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Phylogenetic and phyloclimatic inference of the evolution of Potentilleae (Rosaceae)

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Doctoral Thesis

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In this thesis, I have summarized the content of the five papers I have worked with in the scope of my Ph.D project. The thesis addresses different aspects of the evolution of a group of plants in the Rose family (Rosaceae). The work has concerned the tribe Potentilleae with special emphasis on species in the *Potentilla* clade, as defined by Eriksson et al. (2003). The effect of allopolyploidization on an evolutionary time scale is the central theme around which this thesis is structured. Consequences of past climate change on evolution has also been addressed.

To everyone that have devoted time to read and comment upon the text, thank you! I am truly grateful for having you as my friends.

Vallda Sandö, May 2010

»Natural selection merely modified, while redundancy created.«

Susumu Ohno 1970

CONTENTS

SVENSK SAMMANFATTNING

I denna avhandling har jag sammanfattat innehållet i de fem artiklar jag har arbetat med i mitt doktorandprojekt. Mitt projekt har handlat om evolutionen av en grupp växter i rosfamiljen (Rosaceae). Växtgruppen är tribusen Potentilleae i underfamiljen Rosoide. I denna tribus ingår växter som smultron och jordgubbar *(Fragaria)*, daggkåpa *(Alchemilla)*, Tok *(Dasiphora)* och fingerört *(Potentilla)*. Arbetet har framförallt inriktats på släktet *Potentilla* och den roll som hybridisering har haft under utvecklingen av denna växtgrupp. Hybridisering som leder till att avkomman får dubbel uppsättning av föräldraarternas kromosomer kallas för allopolyploidisering, och är något som man tror har hänt många gånger under utvecklingen av alla blomväxter.

Jag har visat att flera fall av allopolyploidisering troligen skett under utvecklingen av Potentilleae och även kunnat visa hur släktskaps förhållandena i gruppen ser ut. Ett projekt inriktades på en grupp inom *Potentilla*, kallad ivesioid Potentilleae, som troligen utvecklades under en period av klimatförändring i Nordamerika för 25 millioner år sedan. Genom att skapa modeller för det klimat gruppen troligen levde i under olika tidsperioder kunde jag visa att förändringar i klimatet och utvecklingen av gruppen varit knutna till varandra och lett till den geografiska spridning av arter som man kan se idag.

I en annan undersökning fann jag indikationer på att senaste istiden också har påverkat utvecklingen av *Potentilla*, denna gång i Arktis. Området kring Berings sund var isfritt under denna period, och många djur och växter var isolerade i detta refugium under långa perioder. Vi fann tecken på att fler områden fungerat som refugier för växter inom *Potentilla* och att dessa spred sig och korsade sig med varandra när isen drog sig tillbaka.

I ett avslutande projekt har jag undersökt en metod för att skilja olika DNA kopior åt, för att vidare kunna undersöka släktskap och allopolyploidisering inom Potentilleae. Metoden används ofta vid ekologiska undersökningar av mikroorganismer, men visar sig även vara ett användbart verktyg vid undersökning av allopolyploidisering hos växter.

I mitt arbete har jag visat att allopolyploidisering troligen spelat en stor roll för utvecklingen av Potentilleae, och att tider med klimatförändringar också har bidragit till den diversitet som gruppen uppvisar.

ABSTRACT

Polyploidization has occurred many times during the evolution of angiosperms. Allopolyploidization is believed to be the process behind many of these genome duplications, and has resulted in a genetically diverse angiosperm flora. I have investigated the patterns of allopolyploidization in the tribe Potentilleae (Rosaceae), where many species have been proposed to have an allopolyploid origin. I have assessed the extent of allopolyploization in the group, with special emphasis on genus *Potentilla,* by comparing a topology based on nuclear data to one based on plastid data. This led to the identification of several incongruences that supports the notion of a reticulated evolution of the group. However, this has to be confirmed as not all incongruences identified with this method have to be the result of hybridization. Instead, further phylogenetic inference of relationships among the proposed allopolyploid species has to utilise low-copy nuclear genes.

For future prospects, I have therefore evaluated Temperature Gradient Gel Electrophoresis (TGGE) for separating paralogues of low-copy nuclear genes. The method was found to require fewer PCR and sequencing reactions, compared to bacterial subcloning, a method routinely used to separate heterogeneous DNA samples. TGGE was therefore found it to be an efficient and applicable method for separating gene copies for phylogenetic investigations of allopolyploid species.

The work presented in this thesis has also provided new insights into the evolution of Potentilleae. The phylogenetic analysis show that the ivesioid Potentilleae, a morphologically aberrant and diverse group comprising the three North American genera *Ivesia, Horkelia* and *Horkeliella,* form a well-supported clade nested within the *Potentilla* clade. Furthermore, a dated phylogeny of the family Rosaceae finds this clade to have originated approximately 25 Ma, a time when climate change is believed to have reshaped the flora of western North America. The analysis using phyloclimatic modeling of the evolution of the group reveals a close connection to climate change. The results indicate Great Basin as the area of origin and a westward range expansion to Sierra Nevada during Miocene. Several lineages were found to have crossed the mountain range after a Mediterranean type of climate had established in California.

The analysis of microsatellite and AFLP data propose that climate change also have influenced the genetic diversity in Arctic populations. Geographical patterns of this diversity corroborate the hypothesis that Beringia served as a refugium for plants during the Wisconsinan glaciation. Evidence of additional refugia on Banks, Prince Patrick and Melville Islands was also found and further supported by geological data on ice expansion at the last glacial maximum.

From the results of the investigations conducted during my thesis work I therefore conclude that allopolyploidization and climate change have had a great influence on the evolution of Potentilleae.

Keywords: *Potentilla, Ivesia, Horkelia,* allopolyploidization, TGGE, Beringia

PAPERS INCLUDED IN THE THESIS

This thesis is based on the following papers, which are referred to by their Roman numerals.

(I) **Töpel M**, Lundberg M, Eriksson T, Eriksen B. Molecular data indicate several putative hybridization events in the genus *Potentilla* (Rosaceae). Manuscript.

(II) Lundberg M, **Töpel M**, Eriksen B, Nylander JA, Eriksson T. (2009) Allopolyploidy in Fragariinae (Rosaceae): comparing four DNA sequence regions, with comments on classification. Mol Phyl Evol. 51(2). 269-280.*

(III) Eriksen B, **Töpel M**, (2006) Molecular phylogeography and hybridization in members of the circumpolar *Potentilla* sect. *Niveae* (Rosaceae). Am J Bot. 93. 460-469.*

(IV) **Töpel M**, Antonelli A, Yesson C, Eriksen B. Phyloclimatic modeling of the evolution of the ivesioid Potentilleae (Rosaceae). Manuscript.

(V) **Töpel M**, Brosché S, Scheen AC. Temperature Gradient Gel Electrophoresis (TGGE): an alternative to cloning for separation of low-copy gene paralogues. Manuscript.

MT was responsible for writing papers I, IV and V, and a contributor to paper III. Contribution by MT to paper II was in the form of DNA sequences and comments on the manuscript. Responsibility for the laboratory work in paper III was shared between the coauthors but preformed entirely by MT for papers I, IV and V.

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INTRODUCTION

Polyploidy

Polyploidy is the occurrence of more than two complete sets of chromosomes in an organism and is a phenomenon often observed in angiosperms (Otto, 2007). Some studies even suggest that all angiosperms are derived from at least one polyploidization event (Masterson, 1994; Soltis, 2005; Freeling and Thomas, 2006). Two processes are usually recognized to lead to polyploidy. Genome duplication within a species is termed autopolyploidization, and interspecific hybridization followed by genome duplication is called allopolyploidization (Kihara and Ono, 1926, Parisod et al., 2009). This classification is not entirely discriminating and consequently debated by some authors (Ramsey and Schemske, 1998). Furthermore, allopolyploidization is presumably more common than autopolyploidization (Parisod et al., 2009) and is the process studied in this thesis.

Pros and cons of allopolyploidy

The reticulated background of allopolyploid species makes them genetically diverse and has been suggested as one reason for their success. The diverse origins of allopolyploid species make them capable of responding to changing environmental conditions, pathogens etc. It is therefore likely that some individuals of an allopolyploid species will survive under changing conditions (Soltis, 2005).

Hybridization may therefore be an efficient way of creating diverse and vivid populations, but can also have a negative effects on fitness. Hybridization between two species will most often result in a sterile offspring as an effect of incomplete pairing of homologous chromosomes during meiosis (Bell, 1959). Winge (1917) observed that chromosomes in diploid hybrids could be too different to form pairs during meiosis. Polyploidization circumvents this problem by creating duplicate chromosomes. However, homeologous chromosomes (of different parental origin) may still be sufficiently similar and can prevent paring of homologous chromosomes during meiosis. Further rearrangements of DNA may therefore be required for the allopolyploid to form sufficiently dissimilar chromosomes and stabilize the genome (Eckardt, 2001; Wolfe, 2001).

Diploidization is the process whereby a polyploid genome is turned in to a diploid (paleopolyploid) genome. However, the overall mechanisms controlling these chromosomal rearrangements are largely unknown (Ma and Gustafsson, 2009; Gaeta and Pires, 2010) and do not have to be simultaneous for all chromosomes (Wolfe, 2001). Subsequent diploidizations has taken place in independent genetic

lineages, and as a consequence, two different diploid species do not necessarily have the same number of chromosomes. Therefore, whether a plant is to be considered a polyploid or a diploid is only a question of scale and has to be viewed in relation to its closest relatives (Soltis, 2005). Liu and Wendel (2002) went as far as arguing that there are no *bona fide* diploid species in the plant kingdom.

Polyploidy in eukaryotes

Polyploidy is rarely observed in animals but has apparently served as an efficient mechanism for evolution in plants. Ohno (1970) proposed that the early evolution of vertebrates was promoted by polyploidization but that the emergence of the X/Y sex-chromosome system prevented further genome duplications. This system is not so common in plants (but see Grant et al., (1994), and LebeI-Hardenack and Grant (1997) for some exceptions) in which flower morphology, and hence the sex of the flower, is controlled by a number of transcription factors of the MADS gene family (Causier et al., 2009). Ohno (1970) also presented polyploidization as the means by which evolution of new traits can take place. He argued that it is unrealistic to think that point mutations of an essential gene alone could create new functions of a protein. Especially if the new function requires that several functional sites have to be modified. Likewise, Wolfe et al. (1997) showed that the genome of *Saccharomyses cerevisiae* also is of polyploid origin, and hence, demonstrated that polyploidization also occurs in fungi. Polyploidization can therefore be considered an influential factor in the whole eukaryote tree of life, but has been prohibited in certain evolutionary lineages due to arrangements of the DNA at the chromosomal level.

Allopolyploid Species

Whether an offspring from a cross between lineages with different evolutionary histories is considered as a separate (allopolyploid-) species or as part of one of the parental species depends, to a large extent, on the morphological characters being expressed in the offspring. If the morphology of the offspring overlaps with the parental lineages, they may all be treated as a single species with several ploidal levels. *Potentilla argentea* (2n=2x, 4x, 5x, 6x, 7x, 8x, 12x) is an example of the latter, as have been shown in crossing experiments (Holm, 1995; Holm and Ghatnekar, 1996). This species comprise individuals with different ploidal levels that to a large extent share overall morphology, but also significant genetic diversity (Holm, 1995). Intraspecific crosses yield offspring with different ploidal levels. Hence, whether these offspring are allo- or autopolyploids, according to Kihara and Ono's (1926) definition depends on taxonomical decisions.

Based on a study of *Elytrigia repens* and *E. intermedia* (Poaceae) and crosses between the two species, Mahelka et al. (2007) showed that interspecific hybridization is often underestimated when the parental species are morphologically similar and the hybrid exhibits characters that overlap with those of the parental species. Thus, many reticulations may go undetected if only one individual from each species is sampled in an allopolyploidization study.

Allopolyploidization may also result in offspring with a morphology that differs from the parental lineages. Comai et al. (2000) have showed that *in vitro* allotetraploids of *Arabidopsis* (*A. thaliana* x *Cardaminopsis arenosa*) displayed a high level of variation in morphology, and were clearly different from the two parental species in their morphology. The fact that their experiment did not result in a morphologically coherent group of offspring is of particular interest. Whether this is a naturally occurring phenomenon, or is restricted to laboratory experiments, is yet to be investigated. Still, if the different offspring from such crosses form new populations, interpreting relationship among them using morphological data would be difficult.

However, the opposite phenomenon is known to occur in natural populations. Different allopolyploidization events can give rise to morphologically similar offspring. Such individuals are, despite their different origins, most often considered part of the same species. This situation is illustrated by the two allopolyploid species *Tragopogon mirus* and *T. miscellus* that have as many as 9 and 21 origins respectively (Soltis et al., 1995).

As the examples mentioned above illustrates, the sampling strategy can influence the outcome of a study of allopolyploidization. I have for this reason used the fairly broad definition proposed by Harisson (1993) that states that hybridization is *"the interbreeding of individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters"*. Species are not always the biologically important unit in allopolyploidization studies, as exemplified by *Potentilla agrentea*, but they are still very influential on how we view evolution of organismal lineages.

POTENTILLEAE

Several taxonomic re-arrangements in the tribe Potentilleae have preceded the work of contemporary studies (for a review see Eriksson et al., 1998). Resent studies have shoved that Potentilleae form a well supported monophyletic group in the subfamily Rosoidae (Morgan et al., 1994; Eriksson et al., 2003). A formal definition of Potentilleae was coined by Eriksson et al. (2003), which uses stem-based phylogenetic definition for the tribe, and states that *Potentilleae* is *"the most inclusive clade containing Potentilla reptans but not Sanguisorba officinalis, Agrimonia eupatoria, or Rosa cinnamomea"*. The corresponding clade contains the two sub-clades Fragariinae (comprising ten genera) and a clade of species most often recognized as part of the genus *Potentilla* (with the vernacular name Cinquefoil) and the three North American genera *Ivesia* Torr. & Gray, *Horkelia* Cham. & Schltdl. and *Horkeliella* (Rydb.) Rydb., collectivity called the ivesioid Potentilleae (Ertter, 1998).

Fragariinae comprise genera such as *Alchemilla* L. (Lady's mantle), *Fragaria* L. (Strawberries), *Comarum* L. (Swamp Cinquefoil) and *Dasiphora* Raf. (Shrubby Cinquefoil). Several species in the clade have sometimes, as the vernacular names implies, been included in *Potentilla* (Wolf, 1908; Schulze-Mentz, 1964). The clade was first recognized by Eriksson et al. (1998) who also showed that it contains a few species that are still classified in *Potentilla*.

The diversity in the circumpolar genus *Potentilla* is well described and have since the days of Linnaeus been presented in numerous Floras and floristic treatments (e.g. Linnaeus, 1753; Rydberg, 1898 & 1908; Wolf, 1908). Wolf (1908) completed the most extensive taxonomic work to date on the relationships in the genus. He recognized 305 species as well as numerous naturally occurring hybrids in his monograph, and based his classification within the genus on style morphology. This morphological character has in later studies proved to corroborate molecular evidence of relationship in *Potentilla* and the sister clade Fragariinae *(Paper I and II).*

Species in the ivesioid Potentilleae, currently classified in the three genera *Ivesia*, *Horkelia* and *Horkeliella* (Ertter, 1993), have either been included in *Potentilla* or been treated as separate genera (Ryberg, 1898; Jepson, 1936; Keck, 1938). Recent molecular studies have shown that the group forms a well-supported clade nested within *Potentilla* (as defined by Wolf, 1908) as a sister-clade to members of section *Rivales* (Eriksson et al., 1998 & 2003; Dobes and Paule, 2010; *Paper I*). Few chromosome numbers have been determined in the group, but both tetraploid and hexaploid species have been reported (B. Ertter, personal communication). This indicates that allopolyploidization could have influenced the evolution of the group, but this remains to be investigated. The sister clade to the ivesoid Potentilleae clade contains species from section *Rivales*. Two species, *P. norvegica* and *P. biennis*, from this group have been included in the analyses presented in *Paper I* and *IV*. These analyses show that the nuclear- and chloroplast phylogenies yield incongruent results

about the position of *P. norvegica*. This could be interpreted as an indication of hybridization, and is further corroborated by the high and varying ploidal levels in species from section *Rivales*. The rest of Potentilleae also includes many polyploids, along with artificially created hybrids, e.g *Fragaria* x *ananassa* (common garden strawberry) and *P. argentea* (Holm and Ghatnekar, 1996). This makes Potentilleae a suitable group for studies of allopolyploid speciation in plants.

Hybridization in Potentilleae

The chloroplast genome is maternally inherited in most angiosperms (Morgensen, 1996). A phylogenetic analysis using chloroplast molecular markers will therefore only infer the relationships of the maternal lineages. The nuclear genome is inherited from both parents and an analysis using nuclear markers can, depending on what marker is being used, infer either of the maternal or the paternal lineages or both. The nuclear markers ETS and ITS (External- and Internal Transcribed Spacers) which we have used occur in a multitude of copies in the genome and are thought to evolve under concerted evolution. This means that differences in copies are homogenized and that sequence variation within an organism is reduced or completely lost (Álvarez and Wendel, 2003). Therefore, either the maternal or the paternal sequence will be displayed by an allopolyploid, or on occasions both (Soltis and Soltis, 1991 & 1995). Thus, hybridization can lead to incongruent patterns shown by nuclear and chloroplast markers, and can be identified by comparing a nuclear phylogeny to the pattern from chloroplast data. However, not all incongruences identified with this method have to be the result of hybridization. Apart from random errors, phenomena such as incomplete lineage sorting and gene duplication (paralogy) can also lead to incongruences between phylogenies.

The molecular markers used in this study do not have the power to detect all allopolyploidization events, but have the advantage of being easily amplified and sequenced, and are therefore appropriate for the kind of screening we have performed to identify groups for further studies of allopolyploidization. Once groups that are suspected to have been involved in allopolyploidization have been identified, low-copy number nuclear genes can be used to further investigate these reticulations (Popp et al., 2001, Smedmark et al., 2003, Popp et al., 2005). These markers require that the different paralogues of the amplified gene are separated before sequencing, a step often preformed using bacterial subcloning. The protocol commonly employed requires many more PCR and sequencing reactions than direct sequencing of a the PCR product does. It is therefore less suitable for screening large groups of species for indications of allopolyploidization, especially if the ploidal level of the investigated species are unknown.

We have looked for indications of hybridization in *Potentilla* and made a first assessment of the relationships in the genus (*Paper I*). Few phylogenetic investigations of the genus have previously been published (but see Eriksson et. al, 1998 and 2003, Dobes and Paule, 2010). Eriksson et al. (2003) compared a nuclear and a chloroplast phylogeny including 18 species from *Potentilla* and found that six of them belonged in the sister clade Fragariinae, but also reported one or possibly two putative hybridization events. We extended the dataset from Eriksson et al. (2003) to include 64 species of *Potentilla*. Our comparison of the chloroplast and nuclear phylogenies identified six major clades in *Potentilla*, and several cases of incongruence. We therefore hypothesize that interspecific hybridization have occurred in the genus and is a plausible explanation for the variation in ploidal levels found. We also showed that there is a good concordance between the pattern recovered from molecular data and the groups identified by Wolf (1908) based on style type. The analysis also showed that the ivesioid Potentilleae (Ertter, 1998), comprising species from the three genera *Ivesia*, *Horkelia* and *Horkeliella*, form a well-supported monophyletic group in both the nuclear and chloroplast phylogenies.

We used the same approach in *Paper II* and found indications that allopolyploidization also influenced the diversity in the sister clade Fragariinae. The Fragarineae clade comprise ten genera and at least two species that are currently classified in *Potentilla*. Five well-supported incongruences were found, and although data on chromosome counts and hence ploidal levels are sparse from the clade, allopolyploidization is a plausible explanation for many of them.

From the two investigations presented in *Paper I* and *II*, we therefore conclude that denser sampling and the use of low-copy nuclear markers will probably reveal a great deal of reticulations in the Potentilleae clade.

SEPARATION OF PARALOGOUS GENE COPIES

Evolutionary relationships among plant species have often been inferred using the ITS region or chloroplast markers, but as we have seen in *Paper I* and *II*, these markers are not always sufficient to analyze the evolutionary history of hybridizing species. Low-copy nuclear genes are therefore often used (Popp et al., 2001; Smedmark et al., 2003, Popp et al., 2005). Low- or single-copy genes in homozygous allopolyploid species will have one copy of a particular gene inherited from the maternal side and one from the paternal side. By incorporating both copies in a phylogenetic analysis, it is possible to infer both sides of inheritance in a single phylogeny, and hence make inference of the reticulate phylogeny of a group.

Direct sequencing of a PCR product generated from a nuclear DNA region is often problematic if different paralogues and/or genes are amplified. A method for separating unique sequence copies is therefore required. Bacterial subcloning is often used for this but is both time consuming and expensive compared to direct sequencing. In this method is amplified fragments inserted in bacteria that are allowed to multiply on an agar plate. The resulting colonies are presumed to originate from a single cell and therefore only contain one version of the inserted fragment. The different copies are this way efficiently separated and the colonies can serve as template for re-amplification of the desired fragment. The fact that each colony, and hence the PCR template, originates from one sequence copy makes the method prone to PCR artifacts such as PCR recombination and Taq polymerase errors (Cronn et al., 2002).

A method using paralogue specific sequencing primers has also proven to be efficient (Scheen et al., in prep.). The multicopy sample is here sequenced and differences between the copies are identified in the sequence-editing step. Specific primers with 3'-ends matching the different copies are then designed for sequencing of the different copies.

In *Paper V*, we compare a method routinely used in ecological studies, to bacterial subcloning for separation of paralogues of single copy genes. Thermo Gradient Gel Electrophoresis (TGGE) is often used in microbial population analysis (Lessa and Applebaum, 1993; Muyzer, 1998) to separate genes in multiple taxon samples to measure shifts in biodiversity under different environmental conditions. However, it has to our knowledge not been used for separation of paralogues for phylogenetic analyses, but has a number of advantages compared to other methods.

TGGE takes advantage of the fact that a double stranded DNA fragment will move faster through a polyacrylamide gel than a partly or fully melted fragment. The melting temperature is dependent on the base composition of the DNA fragment. By migrating a PCR sample containing different paralogues of a gene over a temperature gradient, each allele will eventually reach a position in the gel

where the temperature equals its melting temperature. When the double stranded DNA fragment starts to melt, the two strands begin to separate, and this "pitch fork" like molecule will move much slower through the gel. The remaining sample will still be in a double stranded conformation and will continue to the next critical temperature in the gradient. The paralogues are thus effectively separated and can be excised from the gel and eluted to serve as templates for re-amplification and subsequent sequencing.

One advantage with TGGE is that the number of PCR reactions needed to successfully recover all amplified paralogues in a sample is much lower than what is necessary for other methods. Rautenberg et al. (2008) calculated the 95% probability of sampling all sequence copies separated by bacterial subcloning. Their calculations showed that sampling of eight colonies from a two copy sample and 13 colonies from a three copy sample is required for a 95% probability of recovering all sequence copies (given that the initial PCR primers used amplify all existing sequence types, and with equal probability). In total, 21 reamplification (PCR) and sequencing reactions would therefore be required for the two samples we used. With TGGE, only five re-PCR and sequencing reactions were needed.

Furthermore, separation of sequence copies with TGGE is also potentially less sensitive to PCR artifacts, because more than one PCR amplicon is amplified and sequenced. The specificity of the PCR product can also be an issue when using other methods for paralogue separation like cloning or allele specific primers. With TGGE, primer dimer or undesired fragments amplified in the initial PCR reaction are efficiently separated from the sought paralogues and accumulated elsewhere in the gel. We therefore believe that the method is particularly useful for large-scale phylogenetic analyses of polyploid taxa.

THE EFFECT OF CLIMATE CHANGE ON EVOLUTION

The Arctic

Climate change often has severe effects on plants and animals and sometimes leads to extinction of species (Pounds et al., 1999) or shifts in spatial distribution (Thomas et al., 2004). These often catastrophic occurrences are key events that have shaped the diversity we observe today and have been important factors in the evolution of organismal lineages.

Erik Hultén (1937) proposed that an area spanning from the river Lena in northeast Siberia, across the Bering straight to river McKenzie in northwest Canada had been ice-free and had served as a refugium for plants and animals during the last glacial maximum (LGM). He further hypothesized that many plants had dispersed from Beringia, as the ice retreated, and recolonized the Arctic in post-glacial time. Refugia are assumed to harbor plants with a greater genetic diversity than adjacent areas (Comes and Kadereit, 1998; Taberlet et al., 1998). This is because organisms within the refugia have had a longer time to accumulate mutations compared to populations formed by postglacial migration. The newly established populations are also prone to founder effect influence if only few or a single individual managed to disperse to the novel area.

We used microsatellite and AFPL data to find evidence for Arctic refugia for *Potentilla* sect. *Niveae* during the Wisconsinan glaciation in *Paper III*. Postglacial migration routs and indications of hybridization was also investigated in the group using these data. Morphological variation is large in the group and species limits are controversial. However, three morphological species complexes have been recognized (Eriksen, 1997), and genetical differences between these complexes were studied.

Microsatellites are short tandemly repeated sequence motifs found in the genome of many species (Weising and Gardner, 1999). Slipped strand mispairing is a mutation that can change the number of times a motif is repeated and thereby change the length of the PCR product generated using primers surrounding the satellite region of the marker. We treated each length of a marker as a unique allele and each unique combination of alleles as a haplotype. The geographical distribution of the identified haplotypes was then compared to geological evidence of the extent of the glaciation at LGM.

Our analysis supported the hypothesis proposed by Hultén (1937) and show that Beringia probably served as a refugium for *Potentilla* sect. *Niveae* during the Wisconsinan glaciation. The results also indicates that at least one more area (Banks Island in the Northwest Territories, Canada) served as a refugium for *Potentilla*. This hypothesis is supported by geological data that show that Banks

Island together with Prince Patrick Island and most of Melville Island, further to the northeast, probably remained unglaciated at the same time as Beringia prevailed (Dyke et al., 2002).

Besides high frequencies of genetic diversity in the two refugia, we found that the diversity was higher in areas corresponding to the edge of the ice sheets at LGM and on Banks Island than in the much larger area corresponding to Beringia. Banks Island and Prince Patrick together with Melville Islands were isolated for ~7000 years (Dyke et al., 2002) and served as two additional refugia in northern Canada. Adjacent areas could therefore have been repopulated by plants from these refugia when the ice retreated. Genetically diverse populations were thereby formed by the union of previously separated genetic lineages. The long isolation of Banks Island together with its intermediate position between Beringia and Prince Patrick/Melville Island has therefore formed the genetically diverse populations we can observe in the area today. .

The deserts of Western North America

In *Paper IV* we studied the evolution of the ivesioid Potentilleae (Ertter et al., 1998), a clade comprising the three genera *Ivesia*, *Horkelia* and *Horkeliella* from Western North America. Many of them grow under extremely dry conditions and have morphological features that have been interpreted as an adaptation to drought (Ertter, 1989). The geographical distribution of these three genera coincide with the arid areas of Western North America, a region that has seen a shift in flora composition with an increase of drought tolerant species during the Neogene (24-1.6 Ma) (Axelrod, 1948). Furthermore, recent phylogenetic studies has showed a sister clade relationship between the ivesioid Potentilleae and *Potentilla* section *Rivales*, a group of species with preference for mesic habitate. This has lead Ertter and Reveal (Flora of North America, in press) to hypothesize that the ivesoid Potentillae radiated from mesic *Potentilla* in response to the late Tertiary development of dry conditions in western North America.

We used phyloclimatic modeling to test if the proposed changes in climate could have had an effect on the evolution of the ivesioid Potentilleae clade. Phyloclimatic modeling uses bioclimatic models (Nix, 1986), dated phylogenies and ancestral state reconstruction to infer the climate preferences of nodes of different ages in a dated phylogeny. The optimized climate model for a node is then projected into a climate scenario for that age to indicate where a suitable environment once could have been found.

Sequences of the maturase K (matK) gene and part of the trnL gene together with the trnL-trnF ingergenic spacer from a representative set of Rosaceae species were used to infer the phylogeny of the family. The dated phylogeny, calibrated with

seven fossils, revealed an age of the family of approximately 100 million years. The Potentilla clade had a stem age of ~75 million years and the ivesioid Potentilleae clade appeared ~25 million years ago. The bioclimatic model for this node shows that the ivesioid Potentilleae probably originates from the foothills of Rocky Mountains and westward into the Great Basin.

We further tested if these variations in predicted geographical areas for different nodes depended on variations in the underlying climate scenarios or in the optimized models by projecting the models into present day climate. By keeping the climate scenario constant, any variation in geographical area prediction will depend on variations in the optimized models. By doing so, we could see if there had been any significant shifts in climate preferences during the evolution of the ivesioid Potentilleae. We could also illustrate the kind of climate the group evolved from, by identifying where this climate is found today.

The analyses proposed that the ivesioid Potentilleae clade evolved in Great Basin in a climate that corresponds to what is now found in and around Sierra Nevada. Furthermore, preferences for this type of climate prevailed as a westward range expansion to Sierra Nevada occurred between ~20-10 Ma. The ivesioid Potentilleae diverged in two clades during this time, but this split cannot be explained by changes in climate preferences. Instead, we suspect that there is an ecological explanation for this diversification. Morphologically the ivesioid Potentilleae species are divided in two groups. One group has narrow stamens and a shallow hypanthium, the other one has flattened upright stamens and a deep hypanthium, a morphology proposed to be an adaptation to bee pollination (van der Pijl, 1982). This adaptation is only found in one of the clades why we suspect that a change in pollination syndrome could have been the driving force of the diversification.

Both clades contains species found in Great Basin and Sierra Nevada, but species adapted to the Mediterranean type of climate found in California is only present in one clade. Preferences for this type of climate was found to have evolved sometimes between ~12-4.5 Ma which coincide with the appearance of this type of climate in California.

Our study therefore corroborated the hypothesis that ivesioid Potentilleae evolved in response to climate change in western North America, and shows that there has been a tight connection between the evolution of this morphologically aberrant and diverse group and past climate change.

CONCLUSIONS

Hybridization and allopolyploidization seems to have occurred frequently in Potentilleae, and are plausible explanations for the multitude of polyploid taxa in the group. The analytical method, that compares a nuclear phylogeny to one from chloroplast data, is suitable for screening large groups for incongruence, but will probably not identify all allopolyploidization events. Therefore, nuclear low-copy number genes have to be used for further investigations of allopolyploidization in the group.

TGGE is found to be a useful method for separating low-copy nuclear genes for phylogenetic investigations. In contrast to bacterial subcloning, the method will clearly show how many paralogues have been amplified in the initial PCR reaction, why the number of PCR- and sequencing reactions necessary to recover all of them are greatly reduced. It is also potentially less sensitive to PCR artifacts, because more than one PCR amplicon is amplified and sequenced. Although only few taxa have been tested, we predict the method to be faster and cheaper than bacterial subcloning for large-scale phylogenetic investigations of polyploid taxa.

Banks Island together with Prince Patrick- and Melville Island probably served as refugia for *Potentilla* sect. *Niveae* during the Wisconsinan glaciation, together with the much larger area of Beringia. Patterns of genetic diversity in present day populations also supports the idea that plants from these refugia migrated and repopulated the Arctic in postglacial time. AFLP-, microsatellite- and morphological data further suggests that hybridization has occurred in *Potentilla* sect. *Niveae* and is a plausible explanation for the taxonomic difficulties exhibited by the group.

The three North American genera *Ivesia*, *Horkelia* and *Horkeliella* form a well-supported clade in the Potentilla clade. The group originates from the western parts of Great Basin and split of from the rest of Potentilla approximately 25 Ma. At approximately 18 Ma, a split not driven by climate change or a shift in climate preferences led to diversification into two clades. A westward range expansion then took place between ~18-10 Ma in Great basin to Sierra Nevada, from where several independent adaptations to a Mediterranean type climate has happened after ~10 Ma.

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