	0.98
BRU2858-14	165891	4.5	63953	6.7	2.61	8.13	0.65
BRU2858-02	151540	6.9	50197	10.8	2.91	9.05	1.20
BRU2858-03	178128	4.3	66611	7.1	2.67	8.32	0.69
AND2865-05	167952	2.6	40846	7.3	3.08	9.58	0.74
AND2865-05	183996	4.7	59210	9.6	3.11	9.66	1.03
AND2865-14	178072	3.2	46638	7.2	2.86	8.90	0.70
AND2865-14	165882	2.8	40861	8.2	3.04	9.46	0.82
ANG2866-01	136911	5.0	48550	11.3	2.77	8.63	1.15
ANG2866-01	144554	5.2	49345	7.5	3.02	9.40	0.78
ANG2866-14	188955	3.8	49665	4.5	2.85	8.86	0.52
ANG2866-15	160332	4.4	47039	6.0	2.55	7.94	0.59
ANG2866-19	134535	5.4	46738	8.9	2.50	7.76	0.93
ANG2866-19	183932	5.4	50129	6.6	2.75	8.55	0.73
ANG2866-21	151253	3.8	44855	8.3	2.53	7.86	0.72
ANG2866-24	143783	3.7	54783	7.5	2.62	8.16	0.68
ANG2866-03	143884	4.1	41010	8.4	2.63	8.17	0.76
ANG2866-04	149901	4.4	42252	6.6	2.66	8.27	0.66
ANG2866-07	149003	3.7	43798	8.7	2.55	7.93	0.75
ANG2866-08	141869	4.1	39190	9.8	2.71	8.43	0.90
ANG2866-09	143521	4.9	48550	11.3	3.00	9.33	1.13
ANG2874-12	145630	4.3	39978	6.3	2.73	8.49	0.65
ANG2874-13	162778	4.6	60033	7.6	2.71	8.43	0.75
ANG2874-14	134235	5.5	47810	8.0	2.81	8.73	0.85
ANG2874-15	131139	5.0	48550	11.3	2.74	8.53	1.04
ANG2874-16	114533	3.6	32368	11.4	2.65	8.24	0.99
ANG2874-19	132475	5.7	46818	7.4	2.83	8.80	0.82
ANG2874-21	106560	4.6	27304	13.6	2.92	9.09	1.31
ANG2874-24	103466	4.6	25855	5.2	3.00	9.32	0.65
ANG2874-03	160599	4.4	47443	5.8	2.54	7.89	0.57

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
ANG2874-04	138387	2.7	39888	7.6	2.60	8.08	0.65
ANG2874-07	141038	5.0	48550	11.3	2.95	9.17	1.12
ANG2874-08	117988	4.1	34175	8.5	2.59	8.04	0.76
AND2877-20	147873	5.4	54664	8.9	2.73	8.48	0.88
AND2877-20	172309	4.4	63457	7.4	2.72	8.44	0.73
AND2877-24	162256	4.7	44831	8.0	2.72	8.46	0.78
AND2877-24	134646	5.3	47927	7.5	2.81	8.74	0.80
BRU2878-06	152430	4.5	45025	8.9	2.54	7.89	0.79
BRU2878-07	112852	5.4	31880	9.5	2.65	8.25	0.90
BRU2878-10	175102	4.7	67401	7.1	3.19	9.94	0.86
BRU2878-16	144608	4.4	48550	11.3	2.82	8.76	1.52
BRU2878-17	129074	4.9	61650	8.4	2.79	8.68	0.84
BRU2878-20	109923	4.9	29295	9.1	2.81	8.74	0.90
BRU2878-20	132240	4.3	35635	8.9	2.78	8.65	0.85
BRU2878-23	184679	3.5	54664	8.9	2.74	8.52	0.93
BRU2878-23	157299	5.4	56525	8.6	2.90	9.02	0.94
ANG2899-19	173583	4.6	63953	6.7	2.71	8.44	0.69
ANG2899-08	152537	6.1	54664	8.9	2.81	8.75	0.94
BRU3072-04	135001	4.3	43731	7.5	2.54	7.90	0.62
BRU3072-10	168477	3.6	50460	6.6	2.50	7.78	0.58
BRU3072-12	154053	4.2	40546	8.5	2.85	8.85	0.84
BRU3072-13	157873	5.0	42288	9.5	2.80	8.70	0.93
BRU3072-20	138376	4.2	44571	6.9	2.56	7.95	0.58
BRU3072-23	135047	3.5	41777	6.6	2.66	8.28	0.56
BRU3072-23	147990	5.1	37838	9.5	2.93	9.11	0.98
BRU3072-24	171569	3.0	43887	9.1	2.93	9.11	0.87
ANG3137-01	164713	3.6	48203	7.3	2.56	7.96	0.65
ANG3137-02	176458	5.4	47407	7.8	2.79	8.67	0.82
ANG3137-03	152424	4.2	41411	8.6	2.76	8.58	0.82
ANG3140-01	160566	5.7	41815	8.7	2.88	8.95	0.93
ANG3141-02	156337	6.0	40373	9.1	2.90	9.02	0.98
ANG3141-03	154791	5.8	40104	10.4	2.89	8.99	1.07
ANG3144-03	141440	3.2	44465	6.6	2.62	8.14	0.54
ANG3144-01	152041	6.3	38328	9.0	2.97	9.24	1.02
ANG3144-02	161196	4.3	46668	7.8	2.59	8.05	0.72
ANG3144-05	153226	5.9	39690	8.1	2.89	9.00	0.90
ANG3144-06	175100	3.7	47717	5.6	2.75	8.55	0.57
ANG3144-06	152133	6.0	39972	8.2	2.85	8.87	0.90
ANG3144-07	159317	4.1	42586	6.9	2.80	8.72	0.70
ANG3144-08	163472	5.2	46603	7.3	2.63	8.17	0.73
ANG3144-09	145998	7.2	36596	10.5	2.99	9.30	1.18
ANG3144-10	141878	5.6	41508	9.8	2.56	7.96	0.90
ANG3144-11	154942	4.3	42723	8.0	2.72	8.45	0.77
ANG3144-12	152947	5.0	39263	9.6	2.92	9.08	0.98
ANG3144-13	167467	3.1	47993	7.2	2.61	8.13	0.64
ANG3144-14	170714	5.0	49439	6.5	2.59	8.05	0.66

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
ANG3144-16	151506	4.4	39485	7.1	2.87	8.94	0.75
ANG3144-17	114339	5.5	37270	9.2	2.30	7.15	0.77
ANG3144-19	143871	5.1	37203	7.6	2.90	9.01	0.82
ANG3144-20	160193	5.0	43088	8.7	2.79	8.66	0.87
ANG3144-20	146166	6.3	37091	10.4	2.95	9.18	1.12
ANG3144-21	138478	4.7	36080	6.7	2.88	8.94	0.73
ANG3144-22	142822	5.7	36564	9.2	2.93	9.10	0.98
ANG3144-22	141597	6.3	35499	9.8	2.99	9.29	1.08
ANG3144-23	147082	6.0	39847	9.2	2.77	8.60	0.94
ANG3150-01	161250	5.6	43696	8.3	2.76	8.60	0.86
ANG3150-02	161238	5.4	45907	8.2	2.63	8.18	0.80
ANG3150-03	159564	3.8	47132	7.2	2.54	7.89	0.64
ANG3150-04	166566	3.0	46666	7.9	2.67	8.32	0.70
ANG3150-05	146473	5.6	38371	8.2	2.86	8.89	0.88
ANG3151-02	169404	3.6	49780	7.2	2.55	7.93	0.64
ANG3151-03	155454	3.9	46350	7.7	2.51	7.81	0.67
BRU3154-01	141036	4.1	43763	7.4	2.65	8.25	0.64
BRU3154-02	157729	3.8	48224	6.8	2.45	7.62	0.59
BRU3154-02	128419	3.8	37840	9.3	2.54	7.91	0.79
BRU3154-03	159925	6.2	43937	8.6	2.73	8.48	0.90
BRU3154-04	146908	8.6	35805	11.5	3.07	9.56	1.37
BRU3154-06	164925	2.7	49501	5.9	2.50	7.76	0.50
BRU3154-06	159887	5.2	43417	8.3	2.76	8.58	0.84
BRU3154-09	123752	4.2	36250	9.9	2.56	7.95	0.86
BRU3154-09	148719	8.2	37741	10.8	2.95	9.18	1.25
BRU3154-10	162435	5.2	45546	8.2	2.67	8.31	0.81
BRU3154-11	122585	4.1	34629	8.6	2.65	8.25	0.79
BRU3154-15	159874	3.7	47208	7.0	2.54	7.89	0.62
BRU3154-16	138058	4.8	36814	9.0	2.81	8.74	0.89
BRU3154-17	160589	5.8	45321	9.0	2.65	8.26	0.88
BRU3154-18	120909	3.9	34921	6.9	2.59	8.07	0.64
BRU3154-18	157995	4.0	44907	7.4	2.64	8.20	0.69
BRU3154-19	117054	4.8	32757	8.9	2.68	8.33	0.84
BRU3154-20	149752	6.3	39219	9.5	2.86	8.90	1.01
BRU3154-21	139852	4.1	43600	7.4	2.64	8.21	0.63
BRU3154-23	125620	4.3	35525	9.2	2.65	8.24	0.84
BRU3154-25	115335	4.8	32833	9.9	2.63	8.18	0.90
BRU3154-26	122903	4.6	37010	8.6	2.49	7.74	0.75
BRU3154-27	123279	4.8	35259	9.6	2.62	8.15	0.87
ANG3161-06	140787	3.2	43972	6.5	2.64	8.20	0.54
ANG2853-14	152749	6.4	50197	10.8	2.93	9.12	1.19
mean					2.74	8.51	0.06
P. borealis							
BOR01-502	225110	4.3	48971	6.0	3.44	10.71	0.79

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
P. bruggemannii							
BRU2906-03	101615	6.2	48550	11.3	2.12	6.61	0.84
BRU2906-04	104306	6.0	48550	11.3	2.18	6.78	0.85
BRU2906-05	170481	3.0	83163	5.3	2.05	6.38	0.39
BRU2906-09	120255	5.2	65841	6.0	2.05	6.39	0.45
BRU2906-14	115123	5.3	58535	6.9	1.97	6.12	0.53
BRU2906-15	105150	4.5	54783	7.5	1.92	5.97	0.52
BRU3063-03	117822	4.7	46130	7.7	1.91	5.95	0.54
BRU3063-04	101366	4.1	44261	6.7	1.89	5.86	0.42
BRU3072-01	101737	5.3	45658	7.4	1.83	5.71	0.47
BRU3072-02	105053	5.5	38690	8.4	2.03	6.33	0.64
BRU3072-07	120500	4.6	49794	7.3	1.81	5.64	0.49
BRU3072-08	106141	6.2	37806	8.5	2.10	6.54	0.69
BRU3072-09	103337	6.3	39071	8.4	1.98	6.16	0.65
BRU3072-14	107916	4.8	40926	8.4	1.98	6.14	0.59
BRU3072-15	104422	5.5	41068	9.1	1.90	5.92	0.63
BRU3072-18	122272	4.2	47395	7.1	1.93	6.01	0.50
BRU3074-02	118695	4.5	47645	7.6	1.87	5.80	0.51
BRU3074-08	80345	6.9	30887	10.2	1.95	6.06	0.75
BRU3075-02	102793	6.2	38175	8.9	2.02	6.29	0.68
BRU3075-03	102768	4.0	44932	6.8	1.88	5.86	0.42
BRU3075-07	106535	5.5	39881	9.1	2.00	6.22	0.66
BRU3075-09	104932	6.6	40721	8.9	1.94	6.02	0.67
BRU3075-13	115650	5.1	44519	8.0	1.95	6.05	0.57
BRU3075-18	95317	4.0	41589	5.9	1.89	5.87	0.38
BRU3075-19	100551	4.8	38050	8.6	1.98	6.16	0.61
BRU3080-05	97972	2.4	44215	6.6	1.82	5.67	0.36
BRU3080-05	100030	5.9	43132	6.8	1.91	5.94	0.49
BRU3080-09	94571	3.5	42450	6.9	1.83	5.70	0.40
BRU3080-09	96061	5.2	41542	7.5	1.90	5.92	0.49
BRU3080-01	130499	6.3	46099	7.5	2.12	6.60	0.65
BRU3080-03	141924	4.7	49012	7.0	2.17	6.75	0.57
BRU3080-08	107578	5.1	40701	10.5	1.98	6.16	0.72
BRU3080-10	121029	4.4	46875	7.3	1.93	6.02	0.51
BRU3080-10	119470	5.5	41760	8.0	2.14	6.67	0.65
BRU3080-16	94640	3.6	42825	6.9	1.82	5.66	0.40
BRU3080-16	99851	5.0	41531	7.2	1.98	6.16	0.49
BRU3080-18	105401	5.0	40099	9.7	1.97	6.12	0.67
BRU3080-21	112001	6.4	39154	8.9	2.14	6.67	0.73
BRU3080-22	116364	6.2	42208	7.8	2.07	6.42	0.64
BRU3080-24	114450	4.6	44846	7.5	1.91	5.95	0.52
BRU3080-25	95704	3.7	43212	6.9	1.82	5.67	0.40
BRU3080-25	101811	5.0	43785	7.1	1.91	5.95	0.47
BRU3083-10	111827	5.2	45329	7.8	1.85	5.75	0.54
BRU3083-02	116361	5.1	46175	7.9	1.89	5.87	0.55

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
BRU3083-06	111226	4.7	44322	7.9	1.88	5.85	0.54
BRU3100-01	105056	5.5	42026	7.9	1.87	5.82	0.56
BRU3100-01	131394	5.4	50129	6.6	1.96	6.11	0.52
BRU3100-02	113999	3.7	41653	6.2	2.05	6.38	0.46
BRU3100-03	103876	5.9	38837	8.1	2.00	6.23	0.62
BRU3100-04	114735	4.0	41854	6.3	2.05	6.39	0.48
BRU3100-09	107992	4.5	39513	6.5	2.05	6.37	0.50
BRU3118	103712	6.0	42649	8.6	1.82	5.67	0.59
BRU3119-01	101521	6.5	39535	9.8	1.92	5.98	0.70
BRU3119-01	108144	5.7	41325	10.7	1.97	6.12	0.74
BRU3144-18	122453	3.6	48939	7.6	1.87	5.83	0.49
BRU3144-24	101242	7.2	36542	9.0	2.08	6.46	0.74
BRU3144-07	113637	4.3	41251	5.8	2.06	6.42	0.46
BRU3161-02	99060	4.2	42739	6.8	1.91	5.93	0.43
BRU3161-04	103798	3.9	44521	6.5	1.92	5.97	0.41
BRU3161-05	98452	4.3	42821	6.3	1.89	5.89	0.41
mean					1.95	6.07	0.04
P. tenella (P. langeana)							
LAN2907-01	48725	6.8	46598	7.4	1.05	3.28	0.33
LAN2907-02	48550	6.2	46598	7.4	1.05	3.27	0.31
LAN2907-03	49914	7.8	51822	8.2	1.00	3.13	0.34
LAN2907-04	58409	6.4	65841	6.0	0.89	2.76	0.24
LAN2907-13	49292	6.0	46598	7.4	1.07	3.32	0.31
LAN3164-01	52121	3.7	45145	4.1	0.95	2.96	0.15
LAN3164-01	53597	4.3	45166	3.9	0.98	3.04	0.16
LAN3164-02	48000	4.1	40501	5.4	0.98	3.03	0.19
LAN3164-02	47824	4.6	39048	5.3	1.01	3.14	0.20
LAN3164-07	49001	4.6	42585	4.6	0.95	2.95	0.17
LAN3164-08	48043	4.8	41837	4.7	0.95	2.94	0.18
LAN3164-08	49708	3.8	42002	5.1	0.97	3.03	0.18
LAN3164-01	39190	5.1	31037	9.4	0.95	2.94	0.31
LAN3164-02	61246	4.3	51378	6.1	0.89	2.78	0.21
LAN3164-03	39536	5.3	31066	9.3	0.95	2.97	0.32
LAN3164-04	58922	5.1	48849	6.9	0.90	2.81	0.24
LAN3164-04	39245	5.2	30661	9.6	0.96	2.98	0.33
LAN3164-05	53535	4.4	42739	5.3	0.94	2.92	0.20
LAN3164-07	49738	6.1	37742	10.0	0.99	3.08	0.36
LAN3164-09	57096	4.0	48072	5.4	0.89	2.77	0.19
LAN3164-10	47558	4.1	41591	4.5	0.94	2.93	0.16
LAN3170-01	47922	4.5	41790	4.7	0.94	2.94	0.17
LAN3170-01	63878	4.2	50540	5.2	1.04	3.24	0.20
LAN3170-02	48332	4.8	41749	4.7	0.95	2.96	0.18
mean					0.97	3.02	0.03

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
P. phryganodes (triploid)							
PHR3169-12	82170	5.2	43102	7.3	1.57	4.88	0.40
PHR3169-17	94271	3.3	45465	6.0	1.71	5.31	0.33
PHR3169-22B	98475	4.2	49652	6.4	1.49	4.62	0.35
PHR3169-09	99675	3.7	49240	6.9	1.52	4.72	0.37
PHR2533-25	81355	5.8	49396	6.5	1.65	5.12	0.45
PHR2818-17	93123	3.8	48287	6.1	1.59	4.94	0.32
PHR2818-17	86324	4.2	43171	6.4	1.65	5.12	0.36
PHR2908-02	80369	4.2	41579	7.3	1.59	4.95	0.38
PHR2908-02	92518	3.8	46269	5.6	1.65	5.12	0.32
PHR2908-03	108165	5.2	65841	6.0	1.64	5.11	0.41
PHR2946-01	80448	9.9	50197	10.8	1.54	4.80	0.73
PHR3163-12	115491	5.1	55261	6.1	1.57	4.87	0.39
PHR3163-14	123439	3.4	51757	5.6	1.79	5.56	0.36
mean					1.61	5.01	0.07
P. phryganodes (tetraploid)							
PHR3123-03	120770	2.4	45498	5.6	2.19	6.80	0.38
PHR3123-04	115323	3.2	45818	5.2	2.07	6.44	0.36
PHR3123-04	118975	2.6	46078	5.5	2.13	6.61	0.37
PHR3123-05	118135	3.3	42240	6.3	2.30	7.16	0.46
PHR3163-01	107260	3.9	43171	6.4	2.05	6.36	0.43
PHR3163-02	114485	5.0	48512	5.6	1.94	6.04	0.41
PHR3163-02	107008	4.2	43992	6.5	2.00	6.23	0.44
PHR3163-04	113008	5.0	47455	5.7	1.96	6.10	0.42
PHR3163-05	113175	5.1	47645	5.6	1.96	6.08	0.42
PHR3163-07	113490	5.3	47508	5.9	1.97	6.12	0.44
PHR3163-07	99640	3.7	41579	7.3	1.97	6.14	0.46
PHR3163-12	101107	3.4	40626	7.0	2.05	6.37	0.45
PHR3163-14	102214	3.5	39294	7.1	1.95	6.06	0.48
PHR3163-15	112709	4.6	46968	6.7	1.98	6.14	0.45
PHR3164-06	111962	3.4	46968	5.4	1.96	6.10	0.35
PHR3164-06	105826	3.5	42539	6.2	2.05	6.37	0.41
PHR3164-06	105395	3.5	42235	5.9	2.05	6.39	0.40
PHR3164-06	115637	2.7	46518	6.1	2.05	6.37	0.39
PHR3164-06	120723	5.7	46029	7.7	1.97	6.13	0.59
mean					2.02	6.31	0.07
P. pumila							
PUM01-342	216379	3.3	60809	7.3	3.56	11.07	0.89
PUM2512B-1	211395	4.4	46721	7.8	3.40	10.58	0.94
PUM2552-08	132693	5.8	27897	9.3	3.56	11.08	1.21
PUM2552-10	129240	5.2	27642	9.1	3.50	10.89	1.14
PUM2552-11	210765	4.5	45117	8.6	3.51	10.92	1.06
PUM2552-11	130505	5.5	26266	8.5	3.72	11.58	1.17

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
PUM2552-12	141625	4.5	30995	7.6	3.42	10.65	0.94
PUM2552-32	183483	3.3	44302	5.9	3.41	10.60	0.65
PUM2563-04	203785	2.4	49040	6.1	3.42	10.64	0.63
PUM2563-04	200996	1.9	45818	5.2	3.61	11.23	0.57
PUM2563-12	214466	4.5	48402	7.8	3.33	10.36	0.93
PUM2563-12	197950	2.2	47589	4.6	3.42	10.65	0.49
PUM2926-20	199215	2.5	45993	6.3	3.57	11.09	0.68
PUM2931-10	218713	5.8	46958	9.0	3.50	10.89	1.16
PUM3216-02	197636	2.3	48126	5.2	3.38	10.52	0.54
PUM3216-16	194157	2.0	45431	4.5	3.52	10.94	0.49
mean					3.49	10.86	0.08
P. banksiensis							
UNK2810-03	50272	6.5	49889	6.4	0.75	2.35	0.21
UNK2810-05	61372	3.7	50129	6.6	0.92	2.85	0.22
UNK2810-05	58670	4.3	45516	6.0	0.97	3.00	0.22
UNK2810-05	98559	4.5	74946	4.9	0.99	3.06	0.20
UNK2810-11	55814	3.8	46355	5.3	0.96	2.99	0.18
UNK2810-13	53618	4.4	45813	4.6	0.88	2.73	0.17
UNK2810-13	49819	5.3	41256	5.6	0.90	2.81	0.22
UNK2810-16	47073	9.8	54664	8.9	0.87	2.70	0.35
UNK2810-17	54124	4.1	44617	5.1	0.97	3.01	0.18
UNK2810-17	53974	3.4	46756	4.0	0.95	2.96	0.14
UNK2810-18	46360	11.4	50197	10.8	0.89	2.77	0.45
UNK2810-20	58447	4.1	47216	6.7	0.99	3.07	0.23
UNK2842-01	52619	8.8	44709	8.0	0.88	2.73	0.44
UNK2842-05	55105	3.9	45136	5.0	0.97	3.03	0.18
UNK2842-05	55916	2.8	49027	4.7	0.94	2.92	0.15
UNK2842-05	52271	3.9	45494	5.1	0.95	2.94	0.17
UNK2842-10	57725	4.3	49837	3.6	0.87	2.70	0.15
UNK2842-13	47807	5.1	38325	7.5	0.93	2.91	0.26
UNK2842-20	46695	5.5	38596	4.3	0.91	2.82	0.20
UNK2842-22	46733	5.9	38805	4.5	0.90	2.81	0.21
UNK2842-23	45192	11.3	48550	11.3	0.94	2.94	0.46
UNK2842-24	54985	4.1	45604	5.0	0.96	2.99	0.18
UNK2842-24	51473	3.1	45208	3.4	0.94	2.92	0.12
mean					0.92	2.86	0.03
P. sp. 2							
UNKP2848-02	179582	3.3	43709	7.4	3.38	10.52	0.78
UNKP2848-03	180325	3.3	44157	6.7	3.36	10.46	0.71
UNKP2848-06	185441	4.8	50047	7.9	3.71	11.52	1.07
UNKP2848-08	189333	3.1	45570	7.4	3.42	10.64	0.78
UNKP2848-09	188830	4.7	51437	7.5	3.67	11.42	1.01
UNKP2848-11	187919	4.4	50737	7.6	3.70	11.52	1.01
UNKP2848-13	179710	4.0	43464	6.9	3.40	10.59	0.77

Appendix B. Continued.

Sample code	Mean fluor.	cv	crbc mean	crbc cv	propdip	pg/2C	sd
UNKP2848-14	188236	4.8	49539	7.4	3.66	11.39	0.99
UNKP2848-14	183117	4.4	51376	7.9	3.70	11.50	1.05
UNKP2848-17	183902	3.3	44226	6.8	3.42	10.65	0.73
UNKP2848-19	202751	4.1	50303	9.0	3.52	10.93	1.18
UNKP2848-19	186457	4.9	57672	6.9	3.71	11.53	0.88
mean					3.55	11.06	0.13
P. vahliana							
VAH2806-11	62954	6.9	57672	6.9	1.09	3.39	0.33
VAH2822-04	59884	6.1	49298	4.4	1.21	3.78	0.28
VAH2822-04	66489	10.3	50197	10.8	1.28	3.97	0.61
VAH2836-01	62824	5.7	57672	6.9	1.09	3.39	0.30
VAH2838-03	62012	5.7	42304	8.5	1.10	3.42	0.35
VAH2838-03	83577	4.6	49011	7.3	1.28	3.97	0.34
VAH2838-06	65520	6.9	44709	8.0	1.12	3.48	0.48
VAH2841-01	95569	4.7	91836	5.2	1.15	3.57	0.23
VAH2841-02	64331	6.7	54783	7.5	1.19	3.71	0.37
VAH3072-21	66620	6.4	41239	8.0	1.21	3.76	0.39
VAH3072-25	67074	7.0	39977	8.4	1.26	3.91	0.43
VAH3073-02	68759	6.2	44533	8.2	1.16	3.60	0.37
VAH3073-05	73368	5.2	46089	9.0	1.19	3.71	0.39
VAH3073-10	66204	3.9	46440	6.0	1.17	3.65	0.24
VAH3073-11	61789	6.8	45087	6.4	1.13	3.51	0.30
VAH3074-07	66630	5.0	48225	6.3	1.14	3.54	0.26
VAH3075-01	66663	5.0	47174	6.6	1.16	3.62	0.27
VAH3075-16	56104	3.8	39011	5.9	1.18	3.68	0.24
VAH3075-16	67731	3.5	45818	5.2	1.22	3.79	0.22
VAH3075-22	69980	4.1	47131	6.2	1.22	3.80	0.26
VAH3075-23	67728	5.8	43120	8.3	1.18	3.66	0.37
VAH3075-23	66010	3.6	47506	4.9	1.14	3.56	0.20
VAH3078-07	62079	4.8	43420	4.3	1.18	3.66	0.21
VAH3080-23	65115	3.6	45427	5.4	1.18	3.67	0.22
VAH3083-01	66145	3.6	46593	5.1	1.17	3.64	0.21
VAH3083-03	67489	6.2	43662	8.8	1.16	3.60	0.39
VAH3084-15	64252	6.6	40295	8.9	1.19	3.72	0.41
VAH3084-09	64136	6.7	39466	7.6	1.22	3.79	0.38
VAH3101-01	71856	4.9	46673	7.3	1.15	3.59	0.32
VAH3101-02	78328	5.2	50129	6.6	1.17	3.64	0.31
VAH3101-02	77420	4.8	48079	7.0	1.21	3.75	0.32
VAH3101-02	72873	3.6	43203	5.9	1.26	3.93	0.27
VAH3101-03	67203	4.8	44025	7.7	1.14	3.56	0.32
VAH3101-04	74509	3.9	45386	5.4	1.23	3.83	0.25
VAH3101-05	70790	4.2	41691	6.2	1.27	3.96	0.30
VAH3102-12	62056	5.5	37521	8.0	1.24	3.85	0.37
VAH3102-16	67992	4.5	48034	6.6	1.17	3.62	0.26
VAH3102-22	62635	6.2	38723	8.9	1.21	3.77	0.41

VAH3102-05	72907	3.9	42697	5.7	1.28	3.98	0.27
VAH3102-06	70331	4.4	42445	6.8	1.24	3.86	0.31
VAH3102-08	70144	4.7	42281	6.7	1.24	3.87	0.32
VAH3117-01	70471	6.2	48713	8.9	1.08	3.37	0.37
VAH3149-06	64089	5.8	40572	8.8	1.18	3.68	0.39
VAH3171-01	68706	8.0	50651	8.0	1.02	3.17	0.36
VAH3171-02	64228	6.8	46816	8.5	1.03	3.21	0.35
VAH3171-02	65901	4.3	47043	5.2	1.15	3.59	0.22
VAH3171-03	65105	4.5	46733	6.0	1.15	3.57	0.24
mean					1.20	3.74	0.06

Appendix C: Table of significance values for generalized linear models for common garden experiments #1 and #2

Tax = taxon; Pop = population. Quantitative characters only in the model. Full list of species and experimental setup given in Table 5.3. Tax, pop(tax), and pop include all species. Significance for contrasts given for specific taxon pairs compared. P-values given only if they are significant. *P. arc.* = *P. arctica*, *P. arc.* agg. = *P. arctica* aggregate (*P. arc.*, *P. agr.*, *P. poa.*), *P. agr.* = *P. agrostidea*, *P. poa.* = *P. poacea*, *P. brug.* = *P. bruggemannii*, *P. ang.* = *P. angustata*, *P. and.* = *P. andersonii*.

1) Common garden experiment #1.

Character	Tax	Pop (tax)	Pop	*P. arc.* (n = 23) vs *P. poa.* (n = 18)	*P. agr.* (n = 20) vs *P. arc.* (n = 23)	*P. agr.* (n = 20) vs *P. poa.* (n=18)	*P.* sp.1 (n = 18) vs *P. arc. agg.* (n = 54)	*P. brug.* vs *P. ang.*	*P. ang.* vs *P. and.*
1. Height	< 0.0001	0.0010	< 0.0001	ns	< 0.0001	0.0020	0.0010	0.0063	ns
6. Ligule length	< 0.0001	ns	< 0.0001	ns	ns	ns	ns	ns	ns
8. Inflorescence	< 0.0001	0.0001	< 0.0001	ns	< 0.0001	0.0002	< 0.0001	ns	ns
10. Branch length	< 0.0001	0.0021	0.0018	ns	ns	< 0.0001	0.0140	ns	ns
11. Distance	0.0010	0.0202	0.0007	ns	ns	ns	ns	ns	ns
12. Internode	< 0.0001	0.0327	0.0024	ns	ns	0.0030	0.0080	ns	ns
14. Pedicel width 1	< 0.0001	0.0001	< 0.0001	ns	ns	ns	ns	ns	ns
15. Pedicel width 2	< 0.0001	ns	< 0.0001	ns	ns	ns	ns	ns	0.0009
16. Spikelet length	< 0.0001	0.0013	< 0.0001	ns	ns	ns	0.0001	ns	ns
21. 1st glume length	< 0.0001	0.0021	< 0.0001	ns	ns	ns	ns	ns	ns
22. 1st glume width	< 0.0001	0.0005	< 0.0001	ns	ns	ns	ns	ns	ns
25. 2nd glume length	< 0.0001	0.0001	< 0.0001	0.0021	ns	ns	< 0.0001	ns	ns
26. 2nd glume width	< 0.0001	0.0008	< 0.0001	ns	ns	ns	0.0006	ns	ns
28. Rachilla length	< 0.0001	ns	< 0.0001	ns	ns	ns	0.0020	ns	ns
29. Rachilla width	< 0.0001	ns	< 0.0001	ns	ns	ns	ns	ns	ns
34. Lemma length	< 0.0001	0.0003	< 0.0001	ns	ns	ns	< 0.0001	0.0040	ns
36. Lemma hair	< 0.0001	0.0001	< 0.0001	ns	ns	ns	< 0.0001	0.0005	0.0036
38. Palea length	< 0.0001	0.0001	< 0.0001	ns	ns	ns	< 0.0001	ns	ns
39. Palea width	< 0.0001	0.0001	< 0.0001	ns	ns	ns	ns	ns	ns
40. Palea hair	< 0.0001	0.0003	< 0.0001	ns	ns	ns	< 0.0001	ns	0.0006
41. Anther length	< 0.0001	ns	< 0.0001	ns	ns	ns	< 0.0001	ns	ns

Appendix C. Continued.

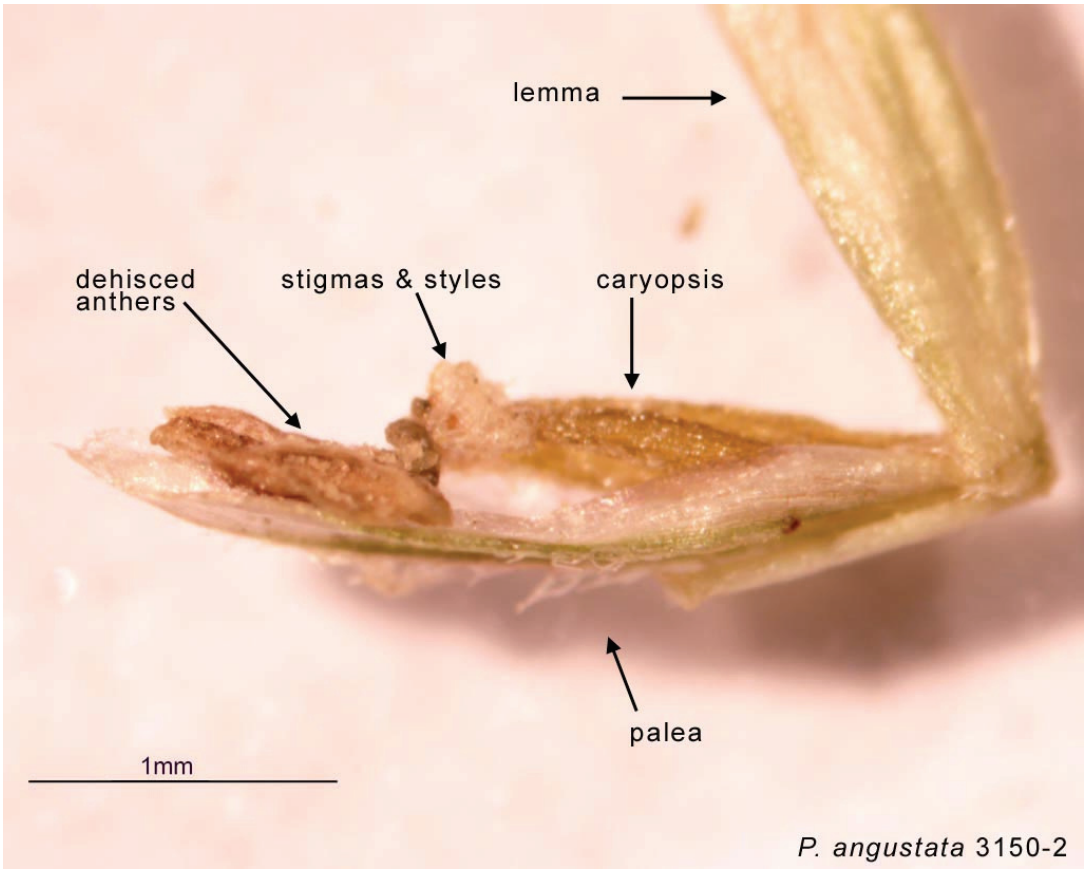
Character	Tax	Pop (tax)	Pop	*P. arc.* (n = 23) vs *P. poa.* (n = 18)	*P. agr.* (n = 20) vs *P. arc.* (n = 23)	*P. agr.* (n = 20) vs *P. poa.* (n=18)	*P.* sp.1 (n = 18) vs *P. arc. agg.* (n = 54)	*P. brug.* vs *P. ang.*	*P. ang.* vs *P. and.*
42. Ratio 1st glume	< 0.0001	0.0001	0.0011	ns	ns	ns	0.0200	ns	ns
43. Ratio 2nd glume	< 0.0001	0.0010	< 0.0001	ns	ns	ns	ns	ns	ns
44. Ratio palea LW	< 0.0001	0.0001	< 0.0001	ns	ns	ns	0.0007	ns	ns

2) Common garden experiment #2.

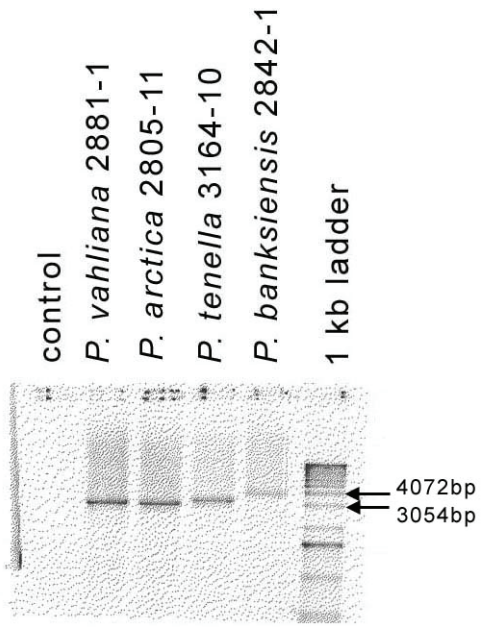
Character	Tax	Pop(tax)	Pop	*P. brug.* vs *P. ang.*	*P. and.* vs *P.* sp. 2
1. Height	0.0005	ns	0.0005	0.0302	< 0.0001
6. Ligule length	ns	ns	0.0025	ns	0.0020
8. Inflorescence	0.0006	ns	0.0034	0.0005	0.0246
10. Branch length	0.0255	ns	0.0407	0.0139	ns
11. Distance	ns	ns	ns	ns	ns
12. Internode	0.0007	ns	0.0056	0.0052	0.0064
14. Pedicel width 1	ns	ns	ns	ns	ns
15. Pedicel width 2	ns	ns	ns	ns	ns
16. Spikelet length	< 0.0001	ns	< 0.0001	ns	ns
21. 1st glume length	< 0.0001	ns	< 0.0001	ns	ns
22. 1st glume width	< 0.0001	ns	< 0.0001	0.0004	ns
25. 2nd glume length	< 0.0001	ns	< 0.0001	ns	ns
26. 2nd glume width	< 0.0001	ns	< 0.0001	0.0045	ns
28. Rachilla length	0.0003	0.0161	< 0.0001	0.0246	0.0480
29. Rachilla width	0.1452	ns	ns	0.0044	ns
34. Lemma length	< 0.0001	ns	< 0.0001	ns	0.0039
36. Lemma hair	0.0043	ns	0.0039	ns	ns
38. Palea length	< 0.0001	ns	< 0.0001	ns	0.0183
39. Palea width	< 0.0001	ns	< 0.0001	0.0031	ns
40. Palea hair	< 0.0001	ns	< 0.0001	ns	ns
41. Anther length	0.0005	0.0020	< 0.0001	0.0032	0.0025
42. Ratio 1st glume	< 0.0001	ns	ns	0.0190	ns
43. Ratio 2nd glume	< 0.0001	0.0300	< 0.0001	0.0038	ns
44. Ratio palea LW	< 0.0001	ns	< 0.0001	0.0001	ns

Appendix D. Photo of floret

Floret with developing caryopsis, stigmas and styles, and dehisced anthers present in same floret. Floret was closed until lemma and palea were dissected apart.

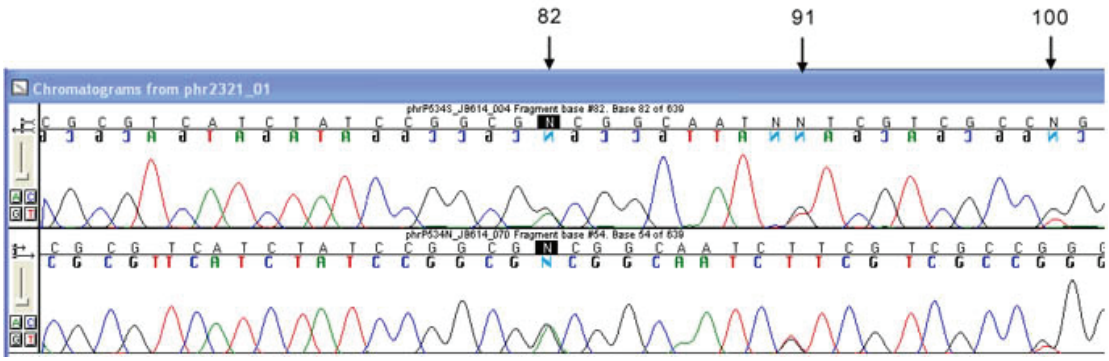


Appendix E. Gel showing amplification of the intergenic spacer region (IGS) of nuclear ribosomal DNA region in several diploid *Puccinellia* species



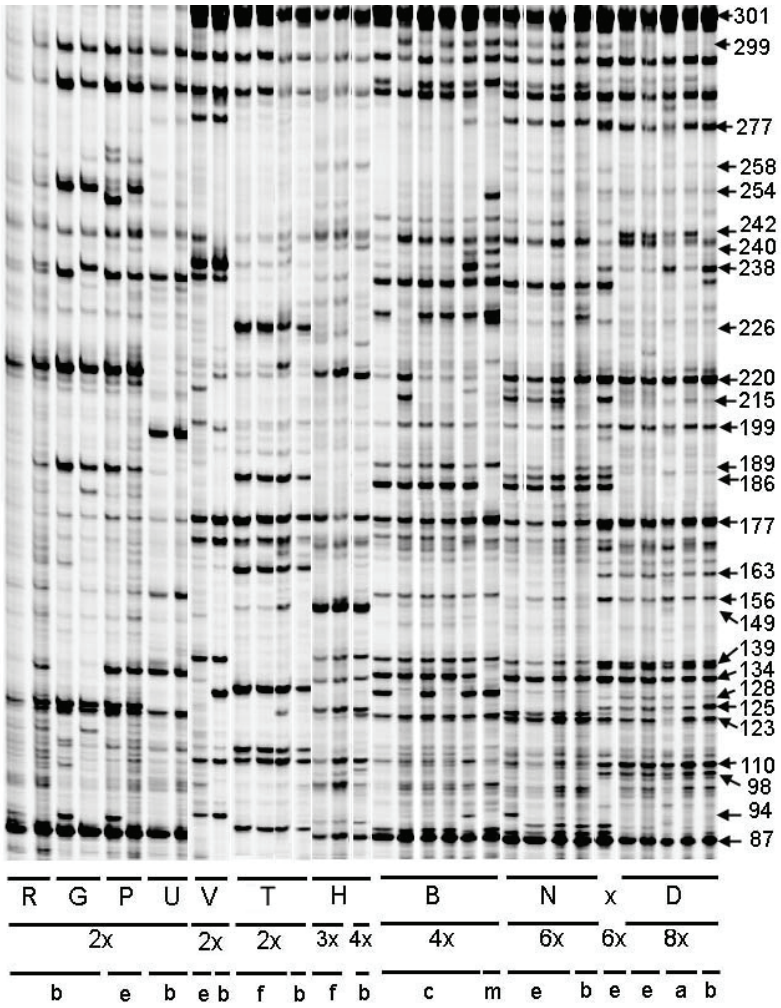
Appendix F. Electropherogram of a *P. phryganodes* ITS sequence, showing examples of multiple peaks in single amplification

The full list of multiple peaks found in *P. phryganodes* is given in Table 6.2. This graph shows the nearly equal double peaks at bases #82, #91, and #100.



Appendix G. Gels of AFLP fragments amplified using selective primer pairs M + CAA / E + ACG, M + CTC / E + ACC, and M + CTT / E + AAG

Lanes (i.e., columns = Col. in tables) on the gel plates of the following pages are individuals for which identifications are given on the pages preceding each gel plate. A sample gel of the primer pair M + CTC / E + ACC is given, showing the differences among the taxa, followed by 13 sample gels. All 31 gels are in an electronic appendix to be deposited in the Department of Plant Science at McGill. The lanes without numbers are size standards. Those labelled in the specimen table with an "f" were failures that were not scored.

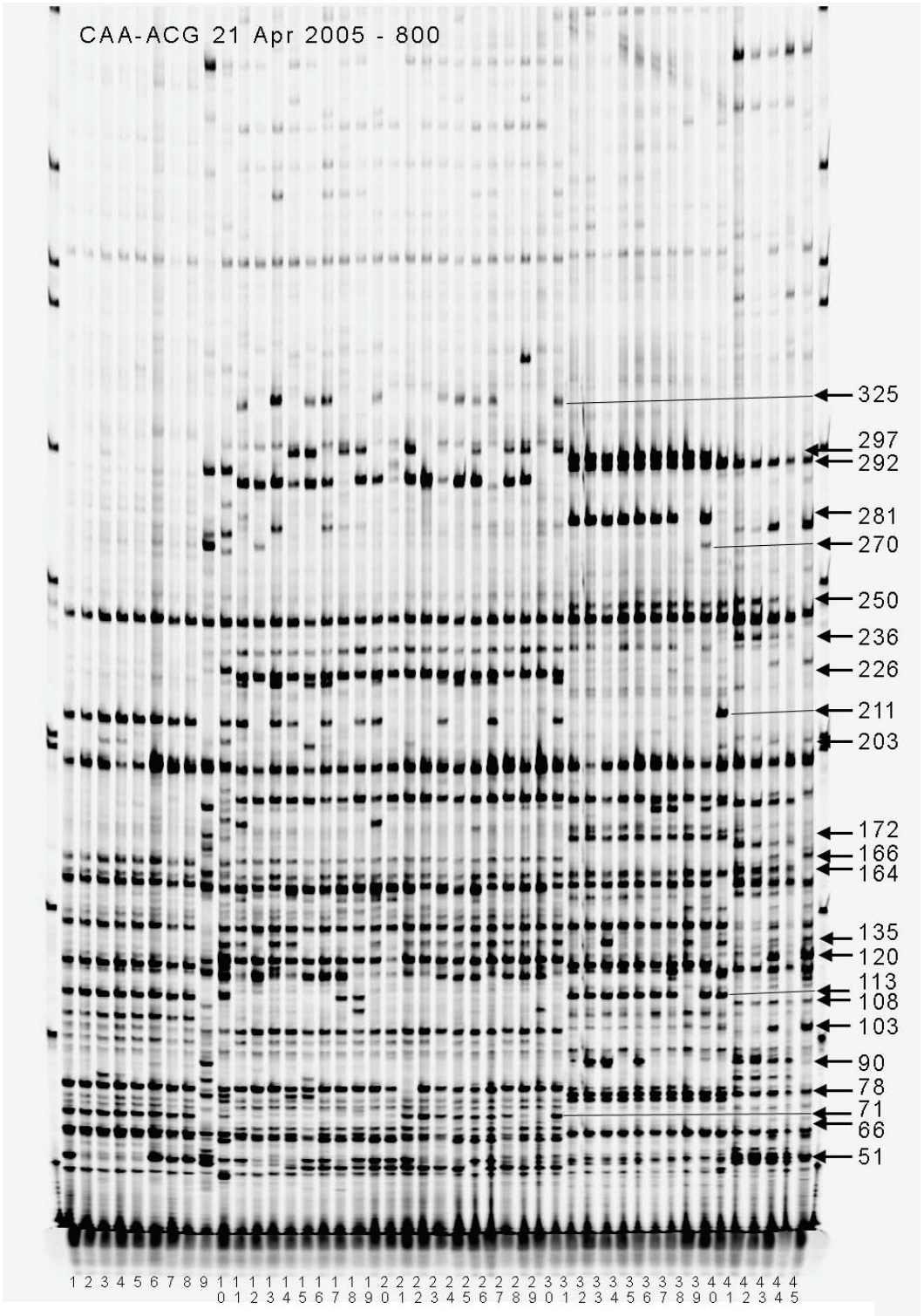


Sample gel from primer pair M + CTC / E + ACC, zoomed to include only 87–300 bp of gel. R = *P. arctica*; G = *P. agrostidea*; P = *P. poacea*; U = *P. banksiensis*; V = *P. vahliana*; T = *P. tenella*; H = *P. phryganodes*; B = *P. bruggemannii*; N = *P. angustata*; D = *P. andersonii*; x = D6 (note it possesses both N and D bands). Lower case a = Axel Heiberg I; b = Banks I; c = Cornwallis I; e = Ellesmere I; f = Baffin I; M = Melville I.

Gel: CAA-ACG 21Apr2005-800

Col.	taxon	specimen
1	*P. banksiensis*	2810-2
2	*P. banksiensis*	2810-9
3	*P. banksiensis*	2810-13
4	*P. banksiensis*	2810-16
5	*P. banksiensis*	2810-18
6	*P. banksiensis*	2842-1
7	*P. banksiensis*	2842-10
8	*P. banksiensis*	2842-20
9	*P. banksiensis*	2856-7
10	*P. banksiensis*	2856-8
11	*P. arctica (P. agrostidea)*	2813-8
12	*P. arctica (P. agrostidea)*	2813-12
13	*P. arctica (P. agrostidea)*	2813-14
14	*P. arctica (P. agrostidea)*	2813-22
15	*P. arctica (P. agrostidea)*	2813-27
16	*P. arctica (P. agrostidea)*	2823-1
17	*P. arctica (P. agrostidea)*	2823-7
18	*P. arctica (P. agrostidea)*	2823-14
19	*P. arctica (P. agrostidea)*	2823-15
20	*P. arctica (P. agrostidea)*	2823-22
21	*P. arctica (P. poacea)*	2864-5
22	*P. arctica (P. poacea)*	2864-7
23	*P. arctica (P. poacea)*	2864-9

Col.	taxon	specimen
24	*P. arctica (P. poacea)*	2864-17
25	*P. arctica (P. poacea)*	2864-23
26	*P. arctica (P. poacea)*	2898-1
27	*P. arctica (P. poacea)*	2898-3
28	*P. arctica (P. poacea)*	2898-14
29	*P. arctica (P. poacea)*	2898-19
30	*P. arctica (P. poacea)*	2898-20
31	*P. vahliana*	2884-1
32	*P. vahliana*	2884-2
33	*P. vahliana*	2884-3
34	*P. vahliana*	2884-4
35	*P. vahliana*	2884-5
36	*P. vahliana*	2836-1
37	*P. vahliana*	2836-2
38	*P. vahliana*	2836-4
39	*P. vahliana*	2836-5
40	*P. vahliana*	2836-6
41	*P. tenella (P. langeana)*	3164-1
42	*P. tenella (P. langeana)*	3164-2
43	*P. tenella (P. langeana)*	3164-3
44	*P. tenella (P. langeana)*	3164-4
45	*P. tenella (P. langeana)*	3164-6


CAA-ACG 21 Apr 2005 - 800
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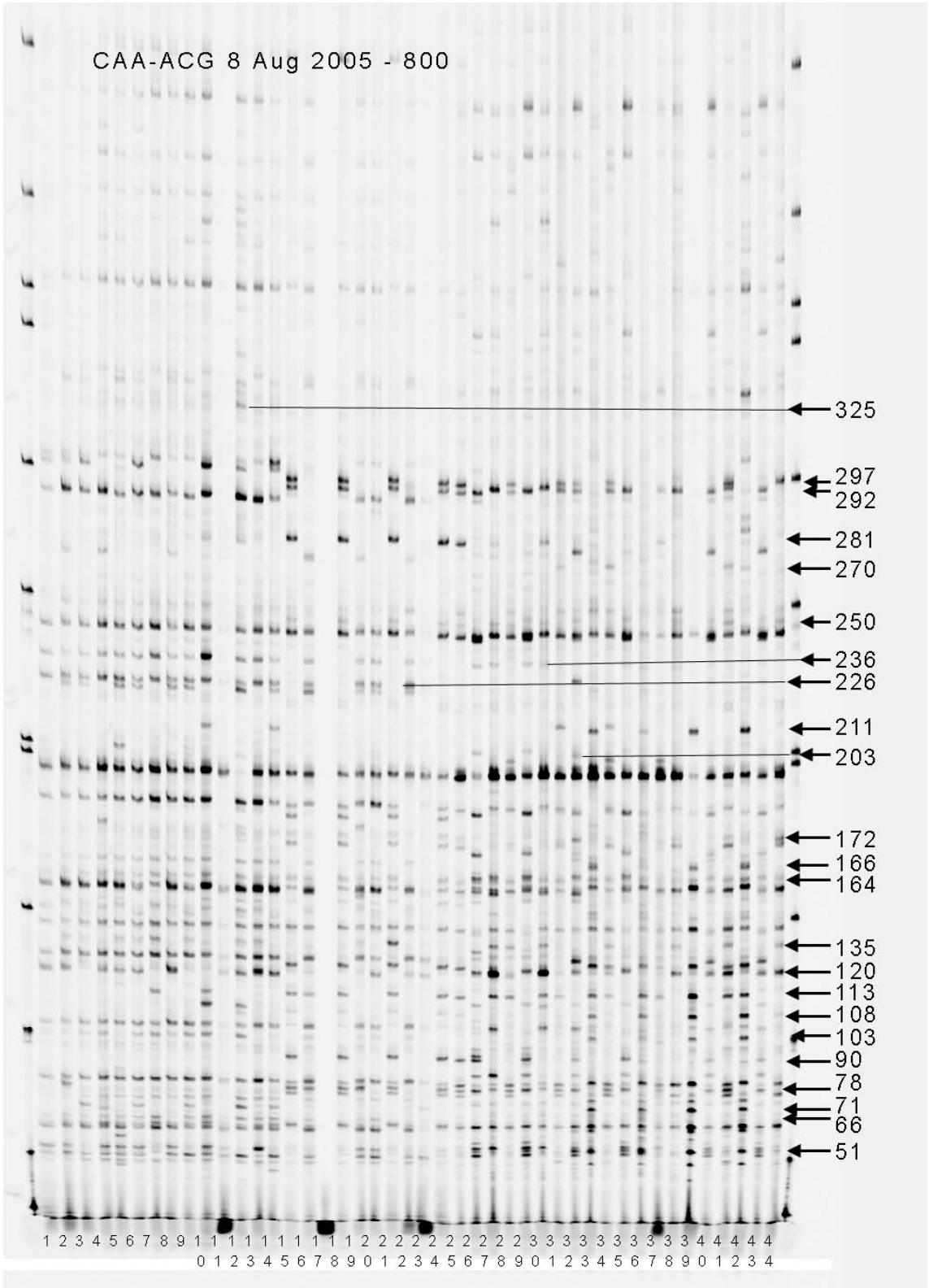
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

CAA-ACG 8 Aug. 2005-800

Col.	taxon	specimen
1	*P. arctica*	2808-4
2	*P. arctica*	2805-11
3	*P. arctica (P. poacea)*	2864-11
4	*P. arctica*	2808-5
5	*P. arctica*	2805-14
6	*P. arctica (P. poacea)*	2864-14
7	*P. arctica*	2808-13
8	*P. arctica (P. agrostidea)*	2823-2
9	*P. arctica (P. poacea)*	2864-15
10	*P. arctica*	2808-14
11	*P. arctica (P. agrostidea)*	2823-3
12	*P. arctica (P. poacea)*	2864-22
13	*P. arctica*	2808-20
14	*P. arctica (P. agrostidea)*	2823-5
15	*P. vahliana*	3102-1
16	*P. arctica*	2805-4
17	*P. arctica (P. agrostidea)*	2823-12
18	*P. vahliana*	3102-5
19	*P. arctica*	2805-9
20	*P. arctica (P. agrostidea)*	2823-13
21	*P. vahliana*	3102-6
22	*P. arctica*	2805-10

Col.	taxon	specimen
23	*P. arctica (P. poacea)*	2864-1
24	*P. vahliana*	3102-8
25	*P. vahliana*	3102-10
26	*P. tenella (P. langeana)*	3164-7
27	*P. alaskana*	bogo1
28	*P. vahliana*	2806-1
29	*P. tenella (P. langeana)*	3164-8
30	*P. alaskana* (f)	bogo2
31	*P. vahliana*	2806-2
32	*P. phryganodes*	3164-9
33	*P. banksiensis*	2842-3
34	*P. vahliana*	2806-6
35	*P. tenella (P. langeana)*	3164-10
36	*P. banksiensis*	2842-4
37	*P. vahliana*	2806-7
38	*P. wrightii*	09-01
39	*P. banksiensis*	2842-5
40	*P. vahliana*	2806-10
41	*P. wrightii*	09-02
42	*P. banksiensis*	2842-11
43	*P. tenella (P. langeana)*	3164-5
44	*P. wrightii*	09-03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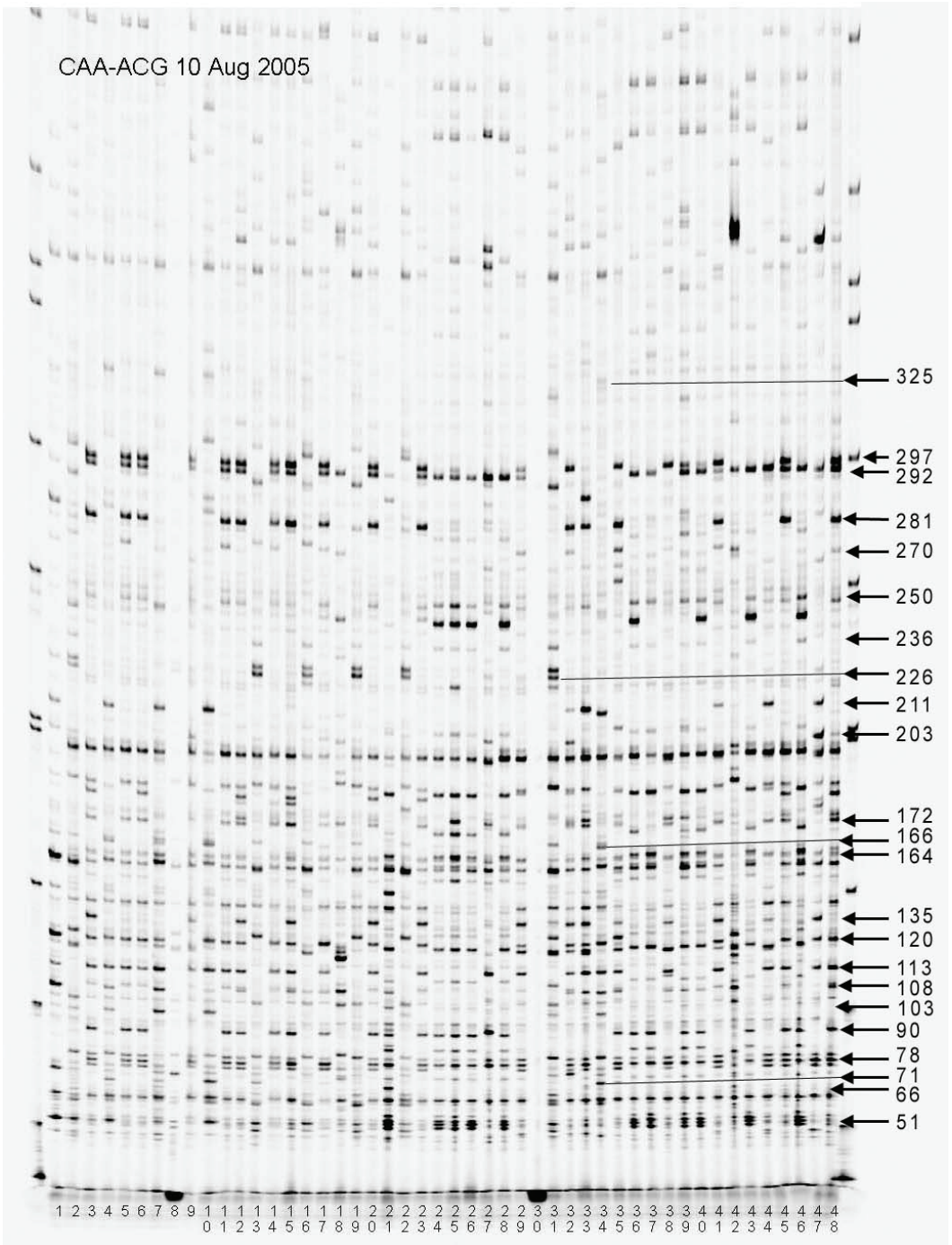
0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4

CAA-ACG 10 Aug 2005

Col.	taxon	specimen
1	*P. banksiensis*	2842-13
2	*P. arctica*	2817-14
3	*P. vahliana*	3073-4
4	*P. banksiensis*	2842-22
5	*P. vahliana*	3149-1
6	*P. vahliana*	3073-7
7	*P. banksiensis*	2842-23
8	*P. vahliana* (f)	3149-2
9	*P. vahliana* (f)	3073-8
10	*P. banksiensis*	2842-24
11	*P. vahliana*	3149-3
12	*P. vahliana*	3073-9
13	*P. arctica*	2817-1
14	*P. vahliana*	3149-4
15	*P. vahliana*	3073-10
16	*P. arctica*	2817-2
17	*P. vahliana*	3149-5
18	*P. lemmonii*	lem2
19	*P. arctica*	2817-6
20	*P. vahliana*	3073-1
21	*P. tenella*	ten1
22	*P. arctica*	2817-12
23	*P. vahliana*	3073-3
24	*P. tenella (P. langeana)*	byl1

Col.	taxon	specimen
25	*P. tenella (P. langeana)*	byl2
26	*P. tenella (P. langeana)*	2907-13
27	*P. vahliana*	2838-8
28	*P. tenella (P. langeana)*	byl3
29	*P. vahliana*	2822-4
30	*P. vahliana* (f)	2838-9
31	*P. arctica*	arcYuk
32	*P. vahliana*	2822-12
33	*P. vahliana*	2838-10
34	*P. banksiensis*	2810-11
35	*P. vahliana*	2822-14
36	*P. tenella (P. langeana)*	2352-7
37	*P. tenella (P. langeana)*	2907-10
38	*P. vahliana*	2822-10
39	*P. tenella (P. langeana)*	2856-21
40	*P. tenella (P. langeana)*	2907-15
41	*P. vahliana*	2822-15
42	*P. parishii*	par1
43	*P. tenella (P. langeana)*	2907-3
44	*P. vahliana*	2838-6
45	*P. vahliana*	3171-1
46	*P. tenella (P. langeana)*	2907-4
47	*P. vahliana*	2838-7
48	*P. vahliana*	3171-2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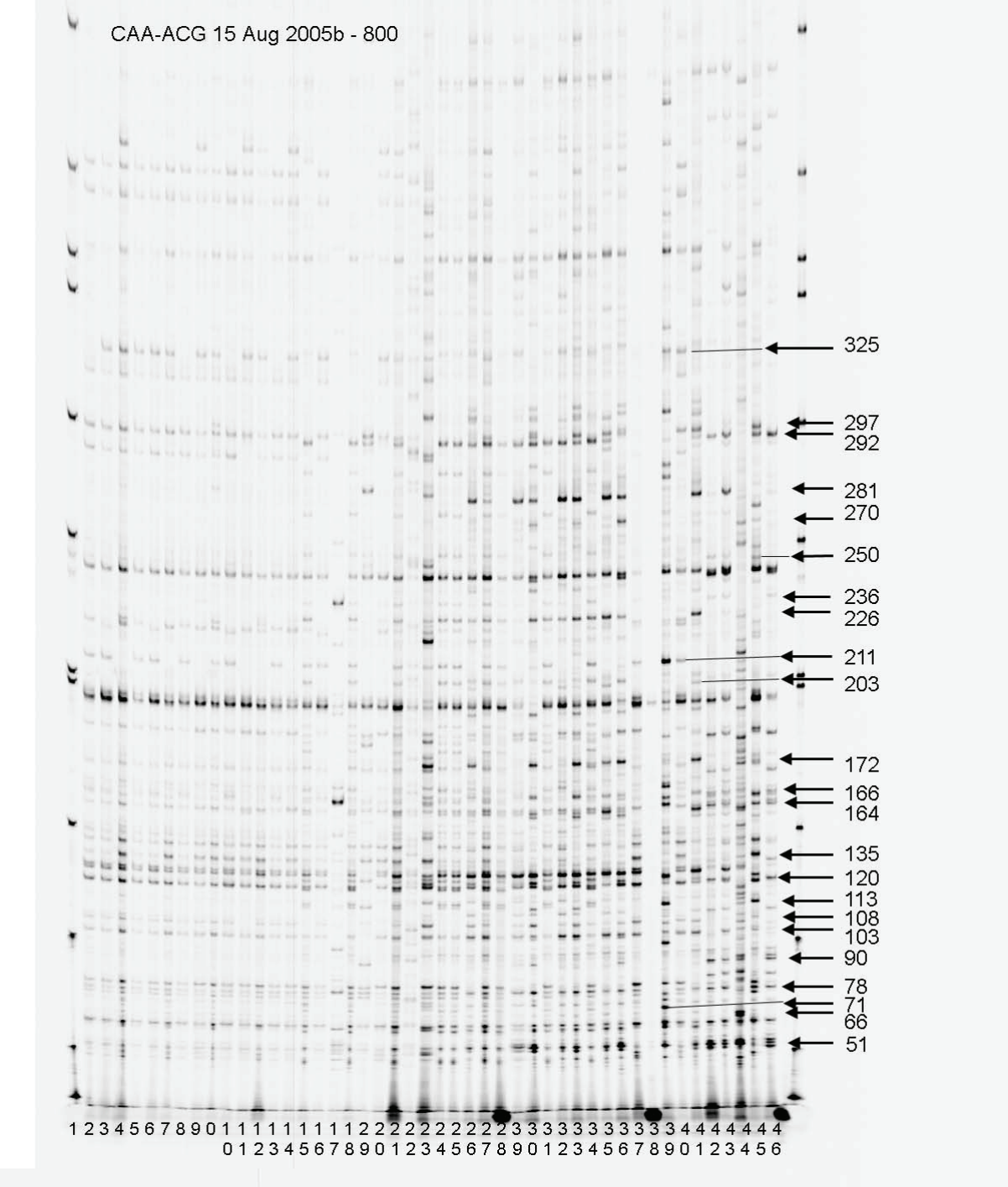
CAA-ACG 15Aug2005b-800

Col.	taxon	specimen
1	*P. bruggemannii*	2906-1
2	*P. vahliana*	3072-14
3	*P. angustata*	2874-13
4	*P. bruggemannii*	2906-3
5	*P. bruggemannii*	3072-18
6	*P. angustata*	2874-14
7	*P. bruggemannii*	2906-4
8	*P. angustata*	2866-1
9	*P. angustata*	2874-15
10	*P. bruggemannii*	2906-9
11	*P. angustata*	2866-9
12	*P. angustata*	2874-19
13	*P. bruggemannii*	2906-14
14	*P. angustata*	2866-11
15	*P. andersonii*	2865-1
16	*P. bruggemannii*	3072-2
17	*P. angustata*	2866-19
18	*P. andersonii*	2865-3
19	*P. bruggemannii*	3072-8
20	*P. angustata*	2866-24
21	*P. andersonii*	2865-9
22	*P. bruggemannii* (f)	3072-9
23	*P. angustata*	2874-7
24	*P. andersonii*	2865-12
25	*P. andersonii*	2865-17
26	*P. phryganodes*	3163-12
27	*P. phryganodes*	2818-17
28	*P. andersonii*	2877-3
29	*P. phryganodes*	3163-14
30	*P. phryganodes*	3123-1
31	*P. andersonii*	2877-5
32	*P. phryganodes*	3163-4

Col.	taxon	specimen
33	*P. phryganodes*	3123-2
34	*P. andersonii*	2877-17
35	*P. phryganodes*	3169-9
36	*P. phryganodes*	3123-3
37	*P. andersonii*	2877-20
38	*P. phryganodes* (f)	3169-22
39	*P. phryganodes*	2842-24
40	*P. andersonii*	2877-24
41	*P. phryganodes*	3169-17
42	*P. tenella (P. langeana)*	2907-13
43	*P. tenella (P. langeana)*	3164-5
44	*P. phryganodes*	2818-1
45	*P. vahliana*	2822-4
46	*P. tenella (P. langeana)*	3164-7


CAA-ACG 15 Aug 2005b - 800
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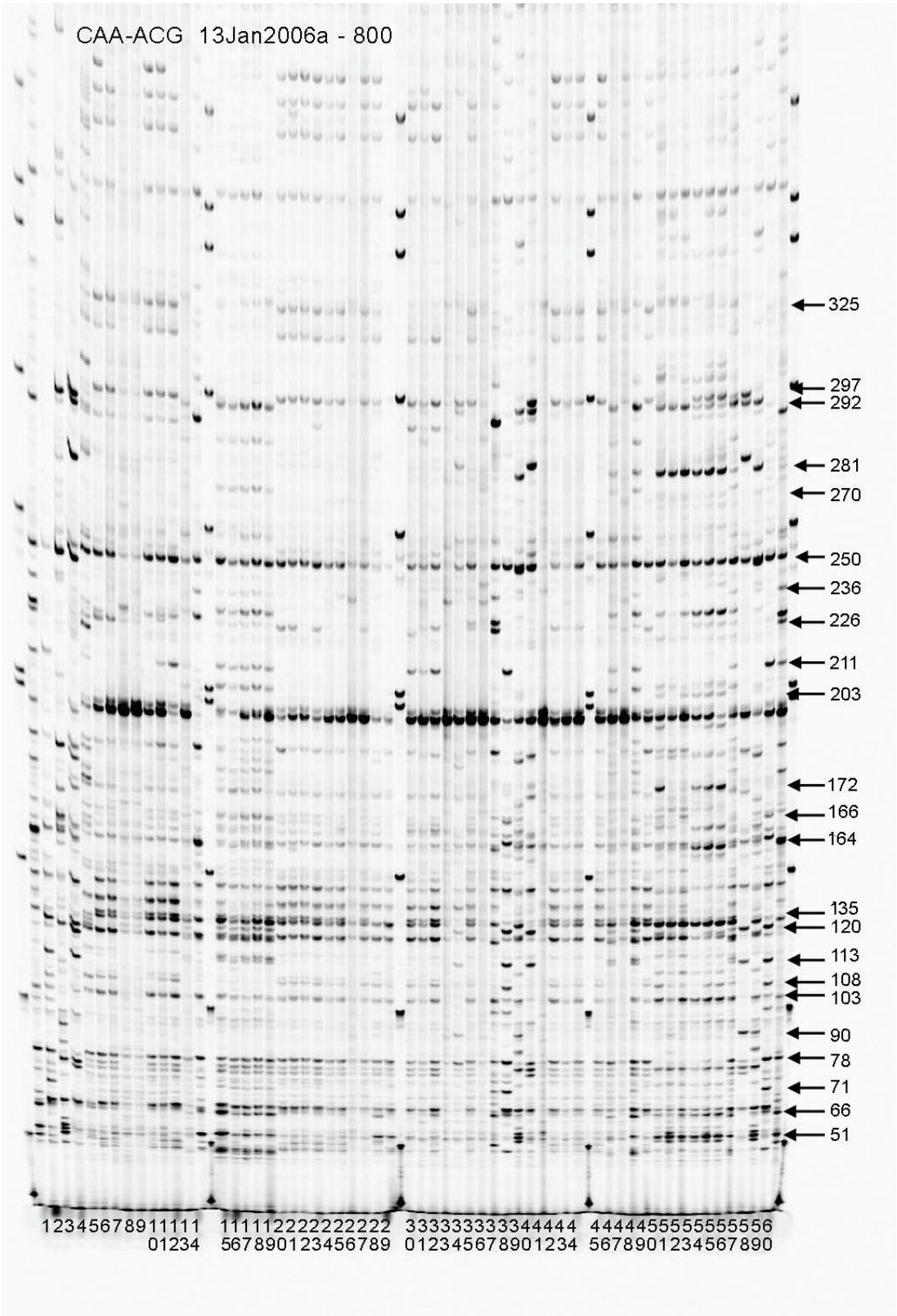
0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6

CAA-ACG 13Jan2006a – 800

Col.	taxon	specimen
1	*P. arctica (P. agrostidea)*	2823-15
2	*P. banksiensis*	2810-13
3	*P. tenella (P. langeana)*	3164-4
4	*P. vahliana*	2836-2
5	*P. angustata*	2874-7
6	*P. angustata*	2874-13
7	*P. angustata*	2874-14
8	*P. angustata*	2874-15
9	*P. angustata*	2874-19
10	*P. angustata*	2878-6
11	*P. angustata*	2878-17
12	*P. angustata*	2878-20
13	*P. angustata*	2878-19
14	*P. arctica*	2808-20
15	*P. andersonii*	2878-10
16	*P. andersonii*	2878-14
17	*P. andersonii*	2878-16
18	*P. andersonii*	2878-9
19	*P. andersonii*	2878-15
20	*P. angustata*	2853-5
21	*P. angustata*	2853-7
22	*P. angustata*	2853-9
23	*P. angustata*	2853-14
24	*P. angustata*	2853-16
25	*P. angustata*	2858-1
26	*P. angustata*	2858-3
27	*P. angustata*	2858-12
28	*P. angustata*	2858-11
29	*P. angustata*	2858-14
30	*P. bruggemannii*	3075-2

Col.	taxon	specimen
31	*P. bruggemannii*	3075-3
32	*P. bruggemannii*	3075-9
33	*P. bruggemannii*	3075-18
34	*P. bruggemannii*	3075-11
35	*P. angustata*	3144-13
36	*P. angustata*	3072-10
37	*P. arctica (P. poacea)*	2864-17
38	*P. banksiensis*	2810-13
39	*P. tenella (P. langeana)*	3164-5
40	*P. vahliana*	2884-4
41	*P. angustata*	3072-12
42	*P. angustata*	3072-13
43	*P. angustata*	3072-23
44	*P. angustata*	3072-24
45	*P. angustata*	2851-1
46	*P. angustata*	2851-4
47	*P. angustata*	2851-9
48	*P. angustata*	2851-3
49	*P. angustata*	2851-18
50	*P. phryganodes*	3163-12
51	*P. phryganodes*	3163-14
52	*P. phryganodes*	3163-4
53	*P. phryganodes*	3169-9
54	*P. phryganodes*	3169-22
55	*P. phryganodes*	3169-17
56	*P. phryganodes*	2818-17
57	*P. vahliana*	2884-5
58	*P. tenella (P. langeana)*	3164-5
59	*P. banksiensis*	2810-9
60	*P. arctica (P. agrostidea)*	2823-15


CAA-ACG 13Jan2006a - 800
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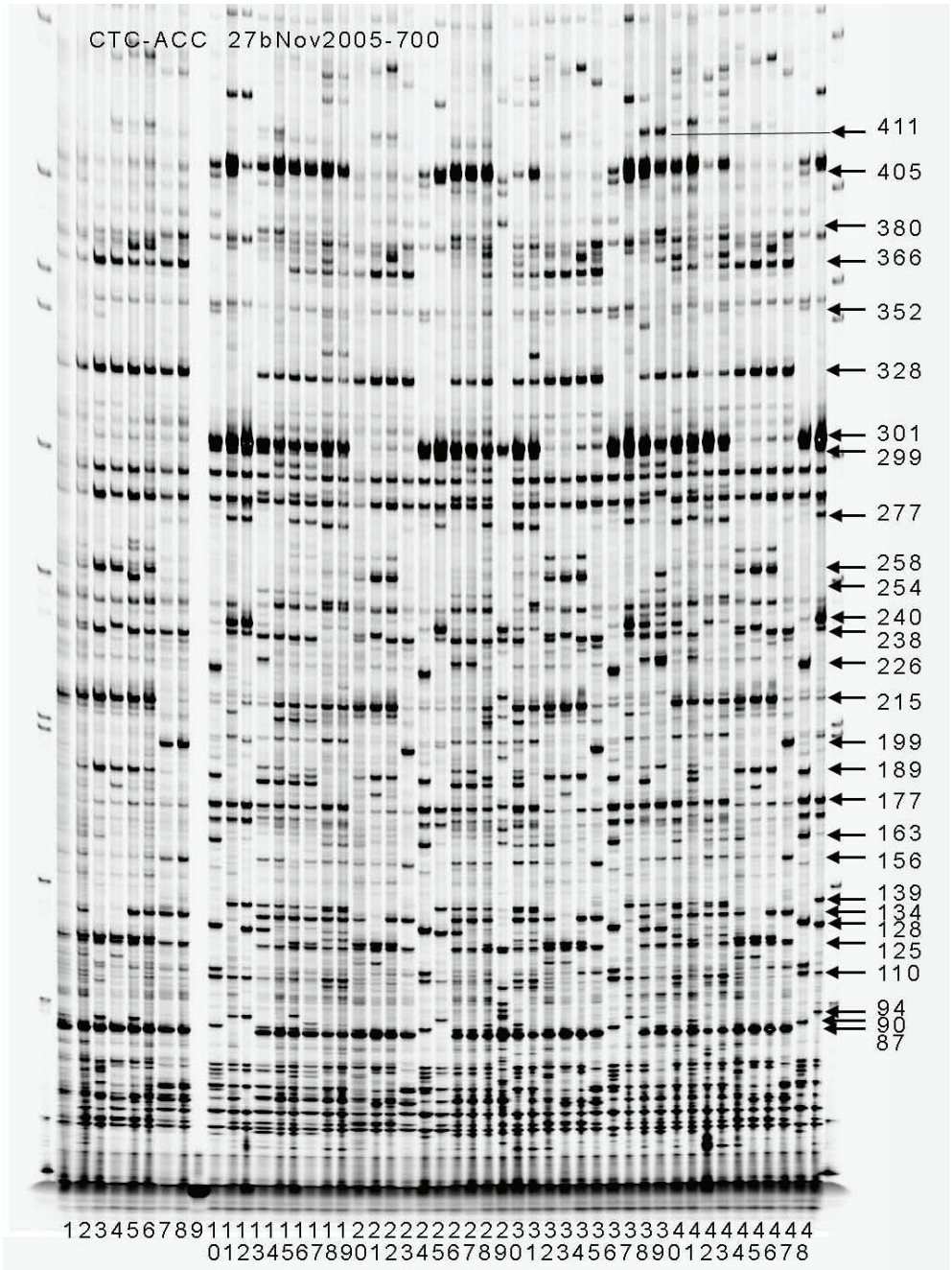
12345678911111 01234
1111122222222222 5678901234567 89
3333333333444444 0123456789012 34
4444455555555556 5678901234567 890

CTC-ACC 27bNov2005 - 800

Col.	taxon	specimen
1	*P. arctica*	2808-13
2	*P. arctica*	2805-4
3	*P. arctica (P. agrostidea)*	2813-14
4	*P. arctica (P. agrostidea)*	2823-1
5	*P. arctica (P. poacea)*	2864-9
6	*P. arctica (P. poacea)*	2898-1
7	*P. banksiensis*	2810-16
8	*P. banksiensis*	2810-18
9	*P. tenella (P. langeana)* (f)	3164-1
10	*P. tenella (P. langeana)*	3164-2
11	*P. vahliana*	2884-4
12	*P. vahliana*	2836-4
13	*P. bruggemannii*	2906-1
14	*P. bruggemannii*	2906-3
15	*P. angustata*	2866-1
16	*P. angustata*	2866-9
17	*P. andersonii*	2865-1
18	*P. andersonii*	2865-3
19	*P. arctica*	2805-4
20	*P. arctica (P. agrostidea)*	2823-1
21	*P. arctica (P. poacea)*	2898-1
22	*P. banksiensis*	2810-18
23	*P. tenella (P. langeana)*	3164-2
24	*P. vahliana*	2836-4

Col.	Taxon	specimen
25	*P. bruggemannii*	2906-4
26	*P. bruggemannii*	2906-9
27	*P. angustata*	2866-11
28	*P. angustata*	2866-24
29	*P. andersonii*	2865-9
30	*P. andersonii*	2865-12
31	*P. arctica*	2805-4
32	*P. arctica (P. agrostidea)*	2823-1
33	*P. arctica (P. poacea)*	2898-1
34	*P. banksiensis*	2810-18
35	*P. tenella (P. langeana)*	3164-2
36	*P. vahliana*	2884-4
37	*P. bruggemannii*	3080-2
38	*P. bruggemannii*	3080-5
39	*P. angustata*	2851-1
40	*P. angustata*	2851-4
41	*P. andersonii*	2878-11
42	*P. andersonii*	2878-14
43	*P. arctica*	2805-4
44	*P. arctica (P. agrostidea)*	2823-1
45	*P. arctica (P. poacea)*	2898-1
46	*P. banksiensis*	2810-18
47	*P. tenella (P. langeana)*	3164-2
48	*P. vahliana*	2836-4


CTC-ACC 27bNov2005-700
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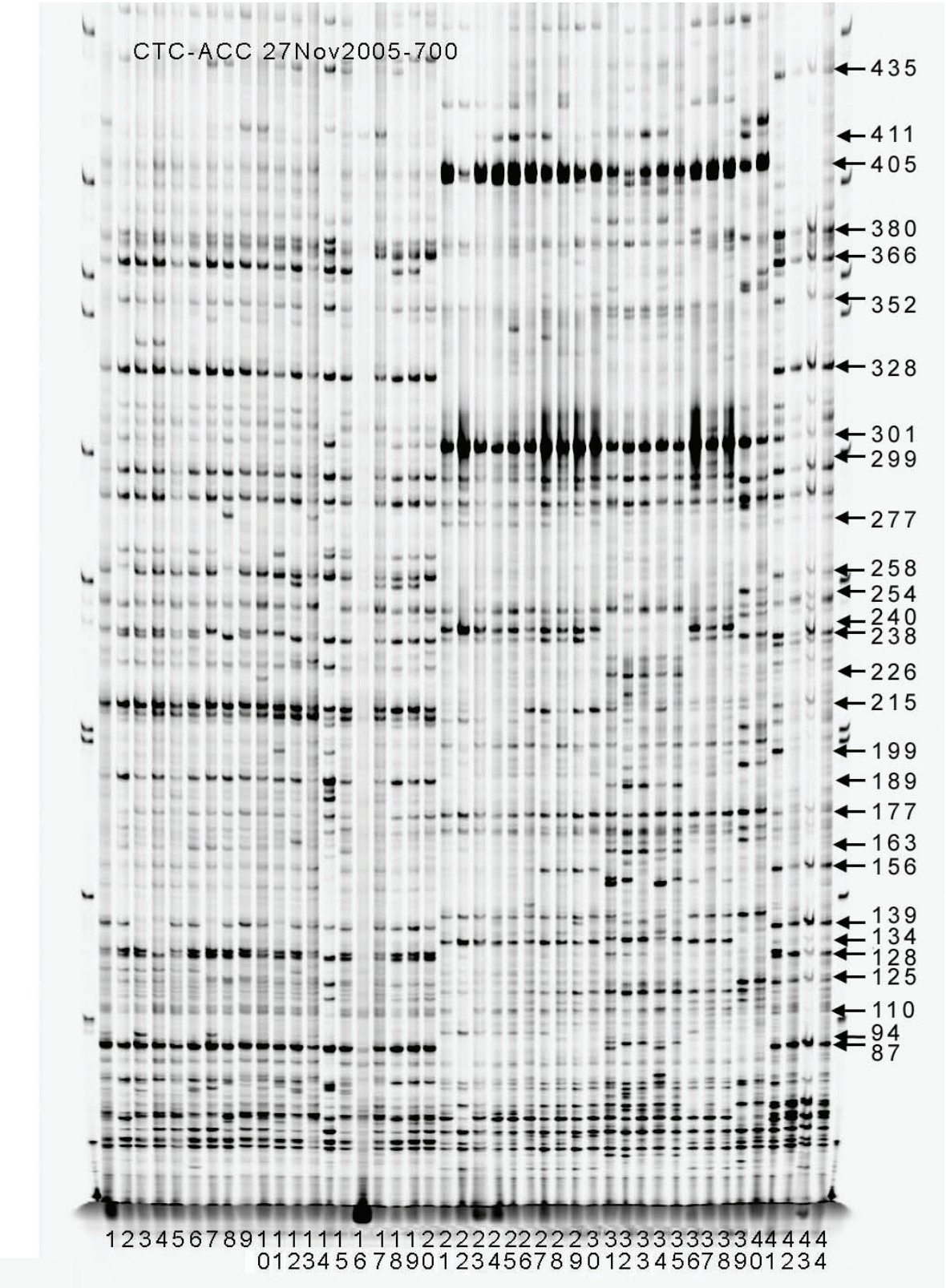
1234567891111111111122222222223333333333444444444
0123456789012345678901234567890123456789012345678

CTC-ACC 27Nov2005 - 700

Col.	taxon	specimen
1	*P. arctica*	2808-4
2	*P. arctica*	2808-5
3	*P. arctica*	2808-13
4	*P. arctica*	2808-14
5	*P. arctica*	2808-20
6	*P. arctica*	2805-4
7	*P. arctica*	2805-9
8	*P. arctica*	2805-10
9	*P. arctica*	2805-11
10	*P. arctica*	2805-14
11	*P. arctica (P. agrostidea)*	2823-2
12	*P. arctica (P. agrostidea)*	2823-3
13	*P. arctica (P. agrostidea)*	2823-5
14	*P. arctica (P. agrostidea)*	2823-12
15	*P. arctica (P. agrostidea)*	2823-13
16	*P. arctica (P. poacea)* (f)	2864-1
17	*P. arctica (P. poacea)*	2864-11
18	*P. arctica (P. poacea)*	2864-14
19	*P. arctica (P. poacea)*	2864-15
20	*P. arctica (P. poacea)*	2864-22
21	*P. vahliana*	3102-1
22	*P. vahliana*	3102-5

Col.	taxon	specimen
23	*P. vahliana*	3102-6
24	*P. vahliana*	3102-8
25	*P. vahliana*	3102-10
26	*P. vahliana*	2806-1
27	*P. vahliana*	2806-2
28	*P. vahliana*	2806-6
29	*P. vahliana*	2806-7
30	*P. vahliana*	2806-10
31	*P. tenella (P. langeana)*	3164-5
32	*P. tenella (P. langeana)*	3164-7
33	*P. tenella (P. langeana)*	3164-8
34	*P. tenella (P. langeana)*	3164-9
35	*P. tenella (P. langeana)*	3164-10
36	*P. wrightii*	09-01
37	*P. wrightii*	09-02
38	*P. wrightii*	09-03
39	*P. alaskana*	bogo1
40	*P. alaskana*	bogo2
41	*P. banksiensis*	2842-3
42	*P. banksiensis*	2842-4
43	*P. banksiensis*	2842-5
44	*P. banksiensis*	2842-6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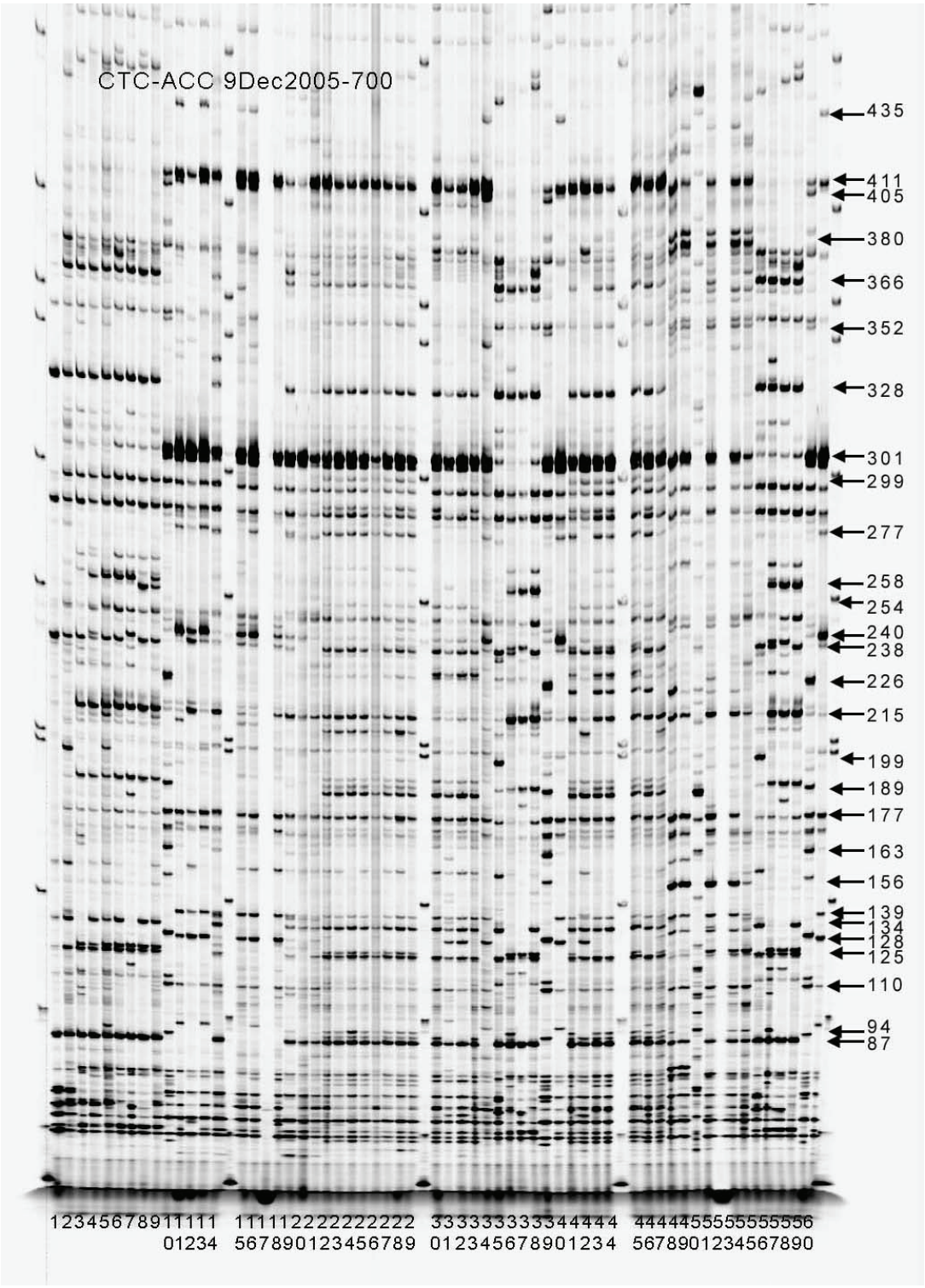
CTC-ACC 9Dec2005-700

Col.	taxon	specimen
1	*P. banksiensis*	2842-10
2	*P. banksiensis*	2810-16
3	*P. arctica (P. agrostidea)*	2813-27
4	*P. arctica (P. agrostidea)*	2823-15
5	*P. arctica (P. agrostidea)*	2823-22
6	*P. arctica (P. poacea)*	2898-3
7	*P. arctica (P. agrostidea)*	2823-1
8	*P. arctica (P. poacea)*	2898-14
9	*P. arctica (P. poacea)*	2898-19
10	*P. tenella (P. langeana)*	3164-1
11	*P. vahliana*	2836-5
12	*P. vahliana*	2836-6
13	*P. vahliana*	2836-4
14	*P. andersonii*	2856-8
15	*P. vahliana*	2822-10
16	*P. vahliana*	2822-15
17	*P. vahliana* (f)	2838-9
18	*P. vahliana*	2838-10
19	*P. angustata*	2878-10
20	*P. angustata*	2878-14
21	*P. angustata*	2878-15
22	*P. angustata*	2853-9
23	*P. angustata*	2853-14
24	*P. angustata*	2853-16
25	*P. angustata*	2858-1
26	*P. angustata*	2858-3
27	*P. angustata*	2858-12
28	*P. angustata*	2858-11
29	*P. angustata*	2858-14
30	*P. bruggemannii*	3075-2

Col.	taxon	specimen
31	*P. bruggemannii*	3075-3
32	*P. bruggemannii*	3075-9
33	*P. bruggemannii*	3075-18
34	*P. bruggemannii*	3075-11
35	*P. banksiensis*	2810-16
36	*P. arctica*	2808-13
37	*P. arctica (P. agrostidea)*	2823-1
38	*P. arctica (P. poacea)*	2898-3
39	*P. tenella (P. langeana)*	3164-1
40	*P. vahliana*	2836-4
41	*P. angustata*	3144-6
42	*P. bruggemannii*	3144-7
43	*P. angustata*	3144-8
44	*P. angustata*	3144-9
45	*P. angustata*	3144-11
46	*P. angustata*	3144-13
47	*P. angustata*	3072-10
48	*P. phryganodes*	3169-22
49	*P. phryganodes*	3169-17
50	*P. phryganodes*	2818-1
51	*P. phryganodes*	2818-16
52	*P. phryganodes* (f)	3123-1
53	*P. phryganodes*	3123-2
54	*P. phryganodes*	3123-3
55	*P. banksiensis*	2810-16
56	*P. arctica*	2808-13
57	*P. arctica (P. agrostidea)*	2823-1
58	*P. arctica (P. poacea)*	2898-3
59	*P. tenella (P. langeana)*	3164-1
60	*P. vahliana*	2836-4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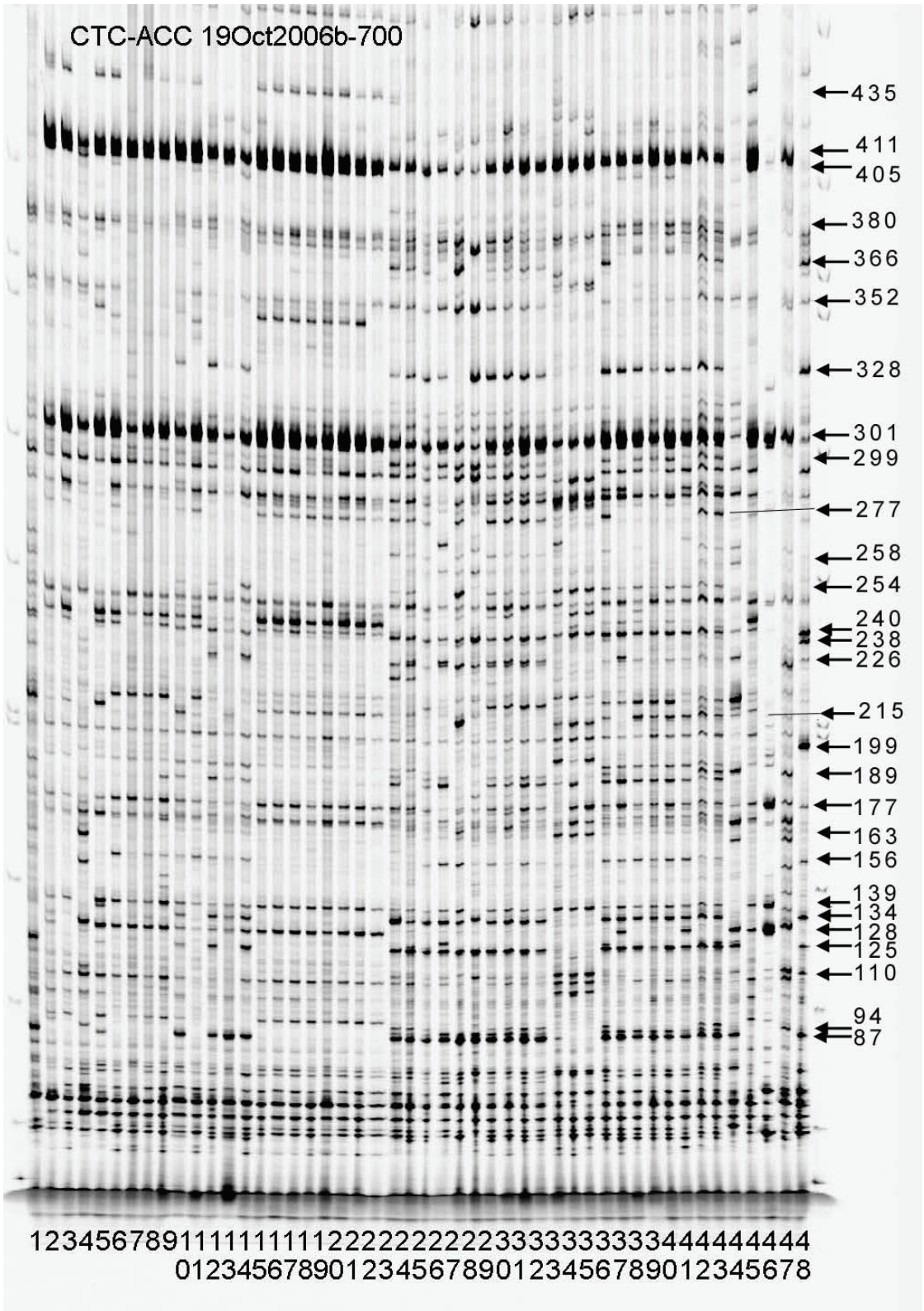
CTC-ACC 19Oct2006b – 700

Col.	taxon	specimen
1	*P. arctica*	2805-4
2	*P. vahliana*	5808
3	*P. vahliana*	2884-4
4	*P. tenella (P. langeana)*	3164-2
5	*P. vahliana*	vah
6	*P. vahliana*	2831-1
7	*P. vahliana*	2831-2
8	*P. vahliana*	2831-3
9	*P. vahliana*	2831-4
10	*P. vahliana*	2831-5
11	*P. vahliana*	3074-1
12	*P. vahliana*	3074-2
13	*P. vahliana*	3074-4
14	*P. vahliana*	3074-8
15	*P. vahliana*	3073-2
16	*P. vahliana*	3073-5
17	*P. bruggemannii*	3075-1
18	*P. bruggemannii*	3075-16
19	*P. bruggemannii*	3075-22
20	*P. bruggemannii*	3075-23
21	*P. bruggemannii*	3083-1
22	*P. bruggemannii*	3083-3
23	*P. angustata*	3140-1
24	*P. angustata*	3140-2

Col.	taxon	specimen
25	*P. angustata*	3140-3
26	*P. angustata*	3150-1X
27	*P. angustata*	3150-2
28	*P. angustata*	3150-3X
29	*P. angustata*	3150-4
30	*P. angustata*	3150-5
31	*P. angustata*	3151-2
32	*P. angustata*	3151-3
33	*P. alaskana*	bogo3
34	*P. alaskana*	bogo4
35	*P. alaskana*	bogo5
36	*P. angustata*	2807-1
37	*P. bruggemannii*	3100-1
38	*P. bruggemannii*	3100-2
39	*P. bruggemannii*	3100-3
40	*P. bruggemannii*	3100-4
41	*P. bruggemannii*	3144-24
42	*P. vahliana*	2849-1
43	*P. vahliana*	2849-5
44	*P. arctica*	2805-4
45	*P. vahliana*	5808
46	*P. vahliana* (f)	2884-4
47	*P. tenella (P. langeana)*	3164-2
48	*P. banksiensis*	2810-9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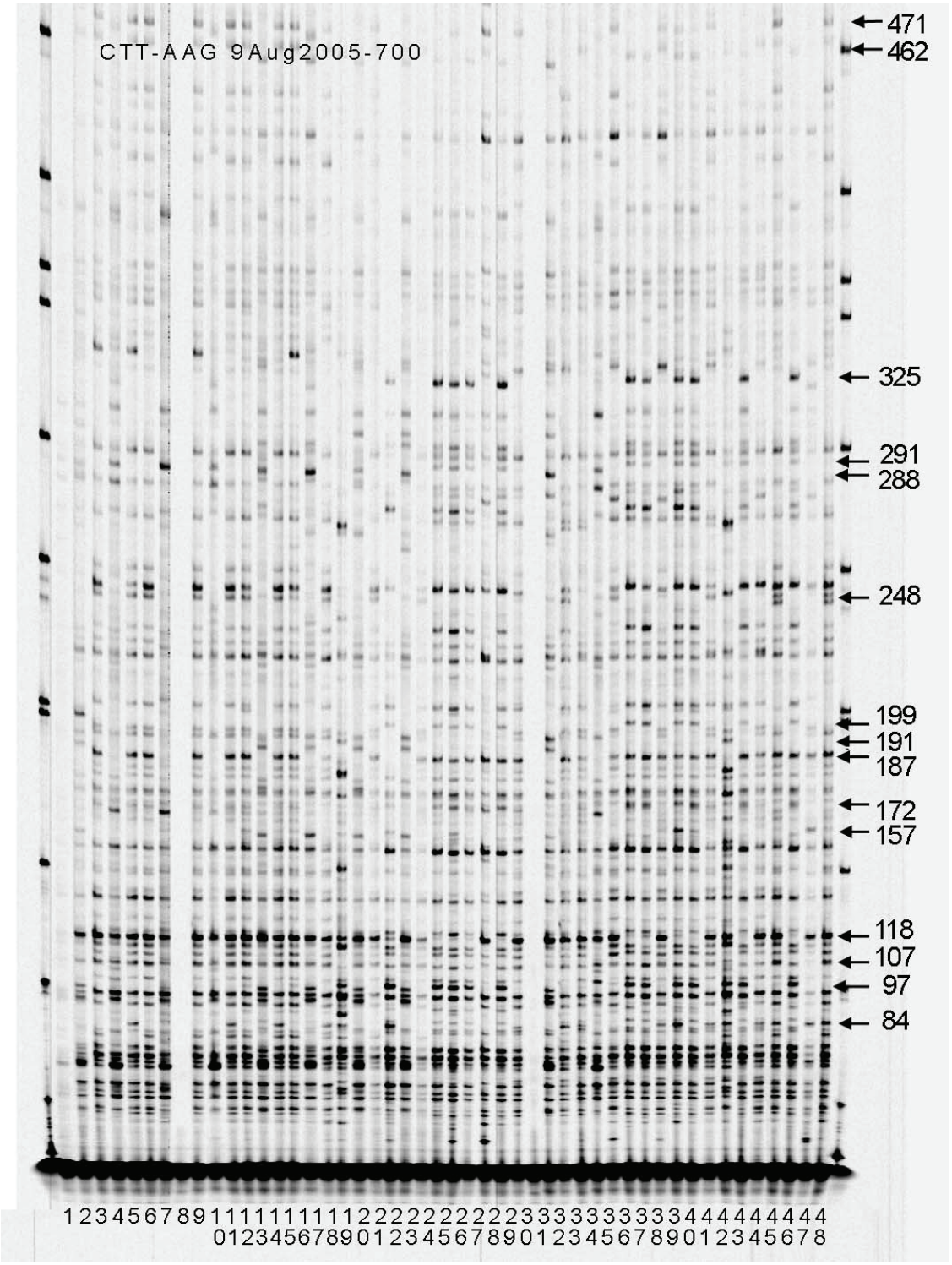
CTT-AAG 9Aug2005 – 700

col.	taxon	specimen
1	*P. banksiensis*	2842-13
2	*P. arctica*	2817-14
3	*P. vahliana*	3073-4
4	*P. banksiensis*	2842-22
5	*P. vahliana*	3149-1
6	*P. vahliana*	3073-7
7	*P. banksiensis*	2842-23
8	*P. vahliana* (f)	3149-2
9	*P. vahliana*	3073-8
10	*P. banksiensis*	2842-24
11	*P. vahliana*	3149-3
12	*P. vahliana*	3073-9
13	*P. arctica*	2817-1
14	*P. vahliana*	3149-4
15	*P. vahliana*	3073-10
16	*P. arctica*	2817-2
17	*P. vahliana*	3149-5
18	*P. lemmonii*	lem2
19	*P. arctica*	2817-6
20	*P. vahliana*	3073-1
21	*P. tenella*	ten1
22	*P. arctica*	2817-12
23	*P. vahliana*	3073-3
24	*P. tenella (P. langeana)*	byl1
25	*P. tenella (P. langeana)*	byl2

Col.	taxon	specimen
26	*P. tenella (P. langeana)*	2907-13
27	*P. vahliana*	2838-8
28	*P. tenella (P. langeana)*	byl3
29	*P. vahliana*	2822-4
30	*P. vahliana*	2838-9
31	*P. arctica* (f)	arcYuk
32	*P. vahliana*	2822-12
33	*P. vahliana*	2838-10
34	*P. banksiensis*	2810-11
35	*P. vahliana*	2822-14
36	*P. tenella (P. langeana)*	2352-7
37	*P. tenella (P. langeana)*	2907-10
38	*P. vahliana*	2822-10
39	*P. tenella (P. langeana)*	2856-21
40	*P. tenella (P. langeana)*	2907-15
41	*P. vahliana*	2822-15
42	*P. parishii*	par1
43	*P. tenella (P. langeana)*	2907-3
44	*P. vahliana*	2838-6
45	*P. vahliana*	3171-1
46	*P. tenella (P. langeana)*	2907-4
47	*P. vahliana*	2838-7
48	*P. vahliana*	3171-2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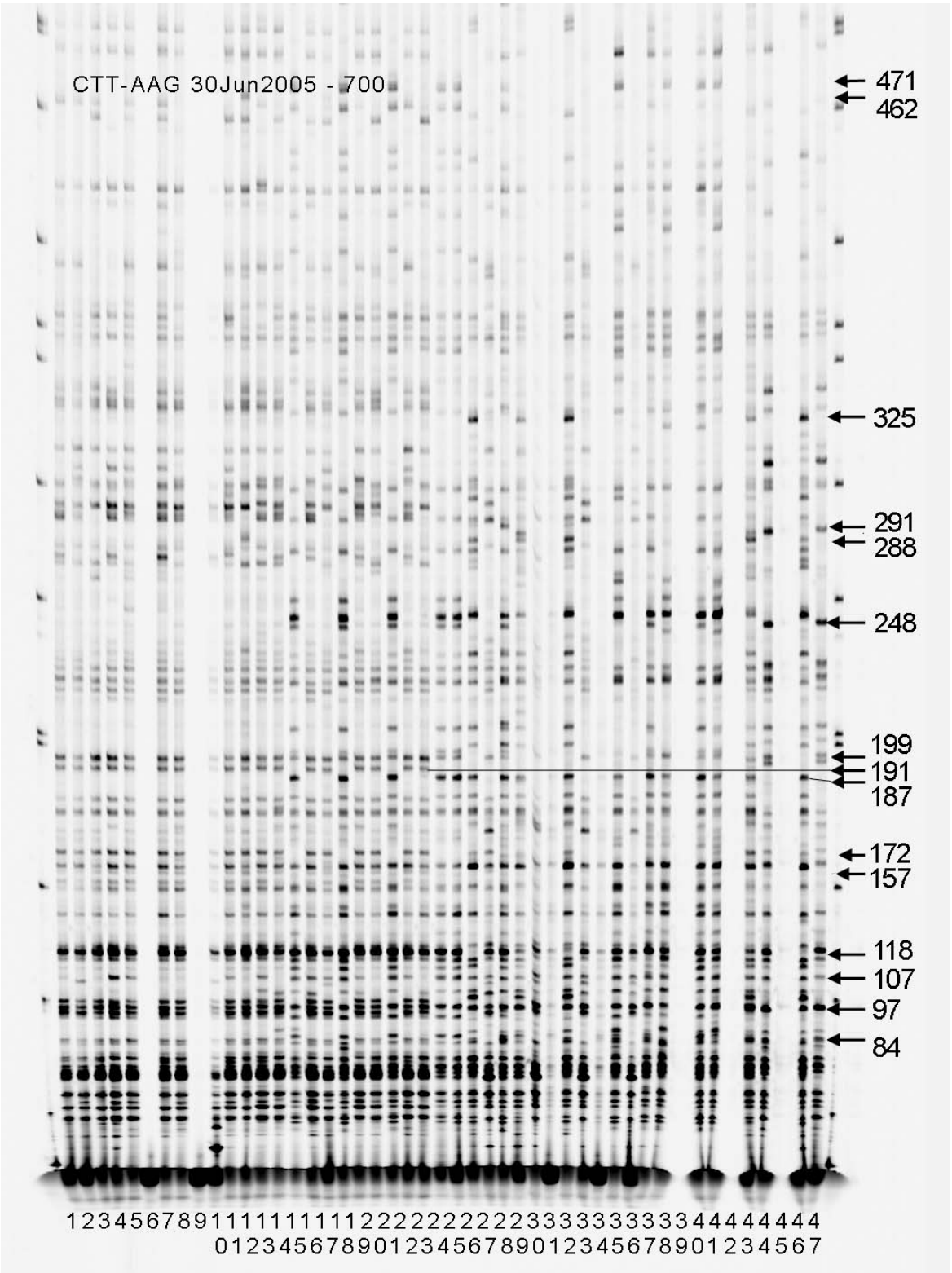
CTT-AAG 30June2005 – 700

Col.	taxon	specimen
1	*P. arctica*	2808-4
2	*P. arctica*	2805-11
3	*P. arctica (P. poacea)*	2864-11
4	*P. arctica*	2808-5
5	*P. arctica*	2805-14
6	*P. arctica (P. poacea)* (f)	2864-14
7	*P. arctica*	2808-13
8	*P. arctica (P. agrostidea)*	2823-2
9	*P. arctica (P. poacea)* (f)	2864-15
10	*P. arctica*	2808-14
11	*P. arctica (P. agrostidea)*	2823-3
12	*P. arctica (P. poacea)*	2864-22
13	*P. arctica*	2808-20
14	*P. arctica (P. agrostidea)*	2823-5
15	*P. vahliana*	3102-1
16	*P. arctica*	2805-4
17	*P. arctica (P. agrostidea)*	2823-12
18	*P. vahliana*	3102-5
19	*P. arctica*	2805-9
20	*P. arctica (P. agrostidea)*	2823-13
21	*P. vahliana*	3102-6
22	*P. arctica*	2805-10
23	*P. arctica (P. poacea)*	2864-1
24	*P. vahliana*	3102-8

Col.	taxon	specimen
25	*P. vahliana*	3102-10
26	*P. tenella (P. langeana)*	3164-8
27	*P. banksiensis*	2842-3
28	*P. vahliana*	2806-1
29	*P. phryganodes*	3169-9
30	*P. banksiensis* (f)	2842-4
31	*P. vahliana* (f)	2806-2
32	*P. tenella (P. langeana)*	3164-10
33	*P. banksiensis*	2842-5
34	*P. vahliana*	2806-6
35	*P. wrightii*	09-01
36	*P. banksiensis*	2842-11
37	*P. vahliana*	2806-7
38	*P. wrightii*	09-02
39	no sample	
40	*P. vahliana*	2806-10
41	*P. wrightii*	09-03
42	no sample	
43	*P. tenella (P. langeana)*	3164-5
44	*P. alaskana*	bogo1
45	no sample	
46	*P. tenella (P. langeana)*	3164-7
47	*P. alaskana*	bogo2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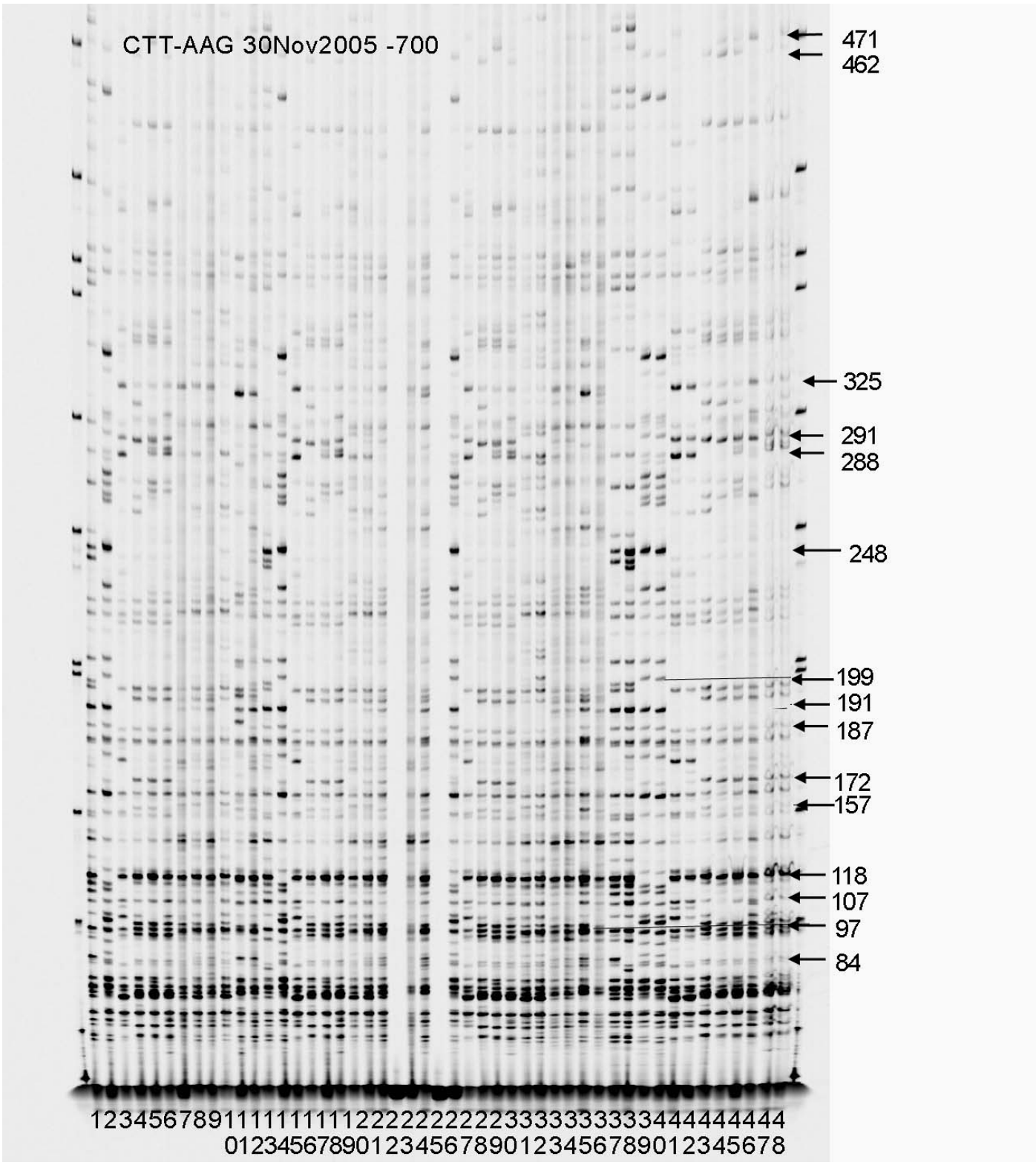
CTT-AAG 30Nov2005 – 700

Col.	taxon	specimen
1	*P. arctica*	2808-13
2	*P. arctica*	2805-4
3	*P. arctica (P. agrostidea)*	2813-14
4	*P. arctica (P. agrostidea)*	2823-1
5	*P. arctica (P. poacea)*	2864-9
6	*P. arctica (P. poacea)*	2898-1
7	*P. banksiensis*	2810-16
8	*P. banksiensis*	2810-18
9	*P. tenella (P. langeana)*	3164-1
10	*P. tenella (P. langeana)*	3164-2
11	*P. vahliana*	2884-4
12	*P. vahliana*	2836-4
13	*P. bruggemannii*	2906-1
14	*P. bruggemannii*	2906-3
15	*P. angustata*	2866-1
16	*P. angustata*	2866-9
17	*P. angustata*	2865-1
18	*P. angustata*	2865-3
19	*P. arctica*	2805-4
20	*P. arctica (P. agrostidea)*	2823-1
21	*P. arctica (P. poacea)*	2898-1
22	*P. banksiensis* (f)	2810-18
23	*P. tenella (P. langeana)*	3164-2
24	*P. vahliana*	2836-4

Col.	taxon	specimen
25	*P. bruggemannii* (f)	2906-4
26	*P. bruggemannii*	2906-9
27	*P. angustata*	2866-11
28	*P. angustata*	2866-24
29	*P. andersonii*	2865-9
30	*P. andersonii*	2865-12
31	*P. arctica*	2805-4
32	*P. arctica (P. agrostidea)*	2823-1
33	*P. arctica (P. poacea)*	2898-1
34	*P. banksiensis*	2810-18
35	*P. tenella (P. langeana)*	3164-2
36	*P. vahliana*	2884-4
37	*P. bruggemannii*	3080-2
38	*P. bruggemannii*	3080-5
39	*P. angustata*	2851-1
40	*P. angustata*	2851-4
41	*P. andersonii*	2878-11
42	*P. andersonii*	2878-14
43	*P. arctica*	2805-4
44	*P. arctica (P. agrostidea)*	2823-1
45	*P. arctica (P. poacea)*	2898-1
46	*P. banksiensis*	2810-18
47	*P. tenella (P. langeana)*	3164-2
48	*P. vahliana*	2836-4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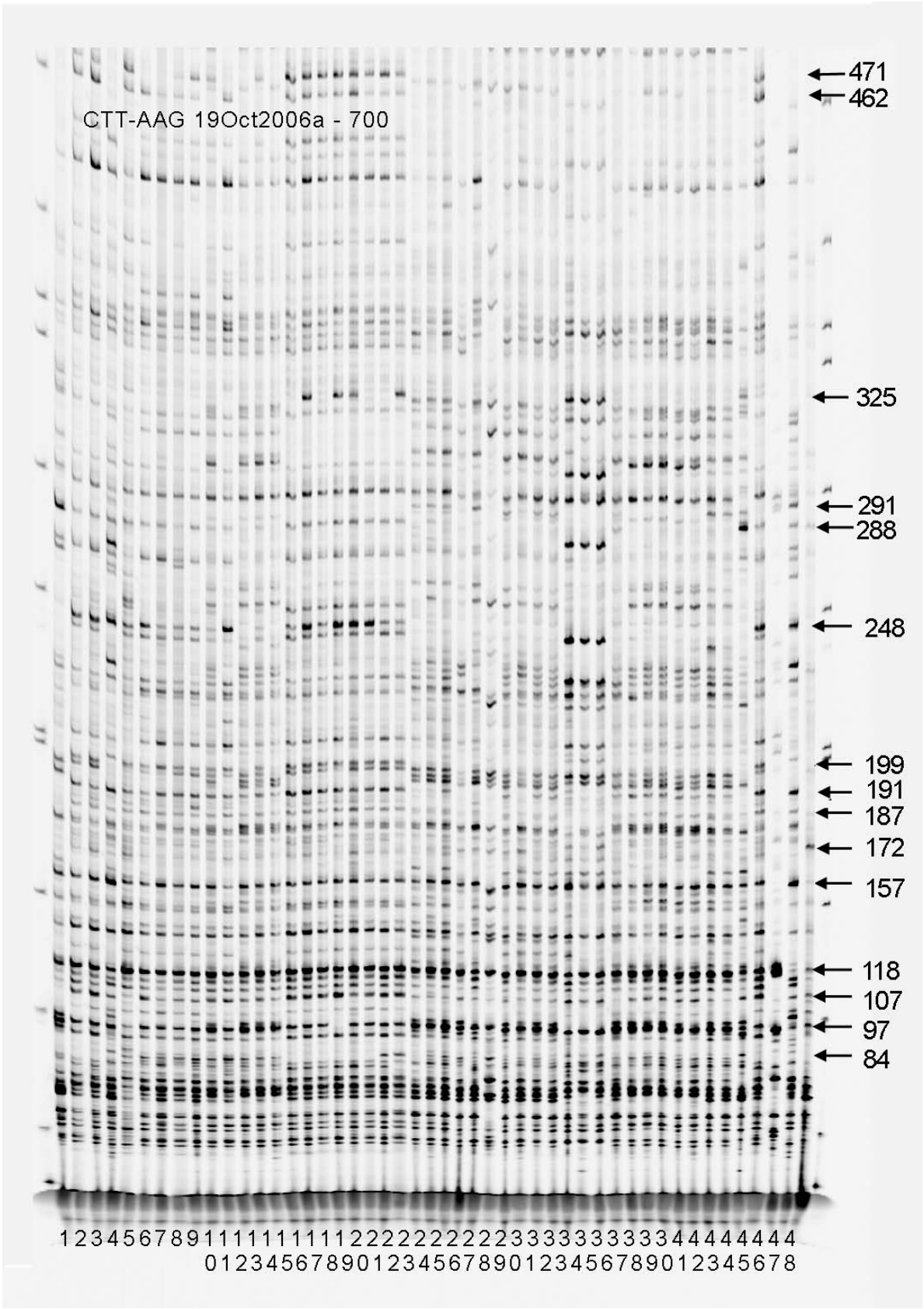
CTT-AAG 19Oct2006a – 700

Col.	taxon	specimen
1	*P. arctica*	2805-4
2	*P. vahliana*	5808
3	*P. vahliana*	2884-4
4	*P. tenella (P. langeana)*	3164-2
5	*P. vahliana*	vah
6	*P. vahliana*	2831-1
7	*P. vahliana*	2831-2
8	*P. vahliana*	2831-3
9	*P. vahliana*	2831-4
10	*P. vahliana*	2831-5
11	*P. vahliana*	3074-1
12	*P. vahliana*	3074-2
13	*P. vahliana*	3074-4
14	*P. vahliana*	3074-8
15	*P. vahliana*	3073-2
16	*P. vahliana*	3073-5
17	*P. bruggemannii*	3075-1
18	*P. bruggemannii*	3075-16
19	*P. bruggemannii*	3075-22
20	*P. bruggemannii*	3075-23
21	*P. vahliana*	3083-1
22	*P. vahliana*	3083-3
23	*P. angustata*	3140-1
24	*P. angustata*	3140-2

Col.	taxon	specimen
25	*P. angustata*	3140-3
26	*P. angustata*	3150-1X
27	*P. angustata*	3150-2
28	*P. angustata*	3150-3X
29	*P. angustata*	3150-4
30	*P. angustata*	3150-5
31	*P. angustata*	3151-2
32	*P. angustata*	3151-3
33	*P. alaskana*	bogo3
34	*P. alaskana*	bogo4
35	*P. alaskana*	bogo5
36	*P. alaskana*	2807-1
37	*P. bruggemannii*	3100-1
38	*P. bruggemannii*	3100-2
39	*P. bruggemannii*	3100-3
40	*P. bruggemannii*	3100-4
41	*P. bruggemannii*	3144-24
42	*P. vahliana*	2849-1
43	*P. vahliana*	2849-5
44	*P. arctica*	2805-4
45	*P. vahliana*	5808
46	*P. vahliana* (f)	2884-4
47	*P. tenella (P. langeana)*	3164-2
48	*P. banksiensis*	2810-9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Appendix H. Gels showing restriction digests of ITS and *rpoB-trnC* amplifications.

Amplifications of ITS cut with *BanII*, *Sau96I*, *AluI*, *BbvI, DpnII,* and of *rpoB-trnC* amplifications cut with *Sau96I.* Letters following individual plant numbers correspond to the sets A, B, and C, which are each comprised of a mixture of species in order to compare the samples side by side. Some gels contain a control for the particular cut instead of a DNA ladder because of space limitations on the gel.

P. alaskana bogo01 , bogo02 (pre), bogo03 (C)

P. andersonii 2856-06 (A), 2856-08 (B), 2856-09 (B), 2856-16 (B), 2865-01 , 2865-03 (B), 2865-09 , 2865-12 , 2865-13 (A), 2865-14 (A), 2865-16 (A), 2865-17 , 2865-18 (A), 2877-03 , 2877-17 , 2878-09 (A), 2878-10 (B), 2878-11 (B), 2878-14 (B), 2878-19 (A).

P. angustata 2849-05 (A), 2851-03 (B), 2851-04 (A), 2851-09 (B), 2851-13 (B), 2851-14 (A), 2851-18 (B), 2853-02 (B), 2853-05 (A), 2853-07 (B), 2853-09 (B), 2853-14 (B), 2853-16 (B), 2856-10 (B), 2856-12 (B), 2858-11 (A), 2858-12 (B), 2866-01 , 2866-03 (A), 2866-11 , 2866-14 (A), 2866-15 (A), 2866-21 (A), 2866-24 , 2873-02 (B), 2874-07 , 2874-12 (A), 2874-13 , 2874-14 , 2874-15 (A), 2874-19 , 2877-20 , 2877-24 , 2878-06 (B), 2878-20 (B), 2878-23 (B), 2899-24 (B), 3072-10 (B), 3072-12 (B), 3072-13 (B), 3072-23 (B), 3072-24 (B), 3144-06 (C), 3144-08 (C), 3144-09 (C), 3144-11 (C), 3144-13 (C), 3154-02 (C), 3154-03 (C), 3154-04 (C), 3154-08 (C), 3154-18 (C), 3154-25 (C), 3154-27 (C).

P. arctica (*P. agrostidea*) 2813-7 (pre), 2813-12 , 2813-22 , 2817-06 (A); (*P. arctica* s.s.) 2805-04 (A).

P. banksiensis 2810-09 (A), 2810-16 , 2814-03 (C).

P. bruggemannii 2906-03 , 2906-04 , 3063-01 (B), 3063-03 (B), 3072-02 , 3072-08 (A), 3072-14 , 3072-18 (B), 3075-02, 3075-03 (B), 3075-09 (B), 3075-10 (B), 3075-15 (A), 3080-02 (pre), 3080-05 (rpo2), 3080-09 (rpo2), 3080-10 (B), 3080-22 (B), 3080-24 (B), 3080-25 (B), 3083-02 (A), 3083-06 (A), 3083-10 (A), 3100-01 (B), 3100-02 (C), 3100-03 (C), 3100-04 (C), 3144-07 (C), 3144-18 (C), 3144-24 (C).

P. hauptiana haupBub (C), haup4106b (C), haup605 (C).

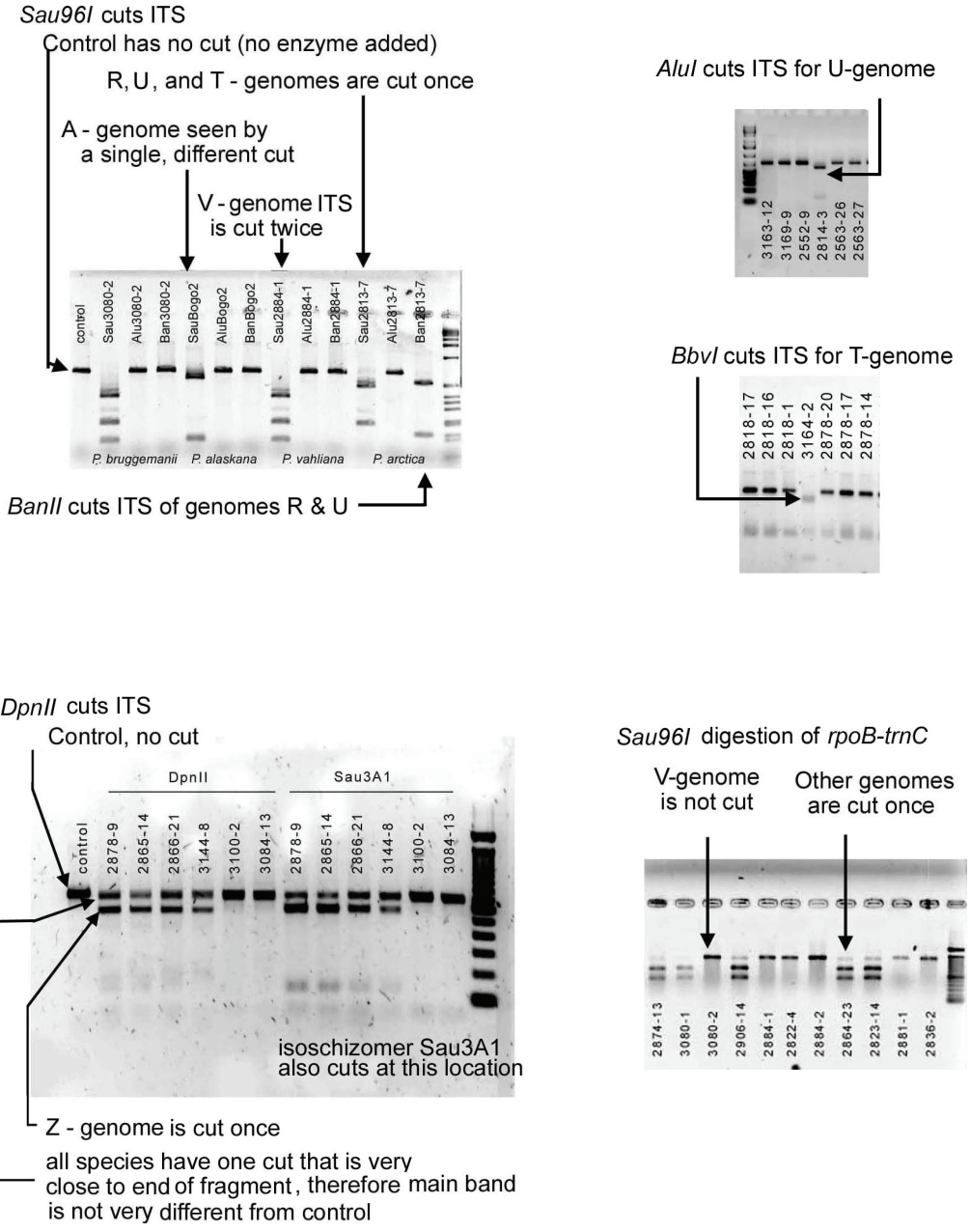
P. phryganodes 2818-01 (B), 2818-16 (B), 2818-17 (B), 2847-01 (A), 2847-02 (A), 3123-01 (C), 3123-02 (C), 3123-03 (C), 3163-04 (A), 3163-05 (C), 3163-12 (C), 3163-13 (C), 3163-14 (C), 3169-02 (C), 3169-05 (C), 3169-09 (C).

P. pumila 2552-09, 2563-26, 2563-27, 2563-28, 2926-01, 2926-19.

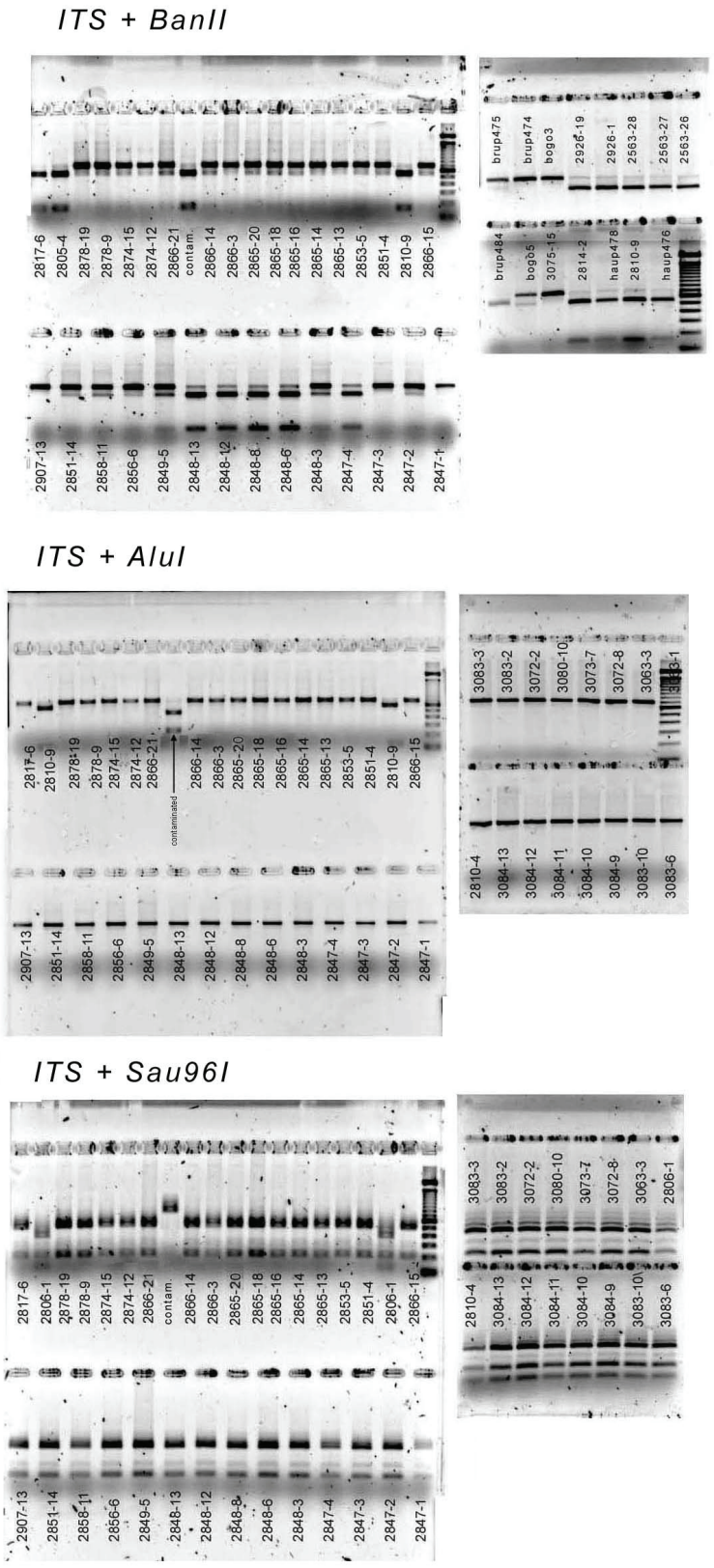
P. vahliana 2836-01 , 3083-03 (A), 3084-09 (A), 3084-10 (A), 3084-11 (A), 3084-12 (A), 3084-15 (C), 3101-01 (B)

P. sp. 2 2848-01 (B), 2848-03 (A), 2848-06 (A), 2848-08 (A), 2848-11 (B), 2848-12 (A), 2848-13 (A), 2848-14 (B), 2848-19 (B).

P. tenella 2907-03 (A), 2907-13 (A), 3164-01 , 3164-02 (A).

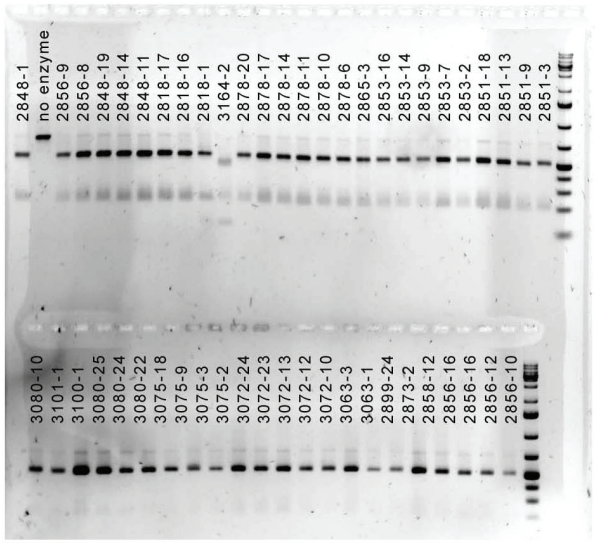


Figures above show the cuts when the amplification products are digested with each of the enzymes.

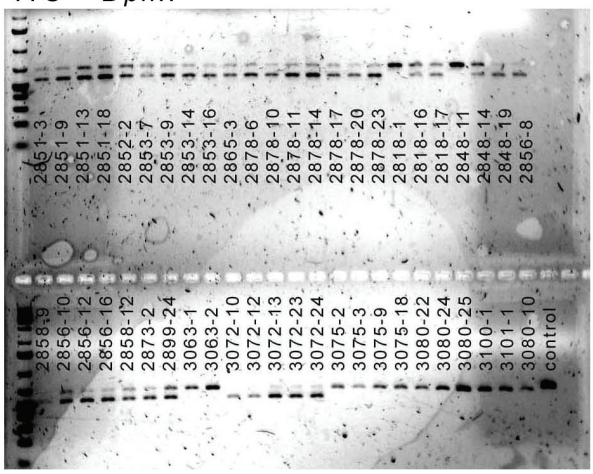


Appendix H1. Sample set A, showing ITS amplification digested with *BanII*, *Sau96I*, and *AluI*.

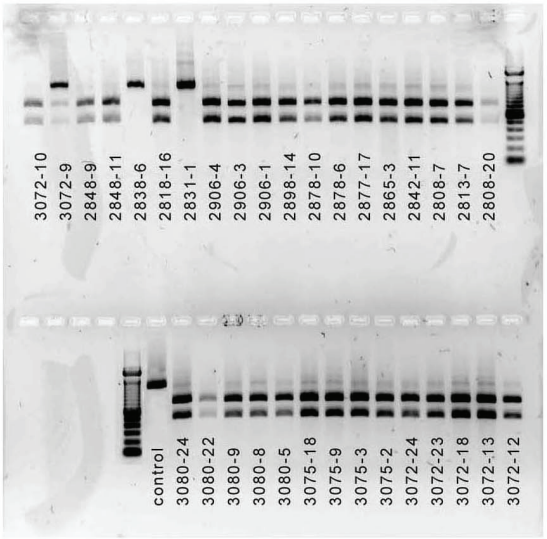
ITS + BbvI



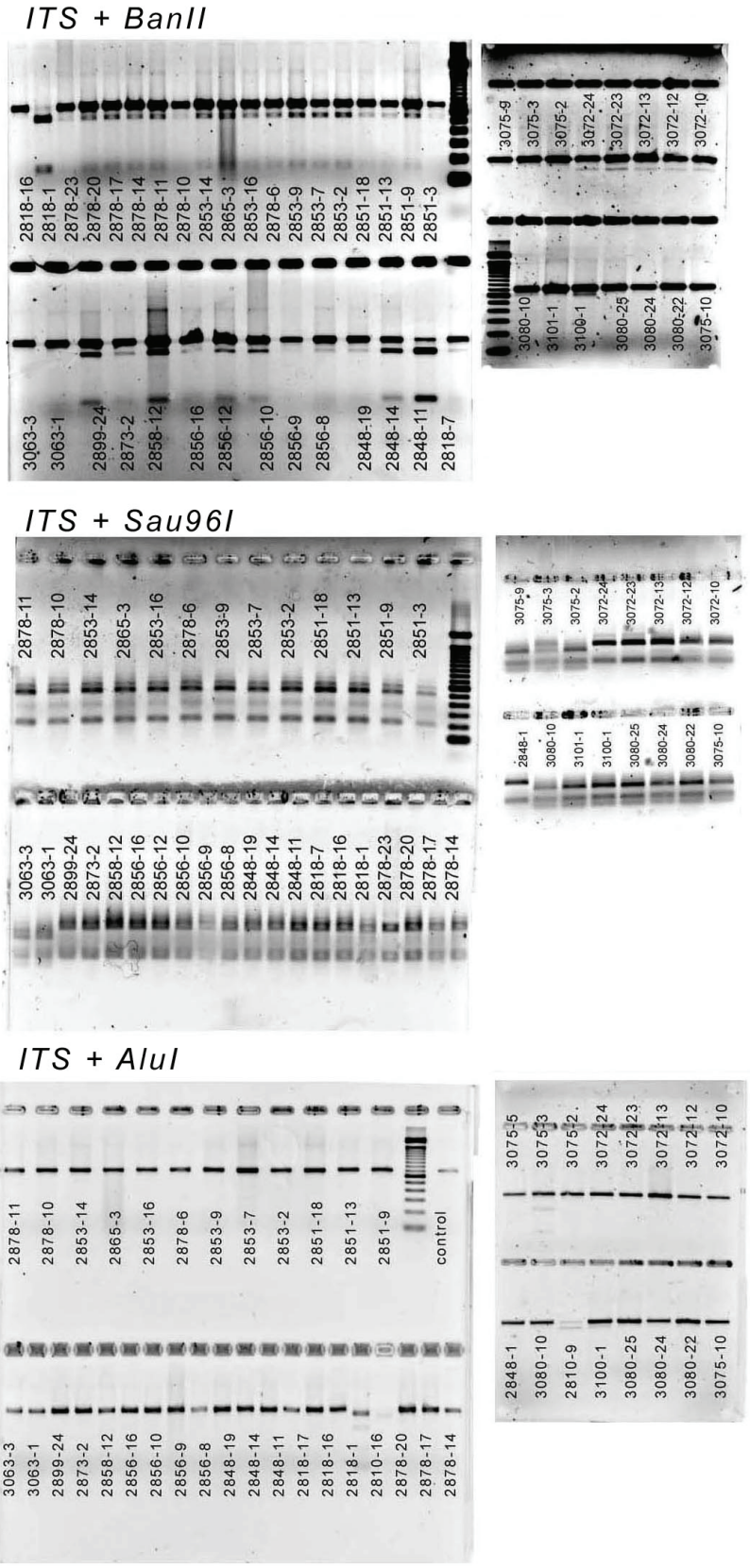
ITS + DpnII



rpoB-trnC + Sau96I

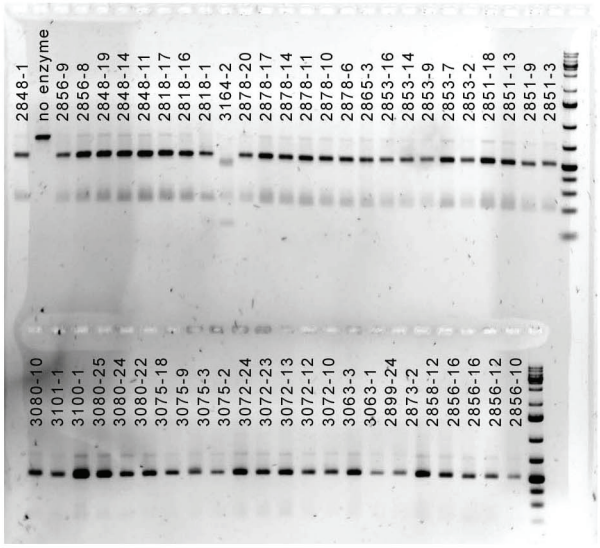


Appendix H2. Sample set A (same sample set as in Appendix H1), showing ITS amplifications digested with *BbvI* and *DpnII* and *rpob-trnC* amplification digested with *Sau96I*.

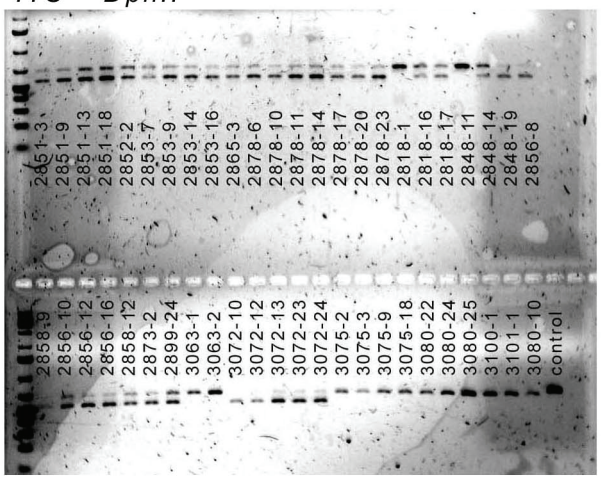


Appendix H3. Sample set B, showing ITS amplifications digested with restriction enzymes *BanII, Sau96I, and AluI*.

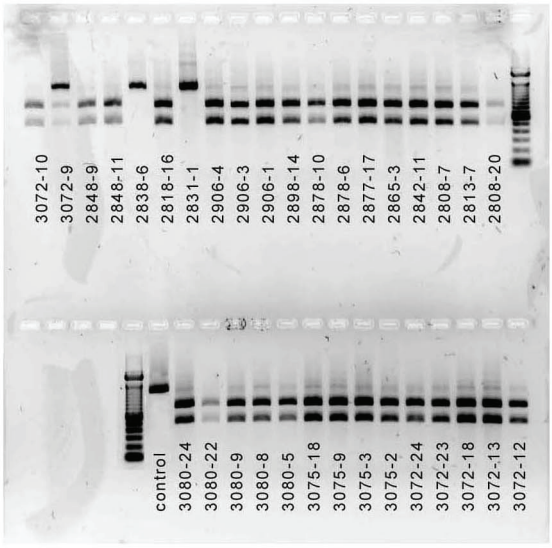
ITS + BbvI



ITS + DpnII

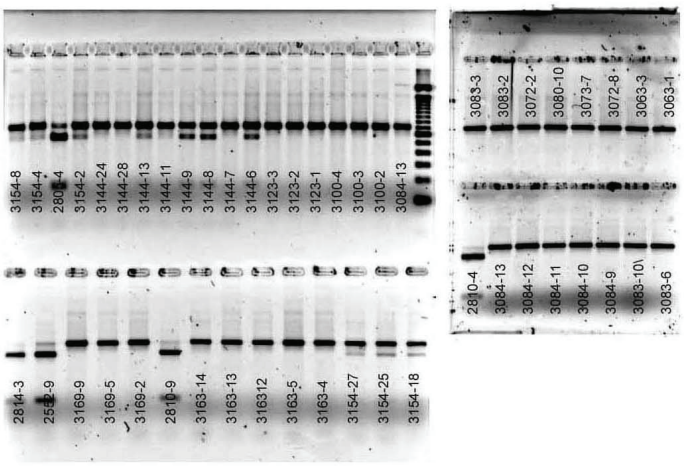


rpoB-trnC + Sau96I

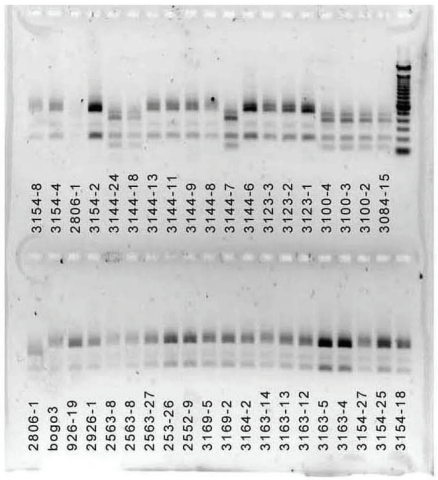


Appendix H4. Sample set B (same as in Appendix H4), showing ITS amplifications digested with *BbvI* and *DpnII* and *rpob-trnC* amplification digested with *Sau96I*.

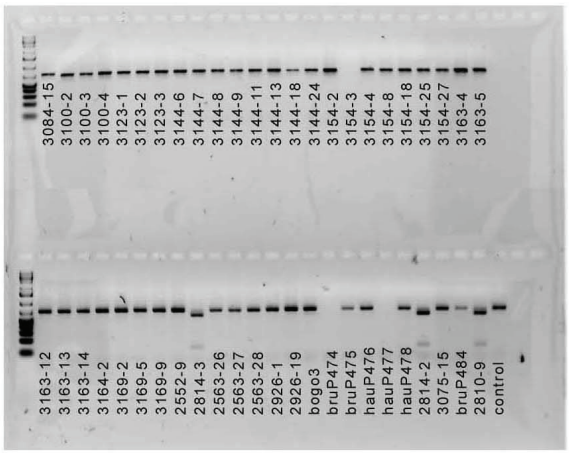
ITS + BanII



ITS + Sau96I

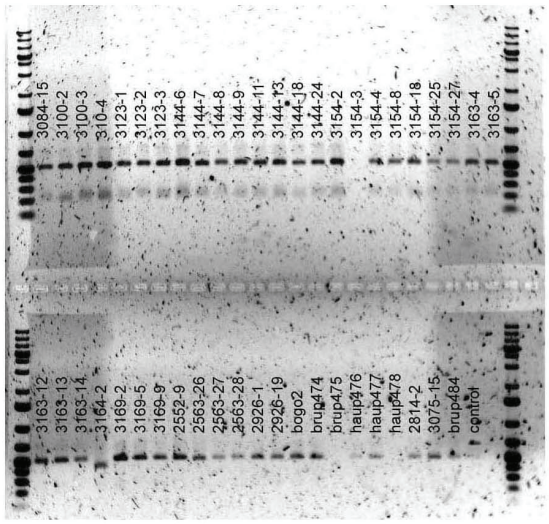


ITS + AluI

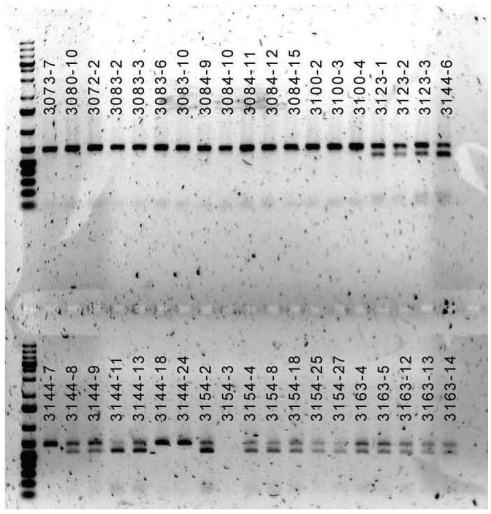


Appendix H5. Sample set C, showing ITS amplifications digested with restriction enzymes *BanII, Sau96I, and AluI*.

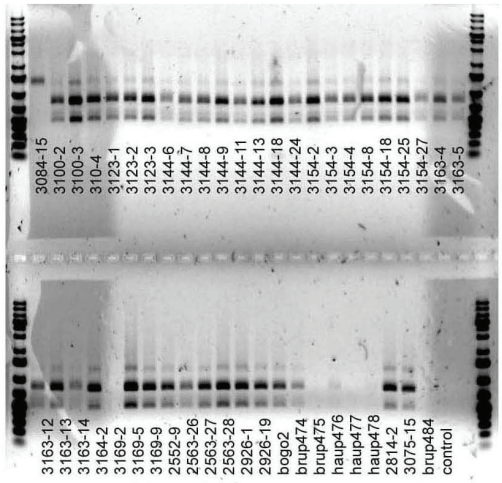
ITS + BbvI



ITS + DpnII



rpoB-trnC + Sau96I



Appendix H6. Sample set C (same as in Appendix H5), showing ITS amplifications digested with *BbvI* and *DpnII* and *rpob-trnC* amplification digested with *Sau96I*.

Appendix I. *Puccinellia* collections and herbarium specimens examined and mapped in this study.

Appendix IA. Collection information for the specimens analyzed for ploidy, AFLP patterns, and morphology in this thesis, in order of collection number. Vouchers at MTMG unless otherwise indicated. Abbreviation "herb" means that DNA was extracted from herbarium specimen.

P. agrostidea

CANADA. NWT, Banks I, De Salis Bay, west end of unnamed lake north of bay, in and around silt/mud ditch, elevation 80 m a.s.l., 71°28.02'N, 121°54.79'W, 20 July 2003, *LLC 2813, LJG, and H. Bickerton*; NWT, Banks I, De Salis Bay, washout plain near Windrum Lagoon, dried silty-sandy soil, elevation 7 m a.s.l., 71°27.27'N, 121°52.10'W, 22 July 2003, *LLC 2823, LJG, and H. Bickerton*; NWT, Banks I, Thomson River Valley, *S.G. Aiken 99-210* (CAN).

P. arctica s.s.

CANADA. NWT, Banks I, Masik R, beside river, frost boiled-silty sand with pebbles, elevation 60 m a.s.l., 71°37.06'N, 123°05.86'W, 16 July 2003, *LLC 2805, LJG, and H. Bickerton*; NWT, Banks I, Masik R, top of ridge, south of unnamed lake, elevation 114 m a.s.l., 71°35.04'N, 123°11.27'W, 15 July 2005, *LLC 2808, LJG, and H. Bickerton*; NWT, Banks I, DeSalis Bay, along coast at shore of Windrum Lagoon just north of bay (not flowering when collected), elevation 4 m a.s.l., 71°28.89'N, 121°48.98'W, 21 July 2003, *LLC 2817, LJG, and H. Bickerton*; Yukon, Herschel I, *W.J. Cody 36154* (CAN).

P. poacea

CANADA. Nunavut, Ellesmere I, Tanquary Camp, Ellesmere Island National Park Reserve, ~1 km s of camp. In dried up stream bed, 81° 24'N, 76°52'W, 19 July 1999, *LLC 2140 & LJG*; Nunavut, Ellesmere I, 2 km E of Eureka, along coast just above sea level in sandy-silty soil, elevation 5-10 m a.s.l., 79°58.35'N, 85°41.72'W, 4 August 2003, *LLC 2864 and K. Faubert*; Nunavut, Ellesmere I, Whitsunday Bay., slope above river valley, elevation 53 m a.s.l., 79°05.22'N, 87°01.76'W, 9 August 2003, *LLC 2894 and K. Faubert*; Nunavut, Axel Heiberg I, Buchanan Lake, shore, gravel and silt, 11 m a.s.l., 79°29.93'N, 87°23.74'N, 11 August 2003, *LLC 2898 and K. Faubert*; Nunavut, Ellesmere I, Tanquary Camp, near head of Tanquary Fiord, Ellesmere Island National Park Reserve, elevation 5–20 m a.s.l., 81°24'N, 76°52'W, 4 August 1994, *LJG 5744 and S.G. Aiken.*

P. banksiensis

CANADA. NWT, Banks I, Masik R, ridge above shore of unnamed lake south of Masik River, elevation 82 m a.s.l., 71°35.64'N, 123°08.94'W, 19 July 2003, *LLC 2810, LJG, and H. Bickerton*; NWT, Banks I, De Salis Bay, beside unnamed lake in cracked brown silty soil topped

with pebbles, 40% ground cover, elevation 9 m a.s.l., 71°28.19'N, 121°53.05'W, 21 July 2003, *LLC 2814, LJG, and H. Bickerton*; NWT, Banks I, Egg River, south facing slope of small creek flowing into Egg River, *Dryas* hummock tundra, on frost boils with *Draba* and *Poa glauca*, elevation 58 m a.s.l., 72°25.65'N, 124°24.39'W, 28 July 2003, *LLC 2842, LJG and H. Bickerton*.

P. vahliana

CANADA. NWT, Banks I, Masik R, solufluction slope above river valley, sparsely vegetated hummocks of moist silty sand with pebbles, elevation 294 m a.s.l., 71°40.05'N, 122°46.61'W, 16 July 2003, *LLC 2806, LJG and H. Bickerton*; NWT, Banks I, De Salis Bay, north of bay, small frost boils, elevation 10 m a.s.l., 71°27.54'N, 121°52.57'W, 22 July 2003, *LLC 2822, LJG and H. Bickerton*; NWT, Banks I, Nelson Head, at edge of cliff, shallow soil on rock adjacent to a wet meadow, south facing slope, elevation 271 m a.s.l., 71°05.55'N, 122°46.76'W, *LLC 2831, LJG and H. Bickerton*; NWT, Banks I, Durham Heights, highest point of land near Nelson Head, elevation 665 m a.s.l., 71°07.67'N, 122°57.78'W, 24 July 2003, *LLC 2836, LJG and H. Bickerton*; NWT, Banks I, Egg River, near camp along creek 100 m from Egg River, north facing slope, turfy soil with moss covering, elevation 75 m a.s.l., 72°26.15'N, 124°23.35'W, 27 July 2003, *LLC 2838, LJG and H. Bickerton*; Nunavut, Axel Heiberg I, slope between McGill field station and Ermine Camp, moist, south facing slope, elevation 316 m a.s.l., 79°25.89'N, 90°43.41'W, 8 August 2003, *LLC 2881 and K. Faubert*; Nunavut, Axel Heiberg I, Expedition Fiord, large river rock field between Colour Lake and Thompson River, elevation 84 m a.s.l., 79°25.161'N, 90°41.48'W, 8 August 2003, *LLC 2884 and K. Faubert*; Nunavut, Beechey I, north shore, poorly drained gravel barrens, 10% ground cover, elevation 5–7 m a.s.l., 74°43.12'N, 91°50.45'W, 12 July 2004, *LLC 3073 and A. Archambault*; Nunavut, Beechey I, northeast shore, silty clay upwellings, 10% ground cover, 5-7 m a.s.l., 74°42.92'N, 91°50.35'W, 12 July 2004, *LLC 3074 and A. Archambault*; Nunavut, Bathurst I, Goodsir Inlet, plain, moist silty soil, hummocks, 30 m a.s.l., 75°45.54'N, 97°58.59'W, 13 July 2004, *LLC 3079 and A. Archambault*; Nunavut, Somerset I, Cunningham Inlet, 42 nautical miles south of Resolute, soil clay-silt, light brown with rock and talus pieces, elevation 63 m a.s.l., 74°06.20'N, 93°51.89'W, 13 July 2004, *LLC 3082 and A. Archambault*; Nunavut, Somerset I, Aston Bay, north facing slope, 20% grade, 10% ground cover, elevation 66 m a.s.l., 73°39.67'N, 94°42.23'W, 13 July 2004, *LLC 3084 and A. Archambault*; Nunavut, Cornwallis I, Resolute, gravel flats 1 km north of PCSP station, elevation 71 m a.s.l., 74°43.51'N, 94°59.44'W, 16 July 2004, *LLC 3102 and A. Archambault*; NWT, Prince Patrick I, Mould Bay, slopes between weather station and water reservoir, west facing slope, silty sand and rock, elevation 32 m a.s.l., 76°14.88'N, 119°11.18'W, 21 July 2004, *LLC 3149 and A. Archambault*; Nunavut, Baffin I, Nanisivik, vicinity of airport, moist stony plateau near airport entrance, 73°02'N, 84°33'W, 26 July 2004, *LLC 3171 and A. Archambault*.

P. tenella subsp. *langeana*

CANADA. Nunavut, Lower Savage I, east shore, just above high tide line, 100–200 m from ocean, 61°49.15'N, 65°42.62'W, 13 August 1999, *LLC 2352, LJG and R.J. Soreng*; NWT, Banks I, Duck Hawk Bluff, sand, with silt/clay underneath, 50 m from shore, elevation just above sea level, 71°57.34'N, 125°49.25'W, 30 July 2003, *LLC 2856a, LJG and H. Bickerton*; Nunavut, Baffin I, Tarr Inlet, rocky coast, 13 m a.s.l., 63°43.73'N, 68°24.42'W, 16 August 2003, *LLC 2907 and K. Faubert*; Nunavut, Baffin I, Tarr Inlet, in sand, in area sometimes submerged at high tide, elevation 0–1 m a.s.l., 63°43.77'N, 68°24.56'W, 25 July 2004, *LLC 3164 and A. Archambault*; Nunavut, Bylot I, *Tremblay 04*.

P. alaskana

USA. Alaska, Bogoslof Island, Aleutian Islands, *R. Meehan #BOGO03* (CAN); USA, Alaska, Little Diomede Island 14–20 Aug 1926, *A.E. and R.T. Porsild 1644,* (CAN) (herb); USA, Alaska, Kiska quad., Rat Islands, *M. Dick 83* (ALA) (herb); USA, Alaska, Kiska quad., Rat Islands, *M. Dick 84*. (ALA) (herb); USA, Alaska, Umnak quad., Bogoslof Island, *B.F. Friedman 80-147*. (ALA) (herb).

P. tenella subsp. *tenella*

RUSSIA. Arctic Jakutia. *Petrovsky 8.IX.1955* (CAN) (herb); Sahka Republic (Yakutia), Polyarka, *Solstad & Elven 04/1156* (ALA) (herb); Chutotka Peninsula, Lavrentiya, 29 Aug 1971, *N.A. Sekretarova et al. s.n.* (ALA) (herb).

P. wrightii

USA. Alaska, *R. Elven and H. Solstad 09*. Alaska, Cape Prince of Wales. 65° 37'N, 168°05'W. *T. Kelso 82-230* (ALA) (herb); RUSSIA. Chukotka Peninsula, Chaplin Cape, *R. Satpuisau s.n.* (ALA) (herb); Chukotka Peninsula., Cape Chaplino, *V. Gavreluk* (ALA) (herb); Chukotka Peninsula, Lavrentiya, *N.A. Sekretarova et al.* (ALA) (herb.); Chukotka Peninsula, Uelen, *N.A. Sekretarova et al.* (ALA) (herb).

P. lemmonii

USA. California, Modoc County, Modoc National Wildlife Refuge, East of US Route 395, 2.4 road mi south of Alturas, N41°27.16', W120°32.92', elevation 1340 m a.s.l., moist alkaline soil, in association with *Puccinellia nuttalliana*, 18 June 1986, *J. I. Davis 425* (BH, MTMG).

P. parishii

USA.New Mexico, Grant County, NW side of New Mexico Hwy 61, ca. 3 rd mi E of U.S. Hwy 180, N32°33.75', W107°58.76', elevation 1564 m a.s.l., in mineralized soil of moderately heavily grazed seepage area downstream from spring, 6 May 1989, *J.I. Davis and P.S. Manos 568* (BH, MTMG).

P. andersonii

CANADA. NWT, Banks I, Worth Point, sandy slope just above coast, and at tundra interface, elevation 4–8 m a.s.l., 72°15.35'N, 125°39.35'W, 29 July 2003, *LLC 2851b, LJG & H.*

Bickerton; NWT, Banks I, Duck Hawk Bluff, sand, with silt/clay underneath, 50 m from shore, elevation just above sea level, 71°57.34'N, 125°49.25'W, 30 July 2003, *LLC 2856b, LJG and H. Bickerton*; Nunavut, Ellesmere I, Eureka, 5.32 km east of airport, sandy, silt habitat at coast, just above high tide line. 79°58.02'N, 85°36.73'W, 5 Aug. 2003, *LLC 2865 and K. Faubert*; Nunavut, Axel Heiberg I, Colour Peak, Big Hill, silty soil, just above sea level, 79°22.79'N, 91°16.28'W, 6 August 2003, *LLC 2877a and K. Faubert*; Nunavut, Axel Heiberg I, Gypsum Hill, elevation 263 m a.s.l., 79°24.737'N, 90°44.340'W, 7 August 2003, *LLC 2878a and K. Faubert*.

P. angustata

CANADA. NWT, Banks I, Masik R, top of ridge, south of unnamed lake, elevation 114 m a.s.l., 71°35.04'N, 123°11.27'W, 15 July 2005, *LLC 2809, LJG & H. Bickerton*; NWT, Banks I, Egg R, approximately 200 m from coast, on bare hummocks which are interspersed with *Dryas*-covered hummocks, elevation 10 m a.s.l., 72°34.27'N, 124°56.56'W, *LLC 2849, LJG, and H. Bickerton*; NWT, Banks I, Worth Point, sandy slope just above coast, and at tundra interface, elevation 4–8 m a.s.l.. 72°15.35'N, 125°39.35'W, 29 July 2003, *LLC 2851a, LJG, and H. Bickerton*; NWT, Banks I, Worth Point, sandy slope just above coast, and at tundra interface, elevation 4–8 m a.s.l.. 72°15.35'N, 125°39.35'W, 29 July 2003, *LLC 2853, LJG, and H. Bickerton*; NWT, Banks I, Duck Hawk Bluff, sand, with silt/clay underneath, 50 m from shore, elevation just above sea level, 71°57.34'N, 125°49.25'W, 30 July 2003, *LLC 2856c, LJG and H. Bickerton*; NWT, Banks I, Sachs Harbour, south facing slope descending west from airstrip towards the town, elevation 95 m. a.s.l., 71°59.51'N, 125°14.49'W, 31 July 2003, *LLC 2858*; Nunavut, Axel Heiberg I, Colour Peak, Big Hill, silty soil, at sea level, 79°22.79'N, 91°16.28'W, 6 August 2003, *LLC 2877b & K. Faubert*; Nunavut, Axel Heiberg I, Gypsum Hill, 263 m a.s.l., 79°24.74'N, 90°44.34'W, 7 August 2003, *LLC 2878b and K. Faubert*; Nunavut, Ellesmere I, Eureka, roadside disturbance, primarily this species with a few *P. poacea*, hard-packed dry silty soil with light salt crust, elevation 81 m a.s.l., 79°59.26'N, 85°43.87'W, 5 August 2003, *LLC 2866 and K. Faubert*; Nunavut, Axel Heiberg I, Colour Peak, Big Hill, to west of main spring, 750 m from water edge, elevation 40 m a.s.l., 79°22.87'N, 91°16.79'W, 6 August 2003, *LLC 2874 and K. Faubert*; Nunavut, Ellesmere I, Blue Man Cape, large permafrost slump, elevation 73 m a.s.l., 79°44.51'N, 85°55.90'W, 11 August 2003, *LLC 2899 and K. Faubert*; Nunavut, Cornwallis I, Resolute, 2 km east of town, 100 m from coast, elevation 21 m a.s.l., 74°40.42'N, 94°55.29'W, 12 July 2004, *LLC 3072b and A. Archambault*; NWT, Prince Patrick Island, Mould Bay, 100 m south of airstrip, 5-10 m from coast, elevation 13 m a.s.l., 76°13.95'N, 119°19.27'W, *LLC 3137 and A. Archambault*; Nunavut, Prince Patrick Island, Mould Bay, SW facing slope 3 km south of Mould Bay Station, 34 m a.s.l., 76°13.54'N, 119°18.10'W, 20 July 2004, *LLC 3141 and A. Archambault*; NWT, Prince Patrick I, Mould Bay, north facing slope 2 km south of airstrip, elevation 35 m a.s.l., 76°13.521'N, 119, 117.98'W, 20 July 2004, *LLC 3144a and A. Archambault*; NWT, Prince Patrick I, Mould Bay, ½ km north of Mould Bay station, elevation 35 m a.s.l., 76°14.89'N,

119°22.18'W, 21 July 2004, *LLC 3150 and A. Archambault*; NWT, Prince Patrick I, Mould Bay, ½ km north of Mould Bay station, elevation 35 m a.s.l., 76°14.89'N, 119°22.18'W, 21 July 2004, *LLC 3151 and A. Archambault*; NWT, Prince Patrick I, Mould Bay, 3 km north of Mould Bay station, around edge of enormous frost boil, dry hummocks of silty soil, elevation 40 m a.s.l., 76°14.89'N, 119°22.18'W, 21 July 2004, *LLC 3154 and A. Archambault.*

P. bruggemannii

CANADA. Nunavut, Cornwallis I, Resolute, *LLC 2906 and K. Faubert*; Nunavut, Cornwallis I, Resolute, barrens 1 km north of PCSP station, elevation 80 m a.s.l., 74°43.80'N, 94°58.93'W, 11 July 2004, *LLC 3063 and A. Archambault*; Nunavut, Cornwallis I, Resolute, 2 km east of town, 100 m from coast, elevation 21 m a.s.l., 74°40.42'N, 94°55.288'W, 12 July 2004, *LLC 3072 and A. Archambault*; Nunavut, Beechey I, northeast shore, silty clay upwellings, 10% ground cover, elevation 5-7 m a.s.l., 74°42.92'N, 91°50.35'W, 12 July 2004, *LLC 3075 and A. Archambault*; Nunavut, Bathurst I, Goodsir Inlet, plain, moist silty soil, hummocks, elevation 30 m a.s.l., 75°45.54'N, 97°58.59'W, 13 July 2004, *LLC 3080 and A. Archambault*; Nunavut, Somerset I, Cunningham Inlet, 42 nautical miles south of Resolute, soil clay-silt, light brown with rock and talus pieces, elevation 63 m a.s.l., 74°06.20'N, 93°51.89'W, 13 July 2004, *LLC 3083 and A. Archambault*; Nunavut, Cornwallis I, Resolute, wet meadows west of PCSP station, elevation 80 m a.s.l., 74°43.08'N, 94°59.59'W, 16 July 2004, *LLC 3100 and A. Archambault*; NWT, Prince Patrick I, Mould Bay, north facing slope 2 km south of airstrip, elevation 35 m a.s.l., 76°13.52'N, 119, 117.98'W, 20 July 2004, *LLC 3144b and A. Archambault.*

P. phryganodes

CANADA. Nunavut, Axel Heiberg I, Expedition Fiord, Colour Peak, near high tide line, 79°23'N, 90°51'W, 9 August 1999, *Dale Anderson (LLC 2321)* (CAN); NWT, Banks Island, De Salis Bay, along coast at shore of lagoon just north of Bay (not flowering when collected), elevation 4 m a.s.l., 71°28.89'N, 121°48.98'W, 21 July 2003, *LLC 2818, LJG, and H. Bickerton*; NWT, Banks Island, Big River, in sand, at shoreline, 72°34.27'N, 124°56.56'W, 29 July 2003, *LLC 2847, LJG & H. Bickerton*; Nunavut, Cornwallis Island, N of Resolute, at coast, 74°44.84'N, 95°03.79'W, 18 July 2004, *LLC 3123 & A. Archambault*; Nunavut, Baffin Island, Tarr Inlet, rocky coast, 13 m a.s.l., 63°43.73'N, 68°24.42'W, 16 August 2003, *LLC 2908 & K. Faubert*; Nunavut, Baffin Island, Tarr Inlet, 20 m from high tide line, reddish sand, gravel and rock, boulders, 10 m a.s.l., 63°43.87'N, 68°25.95'W, 25 July 2004, *LLC 3163 & A. Archambault*; Nunavut, Baffin Island, Tarr Inlet, at coast, sometimes suberged at high tide, 63°43.77'N, 68°24.56'W, 25 July 2004, *LLC 3169 & A. Archambault*.

P. sp.2

CANADA. NWT, Banks I, Big River, in sand just above high tide line, 72°34.27'N, 124°56.56'W, 29 July 2003, *LLC 2848, LJG & H. Bickerton.*

P. pumila

CANADA. Nunavut, Churchill, *S.G. Aiken 01-342* (CAN); Labrador, Nain, near shore at inlet, near sea level, 56°32.46'N, 61°41.59'W, 29 August 2002, *LLC 2512*; Labrador, Edwards Cove, 100 m from mouth of Little Reid Brook, 56°24.89'N, 62°04.88'W, 31 August 2002, *LLC 2552*; Labrador, Anaktalak Bay, coast of Edwards Cove, 2 km west of camp, in sand just above high tide line, *LLC 2926*; Labrador, Anaktalak Bay, coast of Edwards Cove, 1.5 km east of camp, at high tide line, 56°25.22'N, 62°03.37'W, 1 September 2002, *LLC 2563*.

P. hauptiana

USA, Alaska, Palmer, Dutilly, Lepage, and O'Neill 20115 (CAN) (herb); RUSSIA. *Bubnova*, 26 June 1986 (CAN) (herb); Litwinow *4106b* (CAN) (herb); RUSSIA, *Kogelbreckob 605* (CAN) (herb).

Appendix IB. Additional herbarium specimens of *Puccinellia* examined and mapped, listed in order of locality. Vouchers at CAN unless otherwise indicated.

P. andersonii

CANADA. Nunavut, Axel Heiberg I, Expedition Fiord, *Consaul et al. 2298*; *Consaul & Gillespie 2258, 2266*; *Beschel 11104*; Marine sediment and sand flats along NW shore of Flat Sound, *Consaul et al. 2291*; Mokka Fjord, *Parker 73075*; Piling Bay, *Webber & Beschel 655b*; Nunavut, Devon I, Dundas Harbour, W of harbour mouth, *Consaul et al. 2305, 2319*; *Malte 118462*; Nunavut, Ellesmere I, Caledonian Bay, along N side of Danish River, *Consaul & Gillespie 2226, 2234*; Eureka, *Consaul et al. 2277, 2278*; Hazen Camp, on N shore of Lake Hazen, Ellesmere Island National Park Reserve, *Gillespie 5791*; Irene Bay, *Gillett & Shchepanek 18311*; Tanquary Camp, Ellesmere Island National Park Reserve, *Consaul & Gillespie 2130, 2172*; Tuborg Lake, *Consaul & Gillespie 2203*; NWT, Melville I, Sherard Bay, *Maddison M1544*;coastal plain at SW head of McCormick Inlet, *Consaul & Gillespie 2441*; *2442*; Nunavut, Victoria I, Cambridge Bay, *Gillespie & Consaul 6325*; NWT, Holman Island, *A.E. Porsild 17245*; Banks I, east side Ballast Brook, *Hills 11*; Nelson Head, *Manning & Macpherson 99*; Sachs Harbour, *Gillett 18880*; GREENLAND. Disko, 27.7.1913*, M.P. Porsild s.n.*; Nugsuak peninsula, 1902, *M.P. Porsild s.n*.

P. angustata

CANADA. Nunavut, Axel Heiberg I, Expedition Fiord, McGill Univ. Field Camp on N side of Expedition River, *Consaul & Gillespie 2246, 2265*; *Consaul et al. 2282, 2287*; Marine sediment & sand flats along NW shore of Flat Sound, *Consaul et al. 2290*; Mokka Fjord, *Gillespie & Vogel 6082*; S Bastion Ridge, *Beschel 12916*; Striae Hill, *Beschel 12930*; Thompson valley, *Beschel 11487* (DAO); Nunavut, Baffin I, Arctic Bay, *Polunin 2580*; Cape Searle, *Wynne-Edwards 9171*; Clyde Inlet, *Wynne-Edwards 9076*; Head of Clyde Inlet, *Wynne-Edwards 9054,*

9077; Isortoq Fjord, *Webber 340*; Littlecote Channel, *Wynne-Edwards 9339*; Nunavut, Bathurst I, Goodsir Inlet, *Macdonald 557*; Polar Bear Pass, *Consaul & Gillespie 2454*; *Aiken & Maus 92-011*; Nunavut, Beechey I, *Malte 118471*; Nunavut, Broughton I, Island SSW of, *Smith VP134-61*; Nunavut, Cornwallis I, Northwest part of the island, "en surplomb de la riviere Rookery", *Dube 8-15-4* (DAO); Resolute Bay, *A.E. Porsild 21658*; Nunavut, Devon I, Dundas Harbour, *Malte 118463*; NWT, Ellef Ringnes I, Isachsen, *Macdonald 194*; Bache Pen, *Kelsall 59*; Nunavut, Ellesmere I, Caledonian Bay, along N side of Danish River, *Consaul & Gillespie 2222, 2231*; Cape Sheridan, Kelsall, J. P., 35; D'Iberville, *Consaul & Gillespie 2213, 2214*; Dumbell Bay, *Johnson 51*; Eureka, *Consaul et al. 2368,* 2279; *Beschel 11121*; Fosheim Peninsula, Hot Weather Creek, *Consaul et al. 2295*; Ridge Lake, *Consaul et al. 2294*; Franklin Pierce Bay, *Aiken & MacCormac 98-029, 98-033*; Goose Fjord, *Simmons 3436*. Hazen Camp, N shore of Lake Hazen, Ellesmere Island National Park Reserve, *Consaul & Gillespie 2176*; *Gillespie 5786*; Mackinson Inlet, *Balke Jr. 10* (DAO); near John Richardson Bay, *Aiken & MacCormac 98-046, 98-053*; Quiet Lake, *Harington 74*; Slidre Fiord, *Tener 32*; Sverdrup Pass, *Edlund & Roncato-Spencer* 282; Tanquary Camp, Ellesmere Island National Park Reserve, *Consaul & Gillespie 2134, 2171*; *Brassard 1436*; Tuborg Lake, *Consaul & Gillespie 2196, 2207*; Two Basin Lake, 23 km ESE of Eureka airstrip, 100-150 m, *Gillespie 5797*; Fitzwilliam Owen Island, 10-13/07/1968, *Kuc s.n.*; Nunavut, Lougheed I, *Edlund & Nixon 2113*; Nunavut, Victoria I, Cambridge Bay, *Gillespie 5836*; *Porsild 21594*; Diamond Jenness Peninsula, *Edlund 681*; Storkerson Peninsula, *Edlund 330*; NWT, Melville I, Winter Harbour Area, 8–12.8.1971, *Kuc s.n.*; Sabine Pen, 25/26.7.1971, *Kuc s.n.*; Warrington Bay, *Edlund 3918*; Ibbett Bay, *Tener & Harington 294*; McCormick Inlet, *Consaul & Gillespie 2440, 2433, 2445*; Nunavut, Melville Peninsula, Sarpca Lake, *Scott 378*; NWT, Banks I, Ballast Brook, east side, *Hills 11*; Cape Crozier, *Manning & Macpherson 145*; N.E. corner of island, *A.E. Porsild, 17657*; Nelson Head, *Manning & Macpherson 99*; Sachs Harbour, *Gillett 18823, 18827*; NWT, Prince Patrick Island, Mould Bay, *Bruggemann 529* (DAO); *Consaul & Gillespie 2420*; *MacDonald 69*; above reservoir dam, 1 km E of weather station, *Consaul & Gillespie 2417*; Mould Bay Peninsula, east coast, ~11 km E of Mould Bay, *Consaul & Gillespie 2408*; St. Helenes (sic) Island, *Edwards 6326* (DAO); GREENLAND. W Greenland, Pulateriaq, *Beschel 12188*; West Coast, Atanikerdluk, *Erlanson 3224*; East Greenland, Eskimones, *Sørensen 4538*; Hestelselv, *Halliday 238/71*; Kuhn Island, Cape Maurer, *Sørensen 2595*; Nanok, *Sørensen 2598*; Kapp Wynn, *Vaage s.n.*; NW Greenland, North Star Bay, *Ekblaw 101*; Northeast Greenland, Labhen, *Raup 750*; Northernmost Greenland, Heilprin land, Bronlund Fjord, Holmen, *Kjeld 6680*; Northwest Greenland, Polaris Bay, *Powell 245*; Greenandia bor. orient., Danmarks Havn, *Lundager 736*; Greenland, Etah, *Soper 193*; Greenland, John Murray Island, *Wulff s.n.*; Greenland bor-orient., Danmarks Havn, *Lundager 1238*; Greenland Nordkyst, Dragon Point, *Wulff s.n.*; Greenland orientalis, Kjerulf-Fiord, *Dusen s.n.*; Groenl. Occid, Disko, Asuk, *M.P. Porsild, 131154*; Groenl. Orient., Mt. Ramsay & Mt. la Cour, *Seidenfaden 1022*; Groenlandia septentrionalis, Heilbrin Land, Bronlundhus, *Fredskild s.n.*; Northeast Greenland, Kong Oscars Fjord, *H.M & L.G. Raup* 765.

P. arctica

CANADA. Nunavut, Axel Heiberg I, Diana Lake, *A.E. Porsild 18640, 18641*; Gypsum hill, W side of Gibs Fiord, *Consaul et al. 2283*; Marine sediment & sand flats along NW shore of Flat Sound, *Consaul et al. 2289*; NWT, Banks I, De Salis Bay, south coast, *A.E. Porsild 17614*; Masik River, *Mason 146* (DAO); Sachs Harbour, *Aiken 99-241*; *McEwen 313*; Thomsen River, *Aiken 99-237*; Nunavut, Ellesmere I, Caledonian Bay, *Waterston W207*; *Consaul & Gillespie 2221, 2225*; Eureka, *Consaul et al. 2280, 2281*; Fosheim Peninsula, Ridge Lake, *Consaul et al. 2292*; Hazen Camp, N shore of Lake Hazen, Ellesmere Island National Park Reserve, *Consaul & Gillespie, 2174, 2187*; Hot Weather Creek, *Edlund & Roncato-Spencer 219*; Irene Bay, *Waterston W119/72*; Lake Hazen, *Harington 355*; *Soper 8256*; Slidre Fjord, *Tener 34*; Slidre Fjord, Black Top River Valley, *Tener 33*; Tanquary Camp, Ellesmere I National Park Reserve, *Consaul & Gillespie 2135, 2167*; near head of Tanquary Fiord, Ellesmere I National Park Reserve, *Gillespie & Aiken 5756*; Vicinity of field station at the head of Tanquary Fiord, *Haight 6*; Two Basin Lake, 23 km ESE of Eureka airstrip, *Gillespie 5798*, *5800*; Nunavut, Victoria I, Cambridge Bay, *A. E Porsild 21597, 21599*; Namaycush Lake, *Edlund 37*.

P. bruggemannii

CANADA, Nunavut, Bathurst I, Goodsir inlet, *MacDonald, S. D. & Porsild, A. E., 528*; Goodsir River area, *Consaul & Gillespie 2449, 2452*; Polar Bear Pass, *Aiken 3951*; *Consaul & Gillespie 2453*; Nunavut, Beechey I, *Malte 118471*; North side of Beechey Island, *Consaul & Gillespie 2427*; Top plateau of Beechey Island *Consaul & Gillespie 2429, 2430*; Nunavut, Cornwallis I, Resolute Bay, *Consaul et al. 2322*; *Consaul & Gillespie 2455; A.E. Porsild 21659*; SE Cornwallis Island, *Consaul & Gillespie 2431;* Nunavut, King William I, Victory Point, *Cooper 186;* Nunavut, Lougheed I, south end of island, *Edlund & Nixon 2083*; Nunavut, Stefansson I, site 71, *Edlund 260*; Nunavut, Victoria I, Cambridge Bay, *Edlund & Argus 12743*; Minto Inlet, *Edlund 160A*; NWT, Melville I, Bridport Inlet, *Tener & Harrington 164*; Murray Inlet, *Aiken & Edlund 3893*; Sproule Peninsula, *Aiken & Edlund 3855*; Invincible Point, *Steen S84*; McCormick Inlet, ~10 km SW of head of inlet at river delta, *Consaul & Gillespie 2437*, 2438; Sabine Bay, Nias Point, on south coast of Bay, north shore of Melville I, *Consaul & Gillespie 2421*; Sproule Peninsula, *Aiken & Edlund 3847A, 3847B*; NWT, Prince Patrick I, Green Bay, valley at head of north arm of bay, *Consaul & Gillespie 2412*; Intrepid Inlet, West Shore. 8 km N of Disappointment Point, *Consaul & Gillespie 2413*; Mould Bay, *Consaul & Gillespie 2415, 2419*; Mould Bay Peninsula east, ~10 km E of Mould Bay, *Consaul & Gillespie 2402, 2403, 2409, 2410*; Mould Bay, above reservoir dam, 1 km E of weather station, *Consaul & Gillespie 2418.*

P. hauptiana

USA, Alaska, Bendeleben Quad., *D. F. Murray, B. A. Yurtsev, & T. Kelso 11255* (ALA); Seward Peninsula, *D. F. Murray, B. A. Yurtsev, & T. Kelso 10960* (ALA).

P. tenella subsp. *langeana*

CANADA. Nunavut, Baffin I, Arctic Bay, Admiralty Inlet, *Malte 118460*; Beekman Peninsula, *McLaren 129*; Cape Dorset, *Malte 120345*; Head of Clyde Inlet, Pipit Point, *Wynne-Edwards 9068*; Inugsuin Fjord, *Hainault 3967B*; Isortoq Fjord, *Webber 339*; Kangirdluak, *Webber 1303*; Lake Harbour, *Malte 120326*; Lower Savage Islands, Savage Harbour, *Consaul et al. 2352*; Ogac Lake, *Aiken & Leblanc 04-091*; Pangnirtung, *Malte 118458*; Pond Inlet, *Polunin 661*; NWT, Banks I, Cape Kellett, July 1969, *Kuc s.n.*; Sachs Harbour, *Gillett 18880*; Boothia Peninsula, Pelly Bay, *Cooper 130*; Nunavut, Coats I, Cape Pembroke, *Gillett 16890*; NWT, Chesterfield Inlet, *Porsild 6139*; NWT, Hall Beach, *Hainault 4088*; Nunavut, Southampton I, Southampton, *Malte 120655*; Nunavut, Winter I, *Duman 2200-A.*

P. phryganodes

CANADA. Nunavut, Axel Heiberg I, Expedition Fiord, Iceberg Glacier, *Beschel 12855*; Marine sediment and sand flats along NW shore of Flat Sound, *Consaul et al. 2288*; Nunavut, Baffin I, Arctic Bay, *Dutilly 1412*; Cape Dorset, *Malte 120339*; Clyde River, *Dutilly 9389*; Cormack Bay, *Aiken 89-037*; Grant Suttie Bay, *Webber 277a*; Lake Harbour, *Malte 118444*; Lower Savage Islands, V-shaped lake 1.5 km S of Savage Harbour, *Consaul et al. 2353*; Pangnirtung, *Malte 118443*; Pond Inlet, *Malte 118441*; Winton Bay, *McLaren 119*; NWT, Banks I, Sachs Harbour, *Gillett 18920*; Nunavut, Bathurst I, Bracebridge Inlet, *Tener & Harington 76*; Nunavut, Bylot I, *Freudieu 916*; Cornwallis I, *Mackay 3*; Nunavut, Devon I, Dundas Harbour, W of harbour mouth, *Consaul et al. 2307, 2316, 2318*; Nunavut, Ellesmere I, Craig Harbour, *Malte 118449*; Knud Penn, *Gillett & Shchepanek 18216*; Tanquary Fjord, *Brassard 1650*; Igloolik I, *Scott 384*; Nunavut, King William I, Victory Point, *Cooper 183*; Nunavut, Lougheed I, south, *Edlund & Nixon 2060*; Nunavut, Melville I, Murray Inlet, *Aiken & Edlund 3895*; Nias Point, *Edlund 64*; Prince Charles I, south shore, *Baldwin 1915*; NWT, Prince Patrick I, Green Bay, valley at head of north arm of bay, *Consaul & Gillespie 2411*; Mould Bay, *Consaul & Gillespie 2416*; Mould Bay Head, 14 km N of Mould Bay, *Consaul & Gillespie 2414*; Mould Bay Peninsula, east coast, ~11 km E of Mould Bay, *Consaul & Gillespie 2404*; Nunavut, Somerset I, Fort Leopold, *Malte 118452*; Nunavut, Southampton I, Coral Harbour, *Beckett 9*; Nunavut, Stefansson I, North East Corner, *Edlund 405*; Nunavut, Victoria I, Cambridge Bay, *Gillespie 5850*; Cambridge Bay, *Stephens 1207*; Collinson Penn, *Edlund & Argus 12758*; NWT, Victoria I, Holman I, *Porsild 17242*; Minto Inlet, *Edlund 160*; Storkerson Penn, *Edlund 296*.

P. vahliana

CANADA. Nunavut, Axel Heiberg I, Buchanan, *Beschel 11023*; South Fjord, *Beschel & Hegg 11451*; Arctic Bay, *Malte 118467*; Nunavut, Baffin I, Iqaluit, *Aiken 94-024*; Iqaluit, *Gillespie & Soreng 6794*; Savage I, *Wynne-Edwards 7301*; Isortoq River, *Webber 630*; Longstaff Bluff DEW Line Site, *Parmelee & Seaborn 4059, 4096a* (DAO); Lower Savage Islands, Savage Harbour, *Consaul et al. 2346*; NWT, Banks I, Masik River, *Mason 104b* (DAO); Sachs Harbour,

A. E. Porsild 17509; Nunavut, Bathurst I, Allison Inlet, *Aiken & Edlund 3941*; Polar Bear Pass, *Aiken 3950*; Boothia Peninsula, Sanagak River, *Maddison M1358*; Nunavut, Cornwallis I, Resolute, *Gillespie 5812, 5815*; *Aiken & Consaul 91-052*; Nunavut, Devon I, Dundas Harbour, W of harbour mouth, *Gillespie et al. 6682*; *Consaul et al. 2309*; Prince Alfred Bay, *Beschel 10741*; NWT, Ellef Ringnes I, Isachsen: 3.5 mi. WNW of station, *Savile 4372* (DAO); Nunavut, Ellesmere I, Kelsall 58; Caledonian Bay, Waterston 202b/72; *Consaul & Gillespie 2219, 2236*; Canyon Fjord, *Troelsen 18*; Cape Herschel. East coast, *Bridgland 475* (DAO); Craig Harbour, *Polunin 849*; *Soper 111374*; D'Iberville, *Consaul & Gillespie 2211, 2212*; Fosheim Peninsula, Ridge Lake, *Consaul et al.* 2293; Lake Hazen, *Powell 645*; Near John Richardson Bay, *Aiken & MacCormac 98-044*; Otto Fjord, June 18/64, *Hattersley-Smith s.n.*; Snowgoose River, *Longton 2016*; Tanquary Fjord, *Haight 4*; Two Basin Lake, 23 km ESE of Eureka airstrip, 100-150 m, *Gillespie 5808*; Nunavut, Hans I, 18-Jul-72, *Macdonald & Macdonald s.n.*; Nunavut, Igloolik I, Turton Bay, Aug 21/76, *Lewis s.n.*; Karluk I, *Aiken 3939*; Nunavut, Melville I, Ibbett Bay, *Aiken & Edlund 3936*; Marie Bay, *Aiken & Edlund 3877*; Sproule Peninsula, *Aiken & Edlund 3847A*; Nunavut, Melville Peninsula, Committee Bay, *Woodruff 27* (DAO); Hall Beach, *Beschel & Webber 660*; NWT, Banks I, S.W. coast north of Cape Lambton, *A. E. Porsild 17539*; Nunavut, Resolution I, *Dutilly 9251*. Nunavut, Victoria I, Cambridge Bay, *Gillespie 5845*; *Edlund & Argus 12881*; Mount Lady Pelly, *Jones & Hainault 1890* (DAO); Storkerson Peninsula, *Edlund 317*; NWT, Victoria I, Holman, *A.E. Porsild 17244*; Nunavut, Ward Hunt I, *Christie 30*.

P. wrightii

CANADA. NWT, Banks I, Egg River, 24 July 1971, *MacInnes s.n.*

Appendix J: Key to species of *Puccinellia* in the North American Arctic

1. Pedicels smooth or with a few occasional scabrules; lemma apical margins smooth or crenulate.

    2. Lemmas 3–5 mm long; palea keels scabrous distally, frequently with curly hairs on the lower portion; anthers 1.0–2.0 mm long.

        3. Culms 5–15 cm; panicles 2–4 cm long, branches erect to slightly ascending……..……...……..…….. *P. vahliana*

        3. Culms 15–40 cm; panicles 5–8 cm long, branches usually ascending to horizontal..…….……..……*P. wrightii*

    2. Lemmas 2–3 mm long; palea keels glabrous or slightly ciliate; anthers 0.5–0.9 mm long.

        4. Lemmas 2–2.5 mm long, apices obtuse; glume and lemma margins smooth to crenulate; palea keels glabrous…….*P. tenella* subsp. *langeana*

        4. Lemmas 2.5–3.0 mm long, apices acute; glume and lemma margins smooth but not crenulate; palea keels slightly ciliate……..….*P. alaskana*

1. Pedicels slightly to densely scabrous; lemma apical margins scabrous, sometimes with only a few scabrules.

    5. Lemmas (2.1–) 2.3–5.2 mm long; anthers 0.8–2.2 mm long.

        6. Anthers (0.9)1.2–2.2 mm long, roots slender and wavy…..…*P. arctica*

        6. Anthers 0.7–1.2 mm long, roots moderately thick and crinkled.

            7. Inflorescence often less than 4 cm long; lemmas with rounded back and often with an incurved tip; rachilla between first and second lemma 0.8–1.3 mm long; second glume 1.7–2.3 mm long, its length/width ratio 0.5–0.8……..*P. bruggemannii*

            7. Inflorescences often more than 4 cm long; lemma tip not incurved; rachilla between first and second lemma 1–1.7 mm long; second glume 1.9–3.0 mm long, its length/width ratio 0.3–0.6

                8. Hair on lemma extending to 0.7–1.5 mm from base; palea usually with hairs on basal half, hairs extending 1.8–2.8 mm from palea apex; inflorescence branches usually moderately to strongly scabrous…….....*P. angustata*

8. Hair on lemma extending to 0.1–1.0 mm from base; palea never with hairs on basal half, hairs extending to 1.6–1.9 mm from palea apex; inflorescence branches usually slightly scabrous.

9. Lemma apical margin irregularly serrate or moderately to strongly erose, slightly irregularly scabrous; inflorescences usually distinctly exserted from the sheaths……..*P. andersonii*

9. Lemma apical margin entire to slightly erose, strongly and evenly scabrous; infloescences usually barely exserted from the sheaths .....…*P. vaginata*

5. Lemmas 1.5–2.3 (–2.7) mm long; anthers 0.3–0.8 mm.

10. Leaves 3–7 mm wide; palea keels shortly ciliate in proximal half, anthers 0.8–1.0 mm……..…….……...*P. distans*

10. Leaves <3 mm wide; palea keels spinulose in upper half; at least some paleas with curly hair in proximal half or glabrous but not shortly ciliate in lower half; anthers <0.8 mm

11. Palea keels smooth in proximal half; lemmas 1.5–1.8(1.9) mm ……..…….……...…….. *P. hauptiana*

11. Palea keels usually hairy in proximal half; lemmas >1.7 mm

12. Plants 50–80 cm tall; panicles 11–25 mm long, diffuse; branches lax. …..…….……..……. *P. nuttalliana*

12. Plants <50 cm tall; panicles <10 cm long, diffuse or dense, branches erect or lax

13. Plants 20–25 cm tall; leaves 1.5–2 mm wide; panicles 7–10 cm long, dense, branches erect to lax; lemmas 2.3–2.5 (3.0) mm……..……..…….…*P. neglecta*

13. Plants 5–20 cm tall, leaves 0.7–1.2 mm wide; panicles 2–8 cm long, slender, branches usually erect, lemmas 1.7–2.3 mm……..……...*P. banksiensis*

Appendix K. Letters of permission to reprint papers in chapters of this thesis.

Chapter 3. With reference to manuscript: Systematics of North American arctic diploid *Puccinellia* (Poaceae): Morphology, DNA content, and AFLP markers. *Systematic Botany*.

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Chapter 4. With reference to manuscript: A new species of Alkaligrass (Puccinellia, Poaceae) from the western North American Arctic. *Novon.*

16 July 2007

Laurie Consaul
Research Division
Canadian Museum of Nature
P.O. Box 3443, Station D
Ottawa, ON K1P 6P4

Dear Laurie,

Thank you for your email requesting permission to reproduce a version of your manuscript, "A new species of Alkaligrass (Puccinellia, Poaceae) from

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